




Association of a *P2RX7* gene missense variant with brachycephalic dog breeds

R. A. Sophocleous^{*,†}, V. Sluyter^{*,†}, B. L. Curtis[‡], S. J. Curtis[‡], L. M. Jurak^{*,†}, M. Faulks^{*,†},
M. Spildrejorde^{*,†}, S. Gates^{*,†}, E.-J. Proctor^{*,†}, A. Seavers[§], D. Watson^{*,†}, T. Kuit[†], M. Downton^{*,†},
L. Stokes[¶] and R. Sluyter^{*,†} 

^{*}Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia. [†]Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW 2522, Australia. [‡]Albion Park Veterinary Hospital, Albion Park, NSW 2527, Australia. [§]Oak Flats Veterinary Clinic, Oak Flats, NSW 2529, Australia. [¶]School of Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK.

Summary

Missense variants are associated with various phenotypic traits and disorders in dogs. The canine *P2RX7* gene, coding the ATP-gated P2X7 receptor ion channel, contains four known missense variants. The current study aimed to examine the presence of these variants in a random sample of pedigree and mixed-pedigree dogs. Exons 3, 8, 11 and 13 of the *P2RX7* gene, encoding these four respective variants, in 65 dogs were assessed by Sanger sequencing and combined with existing sequencing data from another 69 dogs. The distribution of these variants was then evaluated in all 134 dogs combined and separately within individual breeds including 35 different pure breeds. The rs23314713 (p.Phe103-Leu) and rs23315462 (p.Pro452Ser) variants were present in 47 and 40% of all dogs studied respectively, with the rs23314713 variant associated with brachycephalic breeds. Among pedigree dogs, the rs23314713 and rs23315462 variants were associated with brachycephalic and non-brachycephalic breeds respectively. The rs851148233 (p.Arg270Cys) and rs850760787 (p.Arg365Gln) variants were present only in dogs of Cocker Spaniel and Labrador Retriever pedigrees respectively. No other missense variants were found in exons 3, 8, 11 and 13 of the *P2RX7* gene within the dogs. In conclusion, the rs23314713 and rs23315462 missense variants of the *P2RX7* gene are present in a large proportion of dogs, with the rs23314713 variant associated with a number of brachycephalic breeds. However, the association of this variant with dogs of bulldog ancestry, not brachycephaly *per se*, cannot be excluded.

Keywords canine, DH82 canine macrophages, glioma, MDCK cells, P2X7 receptor, purinergic receptor, single nucleotide polymorphism

The P2X7 receptor is a trimeric ATP-gated cation channel encoded by the *P2RX7* gene (Sluyter 2017). The P2X7 receptor has important roles in inflammation, immunity and cancer (Di Virgilio *et al.* 2017). In dogs, the P2X7 receptor is present on monocytes, lymphocytes, erythrocytes (Sluyter *et al.* 2007; Stevenson *et al.* 2009), kidney epithelial cells (Jalilian *et al.* 2012b; Zuccarini *et al.* 2017) and neurons of the myenteric plexus (Schafer *et al.* 2018). This receptor is also present in normal brain tissue,

including the cerebrum (Lee *et al.* 2005; Truve *et al.* 2016), and in glioblastomas, oligodendrogliomas and astrocytomas (Truve *et al.* 2016) from dogs. Canine P2X7 receptor activation results in interleukin-1 β release from monocytes (Jalilian *et al.* 2012a) and in whole blood (Roman *et al.* 2009; Spildrejorde *et al.* 2014b; Bartlett *et al.* 2017). Moreover, P2X7 receptor activation results in phosphatidylserine exposure and hemolysis in canine erythrocytes (Sluyter *et al.* 2007; Faulks *et al.* 2016). Other cellular functions of the canine P2X7 receptor are yet to be reported, but its activation is likely to mediate events similar to those shown in humans, where it is an emerging therapeutic target in various disorders, including inflammatory bowel disease, osteoporosis, inflammatory pain and cancer (De Marchi *et al.* 2016).

