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L1CAM expression in human gastrointestinal tract development: From tongue to colon-rectum

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Cau Flaviana[1], Piras Monica[2], Congiu Terenzio[2], Murru Raffaele[1], Aimola Valentina[1], Cerrone Giulia[1], Van Eyken Peter[3], La Nasa Giorgio[4], Castagnola Massimo[5], Coghe Ferdinando[6], Orru' Germano[7], Fanni Daniela[1*] and Gerosa Clara[1*]

Abstract

Background: L1CAM (L1 cell adhesion molecule) is a member of the L1 family of neural adhesion molecules, involved in the development of multiple organs and tissues, including kidneys, the enteric nervous system, and adrenal glands. The aim of this study was to analyze, at the immunohistochemical level, the expression of L1CAM in the human tongue, parotid glands, and the different segments of the gastrointestinal tract during human development.

Design and method: Immunohistochemical analysis for L1CAM was performed in the human tongue, parotid glands, and in the different segments of the gastrointestinal tract during development, starting from the 8th up to the 32nd week of gestation.

Results: Our results were given by the expression of the L1CAM protein in different segments of the gastrointestinal tract during development, starting from the 8th week up to the 32nd week of gestation. L1CAM-reactive cells appeared aggregated in small bodies, irregular in shape, showing L1CAM storage in the cytoplasm. L1CAM expressing bodies were frequently found to be connected one to the next by thin fibers, a finding suggestive of the existence of an L1CAM network inside the developing tissue.

Conclusion: Our study confirms that L1CAM is involved in gut development, as well as in tongue and salivary gland development. These findings confirm that the role of L1CAM in fetal development is not restricted to the central nervous system and are necessary for further studies on the role of this molecule in human development.

Keywords

L1CAM, nervous system, gastrointestinal tract, development, immunohistochemistry

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[1]Department of Medical Sciences and Public Health, Division of Pathology, AOU of Cagliari, University of Cagliari, Cagliari, Italy
[2]Department of Medical Sciences and Public Health, Electron Microscopy Laboratory, Division of Pathology, AOU of Cagliari, University of Cagliari, Cagliari, Italy
[3]Department of Pathology, Genk Regional Ziekenhuis, Genk, Belgium
[4]Department of Medical Sciences and Public Health, Hematology Unit, University of Cagliari Cagliari, Italy
[5]Laboratorio di Proteomica, Centro Europeo di Ricerca Sul Cervello, IRCCS, Fondazione Santa Lucia, Roma, Italy
[6]Department of Clinical Laboratory, Azienda Ospedaliero Universitaria (AOU) di Cagliari - Polo di Monserrato, Cagliari, Italy
[7]Molecular Biology Service Laboratory, Department of Surgical Science, University of Cagliari, Cagliari, Italy
*These authors contributed equally to this work.

Corresponding author:
Piras Monica, Department of Medical Sciences and Public Health, AOU, Electron Microscopy Laboratory, University of Cagliari, Cagliari 09124, Italy.
Email: monipiras@hotmail.com

Introduction

L1CAM (L1 cell adhesion molecule) is a member of the L1 family of neural adhesion molecules. This family comprises four different members in vertebrates: L1CAM, Close Homolog of L1 (CHL1), NrCAM, and Neurofascin.[1] L1-type proteins are transmembrane cell adhesion molecules with an evolutionary well-conserved protein domain structure of usually six immunoglobulins and five fibronectin type III domains.[2] By engaging in many different protein-protein interactions, L1CAM is involved in a multitude of molecular functions and is an important player during the formation and maintenance of the metazoan nervous systems.[3] L1CAM plays an essential role in the development of the human nervous system.[4–6] In particular, L1CAM has been shown to play a major role in the initial formation of neurites, interacting with ankyrin-B and actin,[7] as well as in guiding migrating neurons and in axon growing.[8] Research over the last years on L1CAM has confirmed its pivotal role in nervous system development and function.[9]

