G-010

Genes related to labor pain during parturition in dogs

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1. Introduction

The extensive examination of hormonal and functional changes during parturition in dogs has produced invaluable insights [1]. During the final phase of pregnancy, various hormonal and physiological factors that produce oxytocin initiate the complex process of labor [2]. Pain management during labor is essential and demands immediate attention. The primary aim of providing pain relief during labor is to ensure that the individual giving birth experiences minimal discomfort, potential risks and adverse effects to actively participate in the childbirth process and the newborn [3]. Since pain in labor has not been adequately studied in dogs, these findings emphasize the importance of conducting a thorough reevaluation and gaining a comprehensive understanding of labor pain.

2. Background

According to Martínez-Burnes, et al. [3], labor is due to uterine contraction, cervical ripening, dilation and distension, and compression of adjacent structures. There are three stages in labor: I, II, and III. In Stage I, the structures are influenced by the sympathetic nervous system while the parasympathetic nerve system plays a predominant role along with visceral components. When Stage II begins, pain impulses are conducted through the spinal (A-δ and C fibers) during transmission. In the modulation process, the cerebral cortex receives the pain signal from the contractions, and the neural pathways involving spinal cord segment S2–S4 start acting. Contractions and pain begin, leading to delivery and active straining, or a frequent effort to push out the puppies. Additionally, based on Figure 1, when the Pudendal Block at the S2 and S4 levels transmits signals, the pain and pressure on the perineum and adjacent pelvic structures around the vaginal area become somatic (acute and localized). This transformation is attributed to the expansion of the pelvic floor and perineum, involving the nerve fibers that carry sensory information towards the spinal cord and brain. Lastly, in Stage III, the placenta will be removed and the new mom will start to nurse the newborn pups. (See Figure 1)

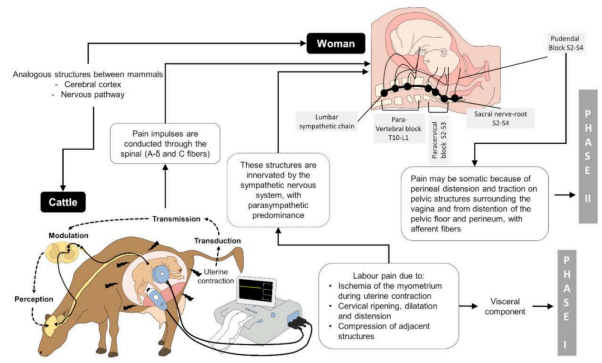

Figure 1. Labor pain leading to phases I and II

Source: [3] (Fig. 1). Reproduced based on Creative Commons Attribution (CC BY) of the original manuscript.

3. Aim

Reviewing articles from PubMed based on Martínez-Burnes, et al. [3] (Table 4, Hypothalamic Pituitary Adrenocortical System) that listed hormones and substances associated with human and veterinary labor pain, found the need to identify genes relative to labor pain in dogs. It is considered that labor pain involves genetic components [4].

Women with the rare Mendelian disorder congenital insensitivity to pain (CIP) due to mutations in *SCN9A* (voltage-gated sodium channel alpha subunit 9 NaV1.7) do not report labor pain or require analgesics during labor [5,6]. Similarly, a homozygous missense variant in *SCN9A*, XM_038584713.1:c.2761C>T or XP_038440641.1:(p.Arg921Cys) in dogs was identified [7]. It is thought that the *SCN9A* gene is predominantly expressed in sensory neurons and plays a role in the generation and conduction of action potentials. In a separate study, Lee et al. (2020) identified a rare allele, c.1255G>A, in the voltage-gated potassium channel (KV) modifier *KCNG4* (KV6.4) SNP rs140124801 in humans. The p.(Val419Met) variant was observed to be over-represented, which suggests that this rare KV6.4-Met419 variant may have an impact on sensory neuron excitability and could potentially reveal a mechanism in uterine nociception. Therefore, we aim to investigate *KCNG4* and *SCN9A* in relation to labor pain in dogs, and the differences in gene expression that are associated with labor pain.

