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ORIGINAL PAPER

Sensory innervation of the human male prepuce: Meissner's corpuscles predominate

Yolanda García-Mesa[1] | Jorge García-Piqueras[1] | Ramón Cobo[1] | José Martín-Cruces[1] | Iván Suazo[1,2] | Olivia García-Suárez[1] | Jorge Feito[1,3,4] | José A. Vega[1,2]

[1]Departamento de Morfología y Biología Celular, Grupo SINPOS, Universidad de Oviedo, Oviedo, Spain

[2]Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, Chile

[3]Servicio de Anatomía Patológica, Complejo Hospitalario Universitario de Salamanca, Salamanca, Spain

[4]Departamento de Anatomía e Histología Humanas, Universidad de Salamanca, Spain

Correspondence
José A. Vega, M.D., Ph.D., Departamento de Morfología y Biología Celular, Facultad de Medicina y Ciencias de la Salud, C/ Julián Clavería, nº 6, 33006 Oviedo, Spain.
Email: javega@uniovi.es

Abstract

Meissner's corpuscles are the most abundant sensory corpuscles in the glabrous skin of the male prepuce. They are type I, rapidly adapting, low-threshold mechanoreceptors, and their function is linked to the expression of the mechanoprotein piezo-type mechanosensitive ion channel component 2 (PIEZO2). Stimulation of genital Meissner's corpuscles gives rise to sexual sensations. It has been recently demonstrated that digital Meissner's corpuscles, Meissner-like corpuscles, and genital end bulbs have an endoneurium-like capsule surrounding their neuronal elements; that is, the axon and glial lamellar cells, and their axons, display PIEZO2 immunoreactivity. It is unknown whether this is also the case for preputial Meissner's corpuscles. Furthermore, the expression of certain proteins that have been found in Meissner's corpuscles at other anatomical locations, especially in the digits, has not been investigated in preputial Meissner's corpuscles. Here, we used immunohistochemistry to investigate the expression of axonal (neurofilament, neuron-specific enolase), glial (S100 protein, glial fibrillary acidic protein, vimentin), endoneurial (CD34), and perineurial (glucose transporter 1) markers in the preputial and digital Meissner's corpuscles of male participants aged between 5 and 23 years. Furthermore, we investigated the occurrence of the mechanoprotein PIEZO2 in male preputial Meissner's corpuscles. Human male prepuce contains numerous Meissner's corpuscles, which may be grouped or isolated and are regularly distributed in the dermal papillae. Lamellar glial cells display strong expression of S100 protein and vimentin but lack expression of glial fibrillary acidic protein. In addition, they show axonal PIEZO2 expression and have an endoneurial capsule, but no perineurial. Our results indicate that human male preputial Meissner's corpuscles share the immunohistochemical profile of digital Meissner's corpuscles, which is considered to be necessary for mechanotransduction. These data strongly suggest that the structure and function of Meissner's corpuscles are independent of their anatomical location.

KEYWORDS
capsule, human, male prepuce, Meissner's corpuscles, PIEZO2

1 | INTRODUCTION

The male prepuce, also known as the foreskin, is a cutaneous structure that covers the glans penis to protect the external genitalia. It is involved in sexual sensations and arousal (Cold & Taylor, 1999). However, a study on the association of penile histology with sexual response found no basis for ascribing sexual function to the prepuce (Cox et al., 2015). The prepuce contains sensory corpuscles related to various modalities of mechanosensitivity. In 1956, Winkelmann summarized all existing knowledge on prepuce innervation and published an excellent study on the cutaneous innervation of the human neonatal prepuce. He described intraepithelial "fibrils," "papillary endings," and Vater-Pacini corpuscles, but not the classical dermal "bodies" such as Meissner's corpuscles, Krause end bulbs, Dogiel's bodies, Golgi-Mazzoni bodies, and genital corpuscles. Later studies confirmed the presence of free nerve endings, Pacinian corpuscles, and Merkel cells, although the most common preputial sensory corpuscles were Meissner's corpuscles (Bath et al., 2008; Cold & Taylor, 1999; Guo et al., 2007; Jiang et al., 2006; Malkoc et al., 2012; Martín-Alguacil et al., 2015; Taylor et al., 1996).

Certain preputial sensory corpuscles, such as Meissner's corpuscles, Pacinian corpuscles, and Merkel cell-neurite complexes, function as mechanoreceptors in human glabrous skin (Cobo et al., 2020, 2021; Zimmerman et al., 2014), mediating various functions related to mechanosensitivity, especially touch (McGlone & Reilly, 2010; McGlone et al., 2014). They have a functional relationship with peripheral processes of primary sensory neurons that encode non-painful mechanical stimuli (low-threshold mechanoreceptors [LTMRs]; Abraira & Ginty, 2013; Fleming & Luo, 2013; Zimmerman et al., 2014). Meissner's and Pacinian corpuscles correspond to type I and type II rapidly adapting LTMRs, whereas Merkel cell-neurite complexes and Ruffini corpuscles correspond to type I and type II slowly adapting LTMRs, respectively (Fleming & Luo, 2013; Rice & Albrecht, 2008; Zimmerman et al., 2014). Slowly adapting LTMRs are involved in the sensation of fine touch, and rapidly adapting LTMRs are involved in the sensations of vibration and motion across the skin (Johnson, 2001; Jones & Smith, 2014; Olson et al., 2016; Owens & Lumpkin, 2014).

