

# The expression of Pannexin 1 in the developing human spinal cord and dorsal root ganglia

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**UNIVERSITY OF SPLIT  
SCHOOL OF MEDICINE**

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**EXPRESSION OF PANNEXIN 1 IN THE DEVELOPING HUMAN SPINAL CORD  
AND DORSAL ROOT GANGLIA**

**Diploma Thesis**

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## ABBREVIATIONS

ATP	Adenosinetriphosphate
ATPase	Adenosinetriphosphatase
C	Cervical
cc	central canal
CNS	Central Nervous System
Co	Coccygeal
COP II	Coat protein complex II
C-terminus	Carboxy-terminus
DAPI	4',6-Diamidino-2-phenylindole
DIL	Dorsal Intermediate Zone
DRG	Dorsal Root Ganglion
DW	Developmental Week
EM	Electron Microscopy
ER	Endoplasmic Reticulum
FM	Fluorescence Microscopy
FP	Floor Plate
GABA	Gamma Aminobutyric Acid
GFAP	Glial Fibrillary Acidic Protein
HIV	Human Immunodeficiency Virus
INL	Inner Layer
IML	Intermediate Layer
L	Lumbar
ML	Marginal Layer
mV	Millivolt
mz	Marginal Zone
nc	Notochord
ne	Neuroepithelium
NF200	Neurofilament 200 kDa
NMDA	N-methyl-D-aspartate
NPC	Neural Progenitor Cell
NS	Nervous System
N-terminus	Amino-terminus
Panx	Pannexin

Panx1	Pannexin1
Panx2	Pannexin2
Panx3	Pannexin3
PBS	Phosphate Buffer Saline
RP	Roof Plate
S	Sacral
SC	Spinal Cord
sg	Sympathetic Ganglion
T	Thoracic
UTP	Uridine Triphosphate
va	Vertebral Arch
vb	Vertebral Body
VIL	Ventral Intermediate Zone

## **1. INTRODUCTION**

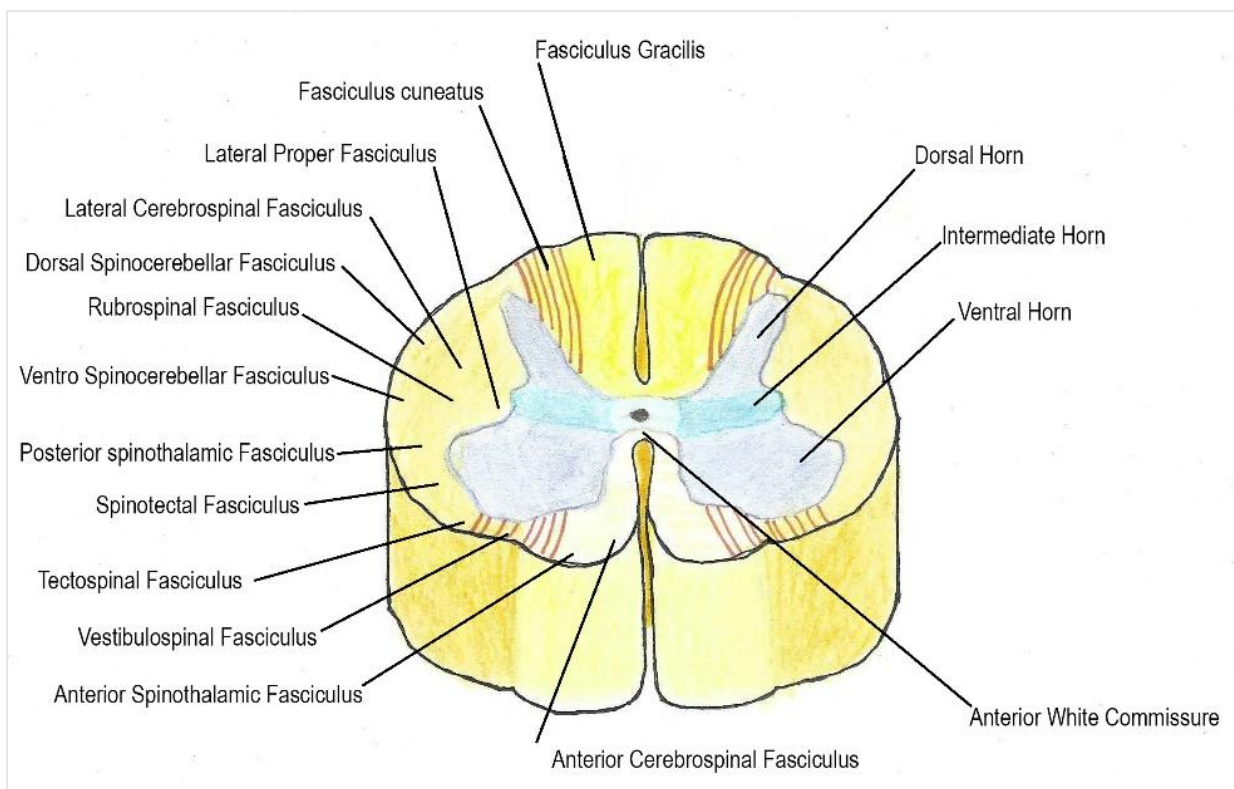


## 1.1. Anatomy

### 1.1.1. Characteristics of the central nervous system

The nervous system (NS) is divided structurally into central NS (CNS) including the brain and spinal cord (SC), and peripheral NS consisting of peripheral nerves and ganglia (1,2). Functionally it is divided into the motor, sensory and autonomous parts (1,2).

The principal function of the CNS consists of transmitting information from peripheral structures to the brain and SC to integrate, coordinate and develop reactions including mental functions such as learning and thinking and react to them (3). The CNS topography is related to functional properties, presented in Figure 1 (1).



**Figure 1.** Cross section of the spinal cord showing main structures: White (yellow) and gray (purple/blue) matter. Schematic presentation of location of tracts connecting spinal structures to cerebrum, cerebellum, thalamus, and midbrain. Created by the author based on sources in parenthesis (1,4).

### 1.1.2. Structure of the spinal cord

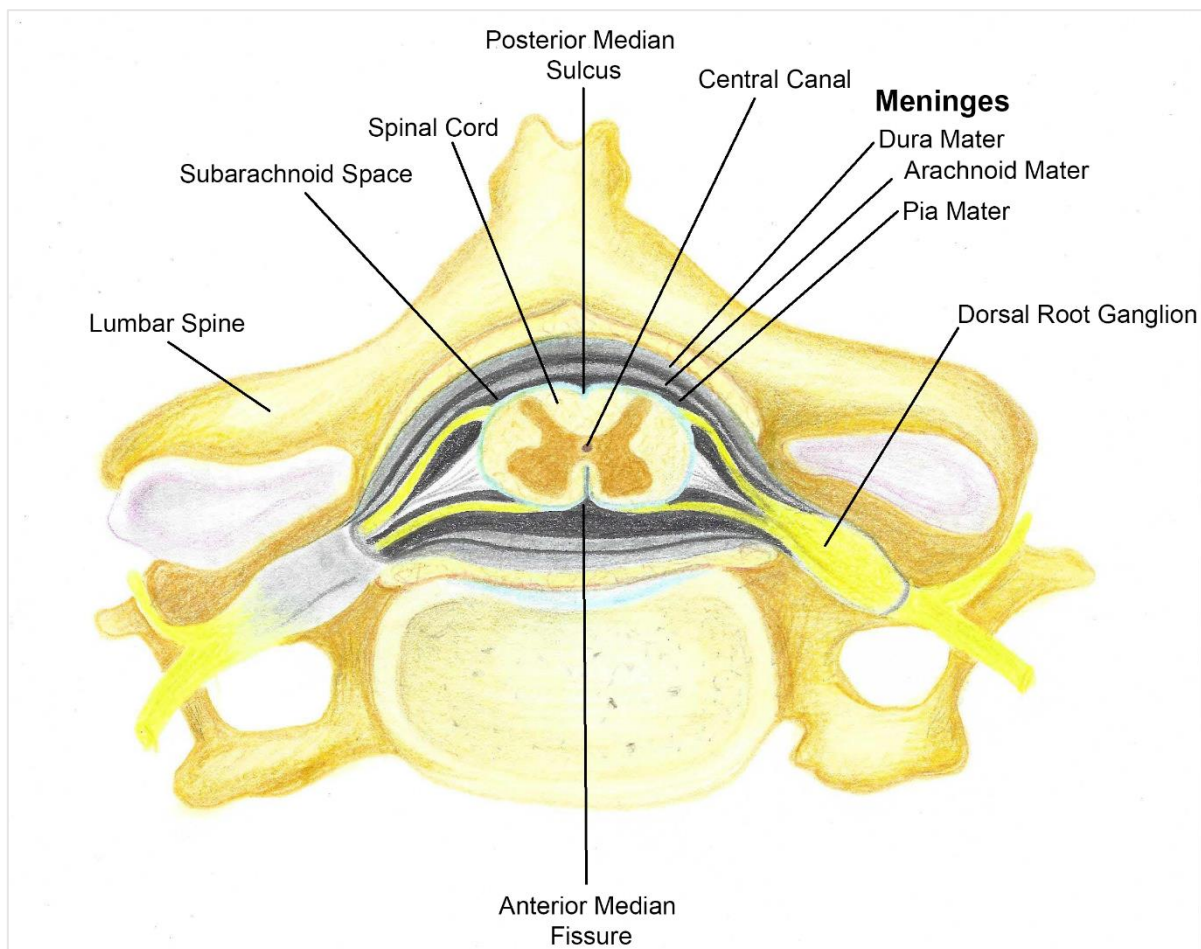
The human SC is approximately 42 to 45 centimeters long, rod-shaped structure that lies within the spine and extends from the medulla oblongata to the lumbar vertebrae 1 and 2 (L1-2) (1). The SC is cranially attached to the foramen magnum and caudally to the *os coccyx* via the filum terminale (1). It terminates caudally as the *conus medullaris* within the cauda equina (1).

The spinal nerves originate bilaterally from the SC and are divided into cervical (C1-8), thoracic (T1-12), lumbar (L1-5), sacral (S1-5) and coccygeal (Co1) nerves exiting the vertebral column via intervertebral foramina (3). On gross examination two thickenings are presented at the level of C4-T1 called cervical enlargement (*intumescentia cervicalis*) and at the level of T10-12 vertebral canal called lumbar enlargement (*intumescentia lumbosacralis*) (1,3). Furthermore, the anterior median fissure and the posterior median sulcus are visible on the surface of SC (1). Three additional sulci are observed: 1) *sulcus anterolateralis*, where the motor neurons exit the ventral horn, 2) *sulcus posterolateralis*, where the sensory fibers enter the dorsal horn and the 3) *sulcus intermedius posterior*, which marks the border between the *fasciculus cuneatus* and *fasciculus gracilis* at the cervical level as demonstrated in Figure 1 (4). On macroscopic examination of a transverse section, the grey matter is medially arranged in the shape of an “H” surrounded by white matter (5). The two anterior horns contain the nuclei of the lower motor neurons and the two posterior horns contain the nuclei of the second sensory neuron (3,5). The first sensory neuron is a pseudounipolar and therefore, the cell body is located in the dorsal root ganglion (6). In the thoracic portion of the SC, a lateral horn also appears, containing the nuclei of the sympathetic nuclei (3). Both, dorsal and ventral horns are made by fusion of the rootlets, exiting the vertebral column and subsequent fusion of the roots forms spinal nerves with motor and sensor fibers (3). Each spinal nerve innervates a specific dermatome and myotome (3,7).

The types of fibers conveyed by spinal nerves are somatic fibers called general sensory and general motor fibers (7). They transmit exteroceptive (pain, temperature, touch, pressure) and proprioceptive sensations from the body to CNS (3,7). Furthermore, there are visceral sensory and visceral motor fibers, which transmit pain, and subconscious visceral reflex sensations or innervate involuntary muscles and glandular tissues, respectively (7).

The autonomous supply of body structures is ensured by sympathetic and parasympathetic innervation (3,7). The nuclei of the presynaptic sympathetic neurons are located in the lateral horn of the grey matter of SC (7). They appear in the level T1-L2/3 (3,7).