The gene encoding L1CAM is located near the telomere of the long arm of the X chromosome in Xq28[10,11] (Several X-linked mental retardation syndromes including X-linked hydrocephalus (HSAS), MASA syndrome, X-linked complicated spastic paraparesis (SP1), and X-linked corpus callosum agenesis (ACC)) are all due to mutations in the L1 gene.[12]

The different forms of L1CAM localize at the plasma membrane as a transmembrane protein, and in the intra- or extracellular environment as cleaved or exosomal forms.[6,13] It has been reported that full-length L1CAM has both intracellular and extracellular targets, including interactions with integrins, and linkage with ezrin.[14] Cellular processing leading to proteolytic cleavage and/or exosome formation results in extracellular soluble forms of L1CAM that may act through similar mechanisms as compared to full-length L1CAM, such as integrin-dependent signals, but also through distinct mechanisms.[15] Moreover, L1CAM has been involved in the molecular pathways underlying epithelial-mesenchymal transition (EMT), during development, in a variety of human carcinomas,[16] and in the senescence of human cells.[17] In recent years, L1CAM has been involved in the development of multiple organs and tissues, including kidneys,[18] the enteric nervous system,[19,20] and adrenal glands.[21] Mutations of the human L1CAM gene have been shown to cause neurodevelopmental disorders such as X-linked hydrocephalus, spastic paraplegia, and mental retardation.[22] The impaired L1CAM function has been also implicated in the etiology of fetal alcohol spectrum disorders, defective enteric nervous system development, and malformations of the renal system.[16]

The aim of this study was to analyze, at the immunohistochemical level, the expression of L1CAM in organs and tissues during human development.

Design and methods

We performed a retrospective study on different formalin-fixed paraffin-embedded tissue (according to conventional techniques) of human embryos/fetuses' specimens in different organs, to test the immunoreactivity of L1CAM. The human embryos/fetuses were obtained at the Division of Pathology of the University Hospital Agency of Cagliari. Fetal ages were estimated from the gestational age, ranging from 8 up to 32 weeks. Three micron-thick sections were stained with hematoxylin and eosin (H&E) and immunostained with a mouse monoclonal antibody (Sigma-Aldrich, clone UJ127) against L1CAM (mouse IgG1 isotype). The ultra-View Universal DAB Detection Kit was used for detecting primary antibodies. Slides were incubated for 20 min at room temperature at 1:100 dilution of the monoclonal anti-L1CAM primary antibody. Nervous structures were utilized as internal positive controls for L1cam. As appropriate negative controls, all sections were processed omitting the primary antibody for L1CAM.

To obtain a semiquantitative evaluation of the degree of immunoreactivity for L1CAM, the following semiquantitative scoring system was applied: 0 = no reactivity; 1 = < 50% of immunoreactive cells; 2 = > 50% and < 75% of immunoreactive cells; 3 = > 75% of cells immunostained for L1CAM. From clinical assessment, no evidence of genetic disease or malformations was present in the two embryos and three fetuses analyzed in this study.

All procedures were performed according to ethical national standards of the responsible committee on human experimentation and approved by the Ethic Human Studies Committee of the University Medical Center of Cagliari (N. PG/2020/10914).

Results

In the present investigation, we mainly focused on the evaluation and localization of L1CAM expression in the human tongue, parotid glands, and in the different segments of the gastrointestinal tract during development, starting from the 8th up to the 32nd week of gestation. Given the different expressions of L1CAM in the different organs, we will describe separately every organ.

Tongue

L1CAM was evenly expressed in this organ, with the highest concentrations inside the muscular layers (Figure 1(a)). L1CAM-expressing cells were isolated or aggregated in small clusters intermingled with the muscular cells. No reactivity was found for L1CAM in the superficial epithelium (Figure 1(a)). At high power, L1CAM-reactive cells appeared aggregated in small bodies, irregular in shape, showing L1CAM storage in the cytoplasm (Figure 1(b)). No significant reactivity for the adhesion molecule was detected

