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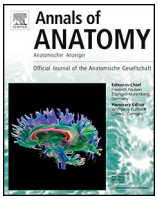

Immunohistochemical detection of PIEZO1 and PIEZO2 in human digital Meissner's corpuscles

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ABSTRACT

Background: The cutaneous end organ complexes or cutaneous sensory corpuscles are specialized sensory organs associated to low-threshold mechanoreceptors. Mechano-gated proteins forming a part of ion channels have been detected in both the axon and terminal glial cells of Meissner corpuscles, a specific cutaneous end organ complex in the human glabrous skin. The main candidates to mechanotransduction in Meissner corpuscles are members of the Piezo family of cationic ion channels. PIEZO2 has been detected in the axon of these sensory structures whereas no data exists about the occurrence and cell localization of PIEZO1.
Methods: Skin samples (n = 18) from the palmar aspect of the distal phalanx of the first and second fingers were analysed (8 female and 10 males; age range 26 to 61 26–61 years). Double immunofluorescence for PIEZO1 and PIEZO2 together with axonal or terminal glial cell markers was captured by laser confocal microscopy, and the percentage of PIEZOs positive Meissner corpuscles was evaluated.
Results: MCs from human fingers showed variable morphology and degree of lobulation. Regarding the basic immunohistochemical profile, in all cases the axons were immunoreactive for neurofilament proteins, neuron specific enolase and synaptophysin, while the lamellar cells displayed strong S100P immunoreactivity. PIEZO1 was detected co-localizing with axonal markers, but never with terminal glial cell markers, in the 56% of Meissner corpuscles; weak but specific immunofluorescence was additionally detected in the epidermis, especially in basal keratinocytes. Similarly, PIEZO2 immunoreactivity was found restricted to the axon in the 85% of Meissner corpuscles. PIEZO2 positive Merkel cells were also regularly found.
Conclusions: PIEZO1 and PIEZO2 are expressed exclusively in the axon of a subpopulation of human digital Meissner corpuscles, thus suggesting that not only PIEZO2, but also PIEZO1 may be involved in the mechanotransduction from low-threshold mechanoreceptors.

1. Introduction

The cutaneous end organ complexes (CEOCs; Handler and Ginty, 2021), also known as cutaneous sensory corpuscles or cutaneous sensory nerve formations (see Cobo et al., 2021), are specialized sensory organs associated to low-threshold mechanoreceptors (LTMRs; Zimmerman et al., 2014; Handler and Ginty, 2021). Structurally, they consist of one axon that contacts specialized glial or epithelial cells, i.e., terminal glial cells (Suazo et al., 2022) or Merkel cells (Bataille et al., 2022), respectively.

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The human glabrous skin is typically filled with Meissner's corpuscles (MCs) differing in both size and relative density among anatomical regions (Kim and Lee, 2020; Ciano and Beatty 2022; Cuendias et al., 2023). MCs are placed at dermal papillae, show variable morphology and size that undergo age-related variations (García-Piqueras et al., 2019). MCs consist of a peripheral axon from an Aβ LTMR, non-myelinating terminal glial cells called lamellar cells, and a thin capsule of CD34-positive endoneurial cells (Cobo et al., 2021). Functionally, MCs are associated to type I rapidly adapting LTMRs (Zimmerman et al., 2014).

Inside MCs, the mechanotransduction, i.e., the process whereby mechanical stimuli are converted into an action potential, is mediated by mechanosensitive ion channels, especially members of the Piezo family of cationic ion channels (Coste et al., 2010; Ranade et al., 2014; Woo et al., 2014; Handler y Ginty, 2021; Villarino et al., 2023). Consistently, Piezo channels are expressed in dorsal root ganglia (DRG; Coste et al., 2010; Roh et al., 2020; Shin et al., 2021, 2023) and PIEZO2 is present in the axon of CEOC (Coste et al., 2010; Ranade et al., 2014; García-Mesa et al., 2017, 2022; Moayedi et al., 2018; Handler et al., 2023) and Krause corpuscles from murine clitoris and penis (Qi et al., 2023). Furthermore, Shin et al. (2021); (2023) reported the occurrence of PIEZO1 and PIEZO2 in rat CEOCs, and Yamanishi and Iwabuchi (2023), PIEZO2 expression in both the axon and the terminal glial cells of human lanceolate nerve endings.

Regarding human CEOCs, the occurrence of PIEZO1 was never investigated, while PIEZO2 has been detected immunohistochemically in CEOCs from different anatomical localizations, i.e., digital skin, lip vermillion, foreskin, or clitoris, as well as in Merkel cells (García-Mesa et al., 2017, 2021a,b; García-Piqueras et al., 2019; Martín-Cruces et al., 2023).