Address for correspondence

R. Sluyter, Illawarra Health and Medical Research Institute
Wollongong, NSW 2522, Australia.
E-mail: rsluyter@uow.edu.au

Accepted for publication 06 November 2019

In a cohort of 69 dogs comprising 68 blood samples and MDCK cells (derived from a Cocker Spaniel), we previously identified four missense variants in the canine *P2RX7* gene: rs23314713 (p.Phe103Leu), rs851148233 (p.Arg270Cys), rs850760787 (p.Arg365Gln) and rs23315462 (p.Pro452Ser) (Spildrejorde *et al.* 2014a). Another group subsequently found that the rs23314713 variant is associated with susceptibility to glioma (Truve *et al.* 2016), a disease more frequent in several brachycephalic breeds (Hayes *et al.* 1975; Snyder *et al.* 2006; Song *et al.* 2013). Brachycephaly in dogs results in the shortening of the muzzle, flat facial conformation and widening of the skull, and is present in breeds such as the Bulldog, Mastiff and Staffordshire Bull Terrier (Bannasch *et al.* 2010; Schoenebeck *et al.* 2012; Packer *et al.* 2015; Marchant *et al.* 2017). To extend these findings, the presence of the four previously described missense variants was evaluated in an additional 65 dogs. These data were then combined with existing data (Spildrejorde *et al.* 2014a) to determine the distribution of these variants in a random sample of the canine population.

Dog samples were collected in accordance with the Animal and Human Ethics Committees (University of Wollongong). Peripheral blood, drawn into VACUETTE lithium heparin tubes (Greiner Bio-One), was collected from 64 dogs presented at local veterinary clinics with informed consent from pet owners. DH82 cells (derived from a Golden Retriever) were obtained from the European Collection of Cell Cultures. These 65 samples including the DH82 cells combined with our previously published cohort of 69 dogs (Spildrejorde *et al.* 2014a) represent 97 pedigree dogs (comprising 35 different pure breeds) and 37 mixed-pedigree dogs (Table S1). The veterinarians in consultation with pet owners assigned the breed of each dog. Pedigree dogs were classified as brachycephalic as reported in other studies (Bannasch *et al.* 2010; Schoenebeck *et al.* 2012; Packer *et al.* 2015; Marchant *et al.* 2017). Mixed-pedigree dogs were assigned as brachycephalic based on known ancestry and/or display of brachycephalic characteristics (shortening of the muzzle, flat facial conformation and widening of the skull). No dogs suffered from glioma at the time of presentation and were not followed up for this cancer post sampling.

DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. Primer pairs (Table S2) were used to amplify and sequence exons 3, 8 and 11 and the first half of exon 13 of the canine *P2RX7* gene by PCR as described previously (Spildrejorde *et al.* 2014a). No other exons of the *P2RX7* gene were sequenced. Resulting sequences were compared with the NCBI Reference Sequence NM_001113456.1, which is from a non-disclosed pedigree. Notably, the alleles in the reference sequence for each of the four missense variants are conserved across various mammalian species (Jiang *et al.* 2013). For some dogs, not all exons could be amplified or sequenced despite repeated

attempts and having successfully achieved this with other exons from the same DNA sample (Table S1). This resulted in different dog numbers being reported below for each exon studied. Differences in heterozygosity, homozygosity, variant prevalence and mutant allele frequency between groups were compared by the Fisher's exact test using Prism 5 for Mac OS X (GraphPad Software) with $P < 0.05$ considered significant. P -Value calculations for prevalence include heterozygous and homozygous dog numbers combined. P -Value calculations for allele frequency include total allele numbers. Analyses did not correct for relatedness of breeds.