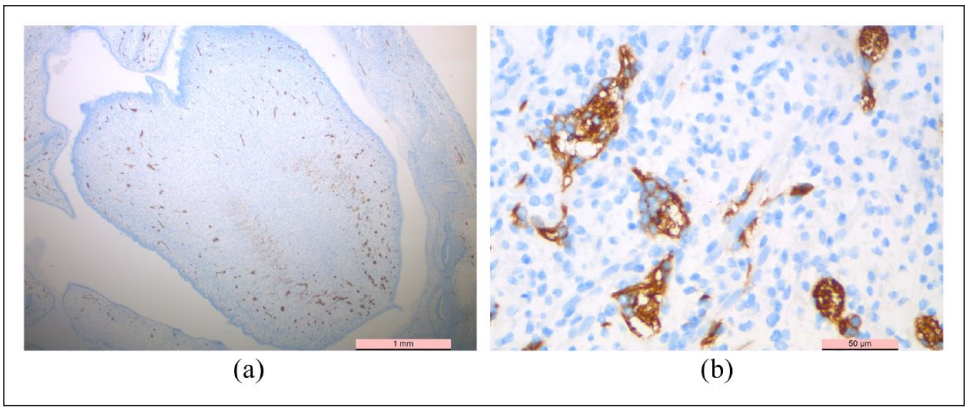

Figure 1. (a) Tongue 11 weeks of gestation (25×). Immunostaining reveals the abundant nervous component inside the muscular layers of the tongue. Nervous fibers and aggregates of cells are more abundant in the apex. (b) Tongue 11 weeks (200×). At higher power nervous structures appear composed of L1CAM reactive cells, surrounding L1CAM negative cells.

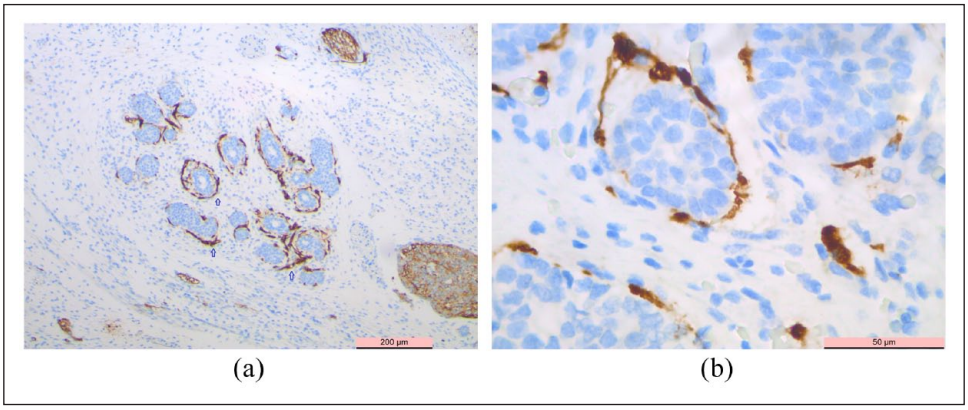

Figure 2. (a) Parotid glands 10-week gestation (50×). At low power, immunoreactive for L1cam is restricted to the periphery of the developing acinar ducts. On the right, nervous ganglions strongly immunostained for L1CAM (arrow). (b) Parotid glands 11 weeks (630×). At higher power, nervous cells surrounding developing ducts show strong reactivity for L1CAM.

in the nuclei. L1CAM expressing bodies were frequently found to be connected one to the next by thin fibers, a finding suggestive of the existence of an L1CAM network inside the developing tongue. Moreover, single spindle cells expressing L1CAM were frequently observed scattered throughout the tongue. Occasionally, L1CAM positive fibers were found to be strictly connected with the surrounding muscle cell precursors, suggesting the existence of functional connections between the developing nervous structures and the muscular component of the tongue. No significant change in L1CAM expression was observed at the different gestational ages analyzed in the present study.

Parotid glands

In the developing parotid glands, immunoreactivity for L1cam was expressed at the periphery of the epithelial buds, which originate solid cords that are subsequently canalized, forming, the early salivary ducts (Figure 2(a)). At higher power, L1CAM-expressing cells showed an elongated cytoplasm, giving rise to a network and circling the developing ducts (Figure 2(b)). These findings suggested the early development of nervous fibers around acinar and ductal developing salivary structures.

