R270C polymorphism leads to loss of function of the canine P2X7 receptor

Mari Spildrejorde,1,2 Rachael Bartlett,1,2 Leanne Stokes,3,4 Iman Jalilian,1,2 Michelle Peranec,1,2 Vanessa Suyter,1,2 Belinda L. Curtis,5 Kristen K. Skarratt,4 Amanda Skora,1,2 Tahani Bakhsh,1,2 Aine Seavers,6 Jason D. McArthur,1,2 Mark Dowton,1,2 and Ronald Suyter1,2

1School of Biological Sciences, University of Wollongong, Wollongong, Australia; 2Illawarra Health and Medical Research Institute, Wollongong, Australia; 3Health Innovations Research Institute, School of Medical Sciences, RMIT University, Bundoora, Australia; 4Sydney Medical School Nepean, University of Sydney, Nepean Hospital, Penrith, Australia; 5Albion Park Veterinary Hospital, Albion Park, Australia; and 6Oak Flats Veterinary Clinic, Oak Flats, Australia

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Some 40 years ago, Parker and Snow (37) demonstrated that the P2X7 receptor is a trimeric ligand-gated ion channel present on various cell types, with high amounts present on the cell-surface of monocytes (34). Upon activation by its natural ligand ATP, the P2X7 channel rapidly opens and dilates to form a pore to allow the flux of organic cations and anions including fluorescent dyes (11). P2X7 activation also results in a variety of downstream events including the release of proinflammatory mediators such as IL-1β, PGE2, and CXC chemoattractant ligand 16 (4, 38, 40). Conversely in the absence of ATP, P2X7 may function as a scavenger receptor to facilitate the phagocytosis of bacteria and apoptotic cells (23). As a result of these and other observations, P2X7 is attracting considerable interest as a therapeutic target in various immune-mediated disorders and pain (36). P2X7 activation is also important in physiological systems other than the immune system such as bone homeostasis (52) and carbohydrate and lipid metabolism (2, 6).

The human, canine, rhesus macaque, rat, and murine P2X7 subunits have been cloned (10, 12, 41, 42, 51). Each of these mammalian P2X7 subunits is 595 amino acids in length and comprise intracellular NH2 and COOH termini, two transmembrane domains, and a large extracellular loop. The P2X7 subunit is encoded by the P2RX7 gene, which comprises 13 exons and is highly polymorphic in humans, resulting in at least 12 loss-of-function and three gain-of-function receptor variants (45). A loss-of-function SNP, P451L (rs48804829), has also been described in mouse P2X7 (1), but descriptions of SNPs in the P2RX7 gene from other species are limited.

Some 40 years ago, Parker and Snow (37) demonstrated that extracellular ATP could increase the membrane permeability of Beagle erythrocytes to cations. Nearly 30 years later, our group using erythrocytes from English springer spaniels, identified that this effect was mediated by P2X7 (44), providing the first direct evidence that functional P2X7 receptors are present within dogs. Subsequently our group demonstrated that P2X7 was also present on monocytes, B cells, and T cells from this species. P2X7 is also highly polymorphic between dogs and this has been used to help elucidate the mechanisms involved in similar disorders within humans. One study that supports this approach has been a genome-wide association analysis, which identified a SNP within the gene coding canine Cu/Zn SOD in breeds susceptible to degenerative myelopathy (3), a disease similar to amyotrophic lateral sclerosis in humans, which is also associated with SNPs in the gene coding human Cu/Zn SOD (43). This approach, however, is currently restricted. First, presumably not all SNPs have been identified in the some 350 breeds that exist. Second, as for humans the molecular and cellular effects of most canine SNPs identified to date are yet to be fully elucidated. Thus, there is a need to identify novel SNPs within the dog and to define how these and existing SNPs influence canine biology.