The conversion of mechanical stimuli into electrical signals in sensory corpuscles involves mechano-gated ion channel activation. Recent studies have shown that piezo-type mechanosensitive ion channel component 2 (PIEZO2), a vertebrate stretch-gated multipass transmembrane protein that is a component of a certain mechanically gated ion channel, is required for mechanotransduction in mammalian cells (Coste et al., 2010; Honoré et al., 2015; Ranade et al., 2014). It is expressed in the dorsal root ganglia (Coste et al., 2010; Ranade et al., 2014), Merkel discs, isolated Merkel cells, and Meissner's corpuscles in murine and human skin (García-Mesa et al., 2017; García-Piqueras et al., 2019; Ikeda et al., 2014; Maksimovic et al., 2014; Ranade et al., 2014; Woo et al., 2014). To the best of our knowledge, the expression of PIEZO2 in preputial mechanoreceptors has not been investigated. However, Chen et al., (2020) studied the role of PIEZO2 in the mechanism underlying premature ejaculation in rats and reported that PIEZO2 expression was significantly increased in the penile head and dorsal root ganglia of these rats.

This study aimed to meticulously investigate the types of sensory corpuscles present in the human male prepuce and determine whether they expressed PIEZO2 mechanoproteins. The main aim of this study was to elucidate the molecular mechanisms underlying mechanosensitivity in sexual organs.

2 | METHODS

2.1 | Tissues

Prepuce samples were obtained from 32 boys and men (aged 5–23 years) who underwent routine circumcision due to phimosis or redundant prepuce. To reflect sexual maturation, the participants were divided into three groups based on age: <10 years (pre-puberty, n = 3), 10–20 years (puberty, n = 17), and >20 years (post-puberty, n = 12). The samples were fixed in 4% buffered formaldehyde and routinely processed for paraffin embedding. These materials were obtained from our laboratory (Registro Nacional de Biobancos, Sección Colecciones, Ref. C-0001627). This study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. Celm, Past: Proyecto 266/18), and tissue samples were obtained in accordance with Spanish law (RD 1301/2006; Ley 14/2007; DR 1716/2011; Orden ECC 1414/2013).

2.2 | Immunohistochemistry

Deparaffinized and rehydrated sections were processed for indirect immunohistochemistry using a Leica Bond Polymer Refine Detection Kit (Leica Biosystems™) following the manufacturer's instructions. Immunohistochemistry was performed to explore the expression of antigens related to axons (neurofilament proteins, neuron specific enolase [NSE]), corpuscular glial cells (S100 protein [S100P], glial-fibrillary acidic protein [GFAP], vimentin), the endoneurium (CD34 antigen), the perineurium (glucose-transporter 1 [GLUT1]), and the mechanoprotein PIEZO2. Table 1 summarizes the primary antibodies used in this study. Indirect immunohistochemistry included several negative and positive controls, as well as internal and external controls.

2.3 | Double immunofluorescence staining

Double immunofluorescence staining was performed to investigate PIEZO2, S100P, and NSE expression. Non-specific binding was reduced in the deparaffinized and rehydrated sections through incubation for 30 min with a solution of 5% bovine serum albumin in Tris-buffered saline (pH 7.4). The sections were then incubated overnight at 4°C in a humid chamber with a 1:1 (v/v) mixture of anti-S100P

TABLE 1 Primary antibodies used in this study

Antigen	Origin	Dilution	Supplier
CD34 (clone QB-END/10)	Mouse	Prediluted	Master Diagnostica[a]
Glial-fibrillary acidic protein (clone G-A–5)	Mouse	1:500	Boehringer Mannheim[b]
GLUT1	Rabbit	0.5 μg/ml	Cell Marque[c]
NSE (clone BBS/NC/VI-H14)	Mouse	1:1000	Dako[d]
PIEZO2[g]	Rabbit	1:200	Sigma-Aldrich[e]
S100 protein (clone 4C4.9)	Mouse	1:1000	Thermo Scientific[f]
S100 protein	Rabbit	1:1000	Dako[d]
Vimentin (clone 3B4)	Mouse	Prediluted	Dako[d]

Abbreviations: ASIC2, acid sensing ion channel subunit 2; GLUT1, glucose transporter 1; NSE, neuron specific enolase; PIEZO2, piezo-type mechanosensitive ion channel component 2.

[a]Granada, Spain.

[b]Indianapolis, Indiana, USA.

[c]Seattle, WA, USA.

[d]Glostrup, Denmark.

[e]Saint Louis, MS, USA.

[f]Freemont, CA, USA.

[g]Amino acid sequence: FEDENKAAVRIMAGDNVEICMNLDAASFSQHNP.

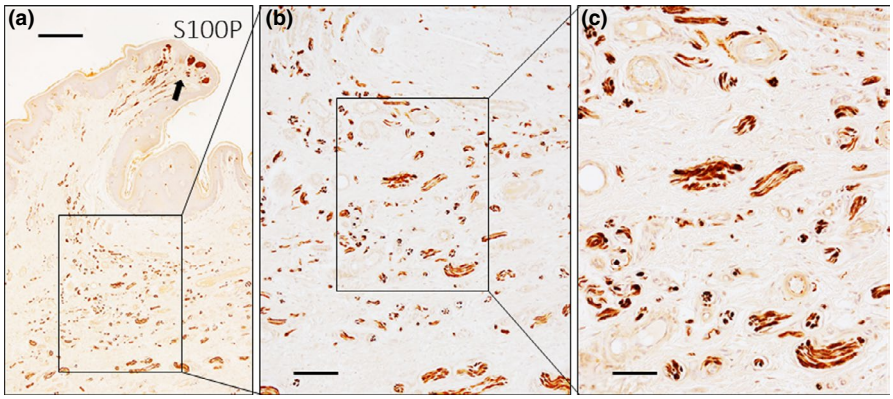

FIGURE 1 Immunohistochemical detection of S100 protein in the human male prepuce. The prepuce contains a dense network of nerve fibers of varying diameters in the deep dermis (image magnification: a → c). Most of the dermal papillae do not show nerve profiles, except for a few Meissner's corpuscles. Arrows in (a). Scale bar: 250 (a), 150 (b), and 80 μm (c) [Colour figure can be viewed at wileyonlinelibrary.com]