Since no data exists on the presence of PIEZO1 in human digital CEOCs, we have conducted a study on human digital MCs using immunofluorescence associated to confocal laser microscopy. The study was aimed to examinate whether PIEZO1 is present in human CEOCs and determine the precise cellular location within them and confirm the occurrence of PIEZO2 in these sensory formations.

2. Methods

2.1. Material

Skin samples were obtained from the palmar aspect of the distal phalanx of the first (n = 8) and second (n = 10) fingers at the Human Tissue Collection (National Registry of Biobanks, Collections Section; Ref. C-0001627, OG-S) of the Peripheral Nervous System and Sense Organs Research Group (SINPOs) at the University of Oviedo. Tissues were dissected from incidental finger amputations of subjects (8 females and 10 males; age ranged from 26–61 years old), free of neurological diseases, and collected within 6 h after incident. The biological material was obtained in compliance with the Spanish Legislation (RD 1301/2006; Law 14/2007; RD 1716/2011; Order ECC/1404/2013) and in agreement with the guidelines of the Declaration of Helsinki II. This study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. Celm. Past: Proyecto 266/18).

Skin samples were fixed in 10% formalin in 1 M PBS (pH 7.4) for 24 hours. Subsequently, they were embedded in paraffin and cut into 10 μm thick sections, perpendicular to the skin surface, and mounted on gelatine-coated microscope slides.

2.2. Double immunofluorescence

The main constituents of MCs were identified using antibodies against neurofilament proteins (NFP), neuron-specific enolase (NSE) and synaptophysin (SYN) to immunolabel the axon, and against S100 protein (S100P) to immunolabel the lamellar cells. Sections were deparaffinized, rehydrated and washed in phosphate buffered saline-Tris PBS-T, pH 7.4, for 20 minutes. Then, they were incubated overnight at 4°C in a humid chamber with a 1:1 mixture of two primary antibodies for the simultaneous detection of two antigens: PIEZO1 (raised in rabbit against a synthetic peptide C-EDLKPQHRRHISIR, corresponding to amino acids sequence 1863–1876 of rat PIEZO1; used diluted 1:200; Alomone, Jerusalem, Israel) plus NFP (neurofilament proteins; mouse clone 2F11; prediluted; Roche, Vienna, Austria) or NSE (neuron-specific enolase; mouse clone BBS/NC/VI-H14; used diluted: 1:200; DAKO, Glostrup, Denmark) or SYN (synaptophysin; mouse clone DAK-SYNAP; prediluted, Dako) or S100P (S100 protein; mouse clone 4C4.9; prediluted; Roche). Identical experiments were conducted using PIEZO2 (against a synthetic peptide of human PIEZO2 with the sequence: VFGFWAFGKHSAAADITSSLSEDQVPGPFLVMVLIQFGTMVVDRALY LRK; raised in rabbit; Sigma-Aldrich, Saint Louis, MS, USA) instead of PIEZO1. After rinsing with TBS, sections were incubated with secondary antibodies for 90 minutes each one: first, Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:100; Serotec™; Oxford, UK) and then, Cy3-conjugated donkey anti-mouse IgG (1:200; Jackson-ImmunoResearch™; Baltimore, MD, USA). Both steps were performed at room temperature in a dark humid chamber. A PBS-T wash was performed between both incubations. Finally, sections were stained with DAPI (4′,6-diamino-2-phenylindole; 10 ng/ml) to contrast 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 immunoreaction controls were performed in the same way as for simple immunohistochemistry. Additional controls omitting both antibodies were conducted to confirm the absence of tissue autofluorescence or produced by the fixation process.

2.3. Quantitative study

The density of MCs and the relative density of PIEZO1- or PIEZO2-positve MCs were established analysing 10 sections per skin sample, separated from each other by 200 μm and processed for the simultaneous detection of S100P + PIEZO1 or S100P + PIEZO2 as follows: ten fields per sections enlarged x20 were captured the number of MCs was 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 MCs was calculated; data were expressed as mean ± standard deviation/mm^2. MCs displaying S100P immunoreactivity were considered 100%, and those displaying S100P+PIEZO1 or S100P+PIEZO2 were subtracted to determine the percentage of MCs expressing PIEZO1 or PIEZO2. In addition, the MCs index was calculated on the same sections to establish the number of MCs in relation to the total number of dermal papillae (for details see García-Piqueras et al., 2019). Results are presented again as mean ± standard deviation/mm^2.