The P2X7 receptor is a trimeric ligand-gated ion channel present on various cell types, with high amounts present on the cell-surface of monocytes (34). Upon activation by its natural ligand ATP, the P2X7 channel rapidly opens and dilates to form a pore to allow the flux of organic cations and anions including fluorescent dyes (11). P2X7 activation also results in a variety of downstream events including the release of proinflammatory mediators such as IL-1β, PGE2, and CXC chemoattractant ligand 16 (4, 38, 40). Conversely in the absence of ATP, P2X7 may function as a scavenger receptor to facilitate the phagocytosis of bacteria and apoptotic cells (23). As a result of these and other observations, P2X7 is attracting considerable interest as a therapeutic target in various immune-mediated disorders and pain (36). P2X7 activation is also important in physiological systems other than the immune system such as bone homeostasis (52) and carbohydrate and lipid metabolism (2, 6).

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LOSS-OF-FUNCTION POLYMORPHISM OF CANINE P2X7

Materials. Ficoll-Paque PLUS was from GE Healthcare Bio- 
sciences (Uppsala, Sweden). YO-PRO-1 iodide solution, ExoSAP-IT,  
DMEM/F12 medium, penicillin-streptomycin, OPTI-MEM Reduced 
Serum Medium, and Lipofectamine 2000 were from Invitrogen (Grand 
Island, NY). ATP, 2′(3′)-O-(4-benzoyl)benzoyl ATP (BzATP), adenosine 
5′-O-(3-thio)triphosphate (ATP\(_3\)) was from Alexis Biochemicals (Lausen, Switzerland). 
AZ11645373 were from Tocris Bioscience (Ellisville, MO). KN-62 
from Alomone Labs (Jerusalem, Israel). A438079, AZ10606120, and 
Ethidium bromide was from Amresco (Solon, OH). Rabbit anti-mouse 
Not Fidelity DNA polymerase was from Stratagene (La Jolla, CA). 
from Sigma Life Science (Castle Hill, Australia). PfuUltra High- 
polymerase was from Bioline (London, UK). Primers for cloning were 
end (San Diego, CA). SPHERO Rainbow Fluorescent Particles were 
conjugated anti-canine CD14 MAb (clone M5E2) was from BioLeg- 
from Sigma Chemical Co (St. Louis, MO). Allophycocyanin (APC) 
19251/H9252/methylene ATP, brilliant blue G (BBG), and propidium iodide were 
311740-60.0 
263 58.7 
368 63.1 
423 64.1 
393 63.1 
420 64.1 
368 65.3 
263 58.7 
170 62.2 
346 64.5 
353 64.1 
368 65.3 
263 58.7 
170 62.2 
300 60.0 
600 64.1 
515 58.6

Table 1. Primers used to amplify and sequence the canine P2RX7 gene

Size of amplicon is in base pairs (bp). Temperature (Temp.) indicates annealing temperatures used for amplification of exons. *Two overlapping primer pairs were used to amplify exon 13.

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Fig. 1. Relative monocyte P2X7 function varies between dogs. Peripheral blood mononuclear cells (PBMCs, from 52 dogs as indicated) or Madin-Darby canine kidney (MDCK) cells were incubated in the absence or presence of 1 mM ATP for 5 min at 37°C. Incubations were stopped by addition of MgCl₂ medium and centrifugation, and YO-PRO-1² uptake into CD14⁺ monocytes or MDCK cells determined by flow cytometry. ATP-induced YO-PRO-1² uptake was defined as the mean difference (from duplicate samples) in mean fluorescent intensity in the presence and absence of ATP for each individual. Symbols represent individual dogs; bars represent group means; broken line represents mean of all samples.

Resulting sequences were compared with the NCBI Reference Sequence NM_001113456.1, which was defined as wild-type canine P2X7.

Cloning of canine P2X7. cDNA from PBMCs of an English springer spaniel (University of Sydney, Sydney, Australia) was used as a template to clone canine P2X7 by standard techniques. Canine P2X7 cDNA was amplified by PCR using PfuUltra High-Fidelity DNA polymerase and the product containing canine P2X7 by standard techniques and confirmed the presence and absence of ATP for each individual. Symbols represent individual dogs; bars represent group means; broken line represents mean of all samples.