After exiting the intervertebral foramina, these fibers separate from the spinal nerve via the white communicating rami to synapse with the postganglionic sympathetic neuron in the paravertebral ganglion (3,7). Twenty-one bilaterally arranged paravertebral ganglia and a single caudal placed ganglia form the sympathetic trunk (7). The axon of the postsynaptic ganglion neuron exits the trunk via the grey communicating rami and travels along with the spinal nerve to their target organ (7).



**Figure 2.** Cross section of the spinal cord and vertebra in the upper lumbar region. The picture shows the main structures present in the finally developed human spinal cord, including the meninges (dura mater, arachnoid mater, and pia mater). Additionally, presenting the posterior median sulcus, anterior median fissure, and dorsal root ganglion. Created by the author based on sources in parenthesis (1,4).

### **1.1.3. Meninges**

Membranous layers surround the brain and include the pia mater, which is the innermost layer, followed by the arachnoid mater and outermost dura mater, presented in Figure 2 (4). These layers serve as mechanical barriers to maintain the blood-brain barrier (8). An additional protective element is the epidural fat, situated in the space between the dura mater and the vertebrae. This unique space only exists around the SC and disappears cranial of the foramen magnum (1).

### **1.1.4. Blood supply**

The blood supply of the SC is divided into vertical and horizontal portions, which are highly anastomosed (1,2). The vertical supply is ensured via the anterior spinal artery (ventral 2/3) and two posterior spinal arteries (dorsal 1/3), which are supplied by the vertebral artery, deep cervical and ascending cervical artery in the cervical region, by intercostal arteries from the thoracic aorta, via the lumbar arteries via the abdominal aorta or by the iliolumbar and median sacral artery via the internal iliac artery (1,2).

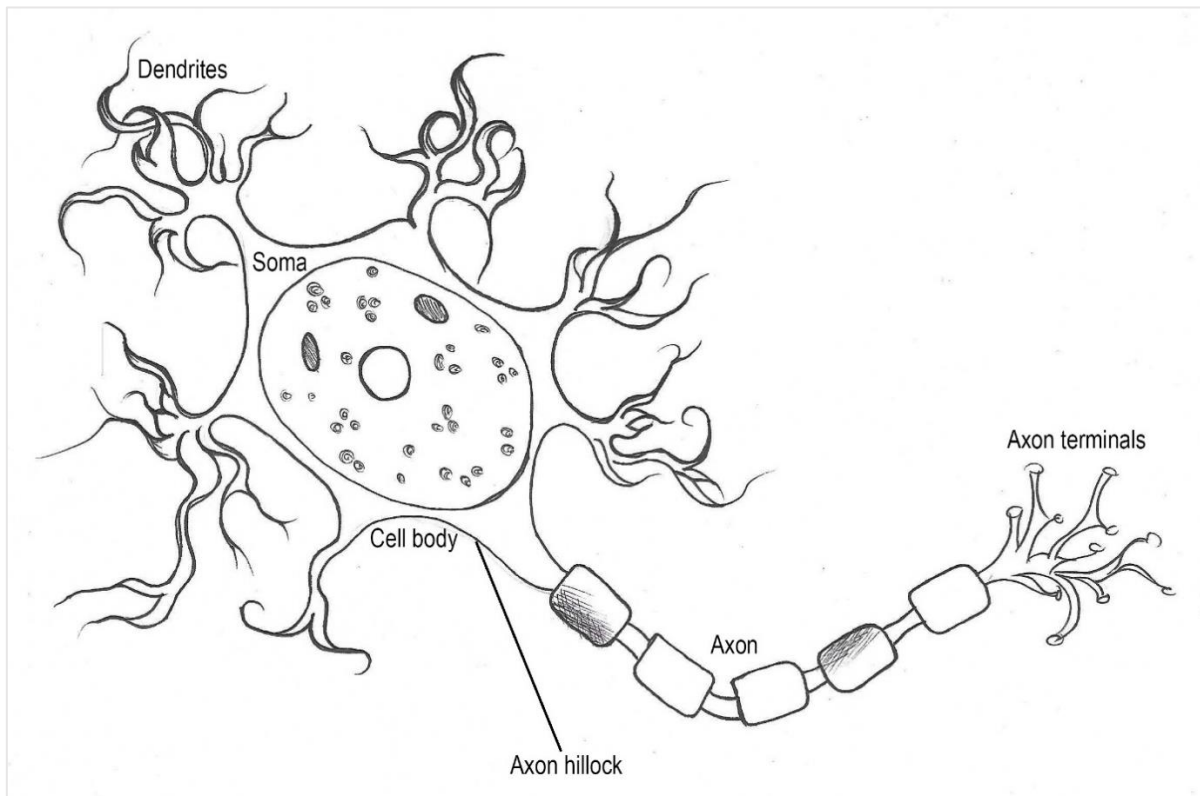
The venous drainage is analogous to the arterial supply. The blood is mostly drained via the horizontal system segmental into the great vessels (superior vena cava, inferior vena cava) (1,2).

## 1.2. Histology

The NS is composed of neural tissue which can be divided into neurons and neuron-supporting cells, namely neuroglia (1,5).

### 1.2.1. Neurons

Neurons are the smallest functional unit of the nervous system. These highly specialized cells are composed of the cell body, the dendrites, and the axon, as shown in Figure 3 (7,9,10).



**Figure 3.** Schematic presentation of neuron, the smallest functional unit of the nervous system. Created by the author based on sources in parenthesis (5,7).

First, the cell body contains the nucleus and nucleoli, Nissl granules, and *perikaryon* (soma) (5,10). At the location where the axons exit the cell body, all excitatory and inhibitory information will be added and one signal containing the information will be transmitted (11). Furthermore, the cytoskeleton is composed of neurofilaments, neurotubuli, and actin filaments which are necessary to support the structure of the cell and ensure trafficking options within the cell (5,10).

Secondly, the dendrites are long protrusions, or spines, whose many foldings increase the surface areas of the cells, enabling multiple synaptic interactions (11).

Finally, the axon is composed of three parts. The initial segment, called axon hillock, is unmyelinated and has many sodium voltage-gated channels therefore allowing an action potential to be easier triggered (6). The middle part-of the axon is myelinated to ensure faster transmission of action potentials (7). The terminal part contains thicker portions for the formation of a synapse (7). The cell membrane and cell plasma are called *axolemma* and *axoplasma*, respectively (10,11).

### **1.2.2. Neuroglia**

Glia cells support neurons and are 10 times more common (12). Glial cells differ in their function depending on their location. In the CNS the following glia cells are present: astrocytes, oligodendrocytes, microglia cells (Hortega cells), and ependymal cells (13).

Astrocytes are the most common glia cells in the CNS and lie tight over the capillaries (6). They are connected to oligodendrocytes via gap junctions, which form a functional syncytium (13). These cells contribute to the majority of glial fibrillary acidic protein (GFAP) production (6). Their functions include architectural support, scar formation after injury, formation of the blood-brain barrier, uptake of metabolites, supply and regeneration of neurons, and maintenance of electrical environment by uptake of potassium and elaboration of chemical transmitters for cell-cell signaling (gliotransmission) (13).

Oligodendrocytes have short processes and are located within the white and gray matter of CNS (6). They form myelin sheaths around the neuronal axons and therefore ensure fast transmission of information (6,13).

Microglial cells are specialized macrophages (6). They have movable processes and play a major role in phagocytosis within the CNS (13).

Ependymal cells are a special epithelial cell type lining the interior surfaces of the lateral, 3<sup>rd</sup>, and 4<sup>th</sup> ventricle, their connections, and to the central canal as well as the external limiting lining beneath the pia pater (6). These cells are interconnected via gap junctions (13).

### 1.3. Physiology

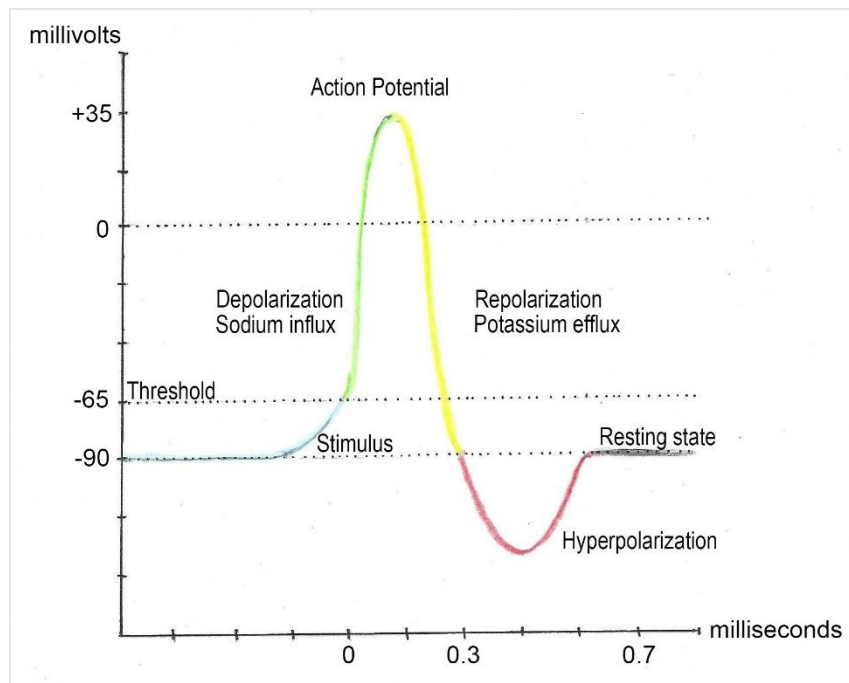
#### 1.3.1. Resting and action potential

A potential describes the electric current present at a semipermeable membrane. It is a result of the different distribution of ions in and outside of the cell. Cations like sodium, potassium, calcium, protons, and anions, mostly chloride, but also negatively charged proteins contribute to the current (14). The resting potential is usually between -70 millivolt (mV) and -90mV, depending on the type of cell. Selective diffusion through certain ion channels (permeable to potassium, and chloride, but not permeable for sodium and anionic proteins) and energy-consuming pumping across the sodium-potassium adenosine triphosphatase (ATPase) ensures the ion difference and, therefore, the electrical current (14). The resting potential for neurons is -70 mV and for glia cells is -90mV (14).

If there are additional stimuli like voltage changes, pressure, or neurotransmitter binding certain ion channels open and change the permeability of ions across the membrane (15). This causes changes in electrical currents and could elicit an action potential, which is composed of the following stages: depolarization, repolarization, and hyperpolarization, as seen in Figure 4 (14).

After a certain stimulus reaches the cell, sodium channels will open, and sodium influx will change the resting potential to become more positive. If the stimulus is strong enough to change the membrane potential above a certain threshold value approximate -65 mV (14). The voltage-gated sodium channels will recognize the reached threshold and open to ensure sodium influx. At the same time voltage-gated potassium channels will close and prevent the efflux of potassium. This elicits finally an action potential and is called the all or nothing principle (6,15). The high upstroke will reach a maximum of +35 mV and is called depolarization, during this phase voltage-gated sodium channels are open and voltage-gated potassium channels are closed (6,14,15). When the maximum is reached, the second phase of repolarization starts. The voltage-gated sodium channels are inactivated, and voltage-gated potassium channels open to ensure potassium efflux and allow the membrane to become negative again (6,15). The third phase is the hyperpolarization, which describes an increased activity of potassium channels. The membrane potential reaches in this phase approximately -90mV (6,15). To return to the starting point known as the resting potential, the sodium potassium ATPase exchanges three sodium for two potassium ions (6,15).

During the described mechanism two refractory periods are present. The absolute and relative refractory periods, in which a full action potential is not or partially elicited, respectively. Here during the depolarization and repolarization, the channels are already open or need time to reset for opening and it is impossible to elicit an additional action potential (14). During the hyperpolarization a greater stimulus is needed to reach the threshold potential, thus the possibility to elicit another action potential is given but a higher influx of sodium for the initial opening is needed (14).



**Figure 4.** Phases of action potential. Presentation of a stimulus reaching the threshold and eliciting via sodium influx an action potential, secondly the repolarization starts due to the efflux of potassium ions and due to the function of the sodium-potassium pump hyperpolarization is reached, followed by the resting state. Created by the author based on sources in parenthesis (5,14).