3. Results

MCs from human fingers showed variable morphology: ellipsoidal mainly (about 67%) but rounded (28%) and irregular (5%) shapes were also found; almost all were compact (around 88%), without lobulations (just about 12%). Regarding the basic immunohistochemical profile in all cases, the axons were immunoreactive for NFP, NSE and SYN, while the lamellar cells displayed strong immunoreactivity for S100P as expected.

3.1. PIEZO1 is expressed in the axon of human digital Meissner corpuscles

PIEZO1 immunostaining was observed in MCs (Figs. 1 and 2). In

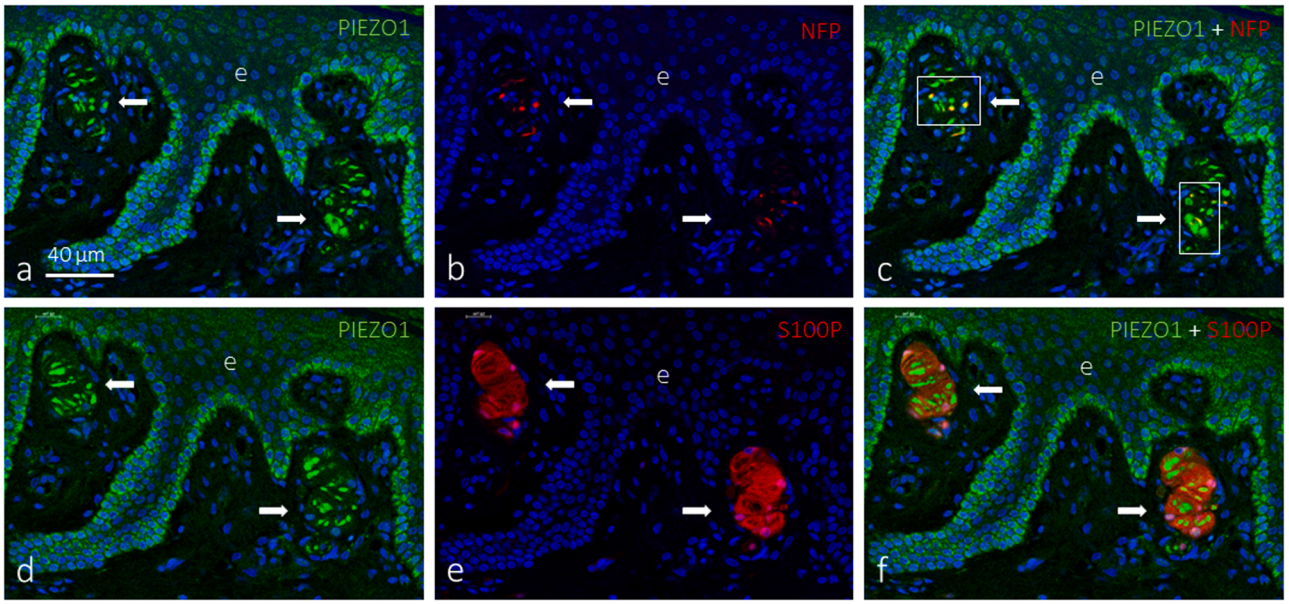

Fig. 1. - Immunofluorescence for PIEZO1 (green fluorescence, in a and d), NFP (b) and S100P (e), and mergences (c and f) in Meissner corpuscles (white arrows). PIEZO1 was localized in axons and never in the lamellar cells. Cell nuclei were labelled with DAPI (blue fluorescence). Objective: 63X/1.40 oil; pinhole: 1.37; XY resolution: 139.4 nm; and Z resolution: 235.8 nm. e: epidermis. Scale bar is the same for all images.