4. Method/Results

KCNG4 and *SCN9A*, humans and dogs are in different speciation nodes on the genetic tree. *KCNG4* has a separation at the placental node into primates for humans and placentals for dogs; *SCN9A* has a separation at the placental node into primates and rodents for humans, and laurasiatherian mammals for dogs [8]. *KCNG4* is most highly expressed in amygdala (50.97), substantia nigra, midbrain, and hypothalamus in humans, and hypothalamus (48.17), saliva-secreting gland, oral gland, and brain gray matter in dogs [9]. *SCN9A* is most highly expressed in SCN9A are dorsal root ganglion (88.05), trigeminal ganglion, colonic epithelium, and epithelium of large intestine in humans, and testis (73.52), hypothalamus, spleen, and pancreas in dogs [9].

4.1 Protein BLAST

For evaluating equivalence and differentiating protein sequences in humans and dogs, protein BLAST was performed for *KCNG4* and *SCN9A* in Homo sapiens against Canis lupus familiaris. In general, the conditions for being recognized as a useful gene candidate (homolog) are when the E-value is less than 1e-10 and when identities and coverage (hit region sequence length divided by total query sequence length) are 30% or more. Based on Table 1, the highest percentage of query cover is 98% and the percentage identical is 80.19 (potassium voltage-gated channel subfamily G member 4 isoform X2). Based on Table 2, the highest percentage of query cover is 99% and the percentage identical is 94.42 (sodium channel protein type 9 subunit alpha isoform X1). Therefore, KCNG4 and SCN9A genes are homologous in humans and dogs.

Table 1. PBLAST of *KCNG4* alignment description (*KCNG4* in Homo sapiens against Canis lupus familiaris)

Description	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
subfamily G member 4 isoform X2 [Canis lupus familiaris]	819	819	98%	0	80.19	506	XP_038522815.1
subfamily G member 4 isoform X1 [Canis lupus familiaris]	819	819	99%	0	80.19	551	XP_038522814.1
subfamily G member 4 isoform X6 [Canis lupus familiaris]	818	818	98%	0	79.62	506	XP_038394074.1
subfamily G member 4 isoform X1 [Canis lupus familiaris]	818	818	99%	0	79.62	551	XP_038381707.1
subfamily G member 1 [Canis lupus familiaris]	547	547	80%	0	62.91	519	XP_038290028.1

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Note: Human accession number: NP_758857.1; database: Reference proteins (RefSeq protein); algorithm: blastp (protein-protein BLAST); parameters (Max target sequences = 100, expect threshold = 1e-6, word size = 5, max matches in a query range = 0, matrix: BLOSUM62, gap costs = Existence: 11 Extension: 1, compositional adjustments: Conditional compositional score matrix adjustment) Analysis performed on NCBI Blast (version BLAST+ 2.15.0) [10,11] Accessed 07/09/2024.

Table 2. PBLAST of *SCN9A* alignment descriptions (*SCN9A* in Homo sapiens against Canis lupus familiaris)

Description	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
sodium channel protein type 9 subunit alpha isoform X1 [Canis lupus familiaris]	3666	3666	99%	0	94.42	1986	XP_038302871.1
sodium channel protein type 9 subunit alpha isoform X2 [Canis lupus familiaris]	3639	3639	99%	0	94.18	1975	XP_038302872.1
sodium channel protein type 9 subunit alpha isoform X4 [Canis lupus familiaris]	3393	3393	92%	0	94.08	1875	XP_022270552.1
sodium channel protein type 9 subunit alpha isoform X3 [Canis lupus familiaris]	3204	3287	93%	0	93.86	1738	XP_022270551.1
sodium channel protein type 2 subunit alpha isoform X1 [Canis lupus familiaris]	3094	3094	99%	0	81.72	2006	XP_013966299.1

Note: Human accession number: NP_001352465.1; database: Reference proteins (RefSeq protein); algorithm: blastp (protein-protein BLAST); parameters (Max target sequences = 100, expect threshold = 1e-6, word size = 5, max matches in a query range = 0, matrix: BLOSUM62, gap costs = Existence: 11 Extension: 1, compositional adjustments: Conditional compositional score matrix adjustment). Analysis performed on NCBI Blast (version BLAST+ 2.15.0) [10,11] Accessed 07/09/2024.