### 1.3.2. Neurotransmitters

Neurotransmitters are chemical substances (16). They need to fulfill certain criteria to be defined as such, this withholds being chemical substances synthesized within neurons and being released at a synapse when nerve terminals are depolarized (usually by calcium ions) (16). They bind to receptors on the postsynaptic cell (16). Therefore, they have an auto- or paracrine function within the neural tissue and play an important role in cell-cell signaling (17). Autocrine secretion is defined by release of a certain substance which binds to receptors on the



secreting cell and influences that certain cell (18). Paracrine secretion involved communication between cells in the same area (18).

Neurotransmitters are classified into the following three major groups: small molecule neurotransmitters, neuropeptides, and gaseous neurotransmitters as demonstrated in Table 1 (16).

**Table 1.** Major classes of neurotransmitters

Class	Group	Substance	Effect
Small molecule neurotransmitters	Excitatory amino acids	Acetylcholine	Active in CNS, PNS, somatic and autonomic parts of NS, additionally plays a role at neuromuscular junction
		Glutamate	Involved in cognitive function of CNS (learning and memory) Opens sodium channels
		Aspartate	Highly selective agonist for NMDAR type glutamate receptors
	Inhibitory amino acids	GABA	Involved in CNS, influenced muscle tone; modulate various ion channels
		Glycine	Involved in CNS and specialized neuronal tissue (retina), increases chloride ion channel permeability
	Biogenic amines: Catecholamines	Dopamine	Production of inhibitory action in CNS, involved in cognition (learning, memory), motivation, increased permeability to potassium ion channels, closure of calcium ion channels
		Norepinephrine	Involved in PNS (sympathetic ganglia neurons in ANS) and specific regions in CNS
		Epinephrine	Involved in various effects in different locations of CNS

*Table continued*

	Indoleamine	Serotonin	Involved in complex brain functions (sleep, appetite, cognition, mood), further modulation of neurotransmitter release
	Imidazole	Histamine	Located in tuberomammillary nucleus, maintain wakefulness and attention, involved in circadian and feeding rhythm and immunity
	Purines	ATP	Involved in neuroprotection, central control of autonomic function, neural-glia interactions, angiogenesis, vasomodulation, pain and mechanosensory transduction
<b>Neuropeptides</b>		beta-endorphin	Prevention of release of pain signaling molecules from neural tissue
		methionine-enkephalin	Involved in immunoregulatory actions
	Opioid peptides	leucin-enkephalin	Involved in regulation of noxious and potentially harmful stimuli
		nociceptin	Involved in pain transmission to the brain via SC
		Substance P	Involved in cognitive functions (learning and memory), involved in modulation of smooth musculature of gastrointestinal system and vasculature
<b>Gaseous Neurotransmitters</b>		NO	Involved in synaptic plasticity via long term potentiation or inhibition, and central regulation of blood pressure

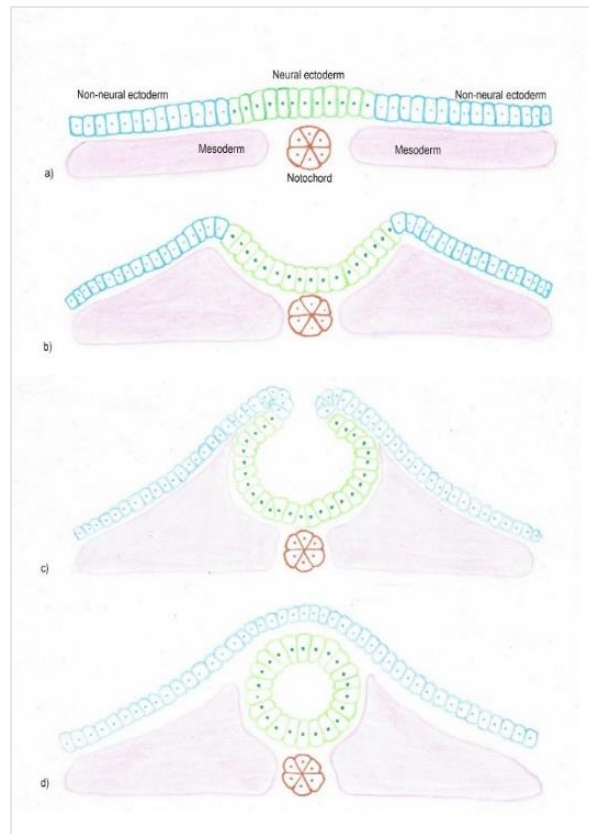
**Table 1.** Major classes of neurotransmitters (continued). Created by the author based on sources in parenthesis (5,16,19–22).

There are neurotransmitters with excitatory characteristics like acetylcholine and neurotransmitters with inhibitory characteristics like gamma aminobutyric acid (GABA) (17,23,24). These different effects are performed by targeting different cellular membrane receptors which cause ion movement across the semipermeable membrane (14). This changes the resting membrane potential and can elicit an action potential or prevent the trigger from reaching the threshold (14). This modification of signal transmission plays an important role in the SC (25). There are neurons transporting information to the brain and into the periphery (7). Moreover, interneurons possess the capacity to modulate neuronal transmission already at the level of the SC (3,16).

#### **1.4. Embryology – Development of spinal cord and dorsal root ganglia**

##### **1.4.1. Formation of neural tube**

In the third week of embryogenesis, the development of the intricate structure of the human CNS starts, as presented in Figure 5 (2,26,27). This process begins with generating a dense layer of neuroepithelium, originating from the ectoderm, known as the neural plate in the anterior mid-dorsal region, located ventral to the primitive node. (2,26). This plate is referred as a large placode, the precursor to sensory organs and ganglia. (2,26). The mechanism of elevation of lateral ends creates neural folds, which are converge each other in cranial and caudal directions and form a cylindrical structure, named the neural tube (2,26). This process is called primary neurulation (2,26). During development the cranial portion becomes broader and caudal part narrower. They are precursors of the future brain and SC, respectively (2,26). This is secondary neurulation and describes the formation of the neural tube just caudal to posterior neuropore, due to the condensation of mesenchymal cells (2,26,28,29).

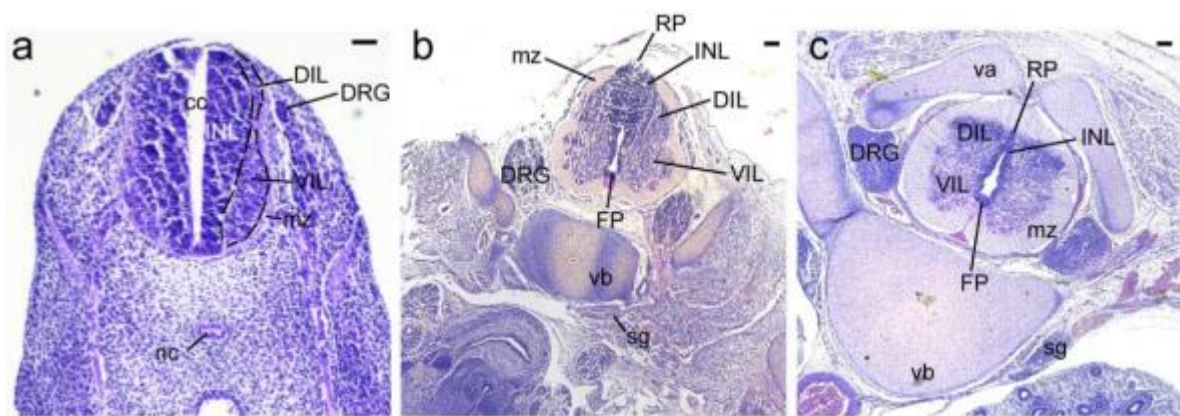


**Figure 5.** Neural tube formation and closure. Created by the author based on sources in parenthesis (5,26).

Moreover, these progenitor cells give rise to multiple types of cells with different functions, like neurons, ependymal, neuroglia, and choroid plexus (30,31). Neural crest cells form future sympathetic and parasympathetic neurons and therefore need to grow to different ganglia located on the dorsal part of the SC and create synaptic connections in the paravertebral and prevertebral region (sympathetic neurons), and in the effector organs (parasympathetic neurons) (32,33).

The neural tube can be divided into three layers, according to the anatomic position: inner layer (IL), intermediate layer (IML) and marginal layer (ML) (26). The inner part of the newly formed cavity lining the developing central canal (cc) contains immature neurons, called neurocytes (26). This dense pseudostratified epithelium, present in the IL, enclosed by the neural tube, can therefore also be called ventricular layer (26). The IML is alternatively named as the mantle layer and includes neuroblasts, which will later develop into the grey matter (26). Lastly, the outer most layer, ML, is commonly denoted as marginal zone (mz) (26). This zone contains axons of these neurons, which will be myelinated in the future and appear later as white matter (26).

In cross-section important landmarks are visible during the proliferation of neurocytes. A pair of grooves, called the *sulcus limitans* appears at approximately halfway along the lateral margins, caused by ventral and dorsal thickening function (26,34). The sulcus limitans presents the border between future sensory and future motoric functions. On the one hand, cells migrating posterior to the sulcus limitans form the alar plate (AP), later providing sensory function (34). On the other hand, cells migrating ventral to *sulcus limitans* form the basal plate (BP), later giving rise to motor function (34). Additionally, the neurons originating from intermediate position to the sulcus limitans will execute autonomic functions (26). The roof plate (RP) and floor plate (FP) are formed dorsally and ventrally, respectively to encircle the neural canal, as demonstrated in Figure 6 (26). Depending on the position and function of the neurons, axons will either form ventral or dorsal roots alongside the dorsal root ganglia and will become efferent or afferent, respectively (26). There are specialized nerve fibers in bundles for sensory information or for innervating voluntary or involuntary effector organs (6,26).



**Figure 6.** Photomicrographs of sections of the human developing spinal cord (SC) and ganglia stained with hematoxylin-eosin staining. Different anatomical structures are marked in (a) 5 weeks (objective magnification – 10x; scale bar = 80 $\mu$ m), (b) 6.5 weeks, and (c) 10 weeks old human conceptuses (both magnification – 4x; scale bars= 200 $\mu$ m). DRG – dorsal root ganglion, INL – inner layer, DIL – dorsal intermediate zone; VIL – ventral intermediate zone; mz – marginal zone; cc – central canal; RP – roof plate; FP – floor plate; nc – notochord; sg – sympathetic ganglion; vb – vertebral body; va – vertebral arch. Pictures were taken with permission from Jurić M, Zeitler J, Vukojević K, et al. Expression of Connexins 37, 43 and 45 in developing human spinal cord and ganglia (35).