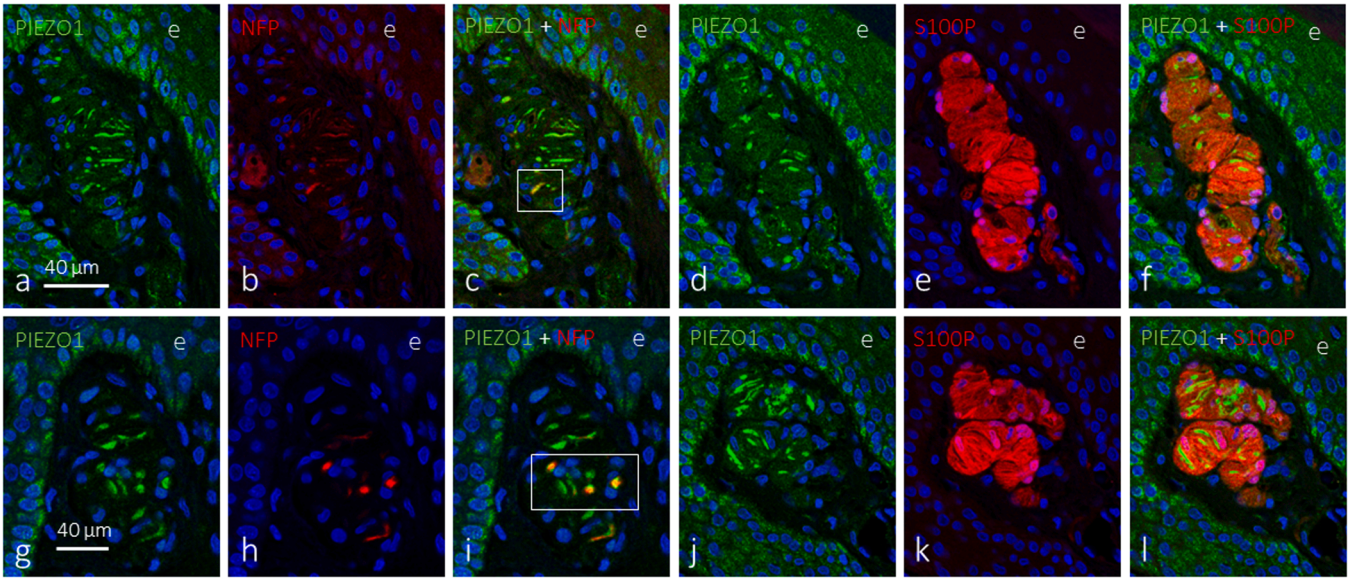

Fig. 2. - Immunofluorescence for PIEZO1 (green fluorescence, in a,d,g and j), NFP (b,h) and S100P (e,k), and mergences (c,f,i and l) in Meissner corpuscles. PIEZO1 was localized in axons and never in the lamellar cells. The blank boxes indicate the convergence zones between PIEZO1 and the NFP axon marker (c, i). Cell nuclei were labelled with DAPI (blue fluorescence). Objective: 63X/1.40 oil; pinhole: 1.37; XY resolution: 139.4 nm; and Z resolution: 235.8 nm. e: epidermis. Scale bar is the same for all images.

consecutive human skin sections, double immunofluorescence between PIEZO1 and specific protein markers for either axonal (NFP) or glial cells (S100P) revealed an axonal pattern within the sensory corpuscle because of co-localization with NFP (Fig. 1, a-c), but not with S100P (Fig. 1, d-f). On the other hand, weak but specific immunofluorescence was detected in the epidermis, especially in basal keratinocytes, as well as in sweat glands (Fig. 1).

3.2. PIEZO1 expression corresponds irregularly to classical axonal immunohistochemical patterns

Since we observed that the used anti-NFP antibody marked slightly the axon path, other experiments were performed to co-localize PIEZO1 with other axon markers such as NSE and SYN (García-Mesa et al., 2021).

PIEZO1 distribution was compared to different axonal protein markers by double immunofluorescence. So, the presence of PIEZO1 in MCs in the axons but not in the surrounding terminal glial cells was ever confirmed: co-localization with NFP (Fig. 2, a-c and g-i) but no co-localization with S100P (Fig. 2, d-f and j-l), respectively. However, immunohistochemical patterns from PIEZO1 and the studied specific axonal proteins were slightly different because of irregular co-localization between them. Regarding the cytoskeletal NFP, full co-localization with PIEZO1 was just observed at some points along the

axon terminal, at the most proximal and distal zones of this sensory corpuscle (Fig. 2, a-c and g-i). As expected, when transversal sections of MC axons were observed, PIEZO1 channel was restricted to the axolemma around the NFP-containing axoplasm. Another axonal protein marker, the glycolytic enzyme NSE, also co-localized with PIEZO1 in the axonal component of MCs (Fig. 3, a-f). In this case, both immunohistochemical patterns were more similar between them than respect to the NFP one. Synaptophysin, an integral membrane protein of synaptic vesicles, was too found co-localizing with PIEZO1 in the axon terminal of MCs (Fig. 3, g-l). Both immunodetections were closely related, even providing a better merged matching respect to NSE or NFP. This co-localization took place along the whole axonal component with no difference.

Therefore, our results demonstrate that MCs in human digital skin express PIEZO1 in the axon but not in lamellar cells.

3.3. *PIEZO2 immunoreactivity*

As described before for PIEZO1, axons in a subpopulation of MCs were immunoreactive for PIEZO2. The level of co-localization of PIEZO2 with different axon markers was variable (Fig. 4, a-i) and co-localization of PIEZO2 with S100 P was never appreciated (Fig. 4, j-l). Some Merkel cells located in the stratum basale of epidermis were also PIEZO2 positive (data not shown), and light immunoreactivity was also detected in the epidermis.