Esophagus

In the developing esophagus, L1Cam showed a complex pattern of reactivity. The highest levels of expression were found in the developing nervous plexuses, localized at the inner part and at the outer part of the tonaca muscularis (Figure 3(a)). Immunostaining for L1CAM revealed a, whereas the inner plexus appeared fragmented and incompletely developed (Figure 3(a)). In some fields, it was possible to find multiple connections between the two esophageal plexuses, suggesting a link between the nervous plexuses (Figure 3(a)). Well-formed external plexus. At high power, another component of L1CAM-reactive cells was detected (Figure 3(b)). Multiple spindle cells appeared scattered in the mucosa and in the sub-mucosa

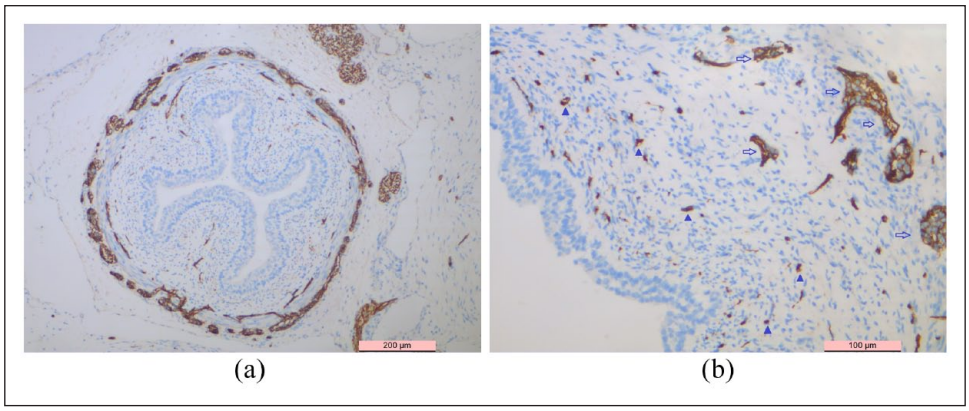

Figure 3. (a) Esophagus, 12 weeks of gestation (50×). L1CAM immunostains both the well-formed external and the internal nervous plexuses. (b) Esophagus 12 weeks, at higher power (200×) immunoreactivity, is not restricted to the plexuses (arrows). Scattered L1CAM reactive cells are detected in sub-mucose (arrowheads).

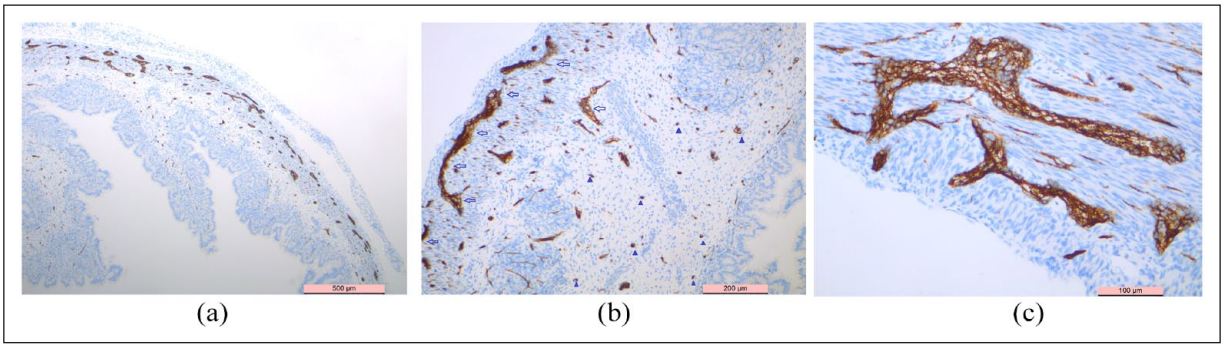

Figure 4. (a) Stomach at 15 weeks of gestation (50×). The image shows a strong positivity of L1CAM in the nervous structures in the muscular layer. Focal immunoreactivity for L1CAM is observed in the mucosa and submucosa. Completely negative the gastric epithelium. (b) Stomach at 15 weeks of gestation (200×). At higher power, L1CAM- reactive cells are organized in fascicles (arrows) in the muscular layers. (Star = gastric lumen). Reactivity for L1CAM is also observed in scattered cells and in fibers (arrowheads) in the submucosa. (c) Stomach 15 weeks, higher magnification (400×) of the developing nervous structures in the gastric muscular layers show an L1CAM-positive network.