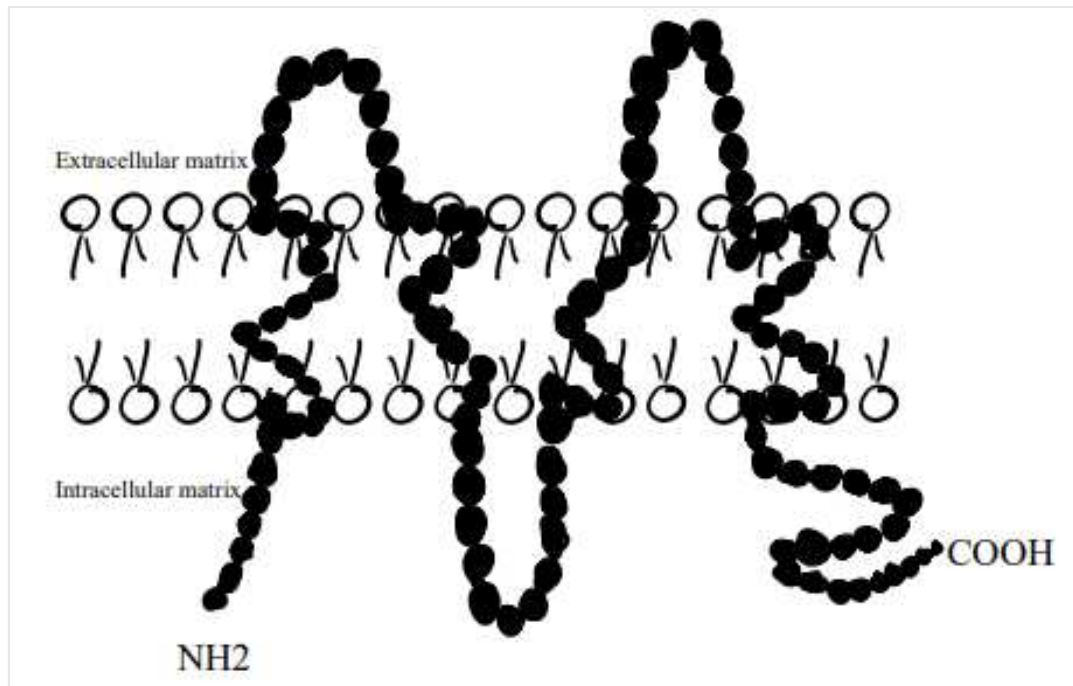
### **1.4.2. Cell differentiation according to position**

In histological differentiation, a neuroepithelial cell is the only cell responsible for differentiating neuroblasts, also known as primitive nerve cells (26). On the one hand, in the anterior aspect of the SC, the transformation of a neuroblast into a fully formed neuron involves two distinct phases: an apolar phase, followed by a subsequent bipolar phase characterized by the emergence of primitive axons and dendrites (26). Successively, multipolar neuroblasts undergo maturation to become fully developed adult nerve cells (26). On the other hand, cells of the sensory dorsal root behave differently, they arise from the neural crest cells and give rise to two distinct processes (32,33). There are centrally located processes entering the dorsal portion of the neural tube and establish synaptic connection with the second neuron in the SC. Simultaneously, some of them ascend to the brainstem, and they are collectively known as dorsal sensory roots of the spinal nerve (26). Additionally, fibers of the peripheral process connect with fibers of the anterior motor roots to create the future spinal nerve and these fibers terminate on sensory receptor organs (6,26).

## **1.5. Pannexin**

### **1.5.1. Structure of pannexin**

Pannexins (Panx) are proteins that belong to a class of membrane channels and ensure the traffic of ions and small molecules across semipermeable membranes, similarly like connexins and innexins (36). The human pannexin family consists of three members: pannexin1 (Panx1), pannexin2 (Panx2), and pannexin3 (Panx3). These types differ in amino-terminus (N-terminus) and carboxy-terminus (C-terminus) and number of monomers (Panx1 has six and Panx2 has eight monomers, Panx 3 not yet determined). The highest variability is seen in the C-terminal (36). These integral membrane proteins have four transmembrane domains, two extracellular loops, three intracellular domains including the N-terminus, the intracellular loop, and a highly divergent C-terminus, as shown in Figure 7 (37).



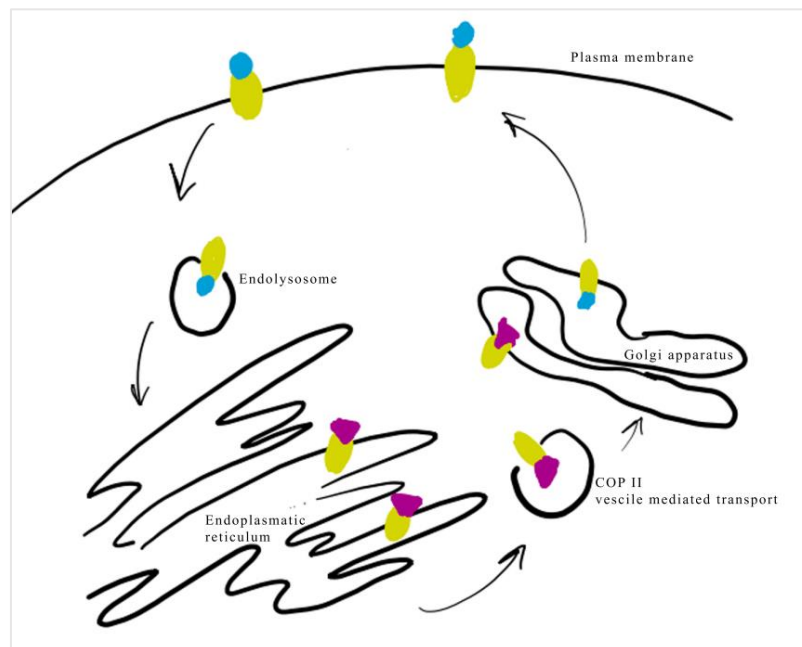
**Figure 7.** Schematic demonstration of the structure of pannexin1 (Panx1). Presentation in the semipermeable plasma membrane of cell with four transmembrane domains, two extracellular loops, and three intracellular domains. Created by the author based on sources in parenthesis (36,37).

On the extracellular domains, there are four conserved cysteine residues, with the option to form disulfide bonds. Additionally, the extracellular residue is glycosylated, which may modify intercellular channel formation and regulation (36). These specific characteristics allow them to communicate via a large pore channel within the extracellular space and cytoskeleton via intercellular connections (36).

### 1.5.2. Distribution and pore positioning

Baranova et al. demonstrated with Northern blot analysis the expression of Panx1 RNA in human tissues like brain, heart, skeletal muscle, skin, testis, ovary, placenta, thymus, prostate, lung, liver, small intestine, pancreas, spleen, colon, blood endothelium and erythrocytes (38). Sosinskiy et al. presented with fluorescence (FM) and electron microscopy (EM) staining that Panx1 and Panx3 are localized throughout the plasma membrane at the apical membrane form polarized cells. Panx1 is also highly expressed in CNS and during embryonal development (39–41). Furthermore, they demonstrated that Panx1 and Panx2 can be concentrated within intracellular compartments (42,43). A subpopulation of Panx is present within the membrane

of the endoplasmic reticulum (ER) and influences the increased leakage of calcium ions. This was especially observed during tumor cell proliferation and osteoblast differentiation (44,45). The location of transmembrane proteins is determined by many factors including N-linked glycosylation, C-terminus modification, and retrograde transport (37). After transcription and translation, further modification of the pre-protein is done in the ER and Golgi apparatus. The glycosylation is critical for the destination of Panxs within the cell (46–48). Glycosylation of the N-terminus will enable the pannexin to reach the cell membrane, as demonstrated in Figure 8 (37).



**Figure 8.** A schematic demonstration of translation and posttranslational modification of Pannexin1 (Panx1) in the endoplasmic reticulum (ER) and Golgi apparatus. Panx1 is translated at the rough ER, followed by the anterograde transport towards the plasma membrane. Therefore, further modification in form of glycosylation with high mannose glycan to asparagine 254 (N254) in its second extracellular loop is done, here demonstrated in purple. Coat protein complex II (COP II) vesicle-mediated transport transfers the protein to the Golgi apparatus for complex glycosylation which is necessary for subsequent translocation to the plasma cell membrane. The complex glycosylated loops are placed extracellular and the -N and highly divergent -C terminals are placed into the cytoplasm. Retrograde trafficking is a complex mechanism, which involves endolysosomal degradation of proteins. Created by the author based on sources in parenthesis (37).



Therefore, alteration of this process, caused by a cerebral vascular incident such as a stroke, can impact the physiologic and pathophysiologic mechanisms (37,49). The C-terminus is important for the correct translocation and is associated with the cytoskeleton and subcellular network for protein trafficking and stabilization (50,51). It directly interacts with actin microfilaments and influences the mobility of the plasma membrane and intracellular accumulation of Panxs. Caspases cleave the C-terminus and have therefore an influence on the translocation of Panxs channels and the functional state of channel protein (52).

The exact mechanism of retrograde transport of Panx is unknown and several proteins have been shown to be involved in internalization (clathrin, caveolin, and dynamin mediated endocytosis) (53). According to Bhalla-Gehi et al. and to Gehi et al., internalization is described independently from clathrin/ caveolin/ dynamin II (51,53). A certain stimulus, such as the increased activity of the channel, can lead to deactivation via internalization and degradation in order to downregulate associated transmembrane signals.

### **1.5.3. Physiologic function and modulation of activity**

Gap junction genes and related protein families like Panxs play an important role in trafficking molecules across the plasma membrane (36). Furthermore, they are involved in development, and it is believed that coupled cells undergo simultaneous differentiation in contrast to uncoupled cells which follow a distinct fate (36). Additionally, Panxs have been shown to act as tumor suppressor genes in gliomas (54).

Under physiological conditions, Panx reacts to external stimuli with increased trafficking of molecules in response to mechanical stretch, strong and prolonged depolarizations, elevated intracellular calcium, and elevated extracellular potassium (52). These physiologic mechanisms are demonstrated in Table 2. It is known that Panx1 can also be activated by G-protein-coupled receptors and other downstream intracellular signaling pathways, i.e., Rho kinase activities (55–57). Their activity can be altered in response to pathological stimuli like oxygen and glucose deprivation, seizure-related N-methyl-D-aspartate (NMDA) receptor activation, and inflammation (58–62). ATP, uridine triphosphate (UTP), and other signaling molecules traverse Panx channels of cells within the pyramidal lobes, the Purkinje cells in the cerebellum, neurons, erythrocytes, T-cells, astrocytes, airway epithelial cells, taste cells, and the cochlear supporting cells (43,63).

**Table 2.** Physiological mechanisms for the modulation of pannexin.

<b>Activation of Panx1 channel</b>	<b>Inactivation of Panx1 channel</b>
Plasma membrane stretch/ strain	High levels of extracellular potassium
Membrane depolarization	High levels of extracellular ATP
Extracellular potassium levels	
Low oxygen tension	
G coupled receptor	
Caspase cleavage	
Ionotropic receptors including P2X	

Created by the author based on sources in parenthesis (52).

### **1.5.3.1. Calcium ion transport**

Calcium ions can traverse the cell membrane via Panx channels and act as second messengers in various cell signaling pathways. This is important for the propagation of calcium ion waves and therefore in the regulation of vascular tone, mucociliary lung clearance, and taste bud function (64).

### **1.5.3.2. ATP transport**

ATP is an important storage form of energy used by the body. Late in the 1920s ATP was isolated from a muscle cell for the first time (52). After additional studies, it was determined that ATP also functions as a signaling molecule in a paracrine manner (52). It is used as a small molecule neurotransmitter (Table 1 and 2) in the SC and is associated with pathomechanisms of epilepsy and physiologic mechanisms such as pain transduction (52). ATP can be released via exocytosis from cells and Panx1 channels also can traffic ATP across the membrane (52). Therefore, Panx1 plays an important role in epilepsy according to its relation to ATP release in neuronal cells (64).

### **1.5.3.3. Receptor-coupled pannexins**

Panx can interact with other receptors like P2X<sub>7</sub> which is involved in inflammatory mechanisms. It releases proinflammatory cytokines in response to receptor stimulation and subsequently activates caspase 1 (52,65). This coupling mechanism of receptor and Panx channels is especially observed in T cell activation, macrophage chemotaxis, and neutrophil regulation (52). It can also trigger Toll-like receptor independent inflammasome and influences extracellular potassium levels in neuronal/astrocytic inflammasomes (58,66).

### **1.5.3.4. Caspase activity**

Caspases not only influence the destination of Panxs by posttranslational modification, but they are also constitutively active channels by cleaving the C terminus of Panx1 (43,52). This happens under cellular stress in Jurkat cells or in relation to the inflammatory response, as described above (43,52).

### **1.5.4. Potential therapeutical impact/ potential**

Panx directly impacts various pathological mechanisms, like cardiovascular diseases, inflammation, and cancer development (67–70). Panx1 plays a role in ischemia induced seizures, tumor formation, and metastasis, hypertension, inflammation, human immunodeficiency virus (HIV) infection, migraine headaches, and neuropathic pain (52,64,71–73). Further investigations have concluded that Panx contributes to protection from ischemic stroke injury, modulation of neuronal excitability and learning, bone development, narcotic withdrawal, the sleep-wake cycle regulation, and behavior (60,74–79). Lifecycles of Panxs are complex and therefore must be understood accurately to use them as a therapeutical target (37).

## **2. OBJECTIVE**

## **Objectives**

Despite their important role in function and development of the NS has been proven by many animal studies, there is no data about the expression of Panxs in the human NS during early intrauterine development. Hence, the objective of this study was to explore the distribution of expression of Panx1 in the SC and ganglia of human conceptus during early phase of development. Therefore, we hypothesized that Panx1 is expressed on specific parts of the SC, including the roof plate, floor plate, alar plate, basal plate, inner and marginal layer, as well as the dorsal root ganglia, notochord, para, and prevertebral ganglia.