Sequence analysis of genomic DNA isolated from 65 new dogs combined with existing sequence data from another 69 dogs (Spildrejorde *et al.* 2014a) revealed (in decreasing order) a variant prevalence of 47, 40, 4 and 3% and a mutant allele frequency of 0.38, 0.26, 0.02 and 0.02 for rs23314713 (p.Phe103Leu), rs23315462 (p.Pro452Ser), rs850760787 (p.Arg365Gln) and rs851148233 (p.Arg270Cys) respectively (Table 1; Table S1). Similar prevalences and allele frequencies for each variant were also observed among pedigree dogs only (Table 1; Table S1). LD analysis (<https://www.broadinstitute.org/haploview/haploview>) revealed that none of the alleles was in strong linkage disequilibrium (results not shown), with the strongest association observed between rs23314713 and rs23315462 ($D' = 0.605$ and $r^2 = 0.088$).

The combined data from pedigree and mixed-pedigree dogs revealed the presence of the rs23314713 (p.Phe103-Leu) variant in 76% of brachycephalic dogs and 31% of non-brachycephalic dogs (Table 2), which differed significantly between these two groups ($P < 0.0001$). The allele frequencies of the rs23314713 mutant allele were 0.65 and 0.23 in brachycephalic and non-brachycephalic dogs respectively (Table 2), and also differed significantly between the two groups ($P < 0.0001$). Among pedigree dogs only, the prevalence ($P < 0.0001$) and allele frequency ($P < 0.0001$) for this variant were also significantly greater in brachycephalic dogs compared with non-brachycephalic dogs (Table 2). Moreover, there was a significantly higher proportion of brachycephalic dogs heterozygous for the rs23314713 variant compared with non-brachycephalic dogs among all dogs ($P = 0.0136$) and pedigree dogs only ($P = 0.0323$). Likewise, there was a significantly higher proportion of brachycephalic dogs homozygous for this variant compared with non-brachycephalic dog among all dogs ($P < 0.0001$) and pedigree dogs only ($P < 0.0001$).

Collectively, the data above indicate that the rs23314713 variant is associated with dogs of brachycephalic pedigree. However, a second possibility is that this variant is associated with dogs of known bulldog ancestry, including the Australian bulldog, American Staffordshire terrier and Staffordshire terrier (Ostrander *et al.* 2017), not brachycephaly *per se*. In support of this, the three bull terriers studied, defined as non-brachycephalic dogs and with bulldog ancestry (vonHoldt *et al.* 2010), were all

Table 1 Prevalence and allele frequency of P2RX7 gene missense variants.

Variant	Amino acid change	Number of dogs (n)	Genotype (n of dogs)			Prevalence (%)	Allele frequency
			Reference sequence	Heterozygous variant	Homozygous variant		
All dogs							
rs23314713	p.Phe103Leu	117	62	20	35	47	0.38
rs851148233	p.Arg270Cys	121	117	4	0	3	0.02
rs850760787	p.Arg365Gln	130	125	5	0	4	0.02
rs23315462	p.Pro452Ser	106	64	28	14	40	0.26
Pedigree dogs							
rs23314713	p.Phe103Leu	88	51	9	27	41	0.36
rs851148233	p.Arg270Cys	89	87	2	0	2	0.01
rs850760787	p.Arg365Gln	93	89	4	0	4	0.02
rs23315462	p.Pro452Ser	79	48	21	10	39	0.26

Table 2 Prevalence and allele frequency of the rs23314713 (p.Phe103Leu) and rs23315462 (p.Pro452Ser) variants in brachycephalic and non-brachycephalic dogs.

Variant	Brachycephalic	Number of dogs (n)	Genotype (n of dogs)			Prevalence (%)	Allele frequency
			Reference sequence	Heterozygous variant	Homozygous variant		
rs23314713 (all dogs)	Yes	42	10	9 ¹	23 ²	76 ³	0.65 ⁴
	No	75	52	11	12	31	0.23
rs23314713 (pedigree dogs)	Yes	28	6	4 ⁵	18 ²	79 ³	0.71 ⁴
	No	60	46	5	9	23	0.19
rs23315462 (all dogs)	Yes	40	29	7	4	28	0.19
	No	66	35	21	10	47	0.31
rs23315462 (pedigree dogs)	Yes	26	21	3 ⁶	2	19 ⁷	0.13 ⁸
	No	53	27	18	8	49	0.32

All statistical comparisons by Fisher's exact test.