(Figure 3(b)). These cells appeared mainly isolated, in the absence of strict connections among them. No expression of L1CAM was observed in the esophageal epithelium.

Stomach

L1CAM immunostaining of the developing stomach showed a marked reactivity in the outer muscular layers, associated with the presence of scattered isolated reactive cells in the submucosa (Figure 4(a)). In the muscular layers, L1CAM highlighted the developing nervous structures (Figure 4). No reactivity for L1CAM was detected in the gastric epithelium. At higher power, the reactivity for L1CAM was marked in the developing nervous fascicles localized in the outer muscular layers, in the sub-serosal zone (Figure 4(b)). Inside the gastric wall, L1CAM was visible in short nervous fascicles and in isolated elongated spindle cells (Figure 4(b)). L1CAM reactive cells were also observed in close proximity to the gastric mucosa (arrowheads). Strong positivity for L1CAM was observed, at high power, in the developing nervous structures in the subserosal zones (Figure 4(c)). In these structures, immunostaining for L1CAM appeared as a network surrounding the nervous cells (Figure 4(c)). At this power, it was possible to observe the presence of multiple L1CAM-positive spindle cells inside the muscular structures.

Ileum

In the small intestine, differently from the esophagus and the stomach, immunoreactivity for L1CAM was also present in the intestinal epithelium. The epithelial reactivity for L1CAM was particularly evident at 15 weeks of gestation (Figure 5(a)). L1CAM was mainly expressed in the wall of the developing ileum, in some places forming a rich network of anastomoses (or connections) of the nerve cells giving rise to the "Meissner's submucosal" (arrows) and "Auerbach's myenteric" (arrowheads) nerve plexuses

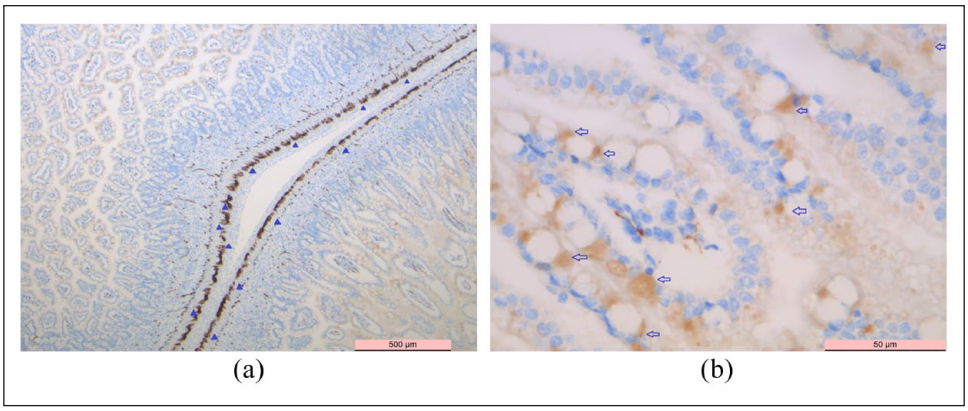

Figure 5. (a) Ileum, at 15 weeks of gestation (50×). L1CAM clearly marks the developing inner and the outer nervous plexuses (arrowheads). (b) Ileum 15 weeks (400×). At higher power, a subset of mucous cells covering the intestinal villi har strongly immunostained for L1CAM (arrows).