and anti-PIEZO2, and anti-NSE and anti-PIEZO2. After rinsing, the sections were incubated for 1 h with Alexa Fluor 488-conjugated goat anti-rabbit immunoglobulin G (Serotec™, diluted 1:1000), rinsed again, and incubated for another hour with Cy3-conjugated donkey anti-mouse antibody (Jackson-ImmunoResearch™, diluted 1:50). Both these steps were performed at room temperature in a dark, humid chamber. The sections were then washed, mounted with Fluoromount Gold (ThermoFisher), and counterstained with 4,6-diamidino-2-phenylindole (10 ng/ml) to label the nuclei. The samples were visualized following triple staining using a Leica DMR-XA automatic fluorescence microscope with Leica Confocal software, version 2.5 (Leica Microsystems, Heidelberg GmbH), and the captured images were processed using ImageJ software, version 1.43 (Master Biophotonics Facility, McMaster University, Ontario, www.macbiophotonics.ca). As controls, representative sections were also processed using the techniques described above, using non-immune rabbit or mouse sera instead of primary antibodies or by omitting the primary antibodies during incubation.

2.4 | Quantitative analyses

Quantitative analyses were performed to determine the density of Meissner's corpuscles using the technique proposed by Verendeev et al., (2015), which has been described in detail in a previous study (García-Piqueras et al., 2019). The Meissner index was determined using the technique proposed by Bhat et al., (2008). The densities of other sensory corpuscles were not calculated because of their infrequent occurrence and irregular distribution in the dermis. Briefly, S100P immunohistochemistry was performed to identify

Meissner's corpuscles in five sections from each skin sample that were obtained from locations 200 μm apart. The sections were scanned using an SCN400F scanner (Leica Biosystems™), and the scans were computed using SlidePath Gateway LAN software (Leica, Leica Biosystems™). Subsequently, Meissner's corpuscles were identified and counted by two independent observers (YG-M and JAV). The average counts were corrected using the Abercrombie formula. The epidermal length (mm) of each section, and the average epidermal length was multiplied by the section thickness (mm) to calculate the surface area (mm^2). Finally, the average number of Meissner's corpuscles (N) was divided by the surface area (mm^2) to calculate the density of Meissner's corpuscles per square millimeter of skin (number of Meissner's corpuscles/mm^2). Subsequently, the average density was calculated from the individual densities for each pre-established age group.

To investigate the relationship between Meissner's corpuscles and dermal papillae, the measurements were standardized according to the length of skin analyzed to compare between the groups. Significant differences among the three pre-established age groups were assessed using the Kruskal–Wallis H test, and p values <0.05, were considered statistically significant.

3 | RESULTS

The prepuce samples were richly innervated by nerve fibers of various diameters that formed a dense network in the reticular dermis. These fibers formed perivascular plexuses, terminated as free nerve endings, and innervated sensory corpuscles of various morphotypes (Figure 1). Human male prepuce is richly innervated by nerve fibers of various diameters that form a dense network in the reticular dermis. These fibers form perivascular plexuses, terminate as free nerve endings, and innervate sensory corpuscles of various morphotypes (Figure 1). Nerve profiles were not observed in most parts of the papillary dermis, except where Meissner's corpuscles were found. Epidermal free nerve endings were scarcely observed (Figure 1a).

3.1 | Meissner's corpuscles are the most common sensory corpuscles in the prepuce

The sensory corpuscles located immediately beneath the epithelium were typical or ovoid Meissner's corpuscles. They displayed a notable tendency to aggregate in the cutaneous folds; their density was high in this region, while they were scarcely present in adjacent areas that were flat or depressed (Figure 2). They showed various morphologies and sizes, were located either in the dermal papillae or the superficial dermis, and displayed the characteristic properties of Meissner's corpuscles (Figure 3a–e). Nevertheless, we also found other corpuscles that were rounded, glomeruloid, or lobulated and were located deep in the dermis; they may have been genital bodies (Figure 2f–h). The morphology, size, and dermal topography of the Meissner's corpuscles were not correlated with age.