Fig. 2. The F103L, R270C, and R365Q substitutions in canine P2X7 identified 4 missense SNPs as indicated. The L440F SNP identified in the cloned English springer spaniel P2X7 (but absent in all other dogs sequenced) is also shown. Boxes represent P2X7 exons (not all 13 exons are represented). B: for each dog, the P2X7 genotype (as indicated) was compared with the relative monocyte P2X7 function (obtained in Fig. 1). *P < 0.05 to 3° corresponding wild type. Symbols represent individual dogs; bars represent group means.
### Table 2. P2RX7 genotype and P2X7 function of dogs

<table>
<thead>
<tr>
<th>Breed</th>
<th>P2RX7 Genotype</th>
<th>P2X7 Function</th>
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<tbody>
<tr>
<td>Alaskan malamute</td>
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<tr>
<td>Cavalier King Charles spaniel and cocker spaniel cross</td>
<td>WT R/C PT PT</td>
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<tr>
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<td>Maltese and shih tzu cross</td>
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<td>Maltese and shih tzu cross</td>
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<td>Mastiff and American</td>
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<td>Poodle and cocker spaniel cross</td>
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<tr>
<td>Pug and fox terrier cross</td>
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<tr>
<td>Rhodesian ridgeback</td>
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<td>Rhodesian ridgeback and Australian cattle dog cross</td>
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<td>Schnauzer (miniature) and bulldog cross</td>
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<td>Shar-pei</td>
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<tr>
<td>Siberian husky</td>
<td>WT WT PT PT</td>
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### Data presentation and statistical analyses

Cells were visualized on a TCS SP5 II confocal imaging system (Leica, Mannheim, Germany) (excitation at 561 nm, emission collected at 575–630 nm).

**Electrophysiology.** Whole cell patch-clamp recordings of HEK293 cells were performed in low divalent solution (145 mM NaCl, 5 mM KCl, 0.2 mM CaCl₂, 13 mM glucose, 10 mM HEPES, pH 7.3, osm 295–310) and recorded with a HEKA (Lambrecht, Germany) EPC10 amplifier as described (7).

**RESULTS**

To determine if the relative P2X7 function varied between dogs, blood was obtained from dogs presented at local veterinary clinics, and the ATP-induced uptake of YO-PRO-1²⁻ uptake into CD14⁺ monocytes [or Madin-Darby canine kidney cells (MDCK)].
exons of this gene from 19 dogs with either low or high P2X7 function, as well as from MDCK cells were sequenced. Sequences were compared with the NCBI Reference Sequence NM_001113456.1, which was designated as the wild-type canine P2X7 sequence. Sequencing confirmed the existence of the two previously reported missense SNPs, rs23314713 (ttc>ctc) in exon 3 and rs23315462 (cct>tct) in exon 13, which code for the amino acid substitutions F103L and P452S, respectively (Fig. 2A, Table 2). Sequencing also confirmed the existence of the previously reported synonymous SNP rs8660017 (aat>aac, N131N) in exon 4 of 14 dogs in either heterozygous (n = 6) or homozygous dosage (n = 8) (results not shown). Finally, sequencing identified two novel missense SNPs, cgt>tgt in exon 8 and cga>caa in exon 11, which code for the amino acid substitutions R270C and R365Q, respectively (Fig. 2A, Table 2). Complete sequences were obtained from all exons except for exon 13, for which the 48 base pairs coding the last 16 amino acid residues of the COOH terminus of P2X7 could not be resolved due to poor signal-to-noise ratio (results not shown).

Next, exons 3, 8, 11, and 13 of the P2RX7 gene were sequenced from the remaining dogs with P2X7 functional data, as well as from 17 other dogs for which genomic DNA was available. The allele frequencies of F103L, R270C, R365Q, and P452S were 0.38, 0.01, 0.03, and 0.39, respectively (Table 2). Notably, the R270C SNP was only observed in a Cavalier King Charles spaniel and cocker spaniel cross and in MDCK cells, which were originally derived from a cocker spaniel (16). In both instances the R270C SNP was present in heterozygous dosage and appeared to be linked with the P452S SNP (Table 2). The R270C SNP was not observed in a second cocker spaniel cross, from which no functional data were available (Table 2). The R365Q SNP was only observed in three Labrador retrievers and in a golden retriever and Labrador retriever.
cross and then only in heterozygous dosage in all four cases; three other Labrador retrievers were wild type at residue position 365 (Table 2).