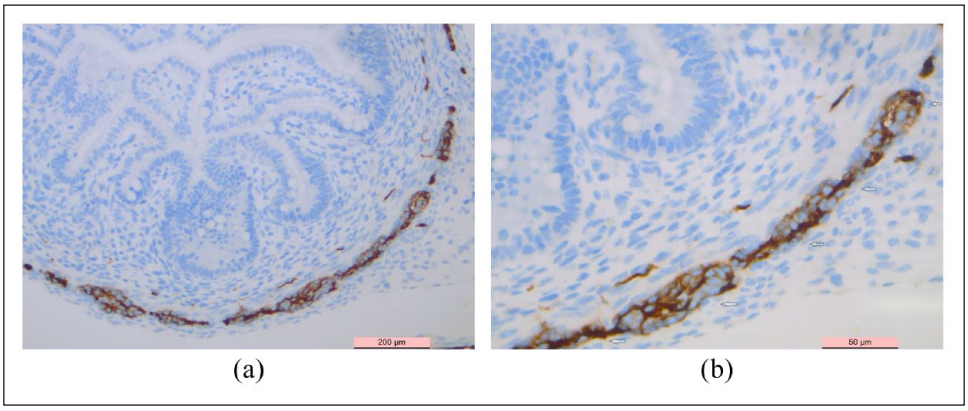

Figure 6. (a) Colon at 11 weeks of gestation (200×). L1CAM is expressed in nervous cells by giving rise to the enteric plexuses. (b) Colon 11 weeks at tiger power (400×), immunostaining for L1CAM is restricted to the cytoplasm of nerve cells. At isolated spindle L1CAM, positive cells were also found in the submucosa (arrowheads).

(Figure 5(a)). At higher power, an interesting immunoreactivity for L1CAM was noted in the cytoplasm of mucous cells covering the intestinal villi (Figure 5(b)). In these cells, L1CAM reactivity was mainly observed in intracytoplasmic globular structures (Figure 5(b)).

Colon

No significant reactivity for L1CAM was detected in the colonic epithelium nor in the submucosa (Figure 6(a)). Immunostaining for L1CAM evidenced the fragmented developing Meissner's submucosal plexus (arrowheads). Moreover, L1CAM was strongly expressed in the subserosal Auerback myenteric plexus cells (arrows) (Figure 6(b)).

Rectum

At low power, L1CAM appeared intensely expressed in the external regions of the rectum (Figure 7(a)). Immunostaining for L1CAM was particularly strong in between the rectum and the developing bladder (Figure 7(b)). At higher power, the disorganized rectal plexuses appeared to be in strict continuity with the abundant nervous structures surrounding the developing rectum (Figure 7(b)) (Arrows). Scattered L1CAM reactive cells were also observed in the rectal wall, in the absence of any significant reactivity in the rectal epithelium (Figure 7(b)).

Discussion

L1CAM is a cell adhesion molecule of the immunoglobulin superfamily, that has been shown to play a major role in the development of the central nervous system (CNS). Recent studies from our group evidenced a high expression of L1CAM in the spinal cord during development.[23] Moreover, L1CAM has been reported to have a major role in the development of multiple organs and tissue, including the digestive system, dental germs, pancreas, and kidneys.[24] More recent studies on L1CAM during renal, development evidenced a restriction of its expression to

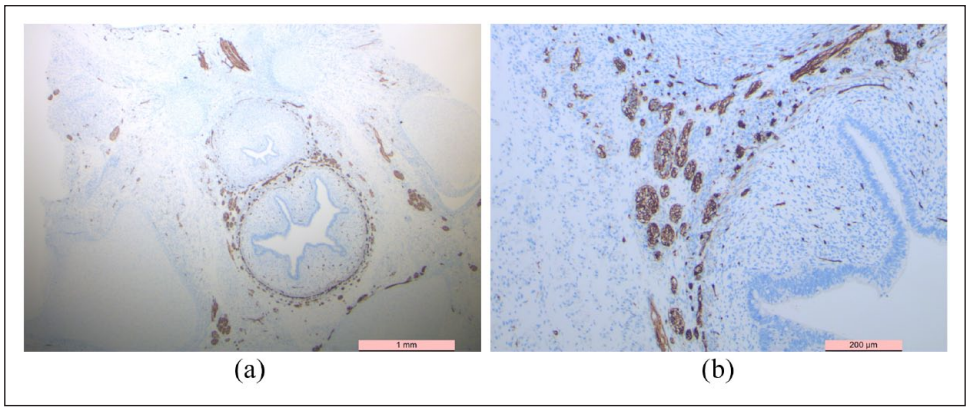

Figure 7. (a) Rectum at 10 weeks of gestation (25×). Immunostaining for L1CAM evidence a strong reactivity in disorganized enteric plexus. (b) Rectum 10 weeks (50×). A higher power, the complexity of developing nervous peri-rectal nervous system is revealed by reactivity L1CAM.

