Article

Not peer-reviewed version

Mechanosensitive Ion Channel-Related Proteins in Terminal Glial Cells of Human Meissner and Pacinian Corpuscles

Irene Amigo , Yolanda García-Mesa , Patricia Cuendias , Jorge Feito , Olivia García-Suárez , Ana Maria Abreu Velez , Iván Suazo , José A. Vega *

Posted Date: 30 April 2026

doi: 10.20944/preprints202604.2199.v1

Keywords: cutaneous end-organ complexes; terminal glial cells; Meissner corpuscles; Pacinian corpuscles; mechanosensitive ion channels; acid-sensing ion channels; transient receptor potential channels; PIEZO channels

 doi:10.20944/preprints202604.2199.v1

Article

Mechanosensitive Ion Channel-Related Proteins in Terminal Glial Cells of Human Meissner and Pacinian Corpuscles

Irene Amigo [1,*], Yolanda García-Mesa [2,3], Patricia Cuendias [2,3], Jorge Feito [2,4,5,6], Olivia García-Suárez [2,3], Ana M. Abreu-Velez [7], Iván Suazo [2,8] and José A. Vega [2,8]

[1] Servicio de Traumatología y Cirugía Ortopédica, Hospital Universitari Son Espases, Palma de Mallorca, Spain
[2] Departamento de Morfología y Biología Celular, Grupo SINPOS, Universidad de Oviedo, Oviedo, Spain
[3] Instituto de Investigación Sanitaria del Principado de Asturias, ISPA, Oviedo, Spain
[4] Servicio de Anatomía Patológica, Complejo Asistencial Universitario de Salamanca, Salamanca, Spain
[5] Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain
[6] Departamento de Biología Celular y Patología, Universidad de Salamanca, Spain
[7] Georgia Dermatopathology Associates, Atlanta, GA 30329, USA
[8] Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Providencia—Área Metropolitana, Santiago, Chile
* Correspondence: javega@uniovi.es

Abstract

Terminal glial cells (TGCs) are integral components of cutaneous end-organ complexes (CEOCs) and have traditionally been regarded as structural and trophic elements. However, increasing evidence suggests their involvement in mechanosensation and mechanotransduction. This study aimed to investigate the expression of mechanosensitive ion channel-related proteins in TGCs and axons of human Meissner and Pacinian corpuscles from glabrous skin at different anatomical sites. Using immunohistochemistry and immunofluorescence, we analysed the distribution of ASICs, ENaC subunits, PIEZO channels, and TRP family members. Axonal terminals showed widespread expression of these proteins, including PIEZO1/2, ASIC2, TRPC6, and TRPV4, consistent with their role in mechanotransduction. In contrast, TGCs displayed a more restricted and heterogeneous profile. ASIC2 and TRPV4 were consistently detected in Meissner corpuscles, whereas PIEZO1/2 and TRPA1 showed site-dependent expression. In Pacinian corpuscles, TGCs were positive for ASIC2, PIEZO2, TRPA1, and TRPV4. Notably, TRPA1 expression in TGCs is reported here for the first time. These findings support the idea that TGCs may modulate mechanosensory input, although their functional role remains to be elucidated.

Keywords: cutaneous end-organ complexes; terminal glial cells; Meissner corpuscles; Pacinian corpuscles; mechanosensitive ion channels; acid-sensing ion channels; transient receptor potential channels; PIEZO channels

1. Introduction

Glial cells associated with cutaneous end-organ complexes (CEOCs), also known as sensory corpuscles, represent a specialized subpopulation of peripheral glia referred to as terminal glial cells (TGCs) [1,2], skin end-organ glia [3] or terminal Schwann cells [4]. In vertebrate CEOCs, the TGCs envelop the peripheral axon terminals of low-threshold mechanoreceptors from development and throughout life [5]. They are variably arranged according to the CEOCs morphotype as "coin stack" (lamellar cells) in Meissner corpuscles, as symmetric hemi "onion bulb" (inner core lamellar cells) in the Pacinian corpuscles or irregularly organized as in Ruffini's corpuscles [6]. Classically, TGCs are

 doi:10.20944/preprints202604.2199.v1

hypothesized to play structural, supportive, and trophic functions (see [2]). However, emerging data suggests that TGCs are fundamental in the process of mechanosensing and mechanotransduction, i.e., the process whereby forces are transformed into receptor potential and action potential [7,8].

The mechanical properties of CEOCs periaxonal cells as well as the differentiations on the axonal membrane, were considered necessary and sufficient to generate the receptor potential and consequently the action potential (see for review [6,9]). Thus, according to this theory the mechanotransduction was regarded as a physical mechanical process. However, the discovery that mechanical forces can modify proteins present in mechanoreceptors opened a new conception about the mechanisms of mechanotransduction. The proteins that can be modified by the action of forces are collectively known as mechanosensors and include transmembrane proteins and membrane ion channels [10], cytoskeletal proteins [11], and components of the extracellular matrix [12]. Mechanosensors are organized into molecular complexes that are responsible for mechanosensing and mechanotransduction pathways.

The mechano-gated ion channels belong to different families, although at present the only mechanotransducers are the PIEZO channels PIEZO1 and PIEZO2 [10,13–15]. Consistently, it is believed that cellular components of CEOCs contain ion channels activated by forces or displacement which are responsible for the first step to transducing mechanical energy into electrical activity. Mechanosensing ion channels have been detected in the mechanoreceptor axon and in TGCs of CEOCs (see [16]). Thus, the presence of mechano-gated ion channels in TGCs supports the idea that they participate in mechanosensing and/or mechanotransduction, as recent studies have demonstrated [17–20]. On the other hand, in addition to the Piezo ion channels, members of other ion channel families have been shown to be involved in the process of mechanosensitivity but not in mechanotransduction. These include members of the transient receptor potential (TRP) superfamily in particular TRPA1, TRPC1, TRPC6, and TRPV4; members of the superfamily of epithelial Na^+ ion channels (ENaC) including the family of acid-sensing ion channels (ASIC) [16]. Some voltage-gated [21–24] or ligand-gated [25,26] channels have also been implicated in touch.

The present study was designed to analyse the presence of mechanosensitive ion channels (ASIC1, ASIC2 and ASIC3, γ- and β-ENaC, PIEZOs, TRPA1, TRPC1, TRPC6,) in cutaneous human CEOCs from the glabrous skin of different anatomical locations. The study aims to contribute to the knowledge of the participation of CEOCs' TGCs in mechanosensitivity and/or mechanotransduction.

2. Results

Negative controls performed by substituting the primary antibody for preimmune serum or by omitting the primary antibody in incubation resulted in a total absence of immunolabeling in controlled sections (data not shown).