The relative P2X7 function obtained above (Fig. 1) was then compared with each missense SNP individually. The P2X7 function of dogs heterozygous for the F103L SNP was similar to that of dogs wild type for F103; however, the P2X7 function of dogs homozygous for this SNP was significantly lower compared with wild-type animals (Fig. 2B). Sample numbers were limited for both the R270C and R365Q SNPs and were only observed in heterozygous dosage, but the mean P2X7 function in dogs with either of these SNPs was about half that of the mean P2X7 function in dogs wild type for R270 or R365 (Fig. 2B). In contrast, mean P2X7 function was similar between dogs wild type for P452 or heterozygous or homozygous for the P452S SNP (Fig. 2B).

Our group has previously characterized P2X7 in erythrocytes and leukocytes from English springer spaniels (44, 48). Therefore, P2X7 was cloned using cDNA from this breed to generate a recombinant P2X7 for site-directed mutagenesis.

Fig. 4. The R270C SNP results in loss of function of canine P2X7. A–C: HEK-293 cells, transfected with wild-type or mutant canine P2X7 plasmid DNA (as indicated), suspended in NaCl medium containing 25 μM ethidium* were incubated in the absence (basal) or presence of 1 mM ATP for 5 min at 37°C. Incubations were stopped by addition of MgCl2 medium and centrifugation. Ethidium* uptake was determined by flow cytometry and expressed as mean fluorescent intensity. Results are mean (SD), n = 3–4; *P < 0.05 or **P < 0.01 to corresponding basal (A–C), ††P < 0.01 to 440F/452S (A), or ††P < 0.05 or ††P < 0.01 to corresponding wild type (B, C). D–F: whole lysates of HEK-293 cells transfected with wild-type or mutant canine P2X7 plasmid DNA (as indicated) were examined by immunoblotting using an Ab against the extracellular epitope of P2X7.

Fig. 5. The R270C SNP results in loss of canine P2X7 channel activity. HEK-293 cells were transfected with wild-type (left), 452S mutant (middle), or 270C/452S mutant (right) canine P2X7 plasmid DNA. Cells were clamped at −60 mV at room temperature, 1 mM ATP in low divalent solution was applied by a fast-flow delivery system, and whole cell currents were recorded: inward current to 1st exposure to ATP (A) and repeated exposures to ATP (B) every 60 s. Black bars indicate ATP exposure (5 s). Traces are representative of 5–7 cells.
The cloned receptor was 595 amino acids in length (results not shown) and was wild type at amino acid positions 103 (F103), 270 (R270), and 365 (R365) (Fig. 3A) compared with the (wild-type) NCBI Reference Sequence NM_00113456.1. In contrast, the cloned receptor differed at amino acid positions 440 (L440F, ttg>H11022 ttt) and 452 (P452S, cct>tct) (Fig. 3A).

To determine if the P2X7 cloned from an English springer spaniel could be expressed heterologously as a recombinant receptor in mammalian cells, whole lysates of HEK-293 cells transfected with English springer spaniel P2X7 plasmid DNA or mock-transfected HEK-293 cells were examined by immunoblotting using an Ab against an extracellular or COOH-terminal epitope of P2X7. Murine EOC13 cells, which express endogenous P2X7 (5), were used as a positive control. Both antibodies detected a major band at 75 kDa, the predicted size of glycosylated P2X7, in HEK-293 cells expressing English springer spaniel P2X7 and EOC13 cells, but not mock-transfected HEK-293 cells (Fig. 3B).

