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Editorial

Polymorphic variants of the P2X7 receptor in the domestic dog

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Due to distinctive features including genome architecture, population diversity, breed structure and breed-specific disorders, the domestic dog (*Canis familiaris*) is becoming an important animal to study the genetics of morphology, behaviour and disease susceptibility in mammals [1]. Further, with around 360 of the some 450 diseases in dogs similar to that of human diseases, dogs provide a valuable model to identify the genetic causes of many diseases in humans [1]. Thus, one of the goals in canine biology is to better define the genetic and phenotypic diversity that exists between the 400 dog breeds [2]. Therefore, our groups have begun to explore the diversity in the gene encoding the canine P2X7 receptor (P2RX7) and its impact on receptor function in this species [3], as well as the role of this receptor in dogs [4-8].

The P2X7 receptor is a trimeric ligand-gated cation channel, which is activated by extracellular adenosine triphosphate (ATP) and important in the release of pro-inflammatory mediators such as prostaglandins and cytokines of the interleukin-1 family [9]. As such, P2X7 has established roles in inflammation, inflammatory disorders and pain [10]. In humans, the gene encoding P2X7 is highly polymorphic with at least 15 non-synonymous (missense) single nucleotide polymorphisms (SNPs) encoding for receptors with either a loss or gain of function [11]. Of note, a number of these SNPs are associated with either increased susceptibility or resistance to behavioural disorders, bone disease, infection, inflammatory disorders or pain [11].

The canine P2RX7 gene is located on chromosome 26, and

like its human orthologue, contains 13 exons [12]. Also similar to humans, the canine P2RX7 gene encodes a receptor subunit 595 amino acid residues in length [3,13] with intracellular N- and C-termini, two trans-membrane domains and an extracellular loop containing ATP binding sites [14]. In contrast to humans, the canine P2X7 subunit contains an additional asparagine residue in the extracellular loop between positions 281 and 282, and lacks one residue in the C-terminus [3,13] corresponding to Thr541 in the human and mouse P2X7 subunits or Iso541 in the rat P2X7 subunit [14] (Figure 1). The functional impacts of these two amino acid differences have not been formally investigated. The additional asparagine residue lies within a key structural region of the extracellular loop, which interacts with the ATP-binding site and undergoes conformational changes during receptor activation [14]. However canine P2X7 is functional in dogs [4,6] and displays pharmacological properties similar to that of the human orthologue [8,13]. Thus, current evidence suggests that this additional asparagine residue has little impact on receptor function. In contrast, the absence of the isoleucine corresponding to residue 541 in rat receptors most likely prevents the calcium-dependent facilitation of canine P2X7-mediated currents. In rat P2X7, a 17 amino acid sequence commencing at Iso541 forms a calmodulin-binding motif, responsible for the calcium-dependent facilitation of rat P2X7-mediated currents [15]. Importantly this isoleucine residue is necessary for both the calcium-dependent facilitation of rat P2X7-induced currents and the binding of calmodulin to this receptor [15]. In contrast, neither of these events occur with human P2X7, which contains Thr541 [16]. Collec-

tively, this indicates that canine P2X7, which lacks Iso541, is also unlikely to undergo significant calcium-dependent facilitation and to bind calmodulin. The physiological relevance of the presence or absence of the calmodulin-binding motif in mammalian P2X7 remains to be established.

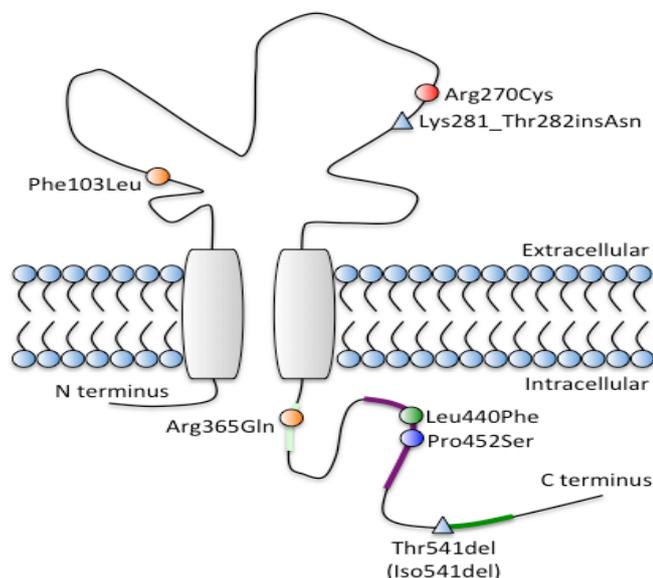


Figure 1. The canine P2X7 receptor subunit. Triangles represent additional (ins) or absent (del) amino acid residues in canine P2X7 compared to human (or rat) P2X7. Circles represent missense single nucleotide polymorphisms in canine P2X7 resulting in either loss of function (red), possible gain (green) or partial loss (orange) of function, or neutral effect on function (blue). Coloured lines represent a calcium-independent facilitation current motif (lime), a pore sensibility and activation domain (purple), or a calmodulin binding site (green) as previously described [20].