On the other hand, it is necessary to point out that it was not always possible to analyze the presence of immunoreactivity for the assessed mechanoproteins because Meissner or Pacinian corpuscles could not be identified in the sections. This fact was particularly frequent in the case of Pacinian corpuscles that were not found in most of the lip and nipple sections and in low numbers in the foreskin and labia minora pudendis. In addition, when positivity is indicated in the terminal glial cells, it should not be understood that it was observed in all corpuscles, so in those cases the percentage is indicated.

As for the intensity of the immunoreaction or immunofluorescence, it was variable from one corpuscle to another, as normally occurs in human materials fixed in formalin and included in paraffin. In addition, it is well known that there is no stoichiometric relationship between the amount of antigen and the intensity of the immunoreactivity.

To illustrate the results, two strategies have been used: selected photographs of the corpuscles in which the TGCs alone, or the TGCs and axons were positive (with different techniques; Figure 1), and summary tables of the Meissner and Pacinian corpuscles in the different anatomical locations sampled in which green means positive immunoreaction, red negative immunoreaction and blue in which no data are provided because the corpuscles were not identified in these histological sections.

 doi:10.20944/preprints202604.2199.v1

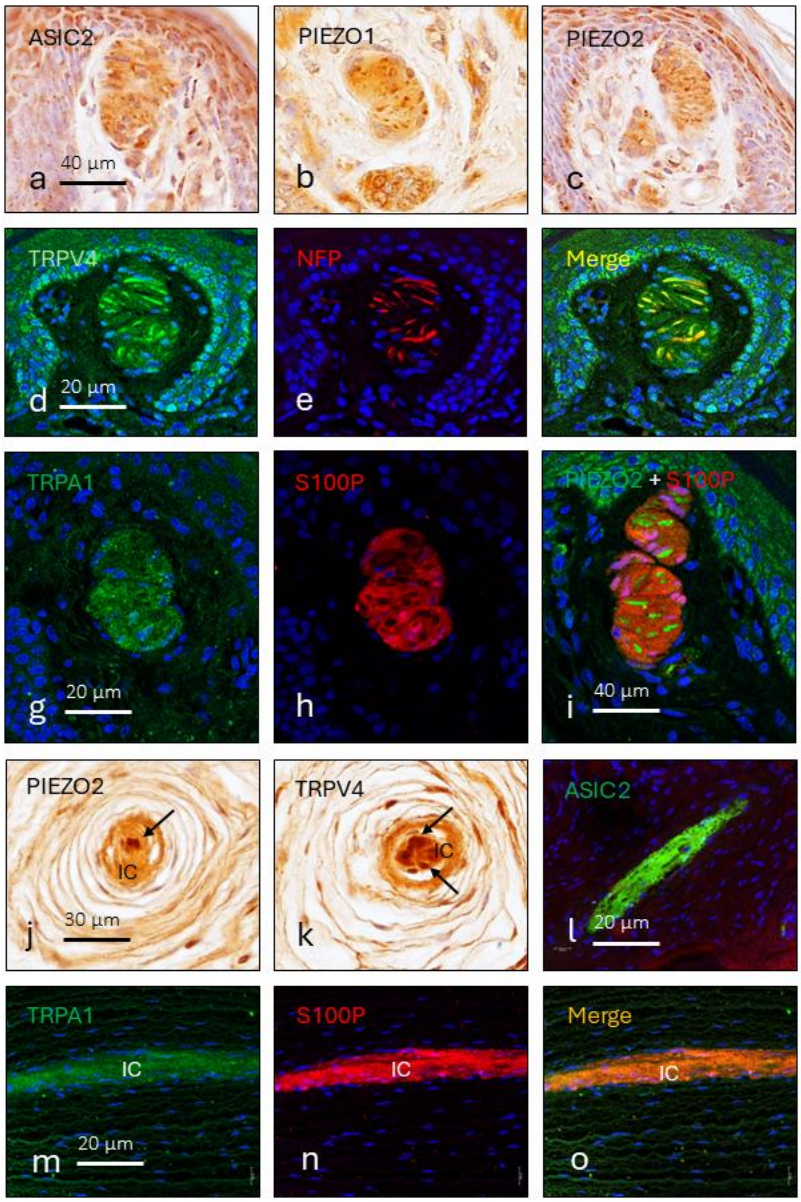

Figure 1. Representative sections of the immunohistochemical detection of different mechanoproteins in Meissner and Pacinian corpuscles of human glabrous skin from different anatomical locations. a) foreskin; **b** and **c)** labia minora pudendis; **e-f)** finger; **g** and **h)** toe; **i)** finger; **j)** finger; **k)** toe; **l)** foreskin; **m-o)** finger. IC: inner core; arrows indicate the axons.

2.1. Detection of Mechanoproteins in the Axon

The investigated mechanoproteins were detected in the axons of Meissner corpuscles: ASIC2, PIEZO2, TRPC6 and TRPV4 in all anatomical locations; β-ENaC was located in the fingers and toes, lips, foreskin and labia minora pudendis; γ-ENaC only in the toes; PIEZO1 in all locations except the lips and foreskin; TRPA1 in the fingers, toes, foreskin, and labia minora pudendis; TRPC1 in the fingers, toes, foreskin, and labia minora pudendis; TRPC6 in all anatomical locations; TRPV4 in all anatomical locations. ASIC1 and ASIC3 were negative (Figure 2).

Regarding the axons of the Pacinian corpuscles, the axons were positive for ASIC1, ASIC2, β-ENaC and γ-ENaC, PIEZO1, PIEZO2, TRPA1, TRPC1, TRPC6 and TRPV4 (Figure 3).

 doi:10.20944/preprints202604.2199.v1

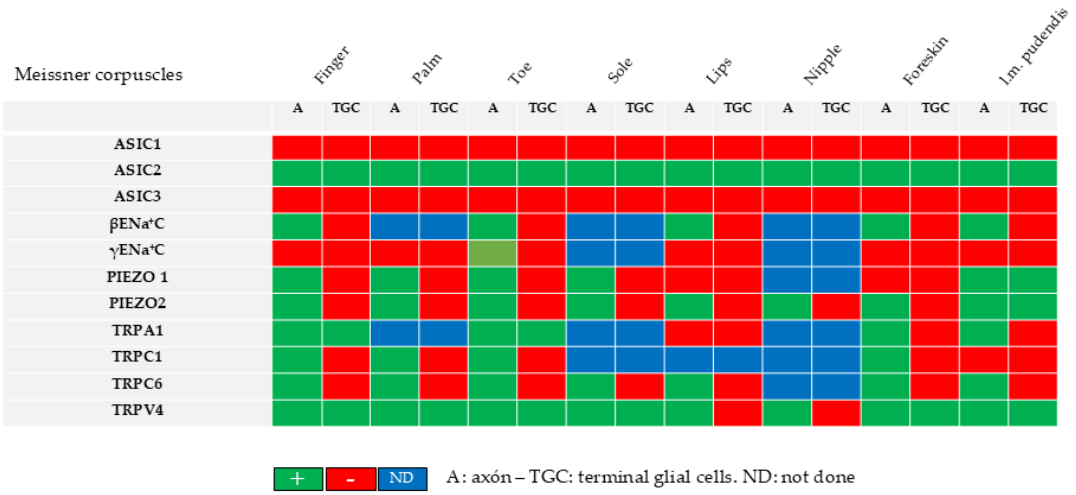

Figure 2. Detection of mechanoproteins in Meissner corpuscles of human glabrous skin from different locations. Green means positive immunoreaction, red negative immunoreaction and blue in which no data are provided because the corpuscles were not identified in these histological sections.