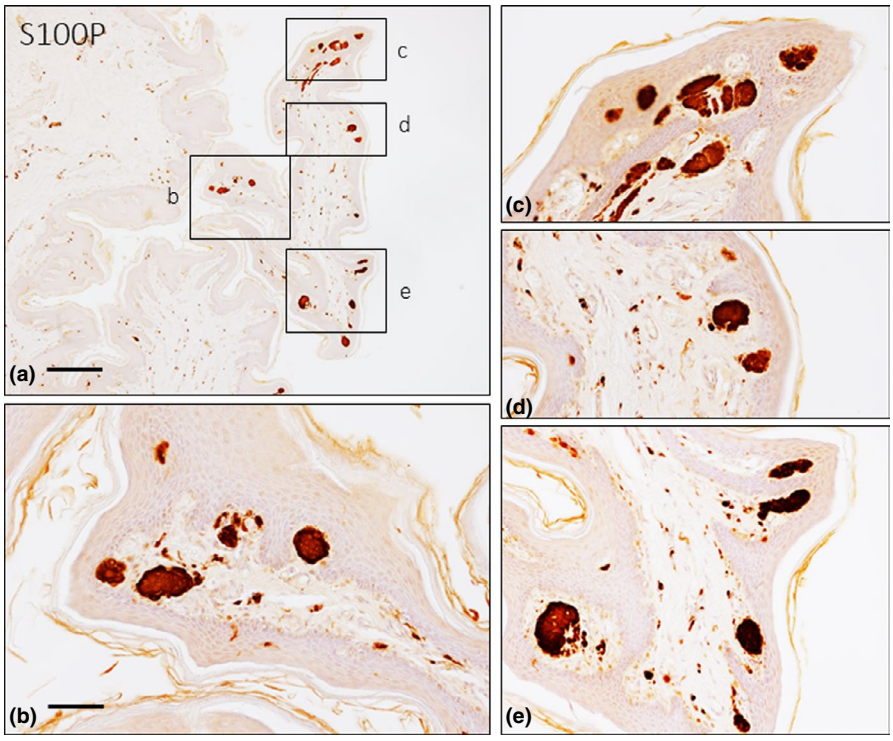

FIGURE 2 Immunohistochemical detection of S100 protein in the sensory corpuscles of the human prepuce. Clusters of Meissner's corpuscles are seen in the cutaneous folds. In contrast, deeper portions of these folds do not show Meissner's corpuscles (a). Images (b)–(e) show details of the Meissner's corpuscles at epidermal excrescences. Scale bar: 250 (a) and 50 μm (b–e) [Colour figure can be viewed at wileyonlinelibrary.com]

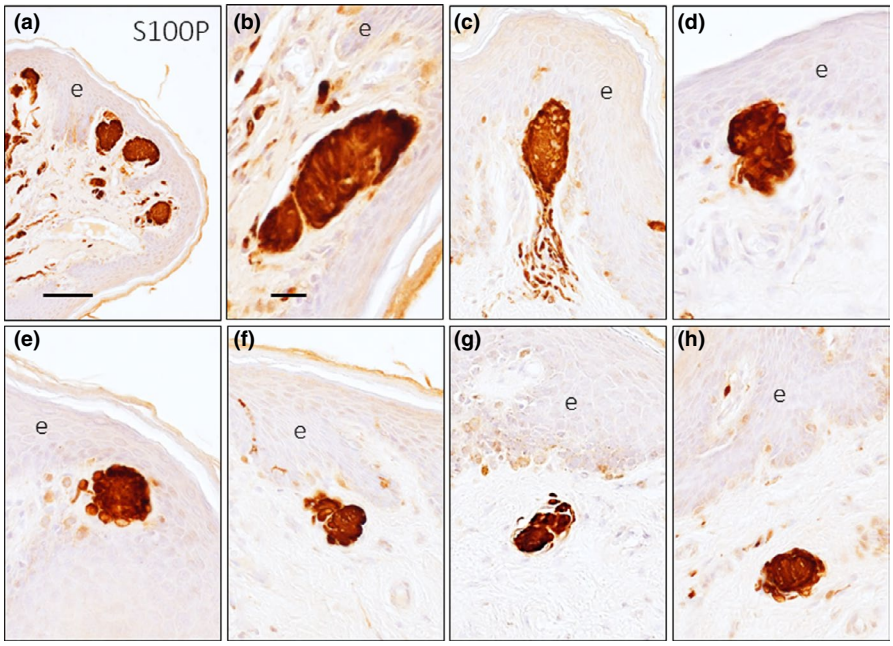

FIGURE 3 Typical Meissner's corpuscles were observed in the dermal papillae (a–e) whereas genital bodies showing rounded, glomeruloid, or lobulated shapes were observed at deeper levels in the dermis (f–h). e: epidermis. Scale bar: 70 (a) and 20 μm (b–h) [Colour figure can be viewed at wileyonlinelibrary.com]

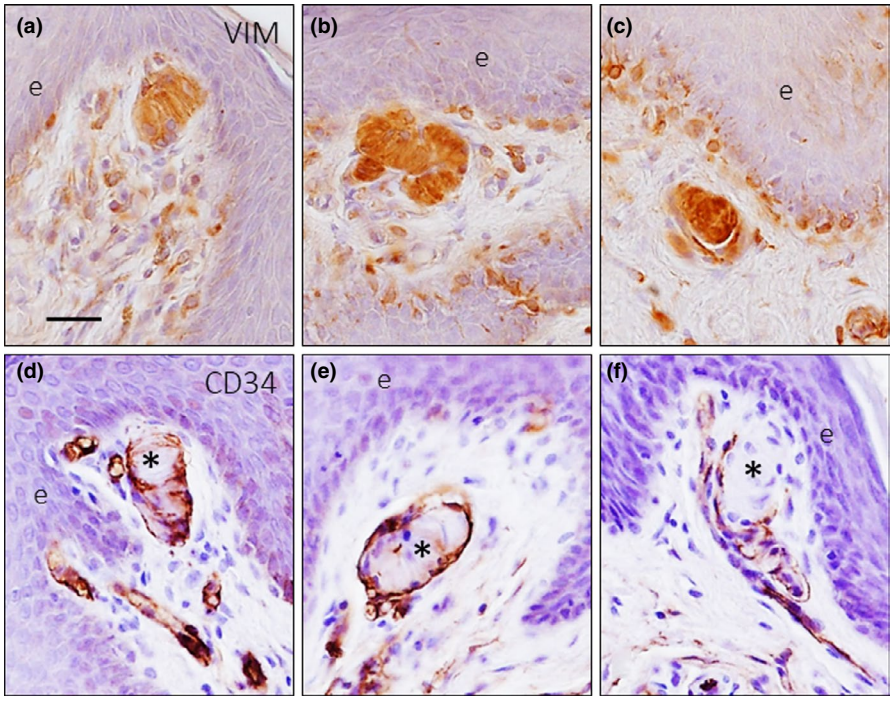

FIGURE 4 The cytoplasm of peripheral glial cells in Meissner's corpuscles and genital bodies displays vimentin immunoreactivity (a–c). Moreover, most of the preputial Meissner's corpuscles and genital bodies are surrounded by a CD34-positive capsule of endoneurial origin (d–f; asterisk: Meissner's corpuscle). e: epidermis. Scale bar: 40 μm (a–f). [Colour figure can be viewed at wileyonlinelibrary.com]