Next, to determine if the P2X7 cloned from an English springer spaniel could form functional receptors when expressed heterologously in mammalian cells, we used a flow cytometric assay to measure ATP-induced fluorescent cation uptake into HEK-293 cells expressing English springer spaniel P2X7 or mock-transfected HEK-293 cells. ATP failed to induce ethidium\(^+\) uptake in mock-transfected HEK-293 cells (Fig. 3C). In contrast, ATP induced robust ethidium\(^+\) uptake into HEK-293 cells expressing English springer spaniel P2X7 compared with corresponding cells incubated in the absence of ATP (Fig. 3C). ATP also induced robust uptake of two other fluorescent cations, YO-PRO-1\(^+\) (375 Da) and propidium\(^+\) (415 Da), as well ethidium\(^+\) (314 Da), into HEK-293 cells expressing English springer spaniel P2X7 (Fig. 3D).

The recombinant English springer spaniel P2X7 expressed heterologously in HEK-293 cells was further characterized with flow cytometric measurements of ethidium\(^+\) uptake. ATP induced ethidium\(^+\) uptake in a concentration-dependent fashion with a half-maximal effective concentration (EC\(_{50}\)) value of 253 nM (Fig. 3E). Moreover, BzATP and ATP\(_S\), agonists of endogenous and recombinant human P2X7 (15, 19), induced ethidium\(^+\) uptake in a concentration-dependent fashion and with EC\(_{50}\) values of 13 and 438 \(\mu\)M, respectively (Fig. 3E). Maximum ethidium\(^+\) uptake was observed with 50 \(\mu\)M BzATP, while ATP and ATP\(_S\) represented partial agonists. In contrast, ADP, UTP, and \(\alpha\beta\)-meATP (each at 1 mM) failed to induce ethidium\(^+\) uptake (Fig. 3E).

The efficacy of five well-known human and rodent P2X7 antagonists was then tested against the recombinant English springer spaniel P2X7. A438079 (35), AZ10606120 (34), AZ11645373 (50), BBG (28), and KN-62 (20) impaired ATP-induced ethidium\(^+\) uptake in a concentration-dependent fashion with half-maximal inhibitory concentration values of 195 nM, 11 nM, 9 nM, 1,000 nM and 16 nM, respectively (Fig. 3F). Moreover, all five antagonists completely impaired ATP-induced ethidium\(^+\) uptake with maximal inhibition observed at concentrations of 0.1 \(\mu\)M for AZ10606120 and AZ11645373, 1 \(\mu\)M for KN-62, and 10 \(\mu\)M for A438079 and BBG (Fig. 3F).

To determine if any of the missense SNPs identified above (F103L, R270C, R365Q, L440F, and P452S) effect P2X7 function, the cloned English springer spaniel P2X7 was mutated as follows. First, the plasmid containing the cloned English springer spaniel P2X7 (which contained both the 440F and 452S SNPs, now termed 440F/452S mutant P2X7) was changed to wild type at position 440 (termed 452S mutant P2X7) by mutating the phenylalanine residue to leucine. Next, the plasmid containing only the 452S SNP was either changed to wild-type P2X7 (termed wild-type P2X7) by mutating the serine residue at position 452 to proline or changed to a double mutant (termed 270C/452S mutant P2X7) by mutating the
arginine residue at position 270 to cysteine. Finally, the wild-type P2X7 plasmid was mutated to contain either the 103L or 365Q SNP (termed 103L and 365Q mutant P2X7, respectively).

Using the above plasmids, we assessed the effect of each SNP on P2X7 function by measurements of ATP-induced ethidium+ uptake into transfected HEK-293 cells. ATP-induced ethidium+ uptake was 24% less in cells expressing 452S mutant P2X7 compared with cells expressing 440F/452S mutant P2X7 (Fig. 4A). ATP-induced ethidium+ uptake into cells expressing 452S mutant P2X7 was similar to that of cells expressing wild-type P2X7 (Fig. 4B). In contrast, ATP-induced ethidium+ uptake was almost completely abolished in cells expressing 270C/452S mutant P2X7 compared with cells expressing either wild-type or 452S mutant P2X7 (Fig. 4B). Finally, ATP-induced ethidium+ uptake was 46 and 37% less in cells expressing either 103L or 365Q mutant P2X7, respectively, compared with cells expressing wild-type P2X7 (Fig. 4C).