2.2. Detection of Mechanoproteins in Glial Terminal Cells

In Meissner corpuscles, immunoreactivity for ASIC2 was detected at all locations (14.2 ± 3.2% in the fingers; 8.6 ± 1.3% in the palm of the hand; 8.8 ± 2.7% in the toes; 4.1 ± 1.1% on the sole of the foot; 100% on the lips; 100% on the nipple; 17.8 ± 2.2% on the foreskin; 11.6 ± 1.9% on the labia minora pudendis) and for TRPV4 in all locations except the lips and nipple (8.6 ± 1.2% on the fingers; 6.2 ± 1.5% on the palm of the hand; 5.7 ± 1.1% on the toes; 6.4 ± 1.9% on the sole of the foot); PIEZO1 and PIEZO2 were only positive in labia minora pudendis (28.6 ± 2.9%); TRPA1 was positive in fingers (18.2 ± 2.9%) and toes (13.3 ± 1.8%); ASIC1, ASIC3, β-ENaC and γ-ENaC, TRPC1 and TRPC6 were negative at all locations (Figure 2).

In the Pacinian corpuscles, the TGCs that form the inner nucleus were positive for ASIC2 in all locations; for PIEZO2 in the hand and skin; for TRPA1 and TRPV4 in the fingers and toes. The rest of the mechanoproteins were not detected (Figure 3).

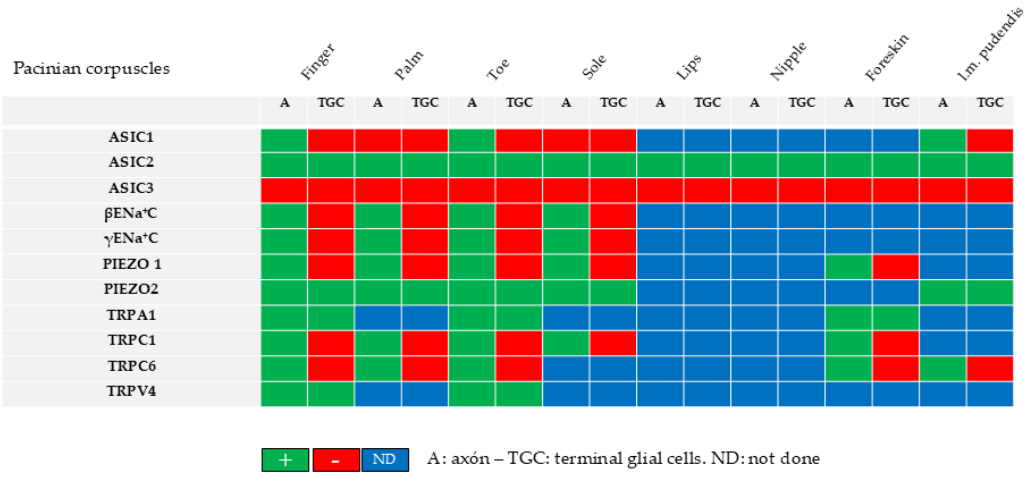

Figure 3. Detection of mechanoproteins in the Pacinian corpuscles of human glabrous skin from different locations. Green means positive immunoreaction, red negative immunoreaction and blue in which no data are provided because the corpuscles were not identified in these histological sections.

 doi:10.20944/preprints202604.2199.v1

3. Discussion

The present study was designed to systematically analyze the presence of mechanosensitive proteins, that are a part of the structure of mechano-gated ion channels, in the TGCs of CEOCs from adult human glabrous skin of different anatomical regions. Two types of CEOCs have been analysed: Meissner and Pacinian corpuscles, which, although morphologically different, share the same cellular elements in their structure and both function as rapidly adapting type I and type II, respectively, low-threshold mechanoreceptors [6,9].

Although the present study focuses on TGCs, it is necessary to briefly comment on the results obtained in axons. As expected, the mechanoreceptor axon terminal that is part of CEOCs expresses most of the ion channel proteins investigated, which is consistent with the view that mechanoreceptors express high densities of mechanosensitive channels [27]. Our findings are consistent with the presence of the mechanoproteins investigated in the dorsal root ganglia neurons of different vertebrate species, including humans [28], although sometimes not or not only in mechanoreceptors. Thus, have been detected in mechanoreceptors ASIC1 and ASIC2 [29–32], subunits ENaC [33], Piezo1 and Piezo2 [34–36], TRPA1 [37], TRPC1 and TRPC6 [38,39], and TRPV4 [40]. In addition, all the mechanoproteins investigated, apart from TRPA1 had already been detected in the axons of different morphotypes of CEOCs in different animal species including men [16,28].

Regarding the presence of mechanoproteins in the TGCs of human CEOCs, in our study we have observed that ASIC2, PIEZO1 and PIEZO 2, TRPA1 and TRPV4 were detected in the TGCs in the Meissner corpuscles, and ASIC2, PIEZO2, TRPA1 and TRPV4 in the Pacinian corpuscles. Previous studies by our group and others had already demonstrated immunoreaction for ASIC2 and TRPV4 in the TGCs of human Meissner corpuscles [30,41] while PIEZO channels were always detected in axons regardless of the anatomical location of the corpuscles [42–44]. To our knowledge, this is the first time that TRPA1 expression has been reported in TGCs. On the other hand, immunoreactivity for ASIC2 was also found in TGCs of the inner nucleus of human [30,45] and mouse [46] corpuscles, but not of *Macaca fascicularis* [47]. TRPV4 had been found in the cells of the inner nucleus of Herbst corpuscles, equivalent to mammalian Pacinian corpuscles [48]. The same as in Meissner corpuscles, this is the first time that an immunoreaction for TRPA1 and TRPC1 has been detected in the TGCs of Pacinian corpuscles.

Most of the available data on the function of TGCs in CEOCs were obtained in Pacinian corpuscles due to their size and the greater ease of experimental manipulation. Classically, it has been proposed that TGCs are involved in the maintenance of the ionic and metabolic microenvironment of CEOCs within the intercellular or interlamellar spaces [49,50] and the mediation of the rapid adaptation [51,52]. However, recent studies suggest that TGCs are active agents in the onset of somatosensory [53,54] especially in mechanosensitivity and mechanotransduction [17,20,27,53,55] since they are mechanosensitive and excitable [17,55–57]. Our observations and the demonstration of the presence of mechanoproteins in TGCs support their active role. However, the function of these mechanosensitive proteins in the TGCs of CEOCs is unknown, apart from the mechanosensitive-mechanotransducers proteins PIEZO. On the other hand, there is no possible explanation in the light of current knowledge that explains the heterogeneity of the presence of mechanoproteins in CEOCs, and it is possible that with more sensitive detection techniques the results are more homogeneous.

