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The acquisition of mechanoreceptive competence by human digital Merkel cells and sensory corpuscles during development: An immunohistochemical study of PIEZO2

Yolanda García-Mesa[a,1], Jorge Feito[a,b,1], Patricia Cuendias[a], Jorge García-Piqueras[a], Antonino Germanà[c], Olivia García-Suárez[a], Benjamín Martín-Biedma[d,2], José A. Vega[a,e,2,*]

[a] Departamento de Morfología y Biología Celular, Grupo SINPOS, Universidad de Oviedo, Oviedo, Spain
[b] Servicio de Anatomía Patológica, Complejo Hospitalario Universitario de Salamanca, Salamanca, Spain
[c] Zebrafish Neuromorphology Lab, Department of Veterinary Sciences, University of Messina, Messina, Italy
[d] Departamento de Cirugía y Especialidades Médico-Quirúrgicas, Universidad de Santiago de Compostela, Spain
[e] Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, Chile

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ABSTRACT

Background: PIEZO2 is a transmembrane protein forming part of an ion channel required for mechanotransduction. In humans, PIEZO2 is present in axon terminals of adult Meissner and Pacinian corpuscles, as well as Merkel cells in Merkel cell-neurite complexes.
Methods: To study the acquisition of functional capability for mechanotransduction of developing type I slowly adapting low-threshold mechanoreceptors, i.e., Merkel cell-neurite complexes, a battery of immunohistochemical and immunofluorescence techniques was performed on human skin specimens covering the whole development and growth, from 11 weeks of estimated gestational age to 20 years of life. In addition, developmental expression of PIEZO2 type I (Meissner's corpuscles) and type II (Pacinian corpuscles) rapidly adapting mechanoreceptors was studied in parallel.
Results: The first evidence of Merkel cells showing the typical morphology and placement was at 13 weeks of estimated gestation age, and at this time positive immunoreactivity for PIEZO2 was achieved. PIEZO2 expression in axons terminals started at 23 WEGA in Pacinian corpuscles and at 36 WEGA in the case of Meissner corpuscles. The occurrence of PIEZO2 in Merkel cells, Meissner and Pacinian corpuscles was maintained for all the time investigated. Interestingly PIEZO2 was absent in most Aβ type I slowly adapting low-threshold mechanoreceptors that innervate MC while it was regularly present in most Aβ type I and type II rapidly adapting low-threshold mechanoreceptors that supplies Meissner and Pacinian corpuscles.
Conclusion: The present results provide evidence that human cutaneous mechanoreceptors could perform mechanotransduction already during embryonic development.

1. Introduction

Merkel cells (MC) are a subpopulation of epithelial cells (Morrison et al., 2009; Van Keymeulen et al., 2009) present in the basal stratum of the epidermis of most vertebrates (Moll et al., 2005; Boulais and Misery, 2007; Abraham and Mathew, 2019). They are especially abundant in touch-sensitive areas of human skin (Lacour et al., 1991; Boot et al., 1992). Most MC establish synaptic-like contacts with Aβ afferents at the dermo-epidermal junction (Halata et al., 2003a; b), and these MC-axon terminal complexes (also called MC-neurite complexes; MC-NC) work as slowly adapting type I low-threshold mechanoreceptors (SAI-LTMRs). MC-NC mediate gentle touch involved in tactile discrimination of shapes and textures (Johnson, 2001; Maricich et al., 2009; Abraira and Ginty, 2013; Maksimovic et al., 2014; Zimmerman et al., 2014). Thus, MC can be regarded as presynaptic cells (Halata et al., 2003a) that contain all components of the presynaptic machinery (active-zone molecules,

* Correspondence to: Departamento de Morfología y Biología Celular, Facultad de Medicina y Ciencias de la Salud, C/ Julián Clavería, 633006 Oviedo, Spain.
E-mail address: javega@uniovi.es (J.A. Vega).
[1] Both authors contributed equally to this paper
[2] Both authors share Senior Authorship

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synaptic vesicle proteins) and release neurotransmitters (Haeberle et al., 2004; Maksimovic et al., 2013; Zimmerman et al., 2014).

Recently, direct evidence has pointed out that MCs are touch-sensitive and essential components of touch receptors in skin, since they transduce mechanical stimuli into electrical signals through the mechanoprotein PIEZO2 and consequently, induce action potentials in SAI-LTMRs (Ikeda et al., 2014; Maksimovic et al., 2014; Woo et al., 2014). PIEZO2 is a mechanically gated multipass transmembrane protein forming part of an ion channel required for mechanotransduction (Coste et al., 2010; Ranade et al., 2014; Honoré et al., 2015).

Pre- and post-natal changes in the morphology and immunohistochemical profile of human cutaneous Meissner and Pacinian corpuscles, which work as rapidly adapting type I (RAI-) and type II (RAII-) LTMRs respectively, have been recently reported in detail (Feito et al., 2018; García-Piqueras et al., 2019), but nevertheless, MC were not investigated in those researchers. Moll et al., (1984, 1986, 1992) studied the appearance and age-related changes in human cutaneous MC density by using immunohistochemistry for cytokeratins (CKs). Much later, Jenkins et al. (2019) analysed the conversion of basal keratinocytes into MC, innervation patterns and signalling pathways required to act as mechanoreceptors. However, when human MC start to express PIEZO2 during pre-natal development has never been considered.