Fig. 5 shows in detail the axon location of PIEZO1 and PIEZO2, based on their co-location with the different axon markers used in the study. In some cases, a characteristic pattern of the axolemma-labelling is shown, while the entire axon is marked in others.

Although a series of preliminary experiments was attempted to study whether PIEZO1 and PIEZO2 co-localize on the same axons, results were unclear and finally removed from the current work.

3.4. *Density of PIEZO1- and PIEZO2-positive Meissner corpuscles*

We analysed the density of MCs in the palmar skin from the distal phalanx of the first and second fingers. A total of 643 MCs, which were identified based on immunoreactivity for S100P expressed by lamellar cells, were evaluated. The mean density of MCs was 8,34 ± 2,82 MCs/mm^2 and the MCs index was 1.9 ± 0,71 MCs/mm^2. On the other hand, the percentage of PIEZO1-positive MCs was about 56%, whereas the percentage of PIEZO2-positive MCs was about 85%.

4. Discussion

The present research was designed to investigate, using immunohistochemistry, the presence of PIEZO1 in MCs of human digital glabrous skin. Parallelly, the presence of PIEZO2 in MCs was studied to confirm previous results (García-Mesa et al., 2017; García-Piqueras et al., 2019). PIEZO1 and PIEZO2 are vertebrate multipass transmembrane proteins forming a part of cationic ion channels, directly involved in mechanotransduction (Coste et al., 2010, 2012; Kefauver et al., 2020). However, while it is well established that PIEZO2 is critical in touch detection, it is unknown if PIEZO1 also contributes to touch sensation. Because MCs are a type of CEOCs specific to human glabrous skin (Cobo et al., 2021) associated with type I rapidly adapting LTMRs (Zimmerman et al., 2014), it is expected that some of their cellular components, especially the axon, may express ion channels related to mechanotransduction, like PIEZO1 and/or PIEZO2.

In agreement with their function, PIEZO1 and PIEZO2 are present in mechanoreceptors and CEOCs (Coste et al., 2010; Roh et al., 2020; Handler and Ginty, 2021; Handler et al., 2023; Shin et al., 2021, 2023). PIEZO2 has been detected in the axon of human MCs (García-Mesa et al., 2017, 2021a,b, 2022; García-Piqueras et al., 2019; Martín-Cruces et al., 2023) and lanceolate nerve endings (Yamanishi and Iwabuchi, 2023), as well as in murine MC-like (Coste et al., 2010; Ranade et al., 2014; Moayedi et al., 2018; Handler et al., 2023; Shin et al., 2023). Additionally, PIEZO2 protein was detected in axons of both Krause's corpuscles from murine clitoris and penis (Qi et al., 2023) and Grandry's corpuscles from birds (Schneider et al., 2017, 2019). In the present study, immunoreactivity for PIEZO2 was found in the axon of a subpopulation of MCs, thus confirming previous data and in total agreement with all previous studies in both humans and non-human vertebrates. Immunoreactivity was never detected in lamellar cells as described by Shin et al. (2021) in murine cutaneous sensory nerve formations. Outside the cutaneous nerves and CEOCs, PIEZO2 immunostaining was