The PIEZO channels present in TGCs deserve special consideration since they are the only ones that have been detected to date in these cells or in cells related to them [5], such as satellite cells of the spinal ganglia [58,59] or Schwann cells [27,60,61], and the TGCs of a subpopulation of Krause corpuscles of the human clitoris [62]. However, the role of PIEZO channels in TGCs is not known.

The precise contribution of TGCs to CEOCS physiology remains unresolved. Again, the data are based on Pacinian corpuscles. Several studies suggest that the role of TGCs in mechanotransduction is as an enhancer, since the axons' connections and anchors establish in axons would activate axon PIEZO2 [27,55,63,64]. In addition, it has recently been shown that it is the axon-TGCs interaction of Pacinian corpuscles, and not the outer nucleus and the capsule classically thought, that is responsible for mechanosensitivity through axon ion channels [57] although for other authors only PIEZO2 is

 doi:10.20944/preprints202604.2199.v1

responsible for the high-frequency vibration detection to stimulus velocity [65]. Finally, although some authors have proposed that Piezo channels are involved in the myelination of glial cells [66] this role cannot be performed in TGCs since they are cells that do not myelinate.

In summary, our findings show that TGCs in human Meissner and Pacinian corpuscles express selected mechanosensitive ion channel-related proteins, supporting their potential role as active modulators of mechanosensitivity. However, the functional relevance of these proteins in TGCs remains to be determined.

4. Materials and Methods

4.1. Materials

The study was performed on glabrous skin samples taken from the hands (distal phalanx of fingers, n =18; palms, n = 6), feet (distal phalanx of toes, n = 11; soles, n = 4), lips (n = 6), nipples (n = 11), and genital organs (clitoris, n = 3; labia minora pudenda: n = 1; and foreskin: n = 12). This skin typically contains different kinds of CEOCs, especially Meissner corpuscles and Pacinian corpuscles (see [6]). The tissues were obtained from the Human Tissue Bank (National Registry of Biobanks, Collections Section; Ref. C-0001627) associated with the research Group SINPOS (Peripheral Nervous System and Sense Organs Group, Spanish acronym) of the University of Oviedo. They corresponded to 64 subjects, 33 female and 31 males, with no clinical history of neurological disease, with an age-range between 26 and 78 years (mean age 57.2). The materials were fixed with 10% neutral buffered formalin (in 1M PBS, pH 7.4), routinely paraffin embedded, and cut at 10 µm thick perpendicular to the skin surface. These materials were obtained in compliance with Spanish legislation (RD 1301/2006; Law 14/2007; RD 1716/2011; Order ECC/1404/2013) and in accordance with the guidelines of the Declaration of Helsinki II. They are being used in different studies, and its use has been authorized by the Research Ethics Committee of the Principality of Asturias (Cod CEIm P Ast: project 266/18).

4.2. Simple Immunohistochemistry

Sections were deparaffinized and rehydrated then permeabilized with 1M PBS at pH 7.6 with 0.5% Tween-20 and endogenous peroxidase activity was blocked with 10% H_2O_2 for 30 minutes. Nonspecific binding was then blocked with 25% foetal bovine serum and samples were incubated overnight at 4º C in a humid chamber with the antibodies listed in Table 1 (identified as IHC). Thereafter, the slides were washed and incubated with the anti-rabbit IgG (used diluted 1:500; Sigma-Aldrich) or anti-mouse IgG (used diluted 1:300;) for 90 minutes. After another washing in TBS-T, the specific antigen-antibody reaction was revealed using 3-3'-diaminobenzidine as chromogen. Finally, sections were counterstained with Mayer's haematoxylin and mounted with Entellan®. Photographs of the samples were taken on a Nikon Eclipse® 80i optical microscope coupled to a Nikon® DS-5M camera.

As controls, representative sections were processed in the same way as described above using non-immune rabbit or mouse sera instead of primary antibodies or while omitting the primary antibodies during the incubation.

Table 1. Primary antibodies used in the study.

Antigen	Origin	Dilution	Supplier	IHC/IF
ASIC1	Rabbit	1:200	Abcam pcl[1]	IHC
ASIC2	Rabbit	1:200	LifeSpan[2]	IHC/IF
ASIC3	Rabbit	1:100	LifeSpan[2]	IHC
β-ENaC	Mouse	1:200	Santa Cruz BT[3]	IHC
γ-ENaC	Mouse	1:200	Santa Cruz BT[3]	IHC/IF

 doi:10.20944/preprints202604.2199.v1

PIEZO1	Rabbit	1:200	Alomone[4]	IHC/IF
PIEZO2	Rabbit	1:200	Sigma-Aldrich[5]	IHC/IF
TRPA1	Rabbit	1:100	Abcam pcl[1]	IHC
TRPC1	Mouse	1:100	Santa Cruz BT[3]	IHC
TRPC6	Mouse	1:200	Novus B.[6]	IHC/IF
TRPV4	Rabbit	1:200	Abcam pcl[1]	IHC/IF
NSE (clone BBS/NC/VI-H14)	Mouse	1:500	Dako[7]	IF
NFP (clone 2F11)	Mouse	1:200	Roche[8]	IF
Synaptophysin (clone 27G12)	Mouse	Prediluted	Leica Biosystems[9]	IF
S100 P (clone 4C4.9)	Mouse	1:500	Roche[8]	IF

[1]Cambridge, UK; [2] Seattle, WA, USA; [3]Santa Cruz, CA, USA; [4]Jerusalem, Israel; [5]St. Louis, MO, USA; [6]Littleton, CO, USA; [7]Glostrup, Denmark; [8]Vienna, Austria; [9]Madrid, Spain. ASIC1: Polyclonal antibody raised in rabbit against an epitope between amino acids 195-297 of human ASIC1. ASIC2: Polyclonal antibody developed in rabbit against a synthetic peptide of the extracellular domain of mouse ASIC conjugated to a carrier protein. ASIC3: Polyclonal antibody developed in rabbit against a synthetic peptide of the localized domain between amino acids 53-102 of human ASIC3. α-ENaC: Monoclonal antibody developed in mice against an epitope located between the amino acids 206-229 of human αENaC. β-ENaC: Monoclonal antibody developed in mice against an epitope located between the amino acids 271-460 of human βENaC. γ-ENaC: Monoclonal antibody developed in mice against an epitope located within the intracellular C-terminal domain of human γENaC.PIEZO1: Polyclonal antibody developed against a synthetic peptide (C)EDLKPQHRRHISIR, corresponding to the amino acids 1863-1876 of rat PIEZO1. PIEZO2: Polyclonal antibody raised in rabbit against a synthetic peptide of human PIEZO2 with the sequence: VFGFWAFGKHSAAADITSSLSEDQVPGPFLVMVLIQFGTMVVDRALY LRK. TRPA1: Polyclonal antibody developed in rabbit against a synthetic peptide corresponding to TRPA1/TSA (internal sequence). TRPC1: Monoclonal antibody developed in mice against the amino acid sequence 689-793 of the C-terminal domain of human TRPC1. TRPC6: Monoclonal antibody developed in mice against a synthetic peptide corresponding to a region close to the carboxyl terminal domain of human TRPC6. TRPV4: Polyclonal antibody developed in rabbit against a synthetic peptide corresponding to the amino acid sequence 720-769 within the intracellular N-terminal region with the sequence VDEVNWSHWNQNLGIINEDPGKNETYQYYYGFSHTVGRLRRDRWSSVVPRVVVVVV.