Therefore, we designed the present study to investigate expression of CK20 and synaptic proteins (synaptophysin -Syn- and chromogranin A -ChrA) in MC during prenatal and postnatal (until 20 years-old) development. Furthermore, we studied the innervation timing of MC and especially, that of PIEZO2 expression, regarded as evidence of mechanotransduction. On the other hand, to complete our previous study (Feito et al., 2018), we analysed parallelly foetal and post-natal (until 20 years-old) timetable for PIEZO2 expression in Meissner and Pacinian corpuscles. Overall, the study aimed to better understand the sense of touch in development.

2. 2. Material and methods

2.1. Material and tissue treatment

Skin samples were obtained from the palmar aspect of the distal phalanx of fingers obtained during autopsy of foetuses (n = 18) and children deceased around birth (n = 5; age range 0–22 days) and from no known neurological diseased children and young adults (n = 26; age range 3 months-20 years-old) suffering partial or total finger amputation. Thus, the sampled age range was from 12 weeks of estimated gestational age (WEGA) to 20 years-old (Table 1). The used material in the present study belongs to the histological collection of the SINPOS Research Group at the University of Oviedo (Registro Nacional de Biobancos, Sección colecciones, Ref. C-0001627, responsible OG-S). It was obtained from the Departments of Pathology of Hospital Universitario Central de Asturias (Oviedo, Spain), Hospital Universitario Donostia (San Sebastián, Spain), Hospital José Molina Orosa (Lanzarote, Spain) and Complejo Hospitalario Universitario de Salamanca (Salamanca, Spain). Specimens were fixed in 10% formaldehyde with 1 M phosphate buffered saline (pH 7.6) for 24–36 h and processed for routinely embedding in paraffin. Pieces were cut into 10-µm-thick sections perpendicularly to skin surface and mounted on gelatine-coated microscope slides. The study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. CElm, PAst: Proyecto 266/18). All of these materials were obtained in compliance with the Spanish Law (RD 1301/2006; Ley 14/2007; DR 1716/2011; Orden ECC 1414/2013).

Table 1
Data on the case material. wega: weeks of estimated gestational age; d: day; m: month; y: year; nd: not done. L/R: left/right. M/F: male/female.

Nº cases	Age	Gender	L-R hand/finger
3	12–13 wega	nd	R/1–5
4	16–18 wega	nd	R/1
2	22 wega	M (1), F(1)	R and L/1–3
4	23 wega	M(2), F(2)	R/1–2–3
2	28–30 wega	M(2)	L/1–2
3	34–36 wega	M(1), F(2)	R/1–2
2	0 d	M	R/1
2	2 d	M	R/1–2
1	22 d	nd	R/1–2
1	3 m	F	R/1–2
3	5–8 m	M(2), nd	R/1–2
4	1–3 y	M(2), nd	R/1–2
5	5–8 y	M(3), F(2)	R/1–2 and L1
6	9–10 y	M(5), F(1)	R/1–2 and L2
4	12–15 y	M(3), F(1)	R/L 1–2 and L1/2
3	18–20 y	M(2), F(1)	R/1–2 and L/1–3

2.2. Immunohistochemistry

Deparaffinized and rehydrated sections were processed for immunohistochemistry using the EnVision antibody complex detection kit (Dako, Copenhagen, Denmark), following the supplier's instructions. Briefly, endogenous peroxidase activity was inhibited with 3% H_2O_2 for 15 min, and non-specific binding was blocked by 25% bovine serum albumin for 20 min. Sections were incubated overnight at 4 °C with the following primary antibodies: mouse monoclonal antibodies anti-cytokeratin 20 (CK20; Dako, Glostrup, Denmark, reference IS777, purchased prediluted), anti-chromogranin A (ChrA; Dako, reference M0869, purchased prediluted), anti-synaptophysin (Syn; Dako; reference M7315, used diluted 1:200); anti-neuron specific enolase (NSE; clone BBS/NC/IV-H14, Thermo Fisher Scientific, Rockford, IL, USA, reference NB100–65648; used diluted 1:200) and rabbit anti-PIEZO2 (Sigma-Aldrich, Saint Louis, MS, USA, reference PA5–72976, amino acid sequence FEDEN-KAAVRIMAGDNVEICMNLDAASFSQHNP; used diluted 1:200). Subsequently, sections were incubated with anti-mouse or anti-rabbit IgG for 90 min at room temperature. Finally, slides were washed in buffer solution before visualizing immunoreaction with diaminobenzidine as a chromogen, washed again, rehydrated, and mounted with Entellan© (Merck, Dramstadt, Germany). To ascertain structural details, sections were counterstained with Mayer´s haematoxylin.

2.3. Double immunofluorescence

Sections were also processed for simultaneous detection of PIEZO2 with either specific axonal (NSE), either Schwann related cells (S100 protein) or Merkel cell (CK-20, ChrA, Syn) proteins (Hartschuh et al., 1989; Llombart et al., 2005; Vega et al., 2009; Fukuhara et al., 2016; Cobo et al., 2021; Carcaba et al., 2022). Non-specific binding was reduced by incubation with a solution of 25% calf bovine serum in tris buffer solution (TBS) for 30 min. Sections were incubated with a 1:1 v/v mixture of polyclonal antibody against PIEZO2 and monoclonal antibodies against NSE, CK-20, ChrA, or Syn in a humid chamber overnight at 4º C. After rinsing with TBS, sections were incubated for one hour with CFL488-conjugated bovine anti-rabbit IgG (diluted 1:200 in TBS; sc-362260, Santa Cruz Biotechnology, Heidelberg, Germany) and then, rinsed and incubated again for another hour with CyTM3-conjugated donkey anti-mouse antibody (diluted 1:100 in TBS; Jackson-ImmunoResearch, Baltimore, MD, USA). Both steps were performed in a dark humid chamber at room temperature. Sections were finally washed, and cell nuclei were stained with DAPI (10 ng/mL). Triple fluorescence was detected by using a Leica DMR-XA automatic fluorescence microscope (Microscopía fotónica y Proceso de imágen, Servicios científico-técnicos, Universidad de Oviedo) coupled with a Leica

Table 2
Density of Merkel cells stablished on the basis of immunoreactivity for age different markers used. w: week; wega: weeks of estimated gestational age; d: day; m: month; y: year; n.i.: cell type not identified.