Additionally, our second hypothesis is, that the distribution of Panx1 is related to following markers in the developing human SC, including PGP9.5, a general neuronal marker, NF200 – a marker for myelinated neurons and nerve fibers, CD 31 – a marker for endothelium, and proliferative marker Ki67.

### **3. MATERIALS AND METHODS**

### 3.1. Tissue procurement and processing

The collection of human conceptus tissue was obtained from the Department of Gynecology and Obstetrics and Department of Pathology and processed with permission of the Ethical and Drug Committee of the University Hospital of Split in accordance with the Helsinki Declaration (class: 003-08/16-03/0001, approval number: 2181-198-03-04-16-0024; 1 August 2020) (35,80). Exclusion criteria were poorly preserved material, and it was therefore ruled out. The age of the conceptus is conducted by assessing the external measurements (crown-rump length), in conjunction with the utilization of Carnegie stages (35,81). They were collected from the University Hospital in Split, after tubal pregnancies or spontaneous abortions. Tissue was fixed in 4% paraformaldehyde in phosphate buffer saline, paraffin-embedded, cut in transversal plane (5 $\mu$ m), and mounted on glass slides (35).

**Table 3.** The human conceptuses analyzed in this study

Age	Crown-Rump Length (CRL) (mm)	No
7 <sup>th</sup> week	21	1
8 <sup>th</sup> week	27	1
10 <sup>th</sup> week	36	1

### 3.2. Immunohistochemistry procedure

The histologic sections of the thoracic segments underwent deparaffinized in xylene, followed rehydrated in 100% ethanol solutions two times for 10 minutes, 95% ethanol solution for 5 minutes, 70% ethanol solutions for 5 minutes, and in distilled water for 5 minutes. After the process of deparaffinization and rehydration, the sections were heated in citrate buffer (pH 6.0) for 30 minutes in a steam cooker. Subsequently, cooling to room temperature, the protein block solution was applied for 20 minutes. The PAP Pen was used to encircle the tissue to prevent spilling. Sections were placed in a humid chamber and incubated with primary antibodies overnight. After washing in phosphate buffer saline (PBS), appropriate secondary antibodies were applied for 60 minutes. Then the slides were washed in PBS, nuclei were stained with 4',6-Diamidino-2-phenylindole (DAPI), the slides air-dried, and the cover slipped. The omission of the primary antibody from the procedure resulted in the absence of staining of the tissue.

For specimen A the primary antibody used targeted Panx1 (1:200 diluted, rabbit antibody), and a neuronal marker PGP9.5 (diluted 1: 500, mouse antibody) was used. For specimen B endothelial marker CD31 (diluted 1:50, mouse antibody) was used. For specimen C Ki67 (diluted 1:50, mouse antibody) was used, and for specimen D neurofilament 200kD (NF200) (diluted 1:100, mouse antibody) was visualized.

The secondary antibody added visualizes the rabbit antibody in green color, and the mouse antibodies in red color, both diluted 1:200.

**Table 4.** Primary and secondary antibodies used

	<b>Antibody</b>	<b>Code no.</b>	<b>Host</b>	<b>Dilution</b>	<b>Source</b>
<b>Primary</b>	Anti-Panx1	ABN242	Rabbit	1:200	Merck KGaA, Darmstadt, Germany
	Anti-PGP9.5	480012	Mouse	1:500	Invitrogen
	Anti-CD31	sc-376764	Mouse	1:50	Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA
	Anti-Ki67	M7240	Mouse	1:50	DakoCytomation, DK- 26000 Glostrup, Denmark
	Anti-Neurofilament 200kD	MAB5266	Mouse	1:100	Sigma-Aldrich, St. Louis, MO, USA
<b>Secondary</b>	Alexa Fluor®488 AffiniPure Anti-Rabbit IgG (H+L)	711-545- 152	Donkey	1:200	Jackson Immuno Research Laboratories, Inc., Baltimore, PA, USA
	Rhodamine Red™- X (RRX) AffiniPure Anti-Mouse IgG (H+L)	715-295- 151	Donkey	1:200	Jackson Immuno Research Laboratories, Inc., Baltimore, PA, USA



### **3.3. Data Acquisition and Analysis**

Using the immunofluorescence microscope (BX61, Olympus, Tokyo, Japan) sections were viewed and captured using a cooled digital camera (DP71, Olympus, Tokyo, Japan). We then captured these using the objectives: UPLFLN10X2, UPLFLN40X, and UPLFLN100XO2 (all Olympus, Tokyo, Japan). Using an objective magnification of 40 visual fields were captured with continuous exposure time. Green granular deposits were interpreted as positive for Pannexin 1 immuno-expression.

The pictures were edited by Image J (National Institutes of Health, Bethesda, MD, USA) and Adobe Photoshop (Adobe Inc., San Jose, California, USA) to present results as clearly as possible. Image J editing included subtraction of red color channels, median filtering by 5.0 pixels, and further threshold by using the triangle threshold algorithm. The collages were created by Adobe Photoshop via the merging of all three layers of captured pictures.

## **4. RESULTS**

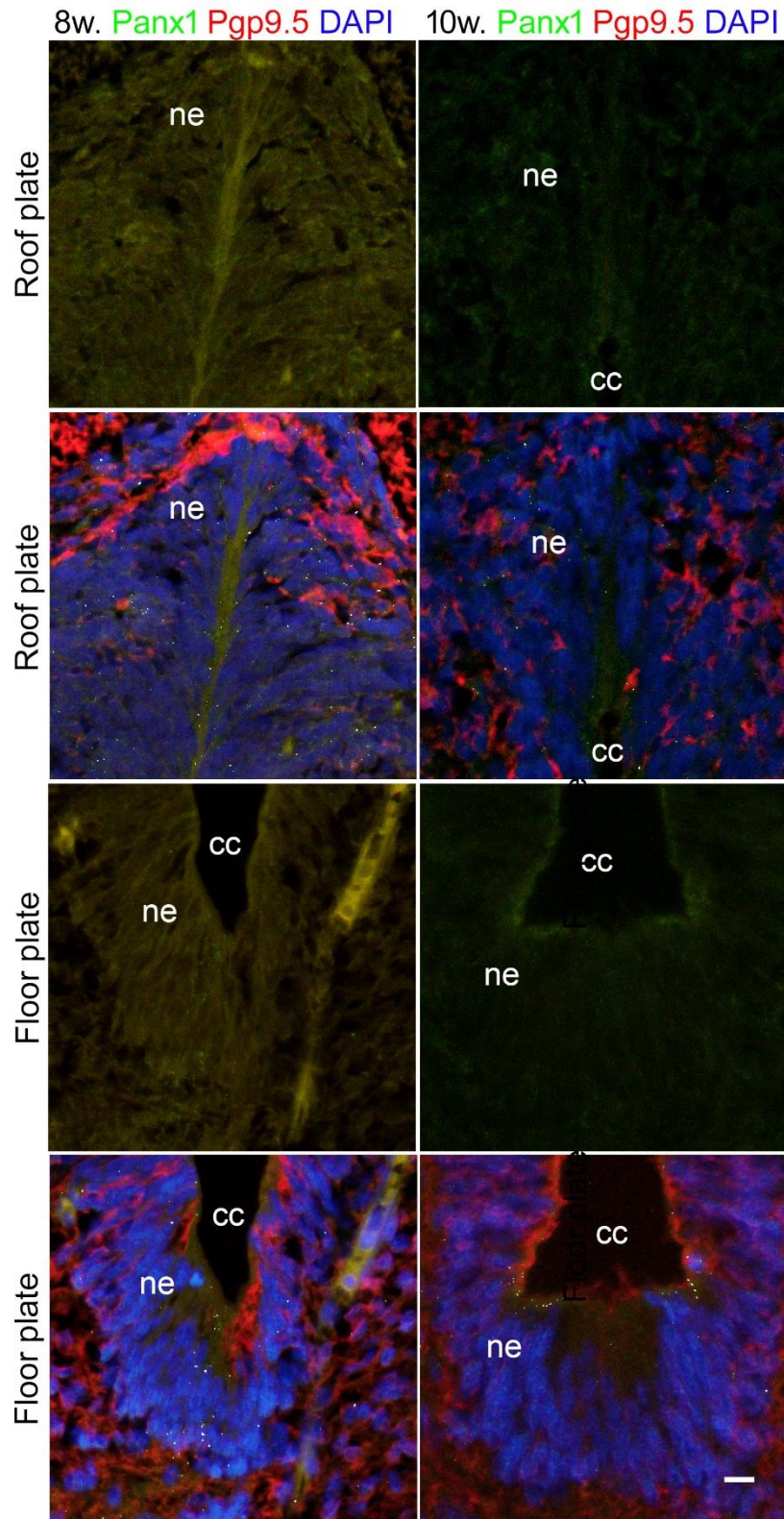
The expression of Panx1 was studied in different parts of the SC during embryonal development. During early human development, we found expression of Panx1 in all areas of interest in the SC (FP, RP, AP, BP, DRG, sg, NC).

Figure 9 shows the expression of Panx1 in the RP and the FP. At the 8<sup>th</sup> and 10<sup>th</sup> developmental week (DW) rare Panx1 immunoreactive puncta were seen in the RP. More Panx1 positive puncta were observed in the FP. However, in the 8<sup>th</sup> DW Panx1 positive puncta FP were more scattered, while in the 10<sup>th</sup> DW, they were concentrated close to the cc. Figure 10 presents the expression of Panx1 in the INL of the SC. Panx1 immunoreactivity was present in both AP and BP. Panx1 immunoreactive puncta were scattered and partially co-localized with PGP9.5 immunoreactivity. They were the densest in the 8<sup>th</sup> DW, where they were denser at the AP in comparison with BP.

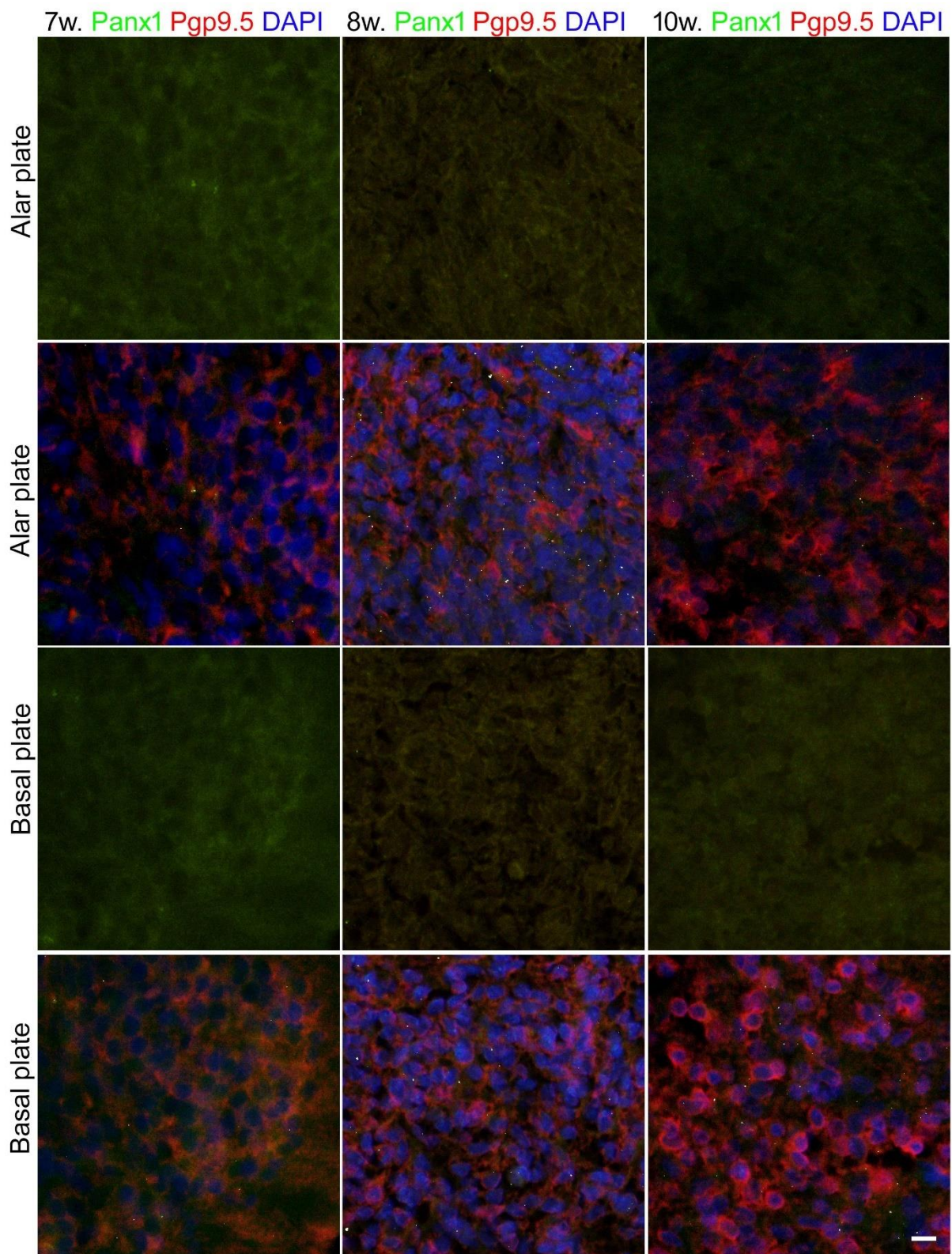
In the INL of developing SC, we found the most prominent expression of Panx1 in 10<sup>th</sup> DW, and Panx1 immunoreactive puncta were mostly concentrated close to the cc. However, in the ML we found very rare Panx1 immunoreactive puncta (Figure 11).