Generally, lamellar cells of sensory corpuscles display strong immunoreactivity for S100P regardless of their anatomical location (Figure 3). As seen in digital Meissner's corpuscles, the preputial Meissner's corpuscles and genital bodies also had lamellar cells displaying vimentin immunoreactivity (Figure 4a–c). No immunoreactivity for GFAP was observed (data not shown). In contrast, approximately 75% of the preputial Meissner's corpuscles were surrounded, partially or completely, by a thin CD34-positive capsule that was

related to endoneurium-derived fibroblasts (Figure 4e,f). Positive GLUT1 immunoreactivity was not detected in the structures resembling sensory corpuscles.

The density and average index of Meissner's corpuscles in the analyzed samples were 8.3 ± 4.1 and 0.14 ± 0.001, respectively. The density of Meissner's corpuscles in participants aged <10 years, between 10 and 20 years, and >20 years was 3.0 ± 1.1, 11.3 ± 4.1, and 10.8 ± 3.7, respectively (Figure 5a). The average Meissner index was 0.02 ± 0.001 in the first decade of life, 0.21 ± 0.01 in the second decade, and 0.28 ± 0.01 in subsequent decades (Figure 5b). Significant differences ($p < 0.001$) were found in both parameters between participants in the pre-puberty group and those in the other two groups, while no significant differences in density or index of Meissner's corpuscles were found between the puberty and post-puberty groups.

3.2 | Meissner's corpuscles of the prepuce show PIEZO2 expression

In this study, intense PIEZO2 immunoreactivity was observed in the axons of Meissner's corpuscles (Figure 6a–d), as well as in scattered Merkel cells (Figure 6e,f). The pattern of axonal PIEZO2 immunostaining was punctate and did not show the entire axonal profile. Double immunolabeling was performed to confirm the localization of PIEZO2 within Meissner's corpuscles. It revealed that PIEZO2 did not co-localize with S100P (thus excluding glial localization) and matched the distribution of neuron-specific enolase well (thus confirming axonal localization, Figure 7).

3.3 | Other morphotypes of sensory corpuscles in the male prepuce

In addition to the characteristic Meissner's corpuscles and Meissner-like sensory corpuscles described above, a few other morphotypes of sensory corpuscles were identified. As a rule, their density was very low, and they showed an irregular morphology. Those that were located in the superficial papillary dermis might have been Krause or Krause-like corpuscles. These corpuscles were easily observable and larger than the previously described superficially located corpuscles (Figure 8). They were generally composed of two glomeruloid elements separated by a hypocellular tissue (Figure 8a), and a capsule surrounded all the neural elements (Figure 8b,c). A few deeply located corpuscles showed the characteristic morphology of Ruffini corpuscles (Figure 9a) and Ruffini-like corpuscles (Figure 9b,c). They were elongated, associated with Schwann-like cells, and surrounded by a nearly developed capsule. In addition, we found a great variety of morphologically undetermined small sensory corpuscles. They were round; composed of several loosely disposed, Schwann-derived cells; and occasionally showed a longitudinal or lamellar structure (Figure 9e–h).

4 | DISCUSSION

Human male prepuce is involved in sensation of diverse mechanical and non-mechanical stimuli. It was found to contain sensory formations of various morphotypes, especially Meissner's corpuscles in the dermal papillae; rounded, deeply located corpuscles that correspond to the genital bodies; and a few sensory corpuscles (Ruffini and Krause corpuscles) of other morphotypes.

The most striking feature of preputial skin is the low degree of innervation of the papillary dermis. While previous studies have not investigated this paucity of innervation, it could be related to site-specific variations within the prepuce sample studied (Martín-Alguacil et al., 2015). This is supported by the fact that there is notable innervation of areas with sensory corpuscles. In our previous study on genital skin (Feito et al., 2018a), we found that Meissner's corpuscles were usually located in the skin folds; this was also the case in the prepuce (Figure 1). These folds probably correspond to the ridged bands and are similar to the vulvar folds; both of these are rich in corpuscles (Cold & Taylor, 1999; Feito et al., 2018a; Sorrells et al., 2007).