Age	CK20 +	ChrA +	Syn +	NSE +	PIEZO2 +
12–13 wega	+ n.i.	+ n.i.	0.9 ± 0.01	+ n.i.	+ n.i.
16–18 wega	+ n.i.	1.2 ± 0.1	1.7 ± 0.3	+ n.i.	+ n.i
22–23 wega	5.3 ± 0.5	4.9 ± 1	5.1 ± 0.3	4.8 ± 0.9	3.1 ± 0.5
28–30 wega	39.8 ± 2.4	38.3 ± 1.6	32.2 ± 2.1	40.1 ± 2.7	25.8 ± 3.1
34–36 wega	56.9 ± 2.2	51.9 ± 1.7	49.9 ± 2.3	52.9 ± 2.8	26.2 ± 1.2
0 d	32.6 ± 0.8	28.7 ± 1.2	32.1 ± 0.6	31.0 ± 1.2	11.8 ± 0.8
3 w	225.4 ± 3,5	216.8 ± 2.9	222.7 ± 2.8	206.9 ± 4.1	54.7 ± 3.5
3–8 m	30.3 ± 2.1	27.4 ± 1.9	30.0 ± 2.2	29.9 ± 2.3	16.9 ± 1.0
1–3 y	33.3 ± 3.1	27.9 ± 2.7	30.9 ± 2.1	31.6 ± 2.1	22.6 ± 1.3
5–10 y	30.6 ± 2.9	28.0 ± 1.9	29.8 ± 1.6	28.7 ± 1.8	27.3 ± 2.2
12–15 y	28.2 ± 0.9	28.1 ± 1	28.3 ± 1.2	26.6 ± 1.1	26 ± 1.9
18–20 y	32.7 ± 0.9	29.3 ± 1.5	31.9 ± 1.1	30.4 ± 1.3	30.8 ± 1.5