¹P = 0.0136 compared with the proportion of heterozygous non-brachycephalic dogs (excludes homozygous dogs).

²P < 0.0001 compared with the proportion of homozygous non-brachycephalic dogs (excludes heterozygous dogs).

³P < 0.0001 compared with the prevalence in non-brachycephalic dogs.

⁴P < 0.0001 compared with the allele frequency in non-brachycephalic dogs.

⁵P = 0.0323 compared with the proportion of heterozygous non-brachycephalic dogs (excludes homozygous dogs).

⁶P = 0.0269 compared with the proportion of heterozygous non-brachycephalic dogs (excludes homozygous dogs).

⁷P = 0.0141 compared with the prevalence in non-brachycephalic dogs.

⁸P = 0.0124 compared with the allele frequency in non-brachycephalic dogs.

homozygous for the rs23314713 variant, whereas four of the five Maltese Shih Tzu studied, defined as brachycephalic dogs and without bulldog ancestry (vonHoldt *et al.* 2010), were homozygous for the reference allele at this position. Thus, it remains to be determined if the rs23314713 variant contributes to brachycephaly, but observations in mice do not support this. Despite well-established roles for P2RX7 activity in bone formation and homeostasis (Agrawal & Gartland 2015), strains of mice with a loss-of-function P2RX7 missense variant (Syberg *et al.* 2012) or global ablation of the P2RX7 gene (Ke *et al.* 2003) do not display facial or skull differences, akin to brachycephaly, compared with mice with normal P2RX7 activity.

It should also be noted that our study, compared with others, is relatively small and represents a limited number of breeds. A study of 576 dogs (representing 62 breeds)

identified a missense variant (p.Phe452Leu) in *BMP3*, which codes for bone morphogenetic protein 3, that is associated with cranioskeletal features of brachycephalic dogs (Schoenebeck *et al.* 2012). Another study of 375 dogs (representing 83 breeds) revealed that a retrotransposon-mediated missplicing of *SMOC2*, which encodes SPARC-related modular calcium-binding protein 2, is associated with brachycephaly (Marchant *et al.* 2017). Both of these proteins have potential roles in bone formation, but how the related genetic variations contribute to brachycephaly remains to be determined (Schoenebeck *et al.* 2012; Marchant *et al.* 2017).

The combined data from pedigree and mixed-pedigree dogs revealed the presence of the second most common variant, rs23315462 (p.Pro452Ser), in 28% of brachycephalic dogs and 47% of non-brachycephalic dogs (Table 2), a difference

that approached statistical significance ($P = 0.0650$). The allele frequencies of the rs23315462 mutant allele were 0.19 and 0.31 in brachycephalic and non-brachycephalic dogs respectively (Table 2), a difference that also approached statistical significance ($P = 0.0547$). Notably, among pedigree dogs, the prevalence ($P = 0.0141$) and allele frequency ($P = 0.0124$) for this variant were significantly greater in non-brachycephalic dogs compared with brachycephalic dogs (Table 2). Collectively, these data revealed that the rs23315462 variant is potentially more prevalent in non-brachycephalic dogs compared with brachycephalic dogs. This difference mirrors the greater prevalence of the rs23314713 variant in brachycephalic dogs, which can be explained by incomplete LD between these two alleles as noted above.