To determine if the differences in ATP-induced ethidium+ uptake in transfected HEK-293 cells related to total P2X7 expression, we examined whole lysates of HEK-293 cells transfected with DNA from wild-type or each mutant P2X7 plasmid by immunoblotting using the Ab against the extracellular epitope of P2X7 and the amount of P2X7 quantified. As above (Fig. 3B), a major band at 75 kDa was detected in lysates of HEK-293 cells expressing wild-type or mutant P2X7 (Fig. 4D–F). The amount of P2X7 was 29% less in cells expressing the 452S mutant compared with cells expressing 440F/452S mutant P2X7 (Fig. 4D). The amount of P2X7 was 22 and 30% less in cells expressing either 452S or 270C/452S mutant P2X7, respectively, compared with cells expressing wild-type P2X7 (Fig. 4E). The amount of P2X7 was 48 and 32% less in cells expressing either 103L or 365Q mutant P2X7, respectively, compared with cells expressing wild-type P2X7 (Fig. 4F).

The above data indicate that the R270C SNP has a major functional impact on canine P2X7. Therefore, this SNP was characterized further in HEK-293 cells expressing either wild-type, 452S, or 270C/452S mutant P2X7. First, patch-clamp measurements revealed that ATP induced robust inward currents in HEK-293 cells expressing wild-type or 452S mutant P2X7, with mean current densities of 49 (19) pA/pF (n = 6) and 39 (24) pA/pF (n = 7), respectively, and which were not significantly different (P > 0.05) (Fig. 5A). In contrast, ATP-induced inward currents in HEK-293 cells expressing 270C/452S mutant P2X7 were significantly smaller [mean current density of 3 (1) pA/pF, n = 5] than those observed in HEK-293 cells expressing wild-type or 452S mutant P2X7 (P < 0.01 and P < 0.05, respectively) (Fig. 5A). In addition, multiple applications of ATP saw wild-type or 452S mutant P2X7 display facilitation of responses over 5 min, whereas 270C/452S mutant P2X7 responses did not display facilitation and remained small (Fig. 5B). Second, immunolabeling with an Ab specific for the extracellular loop of P2X7 and confocal microscopy demonstrated cell-surface expression of P2X7 on HEK-293 cells expressing wild-type, 452S, or 270C/452S mutant P2X7 (Fig. 6). In contrast, immunoreactivity of mock-transfected HEK-293 cells with anti-P2X7 Ab or HEK-293 cells expressing wild-type or mutant P2X7 incubated in the presence of anti-P2X7 Ab with blocking peptide was minimal (results not shown).

**DISCUSSION**

The current study aimed to assess the functional impact of P2X7 variation in a random sample of the canine population. We and others have previously shown that monocyte P2X7 function correlates with P2RX7 genotype in humans (14, 24, 49). Thus, the variable P2X7 function between dog breeds and within individual breeds may in part be caused by SNPs within the canine P2RX7 gene. Of the P2RX7 SNPs identified, F103L, R270C, and R365Q, but not P452S, corresponded to decreased P2X7 function in canine monocytes. However, these SNPs could not explain the majority of differences in P2X7 function between dogs, indicating that other factors contribute to this variability. Such factors may include SNPs in the last 48 base pairs of exon 13 and in the promoter and intronic regions of the P2RX7 gene, differential expression of P2X7 splice variants, or differences in the health status of dogs upon presentation at local veterinary clinics.