The expression of Panx1 was also observed in the DRG (Figure 12). In the 8<sup>th</sup> and 10<sup>th</sup> DW expression was stronger in comparison to the 7<sup>th</sup> DW. Nevertheless, the Panx1 expression was the most prominent in DRG, in comparison to the other neural structures studied. Similarly, as for the SC, Panx1 expression in DRG partially co-localized with PGP9.5 immunoreactivity. The expression of Panx1 in the meninges of the human conceptuses during the 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> DW was strong and denser in comparison to all studied neural structures, including the DRG (Figure 12). Moreover, the expression of Panx1 was found also in DRG, and it was the strongest in 10<sup>th</sup> DW, in comparison to the other stages studied. In the 10<sup>th</sup> DW Panx1 expression was found also in the prevertebral (PRVG) and intramural (IMG) ganglia, where it co-localized with the PGP9.5 staining.

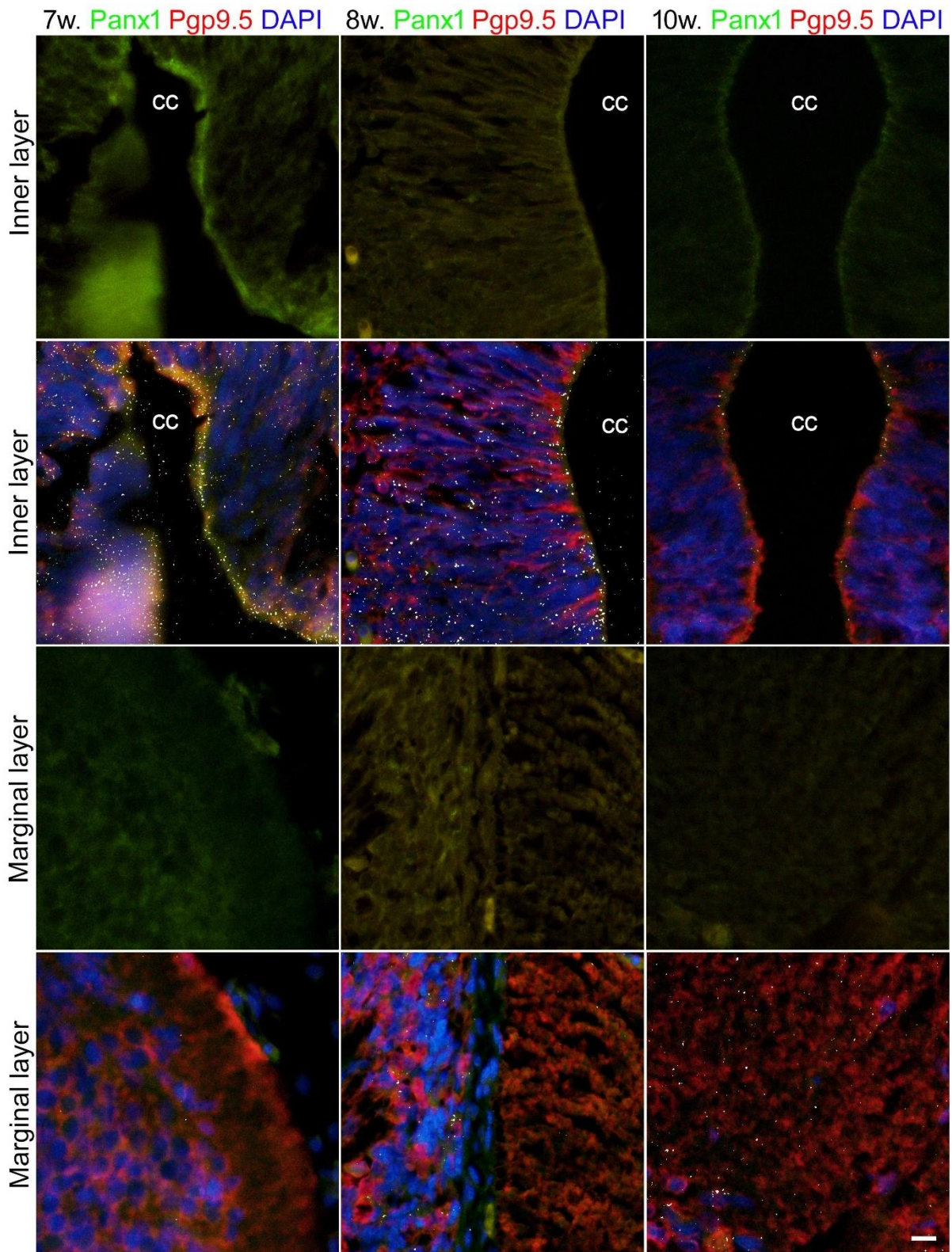
In general, we found co-localization of Panx1 with PGP9.5 in all neural structures studied, which was more pronounced in later stages of development (Figures 9-13). In the 7<sup>th</sup> DW, we found a rare expression of NF200 in the BP of the SC (Figure a and b). Panx1 immunoreactive puncta surrounded these NF200 positive fibers and rare Panx1 puncta co-localized with the NF200. In addition, we found Panx1 immunoreactivity in developing blood vessels in the SC and meninges (Figure 14 c-f). Some of the Panx1 immunoreactive puncta co-localized with a marker for the endothelial cells – CD31, while we also noticed Panx1 positive puncta in CD 31-non-reactive cells of the vascular wall. We found the co-localization of Panx1 with a marker for proliferation – Ki67 in meninges, notochord, INL (cells in contact with the cc), and developing blood vessels (Figure 15 a-e).



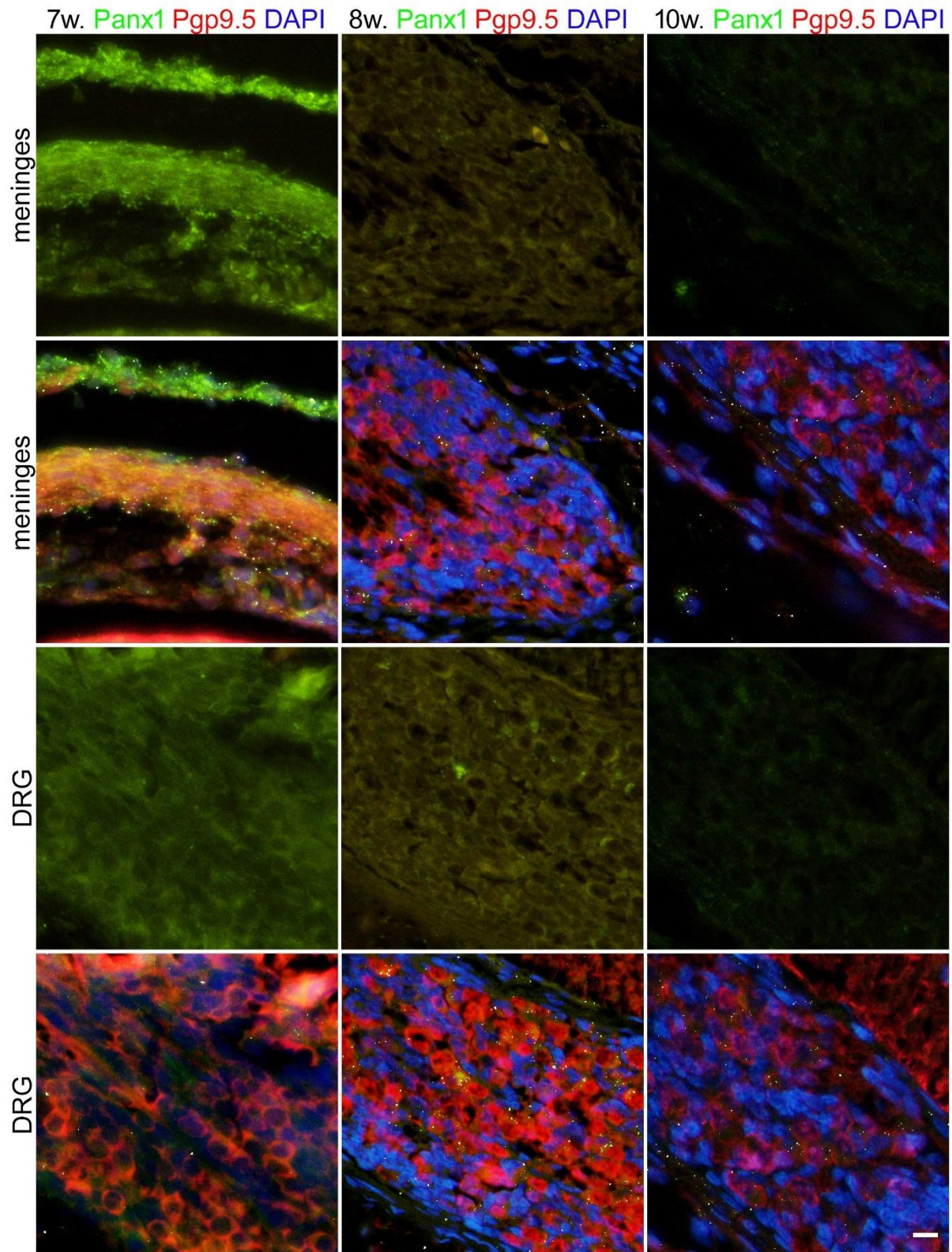
**Figure 9.** Expression of Pannexin1 (Panx1) in the roof and floor plate (RP and FP, respectively) of the spinal cord (SC) in human conceptuses. Panx1 (green) and PGP9.5 (red) staining was performed on the thoracic segments of the SC. The neuroepithelium (ne) in contact with the central canal (cc) could be seen on photomicrographs. Objective magnification – 40x (scale bar= 20 $\mu$ m). DAPI - 4',6-Diamidino-2-phenylindole.