4.3. Simple Immunofluorescence

Sections were deparaffinized and rehydrated then permeabilized with 1M PBS at pH 7.6 with 0.5% Tween-20 and nonspecific binding was then blocked with 10% foetal bovine serum and samples were incubated at 4ºC in a humid chamber with the antibodies listed in Table 1 (identified as IF). Thereafter, the slides were washed with PBS-T and incubated for 90 minutes with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:100; Serotec™, Oxford, UK) followed for 90 minutes, or with Cy3-conjugated donkey anti-mouse IgG (1:200; Jackson-ImmunoResearch™, Baltimore, MD, USA). The incubation was carried out in a dark humid chamber, at room temperature. After washing in PBS, the sections were stained with DAPI (4',6-diamino-2-phenylindole; 10 ng/ml) to contrast the nuclei (blue colour) and mounted with diluted Fluoromount-G mounting medium (Southern-Biotech, Alabama, USA). Immunofluorescence was detected using a Leica DMR-XA automated fluorescence microscope coupled to Leica Confocal Software v2.5 fluorescence capture software (Leica Microsystems, Heidelberg GmbH, Germany), from the Image Processing Service of the University of Oviedo. Specific reaction controls were performed in the same way as for simple immunohistochemistry. Additional controls were conducted to confirm the absence of autofluorescence processes of the tissue or produced by the fixation process omitting both antibodies.

 doi:10.20944/preprints202604.2199.v1

4.4. Double Immunofluorescence

When it was necessary to identify the CEOCs cells in which the ion channel proteins were present, we used double immunofluorescence for those proteins and others that identify the main constituents of Meissner corpuscles like neurofilament proteins, synaptophysin and S100P (see Cobo et al., 2021). Double immunofluorescence was performed exclusively with antibodies that showed positive immunoreactivity with immunohistochemistry or simple immunofluorescence. Sections were deparaffinized and rehydrated using the same procedure described previously. Then, sections were incubated overnight at 4°C in a humid chamber with a 1:1 mixture of NFP, SYN, S100P or CD34 together with the ion channel protein investigated. Subsequently, the sections were washed with PBS-T for 30 minutes and incubated for 90 minutes with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:100; Serotec™, Oxford, UK) followed for 90 minutes with Cy3-conjugated donkey anti-mouse IgG (1:200; Jackson-ImmunoResearch™, Baltimore, MD, USA). Both incubations were conducted in a humid chamber, in the dark and at room temperature. After washing in PBS, the sections were stained with DAPI (4',6-diamino-2-phenylindole; 10 ng/ml) to contrast the nuclei (blue colour) and mounted with diluted Fluoromount-G mounting medium (Southern-Biotech, Alabama, USA). Immunofluorescence was detected using a Leica DMR-XA automated fluorescence microscope coupled to Leica Confocal Software v2.5 fluorescence capture software (Leica Microsystems, Heidelberg GmbH, Germany), from the Image Processing Service of the University of Oviedo. Specific reaction controls were performed in the same way as for simple immunohistochemistry. Additional controls were conducted to confirm the absence of autofluorescence processes of the tissue or produced by the fixation process omitting both antibodies.

4.5. Quantitative Analyses

The percentage of Meissner corpuscles displaying immunoreactivity for the assessed mechanoproteins was established analysing 2 sections per skin sample, separated 200 μm and processed for the simultaneous detection of S100P and each mechanoprotein as follows: 5 fields per sections enlarged x10 were captured using a Leica Confocal Software v2.5 fluorescence capture software (Leica Microsystems, Heidelberg GmbH, Germany) and the number of Meissner corpuscles counted by two independent observers and the results obtained were averaged. Based on the scale bar of the images, the surface of the sections was measured, and the density of Meissner corpuscles was calculated; data were expressed as mean ± standard deviation/mm^2. Meissner corpuscles displaying S100P immunoreactivity were considered 100%, and those displaying S100P+one mechanoprotein were subtracted to determine the percentage of Meissner corpuscles expressing that mechanoprotein. Results are presented again as mean ± standard deviation/mm^2. No calculations were made on Pacinian corpuscles because they were not found in the skin of all anatomical locations and always in small quantities.

Author Contributions: Conceptualization, Y.G.M. and J.A.V.; methodology, I.A. and Y.G.-M.; validation, J.A.V., and Y.G-M.; formal analysis, Y.G-M., P.C., and O.G-S.; investigation, I.A., Y.G-M., and AM.A-V; resources, I.A., J.F., AM.A-V., and I.S.; writing—original draft preparation, I.A. and Y.G-M.; writing—review and editing, J.A.V.; visualization, I.A., and O.G.-S.; supervision, J.A.V.; project administration, P.C., J.F., and I.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and Spanish legislation on biomedical research (RD 1301/2006; Law 14/2007; RD 1716/2011; Order ECC/1404/2013). Human tissues were obtained from the Human Tissue Bank (National Registry of Biobanks, Collections Section; Ref. C-0001627) associated with the research group SINPOS (Peripheral Nervous System and Sense Organs Group, University of Oviedo). The use of these materials was approved by the Research Ethics Committee of the Principality of Asturias (CEIm P. Asturias; project code Amigo).

 doi:10.20944/preprints202604.2199.v1

Informed Consent Statement: Informed consent for tissue donation and use in research was obtained from all subjects or their legal representatives by the Human Tissue Bank (National Registry of Biobanks, Collections Section; Ref. C-0001627), in accordance with Spanish legislation.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ASIC	Acid-sensing ion channel
CEOCs	Cutaneous end-organ complexes
DAPI	4',6-diamidino-2-phenylindole
ENaC	Epithelial sodium channel
β-ENaC	Beta subunit of Epithelial Sodium Channel
γ-ENaC	Gamma subunit of Epithelial Sodium Channel
IF	Immunofluorescence
IgG	Immunoglobulin G
IHC	Immunohistochemistry
NFP	Neurofilament protein
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline with Tween-20
PIEZO1	Piezo-type mechanosensitive ion channel component 1
PIEZO2	Piezo-type mechanosensitive ion channel component 2
S100P	S100 calcium-binding protein P
SYN	Synaptophysin
TBS-T	Tris-buffered saline with Tween-20
TGCs	Terminal glial cells
TRP	Transient receptor potential
TRPA1	Transient receptor potential ankyrin 1
TRPC1	Transient receptor potential canonical 1
TRPC6	Transient receptor potential canonical 6
TRPV4	Transient receptor potential vanilloid 4