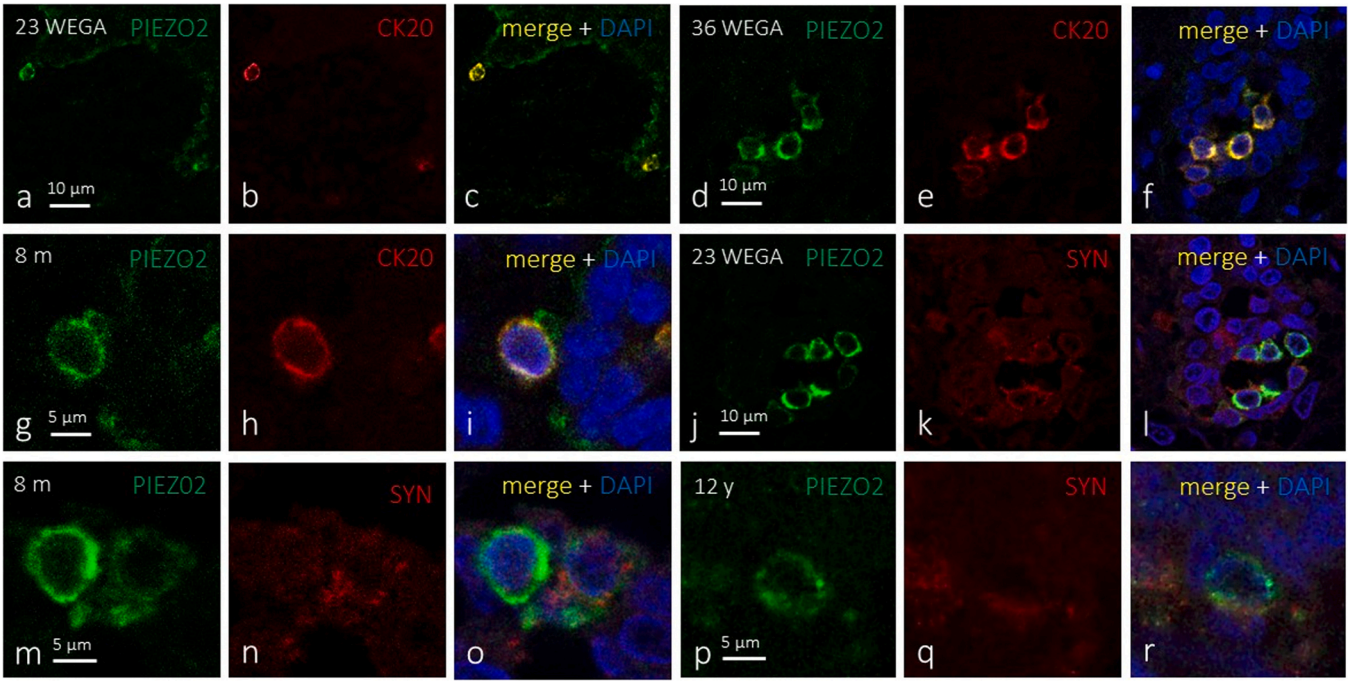

Fig. 1. Double immunofluorescence for PIEZO2 (green; **a, d, g, j, m, p**) and Cytokeratin 20 (CK, red; **b, e, h**) or Synaptophysin (SYN, red; **k, n, q**) in human glabrous skin during development. PIEZO2 totally co-localized with CK20 (yellow) in 23 wega (**c**), 36 wega (**f**) and 8 m (**i**), but did no co-localized with SYN (**l, n, r**). SYN was expressed in the synaptic pole (basal pole) of Merkel cells (**l, n, r**). In the first step of development, numerous clusters of Merkel cells were observed (**d, j**) and immunoreaction of PIEZO2 was positive in all of them. **a-f, j-l:** objective 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm. **g-i, m-r**: objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm.

Confocal Software (version 2.5; Leica Microsystems, Heidelberg GmbH, Germany), and captured images were processed using the software Image J (version 1.43 g; Master Biophotonics Facility, Mac Master University Ontario; www.macbiophotonics.ca, access on 11 January 2021).

For control purposes, representative sections were processed in the same way as described but either not using immune rabbit or mouse sera instead of primary antibodies or omitting primary antibodies when incubation. Furthermore, when available, additional controls were carried out using specifically preabsorbed antisera. Under these conditions, no positive immunostaining was observed (see Supplementary Material).

2.4. Quantitative study

Quantitative analyses were performed to determine densities of digital Merkel cells and sensory corpuscles during development and postnatal life. Number of Merkel cells, as well as Meissner and Pacini corpuscles, were calculated as follows: 4 fields x 5 sections per subject, 50 µm apart, were examined and quantified by microscopy at 10 × by two different observers, and obtained results were averaged. Data are expressed as mean ± sd/mm^2. Additionally, percentages of PIEZO2-positive Merkel cells and PIEZO2-positive sensory corpuscles were estimated, performing quantification with double immunofluorescence as described below: in regard to presumably functional Merkel cells, simultaneous detection of PIEZO2 and CK20 (as a specific marker) in five sections separated from each other by 50 µm; regarding PIEZO2-positive Meissner and Pacinian corpuscles, simultaneous detection of PIEZO2 and S100P in entire sections (for details see García-Piqueras et al., 2019; García-Mesa et al., 2022).

3. Results

3.1. Merkel cells display CK20 immunoreactivity from 22 to 23 WEGA

Immunoreactivity for the cytoskeletal intermediate filament CK20 was first detected in 13-WEGA skin samples and maintained all over the studied time (Fig1. 2b, e and h). However, based on CK20-