Although case numbers were small among all pedigree and mixed-pedigree dogs combined, the current data suggest that the rs851148233 (p.Arg270Cys) and rs850760787 (p.Arg365Gln) variants are limited to dogs of Cocker Spaniel and Labrador Retriever pedigree respectively (Table S3). The rs851148233 variant was present in four dogs of Cocker Spaniel pedigree (two Cocker Spaniels, one Cavalier King Charles Spaniel–Cocker Spaniel cross and one Cocker Spaniel–Poodle cross), but absent in three dogs of Cocker Spaniel pedigree (two Cocker Spaniels and one Poodle–Cocker Spaniel cross). The rs851148233 variant was also absent in two Cavalier King Charles Spaniels, one Maltese–Poodle cross and another 111 dogs of non-Cocker Spaniel pedigree. The rs850760787 variant was present in four Labrador Retrievers and one Golden Retriever–Labrador Retriever cross. This variant was absent in six other Labrador Retrievers and another 119 dogs of non-Labrador Retriever pedigree including four Golden Retrievers.

The current study did not reveal any new missense variants in the *P2RX7* gene in any of the dogs sequenced (results not shown). These data contrast the large polymorphic variation observed in the human *P2RX7* gene (De Marchi *et al.* 2016). Although the possibility remains that other missense variants are present in the remaining exons not sequenced, the limited polymorphic variation of the canine *P2RX7* gene compared with the human *P2RX7* gene is consistent with genome-wide decreases in genetic variation as a result of population bottlenecks associated with domestication, local population history and breed creation (Ostrander *et al.* 2017). Finally, it should be noted that occasional exons could not be amplified or sequenced despite having successfully achieved this with other exons from the same DNA sample. This may be due to sequence variations within the PCR primer binding sites, which were largely intronic, negatively impacting annealing or extension.

In conclusion, the main finding of this study is that the rs23314713 (p.Phe103Leu) and rs23315462 (p.Pro452Ser) variants of the *P2RX7* gene are commonly present in dogs, with the rs23314713 variant associated with a number of brachycephalic breeds; however, the association

of this variant with dogs of bulldog ancestry, not brachycephaly *per se*, cannot be excluded.

Acknowledgements

This work was supported by the American Kennel Club Canine Health Foundation (grants 01265 and 01985) and the Australian Companion Animal Health Foundation. We acknowledge Petra Sluyter (Illawarra Health and Medical Research Institute) for couriering blood samples.

References

- Agrawal A. & Gartland A. (2015) P2X7 receptors: role in bone cell formation and function. *Journal of Molecular Endocrinology* **54**, R75–88.
- Bannasch D., Young A., Myers J. *et al.* (2010) Localization of canine brachycephaly using an across breed mapping approach. *PLoS ONE* **5**, e9632.
- Bartlett R., Stokes L., Curtis S.J., Curtis B.L. & Sluyter R. (2017) Probenecid directly impairs activation of the canine P2X7 receptor. *Nucleosides, Nucleotides & Nucleic Acids* **36**, 736–44.
- De Marchi E., Orioli E., Dal Ben D., & Adinolfi E. (2016) P2X7 receptor as a therapeutic target. *Advances in Protein Chemistry and Structural Biology* **104**, 39–79.
- Di Virgilio F., Dal Ben D., Sarti A.C., Giuliani A.L., & Falzoni S. (2017) The P2X7 Receptor in Infection and Inflammation. *Immunity* **47**, 15–31.
- Faulks M., Kuit T.A., Sophocleous R.A., Curtis B.L., Curtis S.J., Jurak L.M. & Sluyter R. (2016) P2X7 receptor activation causes phosphatidylserine exposure in canine erythrocytes. *World Journal of Hematology* **5**, 88–93.
- Hayes H.M., Priester W.A. Jr & Pendergrass T.W. (1975) Occurrence of nervous-tissue tumors in cattle, horses, cats and dogs. *International Journal of Cancer* **15**, 39–47.
- Jalilian I., Peranec M., Curtis B.L., Seavers A., Spildrejorde M., Sluyter V. & Sluyter R. (2012a) Activation of the damage-associated molecular pattern receptor P2X7 induces interleukin-1 β release from canine monocytes. *Veterinary Immunology and Immunopathology* **149**, 86–91.
- Jalilian I., Spildrejorde M., Seavers A., Curtis B.L., McArthur J.D. & Sluyter R. (2012b) Functional expression of the damage-associated molecular pattern receptor P2X7 on canine kidney epithelial cells. *Veterinary Immunology and Immunopathology* **150**, 228–33.
- Jiang L.H., Baldwin J.M., Roger S., & Baldwin S.A. (2013) Insights into the molecular mechanisms underlying mammalian P2X7 receptor functions and contributions in diseases, revealed by structural modeling and single nucleotide polymorphisms. *Frontiers in Pharmacology* **4**, 55.
- Ke H.Z., Qi H., Weidema A.F. *et al.* (2003) Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption. *Molecular Endocrinology* **17**, 1356–67.
- Lee H.K., Ro S., Keef K.D., Kim Y.H., Kim H.W., Horowitz B. & Sanders K.M. (2005) Differential expression of P2X-purinoreceptor subtypes in circular and longitudinal muscle of canine colon. *Neurogastroenterology and Motility* **17**, 575–84.
- Marchant T.W., Johnson E.J., McTeir L. *et al.* (2017) Canine brachycephaly is associated with a retrotransposon-mediated missplicing of SMOC2. *Current Biology* **27**, 1573–84.e6.