References

1. Reed, C.B.; Feltri, M.L.; Wilson, E.R. Peripheral glia diversity. J. Anat. **2021**. https://doi.org/10.1111/joa.13484
2. Suazo, I.; Vega, J.A.; García-Mesa, Y.; et al. The lamellar cells of vertebrate Meissner and Pacinian corpuscles: Development, characterization, and functions. Front. Neurosci. **2022**, 16, 790130. https://doi.org/10.3389/fnins.2022.790130
3. Kastriti, M.E.; Adameyko, I. Specification, plasticity and evolutionary origin of peripheral glial cells. Curr. Opin. Neurobiol. **2017**, 47, 196–202. https://doi.org/10.1016/j.conb.2017.11.004
4. Hastings, R.L.; Valdez, G. Origin, identity, and function of terminal Schwann cells. Trends Neurosci. **2024**, 47, 432–446. https://doi.org/10.1016/j.tins.2024.03.007
5. Vega, J.A.; García-Mesa, Y.; Cuendias, P.; et al. Development of vertebrate cutaneous end-organ complexes. Curr. Top. Dev. Biol. **2025**, in press. https://doi.org/10.1016/bs.ctdb.2025.03.001
6. Cobo, R.; García-Piqueras, J.; Cobo, J.; Vega, J.A. The human cutaneous sensory corpuscles: An update. J. Clin. Med. **2021**, 10, 227. https://doi.org/10.3390/jcm10020227
7. Xiao, B. Mechanisms of mechanotransduction and physiological roles of PIEZO channels. Nat. Rev. Mol. Cell Biol. **2024**, 25, 886–903. https://doi.org/10.1038/s41580-024-00773-5
8. Cao, R.; Tian, H.; Tian, Y.; Fu, X. A hierarchical mechanotransduction system: From macro to micro. Adv. Sci. **2024**, 11, e2302327. https://doi.org/10.1002/advs.202302327

 doi:10.20944/preprints202604.2199.v1

9. Zimmerman, A.; Bai, L.; Ginty, D.D. The gentle touch receptors of mammalian skin. Science **2014**, 346, 950–954. https://doi.org/10.1126/science.1254229
10. Jin, P.; Jan, L.Y.; Jan, Y.N. Mechanosensitive ion channels: Structural features relevant to mechanotransduction mechanisms. Annu. Rev. Neurosci. **2020**, 43, 207–229. https://doi.org/10.1146/annurev-neuro-070918-050509
11. Ohashi, K.; Fujiwara, S.; Mizuno, K. Roles of the cytoskeleton, cell adhesion and Rho signalling in mechanosensing and mechanotransduction. J. Biochem. **2017**, 161, 245–254. https://doi.org/10.1093/jb/mvw082
12. Humphrey, J.D.; Dufresne, E.R.; Schwartz, M.A. Mechanotransduction and extracellular matrix homeostasis. Nat. Rev. Mol. Cell Biol. **2014**, 15, 802–812. https://doi.org/10.1038/nrm3896
13. Delmas, P.; Coste, B. Mechano-gated ion channels in sensory systems. Cell **2013**, 155, 278–284. https://doi.org/10.1016/j.cell.2013.09.026
14. Gu, Y.; Gu, C. Physiological and pathological functions of mechanosensitive ion channels. Mol. Neurobiol. **2014**, 50, 339–347. https://doi.org/10.1007/s12035-014-8654-4
15. Ranade, S.S.; Syeda, R.; Patapoutian, A. Mechanically activated ion channels. Neuron **2015**, 87, 1162–1179. https://doi.org/10.1016/j.neuron.2015.08.032
16. Cobo, R.; García-Piqueras, J.; García-Mesa, Y.; et al. Peripheral mechanobiology of touch—Studies on vertebrate cutaneous sensory corpuscles. Int. J. Mol. Sci. **2020**, 21, 6221. https://doi.org/10.3390/ijms21176221
17. Nikolaev, Y.A.; Feketa, V.V.; Anderson, E.O.; et al. Lamellar cells in Pacinian and Meissner corpuscles are touch sensors. Sci. Adv. **2020**, 6, eabe6393. https://doi.org/10.1126/sciadv.abe6393
18. Ziolkowski, L.H.; Gracheva, E.O.; Bagriantsev, S.N. Mechanotransduction events at the physiological site of touch detection. eLife **2023**, 12, e84179. https://doi.org/10.7554/eLife.84179
19. Ziolkowski, L.H.; Nikolaev, Y.A.; Chikamoto, A.; et al. The inner core enables transient touch detection in the Pacinian corpuscle. Sci. Adv. **2025**, 11, eadt4837. https://doi.org/10.1126/sciadv.adt4837
20. Chen, Y.T.; de Thomas Wagner, D.; Loutit, A.J.; et al. Lamellar Schwann cells in the Pacinian corpuscle potentiate vibration perception. Sci. Adv. **2025**, 11, eadt5110. https://doi.org/10.1126/sciadv.adt5110
21. Pawson, L.; Bolanowski, S.J. Voltage-gated sodium channels are present on both the neural and capsular structures of Pacinian corpuscles. Somatosens. Mot. Res. **2002**, 19, 231–237. https://doi.org/10.1080/0899022021000009152
22. Laitko, U.; Juranka, P.F.; Morris, C.E. Membrane stretch slows the concerted step prior to opening in a Kv channel. J. Gen. Physiol. **2006**, 127, 687–701. https://doi.org/10.1085/jgp.200509394
23. Morris, C.E.; Juranka, P.F. Nav channel mechanosensitivity: Activation and inactivation accelerate reversibly with stretch. Biophys. J. **2007**, 93, 822–833. https://doi.org/10.1529/biophysj.106.101246
24. Heidenreich, M.; Lechner, S.G.; Vardanyan, V.; et al. KCNQ4 K^+ channels tune mechanoreceptors for normal touch sensation in mouse and man. Nat. Neurosci. **2011**, 15, 138–145. https://doi.org/10.1038/nn.2985
25. Bardoni, R.; Tawfik, V.L.; Wang, D.; et al. Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn. Neuron **2014**, 81, 1312–1327. https://doi.org/10.1016/j.neuron.2014.01.044
26. Chemin, J.; Nargeot, J.; Lory, P. Chemical determinants involved in anandamide-induced inhibition of T-type calcium channels. J. Biol. Chem. **2007**, 282, 2314–2323. https://doi.org/10.1074/jbc.M610033200
27. Handler, A.; Zhang, Q.; Pang, S.; Nguyen, T.M.; Iskols, M.; Nolan-Tamariz, M.; Cattel, S.; Plumb, R.; Sanchez, B.; Ashjian, K.; et al. Three-dimensional reconstructions of mechanosensory end organs suggest a unifying mechanism underlying dynamic, light touch. *Neuron* 2023, *111*, 3211–3229.e9. https://doi.org/10.1016/j.neuron.2023.08.023
28. Del Valle, M.E.; Cobo, T.; Cobo, J.L.; Vega, J.A. Mechanosensory neurons, cutaneous mechanoreceptors, and putative mechanoproteins. *Microsc. Res. Tech.* 2012, *75*, 1033–1043. https://doi.org/10.1002/jemt.22028
29. Page, A.J.; Brierley, S.M.; Martin, C.M.; Martinez-Salgado, C.; Wemmie, J.A.; Brennan, T.J.; Symonds, E.; Omari, T.; Lewin, G.R.; Welsh, M.J.; Blackshaw, L.A. The ion channel ASIC1 contributes to visceral but not