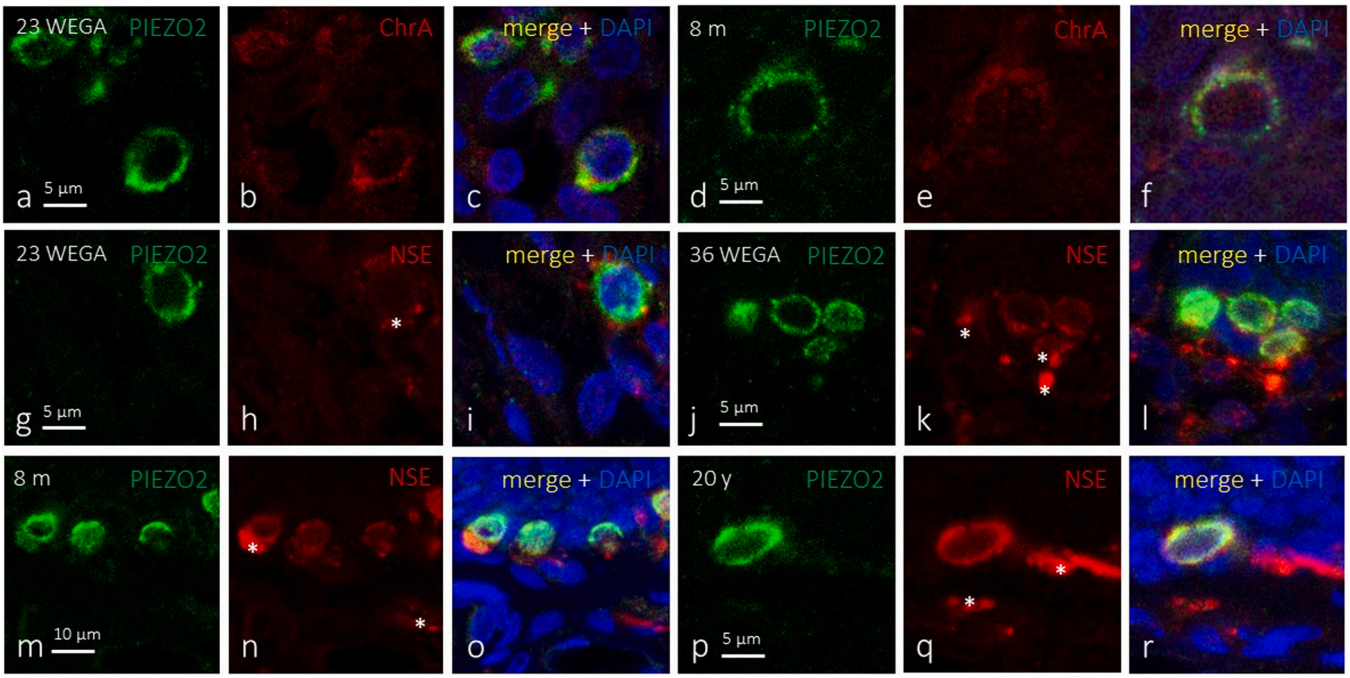

Fig. 2. - Double immunofluorescence for PIEZO2 (green; **a, d, g, j, m, p**) and Chromogranin A (ChrA, red; **b, e**) or Neural Specific Enolase (NSE, red; **h, k, n, q**) at different ages during development. ChrA immunoreaction pattern was diffuse and no-colocalized with PIEZO2 (**c, f**). In the case of the axonal marker NSE, in the 23 wega a diffuse pattern was observed around the positive PIEZO2 halo, corresponding to the Merkel cell (**i**), while in the last prenatal stages (**l**), as well as in the postnatal ones (**o, r**), the immunoreaction for NSE was found both in the Merkel cell and in the axon terminal that reaches the basal pole of the Merkel-neurite complex (asterisks in **h, k, n, q**). In spite of this, there is no co-localization between PIEZO2 and NSE (**i, l, o, r**). Objective 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm.

positive cell placement within epidermis as well as their morphology, they were identified as MCs just from 22 to 23. The immunolabelling pattern was similar in all cases, forming a peripheral ring-like halo. However, density of CK20-positive Merkel cells at different examined timepoints varied, being maximum at 3 postnatal weeks, and remaining rather stable from 3 to 8 months until 20 years-old (Table 2).

3.2. Merkel cells express Syn and ChrA from 22 to 23 WEGA

Syn and ChrA are proteins of presynaptic and secretory vesicles respectively, and both are present in mature human MCs. In the analysed digital skin samples, expression of Syn (Fig. 1k, n and q) and ChrA (Fig. 2b and e) began at 13 and 18 WEGA, respectively. Nevertheless, Merkel cells with typical location and morphology were observed from 23 WEGA onwards. Age-related variations in density of Syn-positive and ChrA-positive Merkel cells are shown in Table 2. Syn expression pattern was fine, granular, non-confluent and arranged in the most peripheral part of the cell; that of ChrA was also granular and formed a peripheral cytoplasmic ring.

3.3. Merkel cells start the expression of PIEZO2 at 22–23 WEGA

PIEZO2 is an essential mechanoprotein for the mechanotransduction process in MC-neurite complexes. Therefore, PIEZO2 occurrence within these cells can be regarded as indirect evidence of the acquisition of mechanosensitivity and mechanotransduction by these sensory complexes. The first evidence of PIEZO2 immunoreactivity in cells located at the basal stratum of epidermis was at 13 WEGA (Fig. 1a); however, just from 23 WEGA, the PIEZO2-positive cells display typical morphology and location of Merkel cells (Fig. 3). From that age and during all the analysed time, MC were PIEZO2-positive (Fig. 1a,d,g,j,m,p; Fig. 1a,d,g,j,m,p; Fig. 3), although their density experienced small variations depending on age (Table 2). Density of PIEZO2-positive MC was roughly equal to that of CK20-positive MC, but percentage of PIEZO2-positive cells increased from 3 weeks up to reach a maximum peak at 18–20 years-old (Table 3). At all ages, the immunolabelling pattern for PIEZO2 in MCs was granular, forming a peripheral cytoplasmic ring.

Another evaluated aspect in the present study was whether PIEZO2-immunoreactive cells are innervated. NSE was used as an axonal marker, although it also is expressed by MCs. This experiment allows us further to analyse whether axon terminals of LTMRs contacting MC express PIEZO2. Indeed, NSE was detected in MC cytoplasm from 13 WEGA to 20 years-old, showing a faint granular pattern at the periphery of the cytoplasm like a ring (Fig. 2h, k,n, and q). In addition, extracellular NSE-positive spots or axon profiles in apparent contact with MCs were detected since 13 WEGA. Occasionally, NSE-positive extracellular structures showed typical morphology of so-called Merkel tactile discs (Fig. 3n). It is important to note that as a rule, PIEZO2 was not detected in axon terminals of MC-neurite complexes (Fig. 3l, or and r).

Moreover, as expected, PIEZO2 co-localized completely or partially in MCs with all of the other investigated proteins (Figs. 1 and 2; Table 2).

3.4. Digital Meissner corpuscles display PIEZO2 immunoreactivity at the ending of the fetal period

In a recent work from our laboratory (Feito et al., 2018), we found that human digital Meissner corpuscles develop from the later prenatal stages. In agreement with those results, now we have observed that from 36 WEGA (Fig. 4a and b) and during the analysed time periods (Fig. 4c to e), axons of Meissner corpuscle are PIEZO2-positive, although the density changed slightly with age (Table 3).

3.5. Pacinian corpuscles display PIEZO2 early in development

Human digital Pacinian corpuscles acquire their typical morphology and basic structure from 24 WEGA (Feito et al., 2018). The