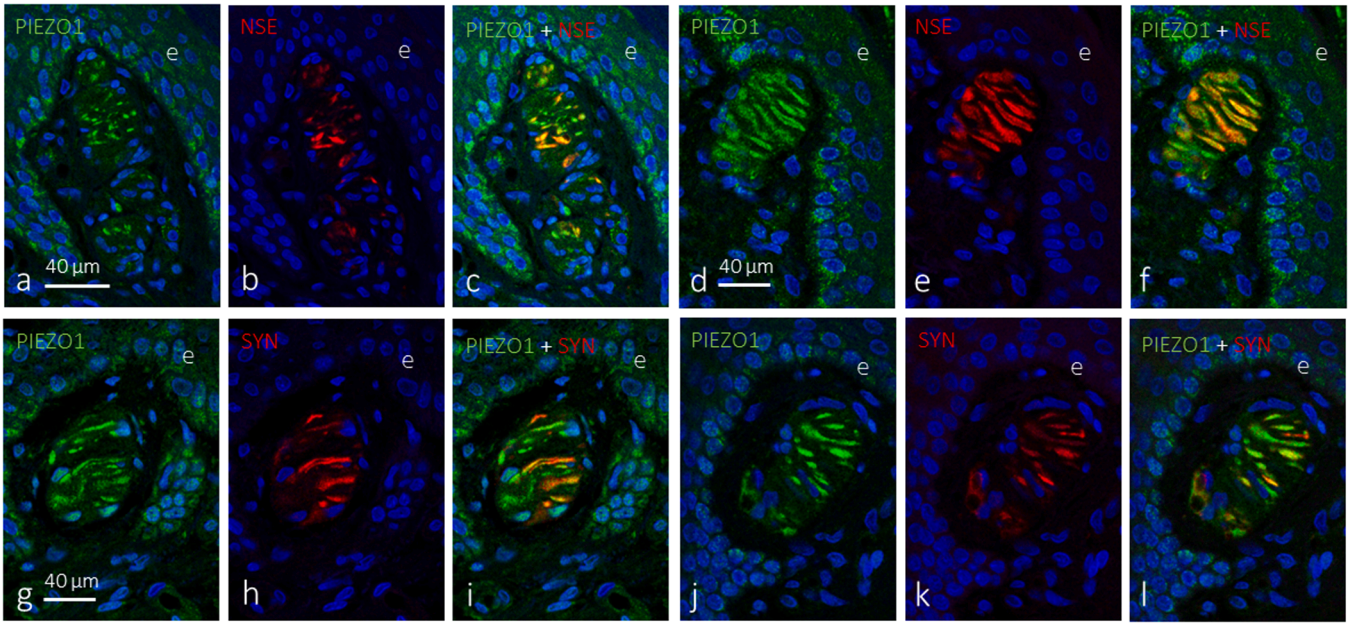

Fig. 3. - Immunofluorescence for PIEZO1 (green fluorescence, in a,d,g and j), NSE (b,e), SYN (h,k), and mergences (c,f,i and l) in Meissner corpuscles. PIEZO1 was localized in axons. Cell nuclei were labelled with DAPI (blue fluorescence). Objective: 63X/1.40 oil; pinhole: 1.37; XY resolution: 139.4 nm; and Z resolution: 235.8 nm. e: epidermis. Scale bar is the same for all images.

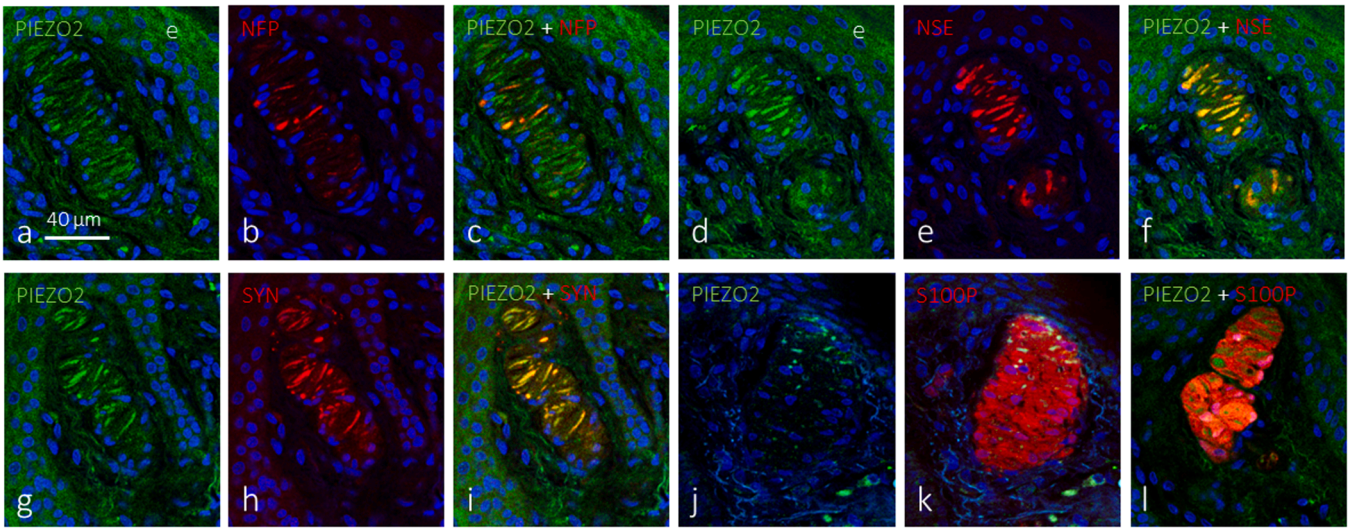

Fig. 4. - Immunofluorescence for PIEZO2 (green fluorescence, in a,d,g and j), NFP (b), NSE (e), SYN (h), S100P (k) and mergences (c,f,i,k and l) in Meissner corpuscles. PIEZO2 was localized in axons and never in the lamellar cells. Cell nuclei were labelled with DAPI (blue fluorescence). Objective: 63X/1.40 oil; pinhole: 1.37; XY resolution: 139.4 nm; and Z resolution: 235.8 nm. e: epidermis. Scale bar is the same for all images.