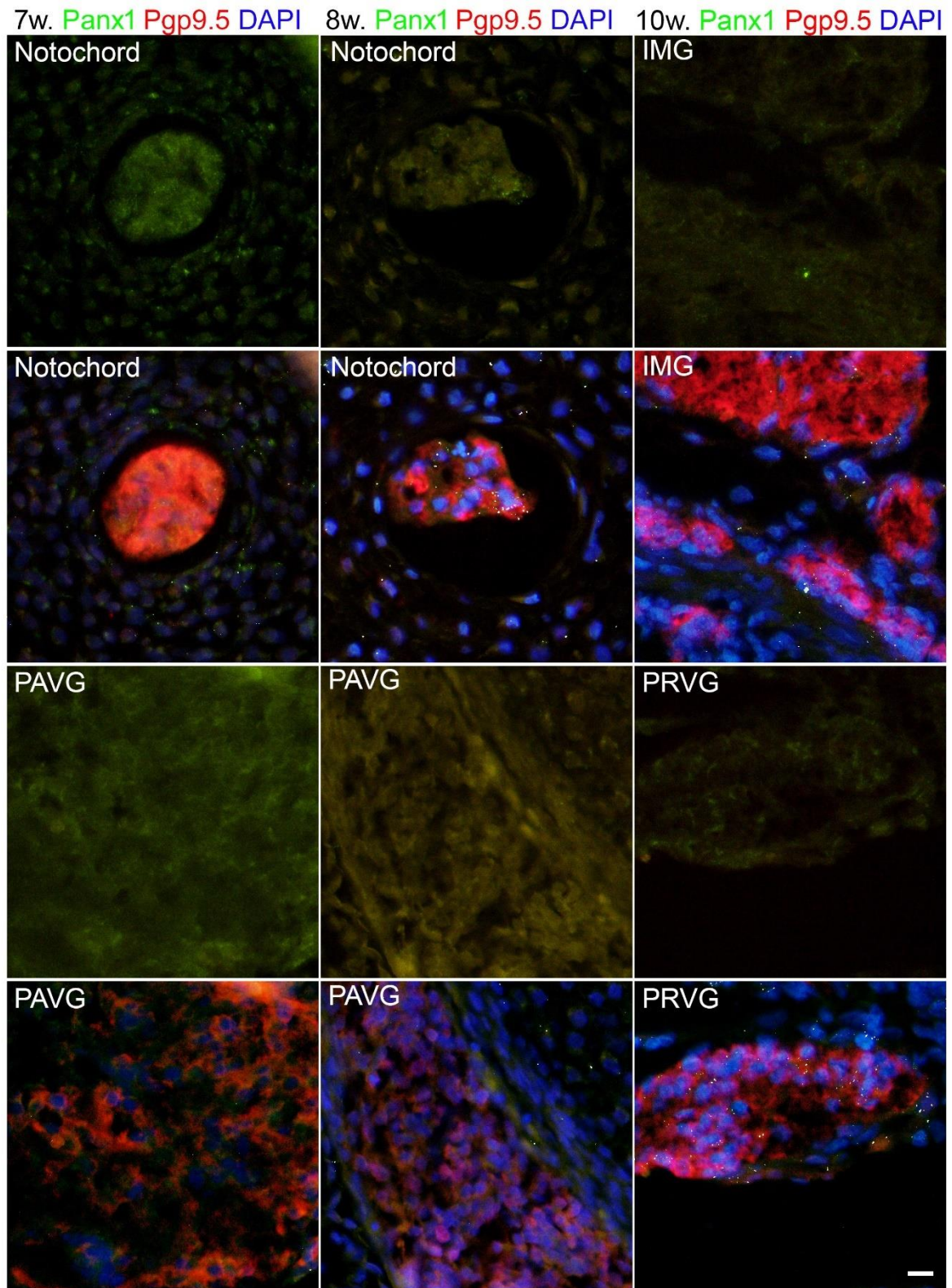
**Figure 10.** Expression of Pannexin1 (Panx1) in the intermediate layer (IML) of the spinal cord (SC) in human conceptuses. Panx1 (green) and PGP9.5 (red) staining was conducted on the thoracic segments of human conceptuses at 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> weeks of development. The alar and basal plate (AP and BP, respectively) were presented on photomicrographs. Objective magnification – 40x (scale bar= 20µm). DAPI - 4',6-Diamidino-2-phenylindole.



**Figure 11.** Expression of Pannexin1 (Panx1) in the inner and marginal layer (INL and ML, respectively) of the developing spinal cord (SC) of human conceptuses. Panx1 (green) and PGP9.5 (red) was performed on the thoracic segments of the SC of 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> week-old human conceptuses. Objective magnification – 40x (scale bar= 20 $\mu$ m). DAPI - 4',6-Diamidino-2-phenylindole.

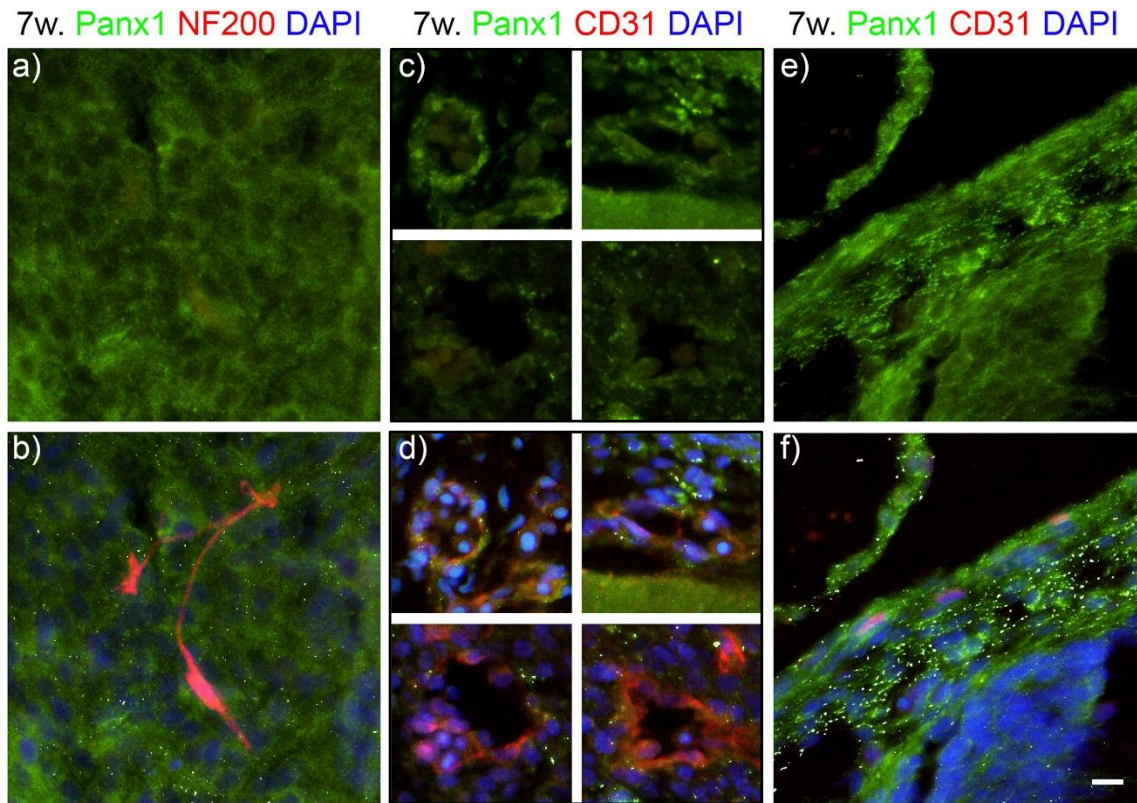


**Figure 12.** Expression of Pannexin1 (Panx1) in developing meninges and the dorsal root ganglia (DRG). Utilizing Panx1 (green) and PGP9.5 (red) staining techniques, the thoracic segments of the SC of 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> week-old human conceptuses were analyzed. DRG and meninges are presented on photomicrographs. Objective magnification – 40x (scale bar= 20µm). DAPI - 4',6-Diamidino-2-phenylindole.

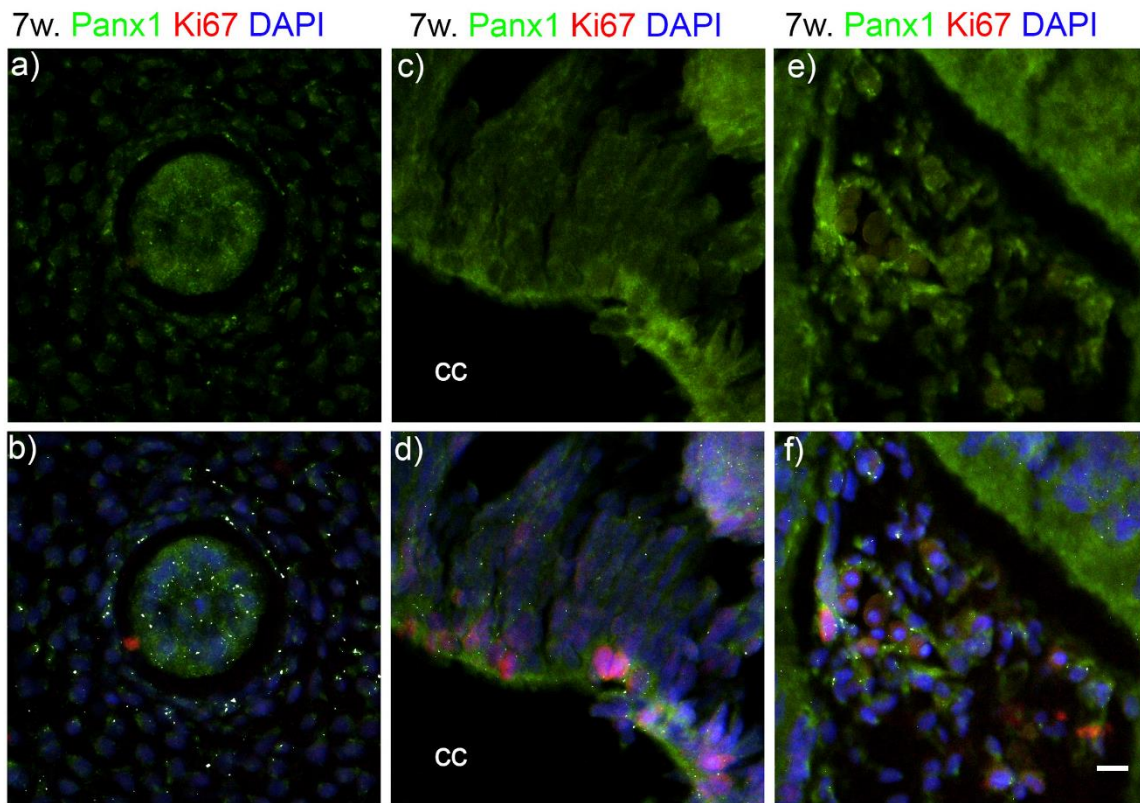


**Figure 13.** Expression of Pannexin1 (Panx1) in notochord and sympathetic ganglia of the human conceptuses. Panx1 (green) and PGP9.5 (red) staining is presented in thoracic segments of the SC of 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> week-old human conceptuses. Photomicrographs illustrate the paravertebral (PAVG), prevertebral PRVG) and intramural (IMG) ganglia. Objective magnification – 40x (scale bar= 20µm). DAPI - 4',6-Diamidino-2-phenylindole.





**Figure 14.** Co-localization of Pannexin1 (Panx1) with (a, b) neurofilament200kD (NF200) in the basal plate (BP) and with developing blood vessels using the endothelial marker CD31 (c, d) and the expression of Panx1 in meninges (e, f) of the human conceptuses. (b) Panx1 (green) and NF200 (red) staining are demonstrated in thoracic segments of the spinal cord (SC) of 7<sup>th</sup> week-old human conceptus. (d, f) Furthermore, Panx1 (green) and CD31 (red) staining were carried out on thoracic segments of SC of 7<sup>th</sup> week old human. In the photomicrographs presents BP, developing blood vessels in the SC and meninges. Objective magnification – 40x (scale bar= 20 $\mu$ m). DAPI - 4',6-Diamidino-2-phenylindole.



**Figure 15.** Expression of Pannexin1 (Pax1) with co-localization with Ki67 in the notochord, INL, and meninges of the human conceptuses. Segments of the thoracic region of the SC of the 7<sup>th</sup> week-old human conceptus were stained for Pax1 (green) and Ki67 (red). Notochord (a, b), INL and central canal (cc) (c, d), and meninges (e, f) are presented on microscopic images. Objective magnification – 40x (scale bar= 20 $\mu$ m). DAPI - 4',6-Diamidino-2-phenylindole.