4.2 RNA-seq analysis

In dogs, pain can be a factor of dystocia [12]. Dystocia is an inability to expel the fetus unassisted through the birth canal caused mainly by uterine inertia and other reasons, such as older age of bitch, small litter size, pregnancies with a large fetus, breed, hypocalcemia, and obstruction. Dystocia can be associated with a higher incidence of congenital conditions [2,3]. Certain dog breeds prone to dystocia were identified [13,14,15]. Based on these observations, 14 female dogs were extracted as Dystocia (D) group from the bioproject number PRJNA188158 in the following breeds: Boxer, Cairn Terrier, Chihuahua, Continental Bulldog, French Bulldog, Golden Retriever, Labrador Retriever, Maltese, Maremma Sheepdog, Miniature Bull Terrier, Old German Shepherd, Pomeranian, Shetland Sheepdog, and Toy Poodles. Other female dogs were in Non-Dystocia (ND) group. RNA-seq aimed at understanding how the two genes affect dystocia in dogs in the 28 dogs (1:1 for the D group and the ND group), comparing KCNG4 and SCN9A expression values. Based on the results in Figure 2, the heatmap of expression values had no significance for *KCNG4* and *SCN9A*. Functional enrichment analysis was performed with the goseq R package [19]. This analysis aims to compare the percentages of significant DE genes annotated to each functional category against whole genes. 100 genes with the lower p-value in the Differential Expression analysis were taken as input for this enrichment analysis. 19 molecular pathways (namely, folate biosynthesis pathway) were statistically significant with a p-value cutoff less than 0.01. These pathways are action series within cells between molecules leading to cellular functions such as lower risk of anovulation and ovulatory infertility.

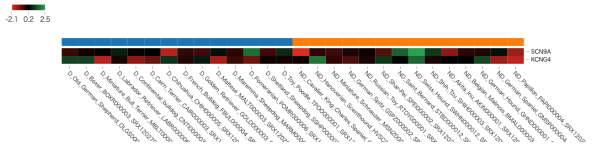

Figure 2 Heatmap of *KCNG4* and *SCN9A*

Note: Heatmap of expression values (normalized as TPMs) of selected genes in each sample. Samples were quantified with Salmon algorithm [16] setting following parameters: paired-end, default parameters. Differential Expression analysis was performed with DESeq2 [17]. Protocol executed by RaNA-seq [18] with following parameters: test: Wald; fitType: parametric. It found 0 significant genes for a cutoff of 0.05. (Note cited from reports generated by RaNA-seq)

5. Conclusion

In conclusion, understanding the labor process in dogs is crucial for ensuring the well-being of both mother and puppies. Recent research has discovered genes related to pain sensitivity in dogs and humans. Specifically, high expression of *KCNG4* and *SCN9A* genes differs in humans and dogs, shedding light on potential similarities in pain perception. Additionally, the study of dystocia, a potential cause of pain in dogs, was conducted across different dog breeds. The results of RNA-seq analysis showed no significant gene expression differences in association with *KCNG4* and *SCN9A*. However, 19 molecular pathways were statistically significant. This study may open the door for future research aimed at understanding how gene mutations affect pain sensitivity in dogs during labor. This line of research has the potential to impact the well-being of both dogs and offspring.

Keywords: *KCNG4, SCN9A*, labor pain, dog, dystocia

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