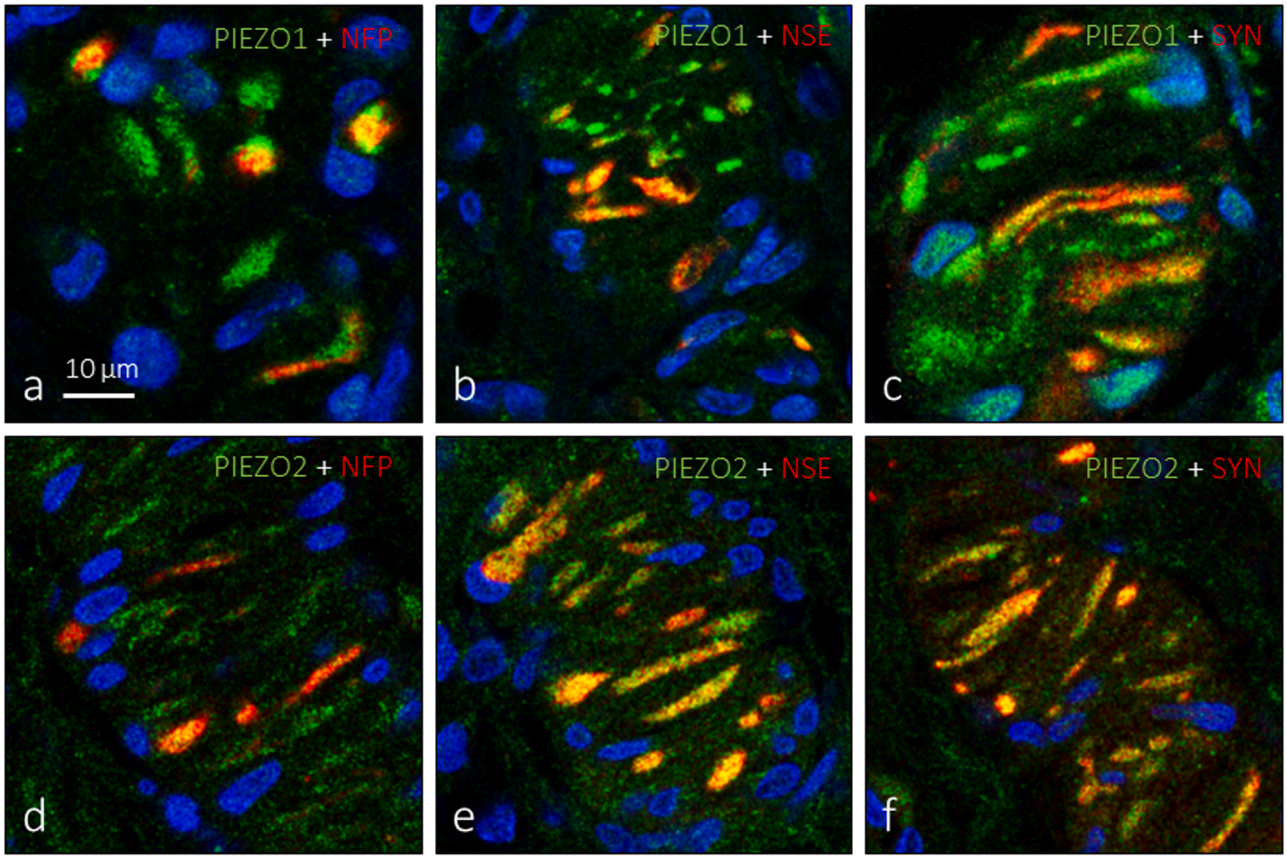

Fig. 5. - Immunofluorescence for PIEZO1 (top row) and PIEZO2 (bottom row) with axonal and synaptic markers. PIEZO1 presents a partial colocalization in segments of the axon pathway (a, b, c), while PIEZO2 colocalizes in most of the axon pathway (see yellow emergence in d, e, f). Cell nuclei were labelled with DAPI (blue fluorescence). Objective: 63X/1.40 oil; pinhole: 1.37; XY resolution: 139.4 nm; and Z resolution: 235.8 nm. e: epidermis. Scale bar is the same for all images.

reported in the epidermis and cutaneous glands, which was especially stronger in Merkel cells. Nevertheless, because of the low protein expression level in the epidermis, it is unlikely that PIEZO2 might be the primary contributor to the remaining keratinocyte mechanical sensitivity (Coste et al., 2010; Hoffman et al., 2018).