Meissner's corpuscles were observed in abundance in the prepuce. They were not morphologically different from those in digital skin, thus being termed Meissner's corpuscles and not Meissner-like corpuscles, which are found in the vulvar labia minora (Feito et al.,

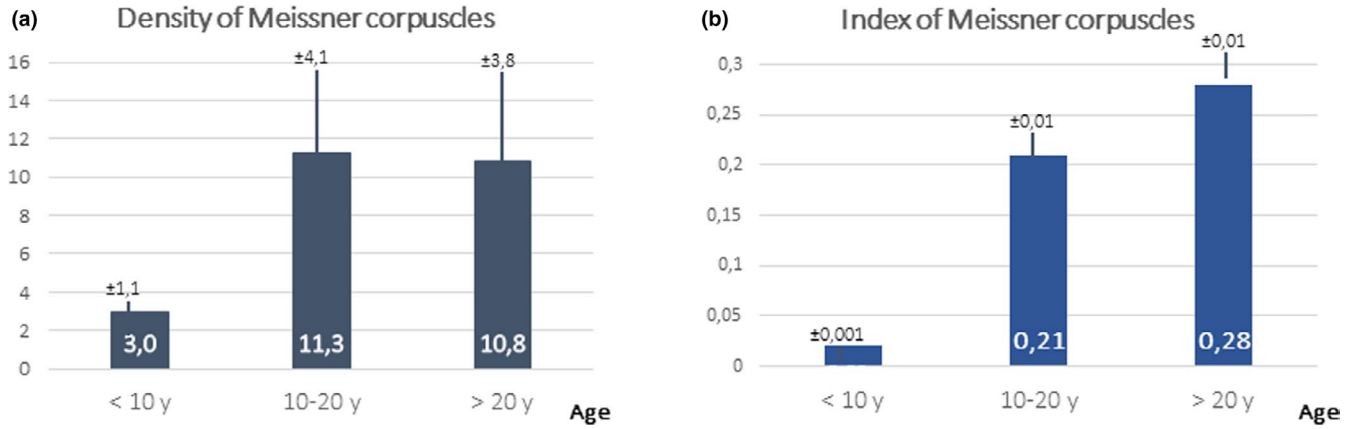

FIGURE 5 Results of the quantitative study [Colour figure can be viewed at wileyonlinelibrary.com]

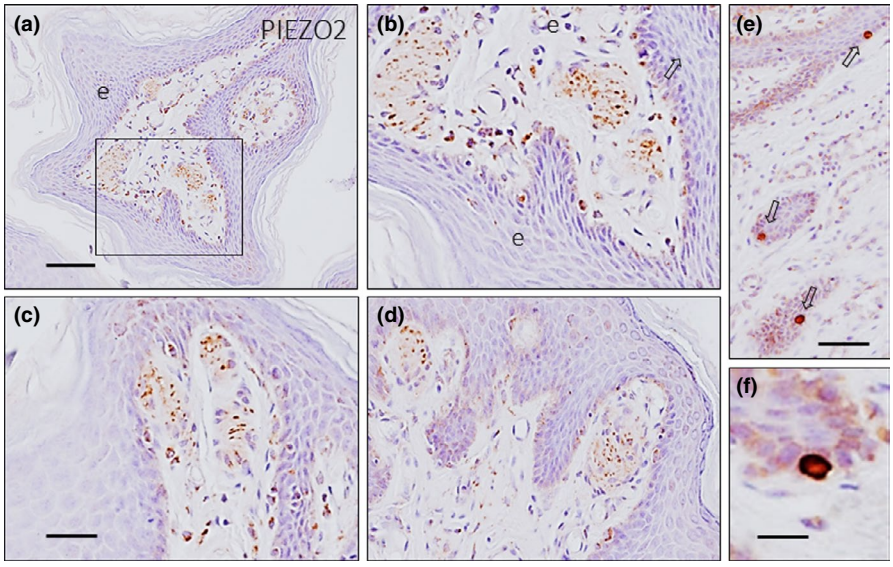

FIGURE 6 Piezo-type mechanosensitive ion channel component 2 (PIEZO2) immunoreactivity of the Merkel cells (e–h) and the axon supplying the Meissner's corpuscles (a–d). In the latter, the pattern of distribution of PIEZO2 is not consistent throughout the axon but is dotted (e–h; e: magnified image of d). Scale bar: 120 μm (a, d, f–h), 30 μm (b, c, e) [Colour figure can be viewed at wileyonlinelibrary.com]