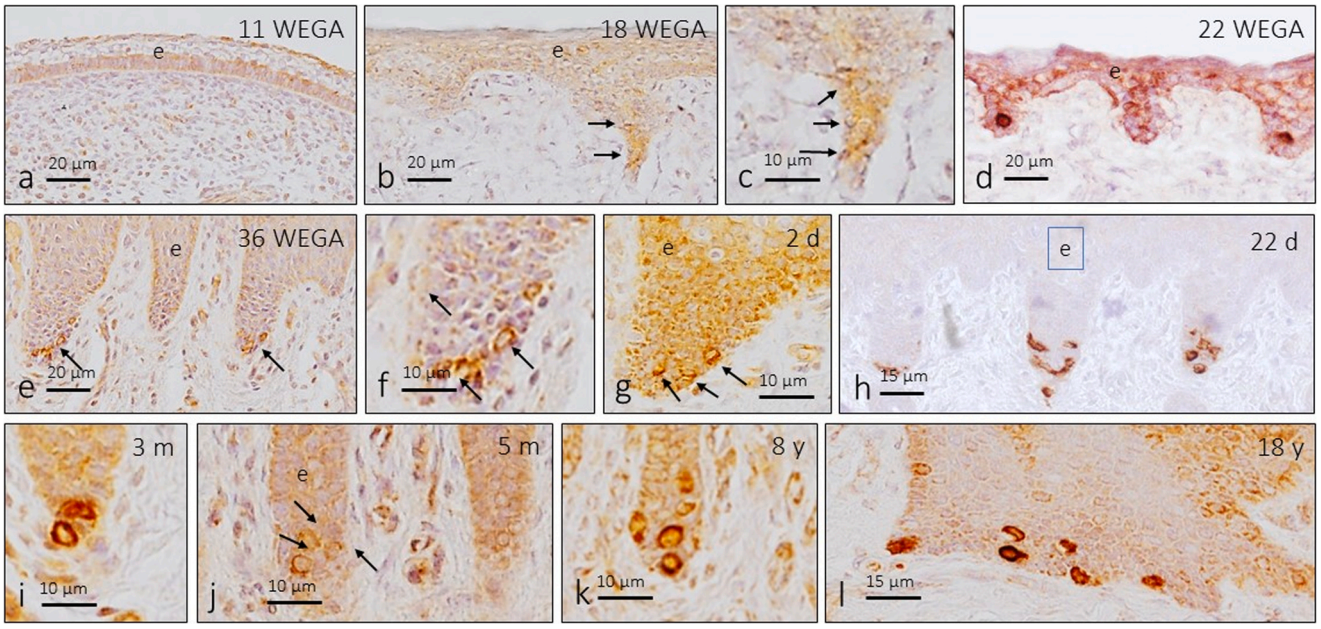

Fig. 3. Single immunohistochemistry for PIEZO2 during development. During the first trimester of development (see 11 wega), PIEZO2 was observed in epidermis (**a**). Merkel cells were first observed at 18 wega (arrows in **b,c**), with a light marking in the basal layer of epidermis, and the immunoreaction gets more intensive from 22 wega (**d**) and the number of Merkel cells increased at 36 wega (**e,f**), appearing clusters (arrows in **e** and **f**). In the postnatal stage, the number of this decreased, being similar during the adult stage (**g-l**), but its morphology change, losing their oval morphology in some cases from 18 years (**l**). e:epidermis.

Table 3
Percentage of Merkel cells, Meissner and Pacinian corpuscles displaying PIEZO2 immunoreactivity. Merkel cells displaying CK20 immunoreactivity were regarded as 100%, and the total number (100%) of Meissner and Pacinian corpuscles was determined in sections processed for detection of S100. n.d: not done; n.i: cell type not identified.

Age	Merkel cells % Merkel c. PIEZO 2 +	MC Corpuscle % MC PIEZO2 +	PC Corpuscles % PC PIEZO2 +
12–13 wega	n.i.	n.d.	n.d.
16–18 wega	n.i.	n.d.	n.d.
22–23 wega	58,49 ± 2.2	n.d.	38.2 ± 5.5
28–30 wega	62.8 ± 1.9	n.d	50.1 ± 4.7
34–36 wega	46.0 ± 1.6	16.3 ± 2.8	69.3 ± 4.7
0 d	36.2 ± 0.6	23.7 ± 2.9	76.3 ± 4.7
3 w	23.9 ± 1.1	22.3 ± 3.2	74.2 ± 3.9
3–8 m	55.7 ± 1.7	22.1 ± 3.1	72.8 ± 5.6
1–3 y	67.2 ± 2.1	33.6 ± 4.1	86.1 ± 5.2
5–10 y	89.1 ± 4.8	76.2 ± 7.3	98.8 ± 2.7
12–15 y	92,5 ± 2,2	98.2 ± 7.3	100
18–20 y	94,1 ± 1.6	96.4 ± 5.1	100

present findings show that the axon of Pacinian corpuscles become PIEZO2-positive at 23 WEGA and maintain its expression throughout all over the studied time (Fig. 4f-h). There were no significant age-related variations in percentage of PIEZO2-positive Pacinian corpuscles.

4. Discussion

We have previously described the temporal pattern as well as the proper immunohistochemical-profile acquisition of human digital Meissner and Pacinian corpuscles during development (Feito et al., 2018) and ageing (García-Piqueras et al., 2019). Now, we extend the research by the present study, analysing the prenatal development and postnatal age-related changes (until 20 years-old) in digital MCs, focusing on the time in which they start to display PIEZO2 immunoreactivity. In the analysis we also include the developmental expression of PIEZO2 by Meissner and Pacinian corpuscles. PIEZO2 occurrence in LTMRs and MCs can be regarded as indirect evidence of mechanosensing and mechanotransduction by these sensory structures because the attributed function to this ion channel (Woo et al., 2014).