Of the SNPs identified in our dog population and our cloned English springer spaniel P2X7 (Fig. 7), the R270C SNP was concluded to be a loss-of-function SNP impairing both P2X7 channel activity and pore formation. To the best of our knowledge this SNP has not been previously reported, but an R270H SNP (rs7958311) has been identified in human P2X7 and confers a partial loss of function (49). Thus, this latter result indirectly supports the concept that R270C, which exchanges a positively charged arginine residue for a polar, uncharged cysteine residue, is a loss-of-function SNP in canine P2X7. Structural modeling of human P2X7 places the R270 residue and the neighboring R276 residue, which is the site for the R276H loss-of-function SNP (49), in the β12 strand of the...
lower body of the extracellular loop and away from the ATP-binding site (27). As such, Jiang and colleagues (27) proposed that the R276H SNP may be involved in conformational changes associated with channel gating or receptor activation/deactivation. Thus by extension, it is possible that the R270C SNP may also act in a similar way to negatively affect P2X7 function. Alternatively, since this SNP introduces a cysteine adjacent to the existing cysteine at position 269, it is possible that the R270C SNP disrupts the potential disulfide bond between C260 and C269 (29) to reduce receptor function by altering the structure of the P2X7 subunit. Disulfide bonds of the extracellular loop of P2X7 are also essential for its trafficking to the plasma membrane (29); however, it appears unlikely that the R270C SNP impairs trafficking of the canine P2X7 to the cell surface as immunocytochemistry showed the presence of cell-surface P2X7 on HEK-293 cells expressing P2X7 containing this SNP.

The missense SNPs F103L and R365Q in canine P2X7 may be partial loss-of-function SNPs. However, the current body of evidence is limited as total P2X7 expression was also reduced by a similar amount in HEK-293 cells transfected with either mutant P2X7 compared with cells transfected with wild-type P2X7. Thus, whether the lower P2X7 function of either mutant receptor is due to reduced P2X7 protein synthesis or half-life or plasmid transfection efficiency remains to be determined.

In contrast to the other missense SNPs in canine P2X7, it is reasonable to conclude that the P452S SNP does not alter P2X7 function. It is noteworthy that the P452S SNP in canine P2X7 is located at the equivalent position to that of the partial loss-of-function P451L SNP in murine P2X7, which is present in some but not all mouse strains (1). This difference in effect between canine P452S and murine P451L on P2X7 function may relate to differences between the hydrophilic and larger hydrophobic side chain of serine and leucine, respectively.

The current study involved the cloning of canine P2X7 from an English springer spaniel for use in site-directed mutagenesis studies to examine the effects of identified SNPs on P2X7 function. This breed was originally chosen following our original studies of endogenous P2X7 in this breed (44, 48). Compared with wild-type canine P2X7 (NCBI Reference Sequence NM_001113456.1), the cloned English springer spaniel P2X7 differed by two amino acid residues, L440F and P452S. The P452S SNP had an allele frequency of 0.39 in our dog population. In contrast, the L440F SNP has not been previously reported and was not observed in any other dogs for which exon 13 was sequenced (results not shown). Whether this variant represents a SNP in English springer spaniels or was introduced as a result of the cloning process remains unknown.

Pharmacological characterization of the recombinant English springer spaniel P2X7 demonstrated that this receptor displays rank orders of agonist and antagonist potencies similar to that of endogenous and recombinant human P2X7 (15, 19, 39). Of note, the EC50 values for BzATP and ATP were similar to that described for endogenous P2X7 in erythrocytes from English springer spaniels (48). Moreover, study of both endogenous P2X7 (48) and the recombinant English springer spaniel P2X7 revealed that ATP was a partial agonist compared with BzATP. These results contrast findings with the previously cloned canine P2X7 of Roman and colleagues (42), who demonstrated that BzATP was a partial agonist of canine P2X7 compared with ATP. The reason for this difference between these studies remains unknown, but one possible explanation is the amino acid at position 103 alters BzATP sensitivity.

In conclusion, the current study identified a missense SNP, R270C, which leads to a loss of function of canine P2X7. It will be of importance in future studies to determine the frequency of this SNP in cocker spaniels and other breeds and to determine whether this SNP disrupts P2X7-mediated physiological processes, such as ATP-induced IL-1β release. Moreover, since SNPs in the human P2RX7 gene are associated with various diseases (17, 21, 22, 32), and since a three base-pair deletion in the gene coding the P2Y12 receptor is associated with postoperative hemorrhage in a greater Swiss mountain dog (8), it will be of interest to determine whether the R270C SNP or other SNPs in the canine P2RX7 gene are associated with disease in dogs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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