 doi:10.20944/preprints202604.2199.v1

cutaneous mechanoreceptor function. *Gastroenterology* 2004, *127*, 1739–1747. https://doi.org/10.1053/j.gastro.2004.08.061

30. Cabo, R.; Alonso, P.; Viña, E.; Vázquez, G.; Gago, A.; Feito, J.; Pérez-Moltó, F.J.; García-Suárez, O.; Vega, J.A. ASIC2 is present in human mechanosensory neurons of the dorsal root ganglia and in mechanoreceptors of the glabrous skin. *Histochem. Cell Biol.* 2015, *143*, 267–276. https://doi.org/10.1007/s00418-014-1278-y
31. Omerbašić, D.; Schuhmacher, L.N.; Bernal Sierra, Y.A.; Smith, E.S.; Lewin, G.R. ASICs and mammalian mechanoreceptor function. *Neuropharmacology* 2015, *94*, 80–86. https://doi.org/10.1016/j.neuropharm.2014.12.007
32. Papalampropoulou-Tsiridou, M.; Shiers, S.; Wang, F.; Godin, A.G.; Price, T.J.; De Koninck, Y. Distribution of acid-sensing ion channel subunits in human sensory neurons contrasts with that in rodents. *Brain Commun.* 2022, *4*, fcac256. https://doi.org/10.1093/braincomms/fcac256
33. García-Añoveros, J.; Samad, T.A.; Zuvela-Jelaska, L.; Woolf, C.J.; Corey, D.P. Transport and localization of the DEG/ENaC ion channel BNaC1alpha to peripheral mechanosensory terminals of dorsal root ganglia neurons. *J. Neurosci.* 2001, *21*, 2678–2686. https://doi.org/10.1523/JNEUROSCI.21-08-02678.2001
34. Anderson, E.O.; Schneider, E.R.; Bagriantsev, S.N. Piezo2 in cutaneous and proprioceptive mechanotransduction in vertebrates. *Curr. Top. Membr.* 2017, *79*, 197–217. https://doi.org/10.1016/bs.ctm.2016.11.002
35. Honoré, E.; Martins, J.R.; Penton, D.; Patel, A.; Demolombe, S. The Piezo mechanosensitive ion channels: May the force be with you! *Rev. Physiol. Biochem. Pharmacol.* 2015, *169*, 25–41. https://doi.org/10.1007/112_2015_26
36. Roh, J.; Hwang, S.M.; Lee, S.H.; Lee, K.; Kim, Y.H.; Park, C.K. Functional expression of Piezo1 in dorsal root ganglion neurons. *Int. J. Mol. Sci.* 2020, *21*, 3834. https://doi.org/10.3390/ijms21113834
37. Talavera, K.; Startek, J.B.; Alvarez-Collazo, J.; Boonen, B.; Alpizar, Y.A.; Sanchez, A.; Naert, R.; Nilius, B. Mammalian TRPA1 channels: From structure to disease. *Physiol. Rev.* 2020, *100*, 725–803. https://doi.org/10.1152/physrev.00005.2019
38. Gottlieb, P.; Folgering, J.; Maroto, R.; Raso, A.; Wood, T.G.; Kurosky, A.; Bowman, C.; Bichet, D.; Patel, A.; Sachs, F.; et al. Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Arch.* 2008, *455*, 1097–1103. https://doi.org/10.1007/s00424-007-0359-3
39. Sexton, J.E.; Desmonds, T.; Quick, K.; Taylor, R.; Abramowitz, J.; Forge, A.; Kros, C.J.; Birnbaumer, L.; Wood, J.N. Contribution of TRPC channels to touch and hearing. *Neurosci. Lett.* 2016, *610*, 36–42. https://doi.org/10.1016/j.neulet.2015.10.052
40. Cui, Y.Y.; Li, M.Y.; Li, Y.T.; Ning, J.Y.; Gou, X.C.; Shi, J.; Li, Y.Q. Expression and functional characterization of TRPV4 in DRG and spinal cord of diabetic rats. *Brain Res. Bull.* 2020, *162*, 30–39. https://doi.org/10.1016/j.brainresbull.2020.05.010
41. Alonso-González, P.; Cabo, R.; San José, I.; Gago, A.; Suazo, I.C.; García-Suárez, O.; Cobo, J.; Vega, J.A. Human digital Meissner corpuscles display immunoreactivity for the multifunctional ion channels TRPC6 and TRPV4. *Anat. Rec. (Hoboken)* 2017, *300*, 1022–1031. https://doi.org/10.1002/ar.23522
42. García-Mesa, Y.; García-Piqueras, J.; García, B.; Feito, J.; Cabo, R.; Cobo, J.; Vega, J.A.; García-Suárez, O. Merkel cells and Meissner corpuscles in human digital skin display Piezo2 immunoreactivity. *J. Anat.* 2017, *231*, 978–989. https://doi.org/10.1111/joa.12688
43. García-Mesa, Y.; García-Piqueras, J.; Cobo, R.; Martín-Cruces, J.; Suazo, I.; García-Suárez, O.; Feito, J.; Vega, J.A. Sensory innervation of the human male prepuce: Meissner corpuscles predominate. *J. Anat.* 2021, *239*, 892–902. https://doi.org/10.1111/joa.13481
44. García-Mesa, Y.; Cuendias, P.; Alonso-Guervós, M.; García-Piqueras, J.; Martín-Biedma, B.; Cobo, T.; García-Suárez, O.; Vega, J.A. Immunohistochemical detection of PIEZO1 and PIEZO2 in human digital Meissner corpuscles. *Ann. Anat.* 2024, *252*, 152200. https://doi.org/10.1016/j.aanat.2023.152200
45. Calavia, M.G.; Montaño, J.A.; García-Suárez, O.; Feito, J.; Guervós, M.A.; Germanà, A.; Del Valle, M.; Pérez-Piñera, P.; Cobo, J.; Vega, J.A. Differential localization of acid-sensing ion channels 1 and 2 in human cutaneous Pacinian corpuscles. *Cell. Mol. Neurobiol.* 2010, *30*, 841–848. https://doi.org/10.1007/s10571-010-9511-2