The first step of our research was to establish from which moment MCs are present in human digital skin. MCs are differentiated epithelial-derived cells that account for less than 5% of the total cell population in epidermis (McGrath and Uitto, 2010; Oss-Ronen and Cohen, 2021) and are found in sensitive cutaneous and mucous areas (Hartschuh and Grube, 1979; Moll et al., 2005). They form sensory complexes with Aβ SA1-LTMRs to encode object features and gentle touch pressure (Johnson, 2001; Maksimovic et al., 2014; Maricich et al., 2012). MC express both epithelial and neuroendocrine markers. Their cytoskeletal intermediate filaments stain positively for low-molecular-weight cytokeratins like CK20 and their cytoplasm, for neuroendocrine proteins such as ChrA, protein gene product 9.5, NSE, and Syn (Halata et al., 2003; for a review see Abraham and Mathew, 2019).

In our hands, although CK20 elements were identified in epidermis from 13 WEGA, typical MCs were only detected from 22 to 23 WEGA. These results differ from those of Moll et al., (1984, 1986) and Moll and Moll (1992), who found CK20-positive MCs at 8 fetal weeks. Similar findings were obtained by Kim and Holbrook (1995), who found CK20-positive cells as early as 56 days of estimated gestational age (8 WEGA) in palmar epidermis. These discrepancies may be due to, at least in part, the anatomical skin sampling, the sample processing or the antibody sensitivity. Regarding the investigated neuroendocrine markers in MC (Hartschuh et al., 1989; Llombart et al., 2005; Fukuhara et al., 2016; Carcaba et al., 2022), they were detected in proper MCs at the same times of CK20 and following a parallel course during the investigated period of time.

As previously reported, we have found that density of MC varies throughout development. With the used method, density of MC in human digital skin increased from 22 to 23 WEGA until 3 postnatal weeks; thereafter, the number decreased abruptly and remained with no change until 20 years old. These results disagree with observations of Moll et al. (1984), Moll and Moll (1992), Boot et al. (1992) and Kim and Holbrook (1995), who found that MCs are considerably more numerous at early stages of development than in older foetuses. The reasons for these discrepancies must be

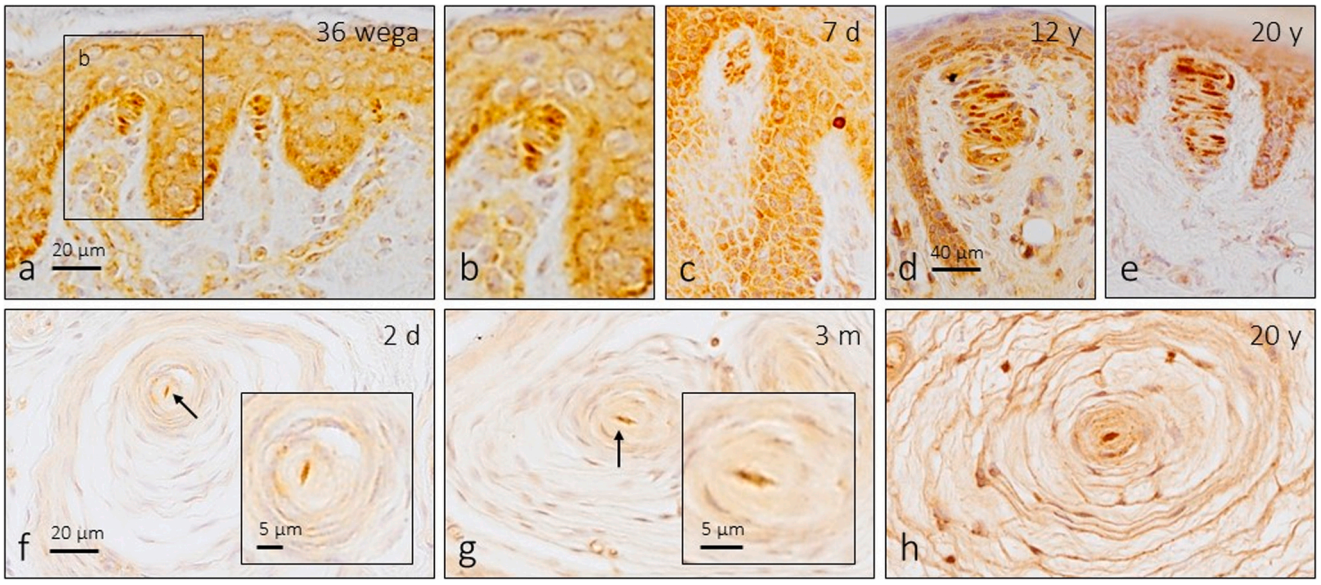

Fig. 4. Immunohistochemical detection of PIEZO2 in human digital skin focusing on Meissner and Pacinian corpuscles at different ages. PIEZO2 was observed from 36 wega in the axon of Meissner corpuscles (**a-e**), with a winding path between lamellar cells, which are slightly positive for PIEZO2 too. In contrast, Pacinian corpuscles were not positive for PIEZO2 until postnatal stage (see 2d; **f**), and the immunoreaction patterns was axonal (**f, g, h**).

reinvestigated, but the anatomical origin of skin samples and sensitivity of the used antibodies might account.

Most MC contact sensory afferents to form MC-neurite complexes in the basal epidermal layer. Therefore, we also investigated the specific time at which MC become innervate. The present results suggest that MC are innervated by the 22–23 WEGA. This time is considerably later than that reported by Narisawa and Hashimoto (1991), in which they recognized nerve-MC relationships in plantar skin of 12-week foetuses by using a combination of MC-specific antikeratin and neurofilament antibodies and showing that MC appearance preceded nerves. Nevertheless, some studies in mouse establish that the location and early differentiation of MCs are independent of nerves (Vielkind et al., 1995). Based on our experiments it is not possible to know if differentiated MCs predate nerves, since both were detected simultaneously at 22–23 WEGA.