## **5. DISCUSSION**

Panx are transmembrane proteins, which form plasma communication pores across the plasma membrane. This enables intercellular metabolic communication between neighboring cells and furthermore coordinated signaling of pathways, including ion or ATP transfer. They seem to contribute to the regulation of glial proliferation, the process of myelination, and differentiation of neuronal stem cells (43,82–84). Panx respond either physiologically to external stimuli, like mechanical stress, prolonged depolarization, and electrolyte changes by increasing molecular trafficking or via more complex intracellular signaling pathways involving Rho kinase activities and calcium ions as a second messenger (52,64). In pathologic conditions like oxygen or glucose deprivation, e.g., in inflammation or seizures, the response of molecule trafficking is altered (58–62). Due to the wide distribution of Panx in the human body and the opportunity to transfer different ions or molecules across the cell membrane, it potentially influences different cascades and furthermore pathologies (43,52,63,64).

The role of Panx and purinergic signaling in the development of the NS has been studied (41,82,85). The expression of Panx1 mRNA was described in developing mouse NS (41). However, there is no data about their expression in developing NS of the human embryo.

Consequently, this descriptive study was undertaken to investigate the expression of Panx1 in SC, ganglia, and surrounding structures of the human embryo during the early phase of development. We hypothesized that their expression might suggest their possible role in embryonal and early fetal development. We detected expression of Panx1 in all areas of interest in the SC, including RP, FP, AP, and BP. In addition, we also found expressed Panx in DRG, sg, NC, and the meninges.

Expression of Panx1 was prominent in the ne of the INL. This finding agrees with previous data that have demonstrated that purinergic signaling has a critical role in the controlling of proliferation of neuronal progenitor cell. Panx channels have been implicated in the mediation of ATP release from neural progenitor cells, as well as nearby neurons and glia cells (82). Released ATP elicits autocrine and paracrine signaling pathways through a diverse range of purinergic receptors on neural progenitor cells (85). In support, we also found co-localization of Panx1 in Ki67 immunoreactive cells in contact with cc of the INL. These ne cells are a source of neuroblasts, which arise exclusively by the division of ne cells (86). Cell proliferation in early embryonic development serves the expansion of neural tissue, during the thickening of the newly formed neural tube, and the proliferation of the cells of the ventricular zone (85). Nucleotides (including ATP, which is released by Panx-channels) activate P2X1 and P2Y2 receptors causing cell proliferation in vitro (82,85).

We found a strong expression of Panx1 in DRG, where it was the strongest in comparison to other neural structures studied and increased in 8<sup>th</sup> and 10<sup>th</sup> DW expression in comparison to 7<sup>th</sup> DW. Here we also found the co-localization of Panx1 with PGP9.5 immunoreactivity. The expression of Panx1 in the 10<sup>th</sup> DW was also found in prevertebral (PRVG) and intramural (IMG) ganglia, where it was co-localized with the PGP9.5 staining. The sensory DRG cells originate from neural crest cells (26). The central processes of these cells form the dorsal sensory root of the future spinal nerve. The spinal nerve is formed by the combination of the peripheral processes and the ventral motor root, which then extend and connect with sensory receptor organs (86). Panx1 transmembrane proteins in the SC and DRG neurons have an important role for transmission of neuropathic pain (87). The strong Panx1 expression in DRG, along with the sympathetic ganglia, may be associated with the previously mentioned function of Panxs proteins in inhibiting neurite outgrowth, as indicated by our findings (88). Furthermore, Panx1 may also be involved in the migration of neural crest cells, akin to its role in cortical neuron migration. While the literature has documented the involvement of Panx1 in facilitating neural progenitor cell proliferation, promoting cell migration and inhibiting neurite outgrowth (82,87,88).

In addition, an expression of Panx1 that we found in the IML/ ML (mantle layer) of developing SC, as well as in developing ganglia, might be also related to their role in gliogenesis. Namely, a role for Panx expression in gliogenesis was suggested earlier by Ray et al. Since it was shown that Panx1 expression in neural crest-like cells is being downregulated as cells adopt a Schwann cell-like glial lineage (82,83). In agreement with that study, we did not find Panx1 in the marginal layer of the SC, in which only neuronal fibers and glia exist. However, it should be cautious with that conclusion, since the developmental stages that we studied are too early for excessive gliogenesis and myelination and we found only rare expression of NF200, a marker for myelinated neurons and nerve fibers (89).

Panax1 expression in the meninges of developing human conceptuses was strong and denser in comparison to all studied neural structures, including the DRG. These findings point out the meaning in cellular paracrine communication in development of human meninges.

In general, we found co-localization of Panx1 with PGP9.5 in all neural structures, which was more pronounced in the later stages of development.

Furthermore, a strong expression of Panx1 in cells of the NC was observed, where it exhibits co-localization with a strong expression of PGP9.5. Considering the crucial role of the NC in initiation of the neural cell differentiation of floor plate cells, motor neurons, and ventral interneurons (43,45,90). It could be assumed that paracrine communication of NC cells is important for the regulation of signaling and specialization of ventral SC cell subtypes (43).

In addition, we found Panx1 immunoreactivity in developing blood vessels in the SC and meninges. Some of the Panx1 immunoreactive puncta co-localized with a marker for the endothelial cells – CD31, while we also noticed Panx1 positive puncta in CD31-non-reactive cells of the vascular wall. In addition, we found occasional co-localization of Panx1 with a marker of proliferation – Ki67 in developing blood vessels. These findings suggest that Panx1 plays a role in the establishment of SC blood circulation during early intrauterine development.

Potential limitations of this study were the limited sample size of specimen investigates (n=3), and the collection of samples being from a single institution. Due to the low number of specimens, it could be argued that the samples are not generalizable at a larger scale. We would therefore encourage further research to support our findings in the future. Furthermore, all specimens were collected at the University Hospital of Split, representing a fraction of total population.

In conclusion, for the first time the spatiotemporal expression pattern of Panx1 during early intrauterine development of human SC and ganglia was characterized. The presence of Panx1 immunoreactivity supports its potential involvement in the processes of neuronal formation, and migration within the SC and ganglia during development. These data might help us to understand potential pathology during development of SC and peripheral NS.

## **6. CONCLUSION**

In conclusion, our study indicates that Panx1 is expressed in various regions of interest during the embryonal development of the human spinal cord (SC) and surrounding structures, including the roof plate, floor plate, alar plate, basal plate, inner and marginal layer, as well as the dorsal root ganglia, notochord, para, and prevertebral ganglia. Panx1 is particularly prominent in the dorsal root ganglia (DRG), suggesting its involvement in processes such as proliferation, migration, and neurite outgrowth inhibition. The expression of Panx1 was prominent in the ne of the INL, which agrees with the major role of purinergic signaling in the modulation of neuronal progenitor cell proliferation.

The expression of Panx1 shows changes in structural arrangement during different stages of intrauterine development during the 7<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> week. In comparison to the 8<sup>th</sup> DW, Panx1 was more concentrated close to the cc in the 10<sup>th</sup> DW, which indicates a role in early embryonal development of human SC.

Additionally, we found rare expression of NF200 in BP of SC and rare co-localization with Panx1. Furthermore, Panx1 is expressed in developing vascular structures and meninges, indicating its role in establishing blood circulation and potential contribution to neuronal progenitor cell proliferation. The co-localization of Ki67 with Panx1 in meninges, notochord, INL, and developing blood vessels indicates the potential role of Panx in cell proliferation.

Overall, these findings contribute to our understanding of the involvement of Panx1 in neuronal development and potential pathological implications in the spinal cord and peripheral nervous system during early intrauterine development. These data might help us to understand potential pathology during the development of SC and peripheral NS.



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## **8. SUMMARY**

**Objectives:** The objective of this study was to explore the expression of Pannexin 1 (Panx1) in the spinal cord (SC) and ganglia of human conceptus during early phase of intrauterine development.

**Material and methods:** The human conceptus tissue was collected from the Department of Gynecology and Obstetrics and the Department of Pathology after tubal pregnancies or after spontaneous abortions. The immunohistochemistry procedure was used to visualize the targeted proteins. The data acquisition and analysis were performed by immunofluorescence microscopy and captured by a cooled digital camera. The further processing of the pictures was done by Image J and Photoshop Adobe.

**Results:** Panx1 is expressed in all areas of interest during the embryonal development of human spinal cord and surrounding structures and can relate to presence of Panx1 in neuronal tissue, as it was partially co-localized with PGP9.5. There is a change in structural arrangement during the different ages of intrauterine development. Furthermore, the Panx1 expression was also found in ganglia, including dorsal root ganglia (DRG), intramural ganglia, pre-, and paravertebral ganglia. Nevertheless, Panx1 expression was most prominent in DRG, in comparison to the other neural structures studied and were stronger in more developed specimens. Additionally, we investigated the co-localization with NF200, CD31 and Ki67 in the 7<sup>th</sup> DW. We found rare expression of NF200 in BP of SC and rare co-localization with Panx1. The developing vascular structures of the spinal cord and developing meninges express Panx1 immunoreactivity. There was a complex pattern, showing the expression of Panx1 in endothelial cells and CD31-non-reactive cells in vascular wall. The co-localization of Ki67 with Panx1 was demonstrated in meninges, notochord, inner layer, and developing blood vessels.

**Conclusion:** The spatiotemporal expression pattern of Panx1 during early intrauterine development of human SC and ganglia was characterized. The presence of Panx1 immunoreactivity supports its possible role in processes of neuronal formation, and migration, in the SC and ganglia during development. These data might help us to understand potential pathology during development of SC and peripheral nervous system.

## **9. CROATIAN SUMMARY**

**Naslov:** Izražaj paneksina 1 u kralježničnoj moždini i spinalnim ganglijima čovjeka tijekom razvoja

**Ciljevi:** Cilj ove studije bio je istražiti izražaj paneksina 1 (Panx1) u kralježničnoj moždini i ganglijima ljudskih zametaka tijekom rane faze intrauterinog razvoja.

**Materijali i metode:** Tkivo ljudskih konceptusa prikupljeno je iz Zavoda za ginekologiju i opstetriciju i Zavodu za patologiju nakon spontanih pobačaja ili nakon tubarne trudnoće. Za vizualizaciju ciljanih proteina korišten je imunohistokemijski postupak. Prikupljanje i analiza podataka provedeni su imunofluorescencijskom mikroskopijom, a snimanje obavljeno hlađenom digitalnom kamerom. Daljnja obrada slika izvršena je korištenjem Image J i Adobe Photoshop softvera.

**Rezultati:** Panx1 je bio izražen u svim područjima od interesa tijekom embrionalnog razvoja ljudske kralježnične moždine i okolnih struktura, što se može povezati s prisutnošću Panx1 u živčanom tkivu, budući da je bio djelomično ko-lokaliziran s PGP9.5. Postoji promjena u strukturnom rasporedu tijekom različitih razdoblja intrauterinog razvoja. Nadalje, izražaj Panx1 također je pronađena u ganglijima, uključujući spinalne ganglije, intramuralne ganglije, pre- i paravertebralne ganglije. Unatoč tome, ekspresija Panx1 bila je najizraženija u spinalnom gangliju, u usporedbi s drugim proučavanim neuralnim strukturama i bila je jača u razvijenijih uzoraka. Osim toga, istražili smo ko-lokalizaciju s NF200, CD31 i Ki67 u 7. DW. Vrlo iznimno pronašli smo izražaj NF200 u bazalnoj ploči (BP) kralježnične moždine, koji je samo rijetko ko-lokalizirao s Panx1. Vaskularne strukture leđne moždine u razvoju i moždane ovojnice u razvoju izražavaju Panx1 imunoreaktivnost. Postojao je složeni uzorak, koji pokazuje ekspresiju Panx1 u endotelnim stanicama i CD31-nereaktivnim stanicama u vaskularnoj stijenci. Ko-lokalizacija Ki67 s Panx1 dokazana je u moždanim ovojnicama, notokordu, unutarnjem sloju i krvnim žilama u razvoju.

**Zaključci:** Okarakteriziran je prostorno-vremenski izražaj Panx1 tijekom ranog intrauterinog razvoja kralježnične moždine i ganglija čovjeka. Prisutnost Panx1 imunoreaktivnosti ukazuje na njegovu moguću ulogu u procesima formiranja i migracije neurona u kralježničnoj moždini i ganglijima tijekom razvoja. Ovi podaci mogu pomoći u razumijevanju potencijalne patologije tijekom razvoja SC i perifernog živčanog sustava