Regarding PIEZO1, as far as we know, it has never been detected in human CEOCs, but was detected in both the axon and terminal glial cells of rat CEOCs (Shin et al., 2023). Here, we demonstrate PIEZO1 immunoreactivity is restricted to the axon and never found in the laminar cells of MCs. As well as for PIEZO2, species-specific differences account for discrepancies respect to results in rats. These data suggest that PIEZO1 may be involved in fine-touch detection. In supporting present results

the PIEZO1 deficient animals have deficits in behavioural responses to both light touch and high-threshold mechanical stimuli (Mikesell et al., 2022).

In addition to MCs, we detected faint but specific immunoreactivity for PIEZO1 in all the epidermis layers, being especially intense in keratinocytes of the basal layer, but did not be able to say whether it is also located in Merkel cells and melanocytes (Shin et al., 2023). The expression of PIEZO1 in mouse keratinocytes was originally reported by Coste et al. (2010) and confirmed subsequently by Holt et al., (2021). It is currently accepted that PIEZO1 is critical for mechanotransduction in keratinocytes, which enables these cells to encode mechanical force and convey this signal to sensory afferent terminals (Mikesell et al., 2022), and aids to orchestrate mechanosensation. Since keratinocytes are sensitive to mechanical forces and contribute to touch sensation, it would be expected they express mechanosensory proteins (see Eid and Kurban 2022). Based on present results, PIEZO1 may be a mechanotransducer in keratinocyte, like in mouse skin (Mikesell et al., 2022). Furthermore, keratinocytes form close 'synapse-like' connections with intraepidermal nerve fibres (Talagas et al., 2020), and PIEZO1 is in free nerve endings contacting keratinocytes (Pereira et al., 2023). On the other hand, keratinocyte activity is critical for normal sensory neuron responses to mechanical stimuli. It was classically accepted that primary sensory neurons were the sole transducers of innocuous stimuli in skin. However, this dogma has essentially been negated by recent works that demonstrate that non-neuronal cells, including keratinocytes o terminal glial cells, are required for the normal detection and coding of somatosensory stimuli in the peripheral nervous system (Abdo et al., 2019; Neubarth et al., 2020; Suazo et al., 2022).

Although we have not investigated the possible co-localization of PIEZO1 and PIEZO2 in MCs, the individual percentages suggest they may represent two independent subsets of MCs that represent primary sensory neurons projecting to the skin. Immunohistochemistry revealed Piezo2 expression in pan primary sensory neurons of DRG in rats (Shin et al., 2021) and about 45% of DRG neurons in rat and human (Szczot et al., 2018; Murthy et al., 2018), while *Piezo1* mRNA and protein were reported in about 60% mouse DRG neurons small- (< 25 μm) and medium-sized (25–35 μm) mouse DRG neurons (Roh et al., 2020; Shin et al., 2023). The medium-sized neurons are regarded as mechanoreceptive, which is consistent with the expression of PIEZO1 and PIEZO2 in murine MC-like. Human studies are needed to know which neuronal populations of the DRG express PIEZO proteins.

Overall, the present study demonstrates than human digital skin contains subpopulations of MCs displaying axonal PIEZO1 and PIEZO2, and thus, both ion channels may be involved in touch. Furthermore, while PIEZO2 seems to mediate specifically light touch originated in MCs, epidermal PIEZO1 appears to be a more general amplifier of cutaneous mechanical stimuli because its presence in MCs and keratinocytes (Mikesell et al., 2022).

Funding statement

No founding.

Ethical statement

This study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. CElm, PAst: Proyecto 266/18).

CRediT authorship contribution statement

Yolanda García-Mesa: Conceptualization, Methodology, Experiments, Writing – original draft. **Patricia Cuendias**: Conceptualization, Methodology, Experiments, Writing – original draft. **Marta Alonso-Güervos**: Methodology, Experiments. **Jorge García-Piqueras**: Conceptualization, Writing – original draft. **Olivia García-Suárez**: Visualization, Investigation. **José A. Vega**: Conceptualization, Methodology, Supervision, Writing – review & editing.

Author statement

Suárez Olivia: Conceptualization, Investigation, Methodology, Writing – original draft. **García-Mesa Yolanda:** Data curation, Investigation, Methodology, Validation. **Cuendias Patricia:** Formal analysis, Investigation, Methodology. **VEGA Jose ANTONIO:** Conceptualization, Writing – original draft, Writing – review & editing. **Martín-Biedma Benjamín:** Data curation, Funding acquisition, Investigation, Project administration, Resources. **Cobo Teresa:** Data curation, Formal analysis, Investigation, Project administration, Resources. **Alonso-Guervós Marta:** Methodology, Software, Visualization. **García-Piqueras Jorge:** Investigation, Methodology, Supervision, Validation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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