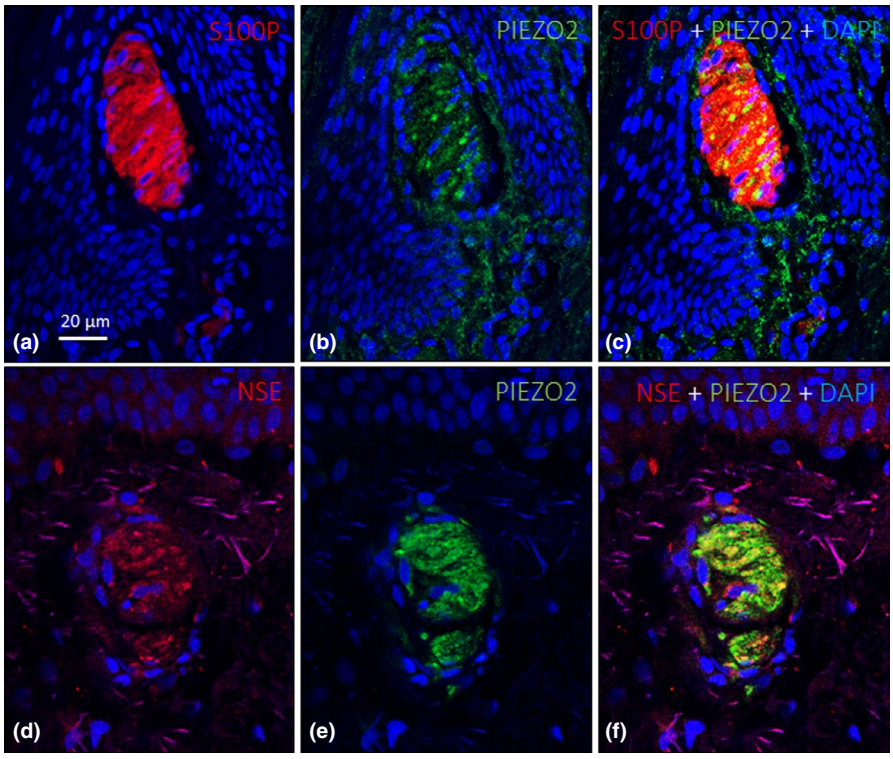

FIGURE 7 Double immunofluorescence for piezo-type mechanosensitive ion channel component 2 (PIEZO2; green fluorescence; b, c, e, and f) and either S100P (red fluorescence; a and c) or neuron specific enolase (NSE; red fluorescence; d and f) confirms the presence of PIEZO2 in the axon (co-localization with NSE, yellow merge; f) and the absence of PIEZO2 in the lamellar cells (no co-localization with S100P, no merge; c). Sections were counterstained with 4,6-diamidino-2-phenylindole to ascertain structural details. Objective: 63×/1.40 oil, pinhole: 1.37, *XY* resolution: 139.4 nm, and *Z* resolution 235.8 nm. Scale bar: 20 μm [Colour figure can be viewed at wileyonlinelibrary.com]

2018a). These results are consistent with those of previous studies (Cold & Taylor, 1999; Halata & Munger, 1986; Martin-Alguacil et al., 2015; Taylor et al., 1996). Furthermore, preputial Meissner's corpuscles and genital bodies share the immunohistochemical characteristics of their digital counterparts, and most of them have a thin capsule of endoneurial origin (Cobo et al., 2021; García-Piqueras

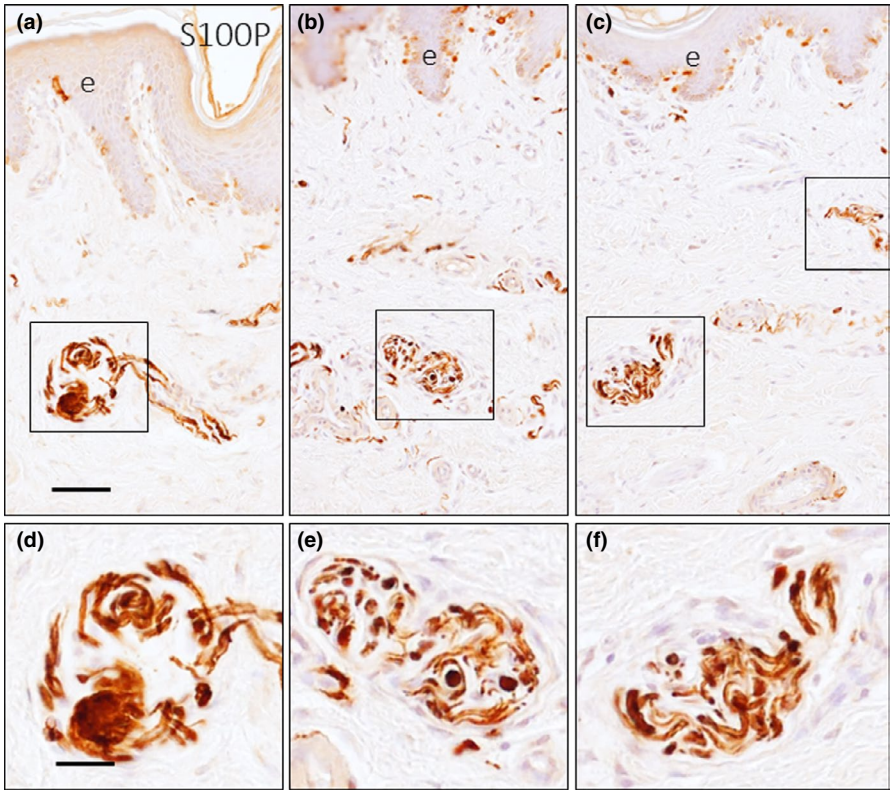

FIGURE 8 The deep dermis contains various morphotypes of sensory corpuscles, including Krause or Krause-like corpuscles. They are observed on immunohistochemistry for S100P. Compared with the Meissner's corpuscles, these are bigger, have a more irregular morphology, and are located deeper in the dermis (a–c). Constitutively, the two neural glomeruloid elements (a) are surrounded by a capsule (b and c). d–f: image magnifications of a–c, respectively. e: epidermis. Scale bar: 50 (a–c) and 20 μm (d–f) [Colour figure can be viewed at wileyonlinelibrary.com]