 doi:10.20944/preprints202604.2199.v1

46. Montaño, J.A.; Calavia, M.G.; García-Suárez, O.; et al. The expression of ENaC and ASIC2 proteins in Pacinian corpuscles is differently regulated by TrkB and its ligands BDNF and NT-4. *Neurosci. Lett.* 2009, *463*, 114–118. https://doi.org/10.1016/j.neulet.2009.07.073
47. Cabo, R.; Gálvez, M.A.; San José, I.; Laurà, R.; López-Muñiz, A.; García-Suárez, O.; Cobo, T.; Insausti, R.; Vega, J.A. Immunohistochemical localization of ASIC2 in cutaneous Meissner and Pacinian corpuscles of *Macaca fascicularis*. *Neurosci. Lett.* 2012, *516*, 197–201. https://doi.org/10.1016/j.neulet.2012.03.081
48. Cabo, R.; Gálvez, A.; Laurà, R.; San José, I.; Pastor, J.F.; López-Muñiz, A.; García-Suárez, O.; Vega, J.A. Immunohistochemical detection of ASIC2 and TRPV4 in avian Herbst corpuscles. *Anat. Rec. (Hoboken)* 2013, *296*, 117–122. https://doi.org/10.1002/ar.22615
49. Bell, J.; Bolanowski, S.; Holmes, M.H. The structure and function of Pacinian corpuscles: A review. *Prog. Neurobiol.* 1994, *42*, 79–128. https://doi.org/10.1016/0301-0082(94)90022-1
50. Dubový, P. Electron microscopical localization of non-specific cholinesterase activity in cat Pacinian corpuscles. *Acta Histochem.* 1986, *80*, 3–12. PMID: 3099532.
51. Pawson, L.; Pack, A.K.; Bolanowski, S.J. Possible glutaminergic interaction in Pacinian corpuscles. *Somatosens. Mot. Res.* 2007, *24*, 85–95. https://doi.org/10.1080/08990220701388364
52. Pawson, L.; Prestia, L.T.; Mahoney, G.K.; Güçlü, B.; Cox, P.J.; Pack, A.K. GABAergic/glutamatergic interaction contributes to rapid adaptation in Pacinian corpuscles. *J. Neurosci.* 2009, *29*, 2695–2705. https://doi.org/10.1523/JNEUROSCI.5974-08.2009
53. Abdo, H.; Calvo-Enrique, L.; Lopez, J.M.; et al. Specialized cutaneous Schwann cells initiate pain sensation. *Science* 2019, *365*, 695–699. https://doi.org/10.1126/science.aax6452
54. Ojeda-Alonso, J.; Calvo-Enrique, L.; Paricio-Montesinos, R.; Kumar, R.; Zhang, M.-D.; Poulet, J.F.A.; Ernfors, P.; Lewin, G.R. Sensory Schwann cells regulate mechanical nociception. *Nat. Commun.* 2024, *15*, 898. https://doi.org/10.1038/s41467-024-44845-8
55. Nikolaev, Y.A.; Ziolkowski, L.H.; Pang, S.; Li, W.-P.; Feketa, V.V.; Xu, C.S.; Gracheva, E.O.; Bagriantsev, S.N. 3D architecture and a bicellular mechanism of touch detection in mechanosensory corpuscle. *Sci. Adv.* 2023, *9*, eadi4147. https://doi.org/10.1126/sciadv.adi4147
56. Ziolkowski, L.H.; Gracheva, E.O.; Bagriantsev, S.N. Tactile sensation in birds: Physiological insights from avian mechanoreceptors. *Curr. Opin. Neurobiol.* 2022, *74*, 102548. https://doi.org/10.1016/j.conb.2022.102548
57. Ziolkowski, L.H.; Nikolaev, Y.A.; Chikamoto, A.; et al. Inner core enables transient touch detection in Pacinian corpuscles. *Sci. Adv.* 2025, *11*, eadt4837. https://doi.org/10.1126/sciadv.adt4837
58. Shin, S.M.; Itson-Zoske, B.; Fan, F.; et al. Peripheral sensory neurons express functional Piezo1 channels. *Mol. Pain* 2023, *19*, 17448069231174315. https://doi.org/10.1177/17448069231174315
59. Shin, S.M.; Moehring, F.; Itson-Zoske, B.; et al. Piezo2 in sensory neurons and non-neuronal cells. *Pain* 2021, *162*, 2750–2768. https://doi.org/10.1097/j.pain.0000000000002356
60. Suttinont, C.; Maeno, K.; Yano, M.; Sato-Numata, K.; Numata, T.; Tsutsumi, M. Role of Piezo2 in Schwann cells. *Cell Physiol. Biochem.* 2024, *58*, 292–310. https://doi.org/10.33594/000000713
61. Yamanishi, H.; Iwabuchi, T. Ultrastructure of lanceolate nerve endings in human scalp. *J. Anat.* 2023, *242*, 1012–1028. https://doi.org/10.1111/joa.13831
62. Cuendias, P.; Vega, J.A.; García-Suárez, O.; Suazo, I.; Cobo, R.; García-Piqueras, J.; García-Mesa, Y. PIEZO1 and PIEZO2 in human clitoral Krause corpuscles. *Int. J. Mol. Sci.* 2024, *25*, 6722. https://doi.org/10.3390/ijms25126722
63. Hu, J.; Chiang, L.-Y.; Koch, M.; Lewin, G.R. Evidence for a protein tether involved in somatic touch. *EMBO J.* 2010, *29*, 855–867. https://doi.org/10.1038/emboj.2009.398
64. Schwaller, F.; Begay, V.; Garcia-Garcia, G.; et al. USH2A is a Meissner corpuscle protein necessary for normal vibration sensing in mice and humans. *Nat. Neurosci.* 2021, *24*, 74–81. https://doi.org/10.1038/s41593-020-00751-y

 doi:10.20944/preprints202604.2199.v1

65. Chikamoto, A.; Meng, M.; Gracheva, E.O.; Bagriantsev, S.N. Velocity sensitivity in mechanotransduction. *Nat. Commun.* 2026, *17*, 2122. https://doi.org/10.1038/s41467-026-69251-0
66. Acheta, J.; Bhatia, U.; Haley, J.; Hong, J.; Rich, K.; Close, R.; Bechler, M.E.; Belin, S.; Poitelon, Y. Piezo channels in myelination. *Glia* 2022, *70*, 2276–2289. https://doi.org/10.1002/glia.24251