Cutaneous MC in human skin displayed PIEZO2 immunoreactivity and density of PIEZO2-positive MC undergo age-dependent changes (García-Mesa et al., 2017; García-Piqueras et al., 2019). Now, we have observed that MC start to show PIEZO2-immunoreactivity earlier during development (22–23 WEGA), and it continues throughout the whole analysed period of time. Considering PIEZO2-positivity, MC density follows roughly a parallel trend to that described by MC when identified by expression of CK20 and other markers. However, not all CM were PIEZO2-positive. Percentage of PIEZO2-positive cells firstly decreased from 28 to 30 WEGA to three weeks and then increased progressively until 5–10 years-old. From this age to 20 years-old, approximately 92% of MC were PIEZO2-positive. In a previous study in older subjects (age range between 21 and 60 years-old), about 80% of MC were PIEZO2-positive (García-Mesa et al., 2017). Recently, Michel et al. (2021) have observed a decline in behavioural cutaneous touch sensitivity and PIEZO2-mediated mechanotransduction in cultured mouse dorsal root ganglia neurons during mouse maturation, but not thereafter.

As far as we know, this is the first study demonstrating PIEZO2 expression in prenatal MC, and the results suggest that MC are able to transduce mechanical stimuli during fetal life since PIEZO2 is an essential component in the mechanotransduction mechanism (Ikeda et al., 2014; Maksimovic et al., 2014; Woo et al., 2014). In support of this view, we observed that most PIEZO2-positive MC are in contact with nerve profiles, thus indicating they are innervated. Nevertheless, this indirect evidence needs to be confirmed by functional studies. In recent years, it has been confirmed a long-debated hypothesis, affirming that CM are mechanosensory cells required for tactile sensation: upon direct touch stimuli, MC display ionic currents mediated by PIEZO2, which induce neurotransmitter release, which in turn, triggers action potential firing of the Aβ SA1-LTMR (Ikeda et al., 2014; Maksimovic et al., 2014; Nakatani et al., 2015; Woo et al., 2014, 2015a; 2015b).

In the present study, we also analysed PIEZO2 expression in Meissner and Pacinian corpuscles from 11 WEGA to 20 years old. Our results are in complete agreement with previous studies from our lab (García-Mesa et al., 2017; García-P í queras et al., 2019), reporting PIEZO2 occurrence in axons of Meissner and Pacinian corpuscles, likewise as in Meissner-like corpuscles from murine skin (Ranade et al., 2014) and palatal mucosa (Moayedi et al., 2018).

There is an interesting result that emerges from our study: while almost all Aβ RAI- and RAII-LTMRs that supply Meissner and Pacinian corpuscles respectively, display PIEZO2 immunoreactivity, very few Aβ SAI-LTMRs that innervate MC are PIEZO2-positive. In contrast, accessory terminal cells of Meissner and Pacini corpuscles, i.e., the glial terminal cells, do not express PIEZO2 and those of the MC-neurite complexes, i.e., the MCs, do it. More studies are needed to explain these facts.

Touch is of capital importance for functional brain maturation (Nicolelis et al., 1996; Lauronen et al., 2006; Pihko et al., 2009; Nevalainen et al., 2014). Somatosensorial system provides information about touch, pressure, temperature, form, sharpness, hardness, undulation, etc. to the brain (Pleger and Villringer, 2013), and this information starts by the seventh gestational month, although the basic mechanisms are functional a little before (Pihko and Lauronen, 2004; Nevalainen et al., 2014). Cortical responses to touch at 2 years-old are similar as in adults (Lauronen et al., 2006; Pihko et al., 2009). In any case, touch plays key roles in the development of neurological (Nevalainen et al., 2014) and social (Croy et al., 2017) brain functions. The present results, together with our previous results (Feito et al., 2018), provide structural basis of the touch at the nervous system periphery. Whether these data can have clinical relevance remains to be determined in future studies. Tactile deprivation studies in hAlterations in somatosensory (touch and pain) behaviors are highly prevalent among people with autism spectrum disorders (ASDs).

However, the neural mechanisms underlying abnormal touch and pain-related behaviors in ASDs and how altered somatosensory reactivity might contribute to ASD pathogenesis has not been well studied. Here, we provide a brief review of somatosensory alterations observed in people with ASDs and recent evidence from animal models that implicates peripheral neurons as a locus of dysfunction for somatosensory abnormalities in ASDs. Lastly, we describe current efforts to understand how altered peripheral sensory neuron dysfunction may impact brain development and complex behaviors in ASD models, and whether targeting peripheral somatosensory neurons to improve their function might also improve related ASD phenotypes.uman, non-human primate, and rodents showed that early life experiences and developmental tactile stimulation are essential for proper brain development, cognition and adult social behaviours. Individuals with autism spectrum disorders display abnormalities in processing tactile modalities and these impairments correlate with deficits in social behaviour (Rogers and Ozonoff, 2005; Mikkelsen et al., 2018; Orefice, 2020). Adults with autism spectrum disorders have been shown to exhibit lower thresholds for tactile perception of vibro-tactile stimuli, suggesting a specific hypersensitivity in Aβ RAII-LTMRs pathways (Blakemore et al., 2006). In concordance with this finding, Cascio and colleagues found that adults with autism spectrum disorders display increased sensitivity to both vibration and thermal pain (see Cascio et al., 2016).

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).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aanat.2022.151953.

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