the collecting tubules.[18] This study is a part of a more general project aimed to describe the role of L1CAM in human organs and tissues during development. In this work, we analyzed L1CAM expression in the gastrointestinal tract, tongue, and parotid glands. Our findings indicate a major role for L1CAM in the development of the tongue, salivary glands, and all segments of the gastrointestinal tract here analyzed. L1CAM by means of immunohistochemistry. appeared highly expressed in all these organs, starting from the 8th to the 32nd week of gestation These data evidence a major role for L1CAM even in gut development.[24] In all the organs here studied, L1CAM was mainly found in cells of the peripheral nervous system. In the gut, the reactivity for L1CAM was restricted to cells of the enteric nervous system, confirming previous studies.[19] Moreover, immunostaining for CD117 (data not shown) evidenced the reactivity of the cells L1CAM-positive for CD117,[25] suggesting their origin from cells of Cajal.

To the best of our knowledge, our data on L1CAM expression in the tongue are original and underline a major role of L1CAM in tongue development. In the tongue, immunoreactivity for L1CAM was present in fetuses examined at all weeks of gestation. No reactivity for L1CAM was found in the epithelial cells of the tongue, nor in muscular precursor cells.

Interesting data emerged from the study of L1CAM expression in the parotid glans.[26] Our preliminary analyses identified a specific differential expression of L1CAM in the salivary glands during fetal development. L1CAM was mainly expressed in spindle cells encircling the epithelial buds, in the absence of any significant reactivity in the epithelial cells. L1CAM-positive spindle cells gave rise to a delicate network surrounding the developing epithelial buds, suggesting a role for L1CAM-reactive cells in the development of the human parotid glands. In the epithelia of the gastrointestinal organs, L1CAM has not been observed in the phases examined; neither in the esophagus nor in the small and large intestine. Despite these results, our IHC data indicate weak L1CAM expression, but only in a few epithelial cells in one ileum at week 15. The positivity of L1CAM, in epithelial tissue at week 15, was concentrated in the goblet cells. These results could be an interesting finding, as they highlight a different maturity of the colonic tissue in embryonic development, as the expression of L1CAM appears to be absent in the intestinal epithelial cells before the 15th week of gestation.

From a practical point of view, the expression of L1CAM appeared very useful for the identification of the neural components proceeding from the esophagus to the anorectum. Immunoreactivity for L1CAM enabled us to easily identify the precursors of the enteric neurons and glial cells, deriving from neural crest cells,[27] and in the circuits of the enteric nervous system (ENS). Mostly L1CAM was present in all the gut regions here analyzed, in the ganglionated networks, the submucosal plexus located adjacent to the mucosal layer, and the myenteric plexus situated between the outer longitudinal and inner circular muscle layers.[28] The reactivity was present in a variety of different subtypes of enteric neurons and glia in the developing and differentiating from neural crest progenitors that undergo extensive proliferation and migration during embryonic and postnatal development.[29,30]

In conclusion, our study confirms that L1CAM is involved in gut development, as well as in tongue and salivary gland development. These findings confirm that the role of L1CAM in fetal development is not restricted to the central nervous system, L1CAM is highly expressed in the peripheral nervous system in multiple organs.[30] Moreover, in this study, we found L1CAM reactivity even in other cell types, indicating the necessity of further studies on the role of this adhesion molecule in human development.

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Declaration of conflicting interests

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ORCID iD

Castagnola Massimo https://orcid.org/0000-0002-0959-7259

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