Recently our group cloned English Springer Spaniel P2X7, and has assessed the functional impact of P2X7 variation in a random sample of the canine population, which included 23 different breeds and a variety of cross breeds [3]. As a result of this study, we identified three novel missense SNPs (Arg270Cys, Arg365Gln and Leu440Phe) and confirmed the presence of two previously reported missense SNPs (Phe103Leu, rs23314713; Pro452Ser, rs23315462) (Figure 1). Of these five SNPs, the Arg270Cys SNP resulted in an almost complete loss of receptor function despite normal surface expression in cells. This SNP was found only in heterozygous dosage with an allele frequency of 0.01, and appeared to be associated with the Pro452Ser SNP and with Cocker Spaniels. Structural modeling of human P2X7 places Arg270 in the lower body of the extracellular loop and away from the ATP-binding site [14], but how the Arg270Cys SNP impairs receptor function remains unknown. A partial loss of function SNP (Arg270His, rs7958311) is present in human P2X7 [17], indicating an important role for Arg270 in functional P2X7 receptors. Of clinical relevance, the Arg270His SNP is associated with reduced post-mastectomy pain in breast cancer patients, as well as with reduced pain in patients with osteoarthritis [18]. Thus, the Arg270Cys SNP may also be associated with reduced pain sensitivity in Cocker Spaniels or

other breeds possibly encoding this SNP.

Of the other canine P2X7 SNPs identified, the Phe103Leu and Arg365Gln SNPs may be partial loss-of-function mutations. However the current body of evidence is limited as total P2X7 expression was also lower in the heterologous cell assay used [3]. This may reflect either differences in transfection efficiency or real differences in protein expression. The Phe103Leu SNP was frequently observed in dogs with an allele frequency of 0.38 [3], and Phe103 is conserved in human and rodent P2X7 suggesting that this residue is important in P2X7 function. Notably the first cloned canine P2X7 (which contains Leu103) displays an agonist profile [13] different to that of the cloned English Springer Spaniel P2X7 (which contains Phe103) [3,8]. In these latter studies ATP was shown to be a partial agonist of English Springer Spaniel P2X7 compared to 2'(3')-O-(4-benzoyl)benzoyl ATP (BzATP) [3,8], while in the former study, BzATP was a partial agonist compared to ATP [13]. Given that residue 103 is in the extracellular loop of the P2X7 subunit, which contains the ATP binding sites, it is possible that this residue contributes to ATP and BzATP sensitivity, and that the Phe103Leu SNP may modulate ATP binding *in vivo*. In contrast to the high frequency of the Phe103Leu SNP, the Arg365Gln SNP had an allele frequency of 0.03, was only found in heterozygous dosage, and appeared to be associated Labrador Retrievers [3]. Arg365, which is conserved in the equivalent positions in human and rodent P2X7, lies within a motif that contributes to calcium-independent facilitation of P2X7-mediated currents [16], however the contribution of Arg365 to this action remains unknown.

Evidence indicated the Leu440Phe SNP may cause a partial gain of P2X7 function, but this SNP was only observed in the cloned English Springer Spaniel P2X7 receptor [3]. Thus, it remains uncertain if this variant is a mutation arising from the cloning process, or a SNP present in this or possibly some other breeds. However Leu440 is conserved in equivalent positions in human and rodent P2X7, and resides within a regulatory region of P2X7 [19], termed the pore sensibility and activation domain by others [20]. Thus, it remains possible that the residue at position 440 may partly modulate or regulate P2X7 function.

In contrast to the other canine P2X7 SNPs, the Pro452Ser SNP had a neutral effect on P2X7 function [3]. This SNP had an allele frequency of 0.39 in our cohort and also resides within the pore sensibility and activation domain. Further, this SNP is (somewhat serendipitously) located at the equivalent position of a partial loss-of-function SNP (Pro451Leu) in murine P2X7 [21]. The different impact of these SNPs on canine and murine P2X7 function most likely reflects the differences in physical properties between serine and leucine residues.

Recent findings therefore demonstrate the presence of at least five missense SNPs in the canine P2RX7 gene, four of which may alter function. Of these SNPs, the Arg270Cys SNP is potentially the most important. This SNP caused an almost complete loss of receptor function and a similar SNP (Ar-

g270His) at the equivalent position in human P2X7 is associated with reduced pain sensitivity. Whether the Arg270Cys SNP has an important pathophysiological role in dogs, such as reduced pain sensitivity, remains to be established. Further, the other SNPs in the canine P2RX7 gene require further investigation before a conclusion can be made about their impact on receptor function and any potential association with disorders in dogs. Nevertheless these recent findings provide a basis from which further investigations into the role of P2X7 and its polymorphic variants in canine biology can be made.

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