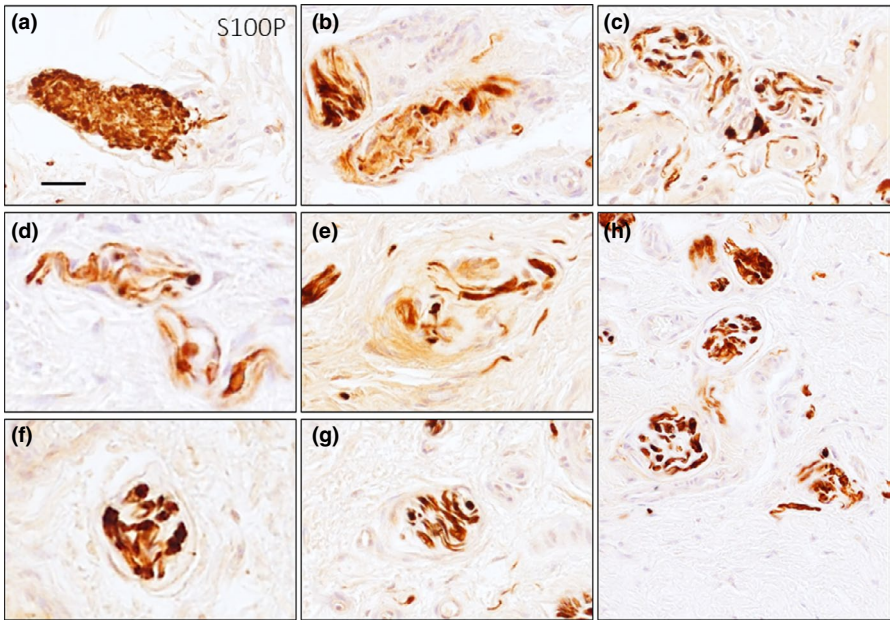

FIGURE 9 S100P immunostaining reveals different morphotypes of sensory corpuscles identified respectively as Ruffini (a) and Ruffini-like corpuscles (b–c). Moreover, other unidentifiable sensory corpuscles with undetermined morphology and loose structures were also observed (d–h). Scale bar: 60 μm (a–h) [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2020; Vega et al., 2009). Interestingly, the density of Meissner's corpuscles increased during adolescence and in early adulthood. This might have been related to the completion of maturation of the Meissner's corpuscles, leading to better identification of these corpuscles (see Feito et al., 2018b). Alternatively, it could be related to pubertal changes in the preputial skin. This is supported by the fact that the labia minora shows widespread changes in three defined phases that correspond to the prepubertal, pubertal, and postpubertal stages (Feito et al., 2018a).

Genital end bulbs are corpuscular structures that are closely related to Meissner's corpuscles. They differ from Meissner's corpuscles in morphology and are located in the dermis (Halata & Munger, 1986). These sensory structures are more deeply located than Meissner's corpuscles and share their immunohistochemical profiles. Ruffini corpuscles are rarely observed in the human prepuce. Some authors have identified them as secondary structures involved in preputial innervation (Halata & Munger, 1986), while others did not observe them (Cold & Taylor, 1999; Martin-Alguacil et al., 2015). Here, we observed typical Ruffini corpuscles and a few Ruffini-like corpuscles. Additionally, we observed rounded sensory corpuscles that might have been Krause corpuscles. Krause corpuscles have been previously identified in the human prepuce (Martin-Alguacil et al., 2015). Unlike other studies (Halata & Munger, 1986; Martin-Alguacil et al., 2015), we did not find Pacinian corpuscles in the human prepuce. In any case, sensory corpuscles other than Meissner's corpuscles are scarcely found in the human prepuce.

The most striking finding of this study was the demonstration of PIEZO2 immunoreactivity in Merkel cells and axons of both preputial Meissner's corpuscles and genital end bulbs, which supports the role of these corpuscles as mechanoreceptors. In fact, in cutaneous mechanoreceptors, the conversion of mechanical stimuli into electrical signals (mechanotransduction) involves mechano-gated ion channels, especially PIEZO2 (García-Mesa et al., 2017; García-Piqueras et al., 2019; Ranade et al., 2014), although other proteins could be involved or indirectly participate in it (Alonso-González et al., 2017; Cabo et al., 2015; Calavia et al., 2010). Here, we observed that the axons of the preputial sensory corpuscles displayed PIEZO2 immunoreactivity similar to that of digital Meissner's corpuscles (García-Mesa et al., 2017; García-Piqueras et al., 2019).

Preputial Merkel cells (Cold & Taylor, 1999; Halata & Munger, 1986) were occasionally observed in our study. They displayed PIEZO2 immunoreactivity. They were not observed in a previous study (Martín-Alguacil et al., 2015). We believe that this is the first time that the presence of mechanoproteins, particularly PIEZO2, in the preputial sensory corpuscles has been reported. These findings are of interest because mechanical stimulation of the male prepuce plays a role in sexual arousal, and PIEZO2 is critical for mechanotransduction (Wu et al., 2017).

Nevertheless, the role of the prepuce in sexual pleasure and orgasm is a matter of debate (Jenkins, 2014; Morris et al., 2019; Poland, 1990). Most of the information on this topic comes from clinical data of individuals undergoing circumcision for religious, social, medical, or preventive purposes (Morris et al., 2013, 2014, 2016, 2019). A recent meta-analysis failed to demonstrate significant clinical alterations associated with circumcision (Tian et al., 2013). Some authors have reported that circumcision decreases penile sensitivity (Bronselaer et al., 2013; Sorrells et al., 2007), while others have failed to demonstrate any difference (Bleustein et al., 2005; Payne et al., 2007) or have disputed the results (Hegarty, 2013; Morris et al., 2013). It is possible that the prepuce has been proposed to play a role in sexual function because of the belief that it must have a particular role (Martin-Alguacil et al., 2015). It has been demonstrated that mechanical stimulation of the prepuce leads to activation of the external urethral sphincter in rats (Juárez et al., 2016). Although the effects of circumcision on penile sensitivity and sexual arousal are probably minor, the prepuce is the most sensitive area of the penis (Bossio et al., 2016), which is indicated by the rich innervation observed on microscopy in this study.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the publication of this paper.

AUTHOR CONTRIBUTIONS

YG-M, RC, and JM-C conducted the experiments. JF and OG-S collected and processed the material in compliance with the ethical guidelines and performed some parts of the experiments. YG-M and JG-P quantified and evaluated the data. IS, JF, and JAV designed the study, analyzed the data, and wrote the manuscript.

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) Informed consent was obtained from either the patient or his parent/legal tutor, and all of these materials used in the study were obtained in accordance with Spanish law (RD 1301/2006; Ley 14/2007; DR 1716/2011; Orden ECC 1414/2013).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

José A. Vega https://orcid.org/0000-0003-1276-0018

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