- Ostrander E.A., Wayne R.K., Freedman A.H., & Davis B.W. (2017) Demographic history, selection and functional diversity of the canine genome. *Nature Reviews Genetics* **18**, 705–20.
- Packer R.M., Hendricks A., Tivers M.S. & Burn C.C. (2015) Impact of facial conformation on canine health: brachycephalic obstructive airway syndrome. *PLoS ONE* **10**, e0137496.
- Roman S., Cusdin F.S., Fonfria E., Goodwin J.A., Reeves J., Lappin S.C., Chambers L., Walter D.S., Clay W.C. & Michel A.D. (2009) Cloning and pharmacological characterization of the dog P2X7 receptor. *British Journal of Pharmacology* **158**, 1513–26.
- Schafer B.T., Silveira M.P., Palombit K., Mendes C.E., Watanabe I.S., Miglino M.A. & Castelucci P. (2018) Morphological characterization of the myenteric plexus of the ileum and distal colon of dogs affected by muscular dystrophy. *Anatomical Record (Hoboken)* **301**, 673–85.
- Schoenebeck J.J., Hutchinson S.A., Byers A. *et al.* (2012) Variation of BMP3 contributes to dog breed skull diversity. *PLoS Genetics* **8**, e1002849.
- Sluyter R. (2017) The P2X7 receptor. *Advances in Experimental Medicine and Biology* **1051**, 17–53.
- Sluyter R., Shemon A.N., Hughes W.E., Stevenson R.O., Georgiou J.G., Eslick G.D., Taylor R.M. & Wiley J.S. (2007) Canine erythrocytes express the P2X7 receptor: greatly increased function compared with human erythrocytes. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **293**, R2090–8.
- Snyder J.M., Shofer F.S., Van Winkle T.J. & Massicotte C. (2006) Canine intracranial primary neoplasia: 173 cases (1986–2003). *Journal of Veterinary Internal Medicine* **20**, 669–75.
- Song R.B., Vite C.H., Bradley C.W. & Cross J.R. (2013) Postmortem evaluation of 435 cases of intracranial neoplasia in dogs and relationship of neoplasm with breed, age, and body weight. *Journal of Veterinary Internal Medicine* **27**, 1143–52.
- Spildrejorde M., Bartlett R., Stokes L. *et al.* (2014a) R270C polymorphism leads to loss of function of the canine P2X7 receptor. *Physiological Genomics* **46**, 512–22.
- Spildrejorde M., Curtis S.J., Curtis B.L. & Sluyter R. (2014b) Extracellular adenosine 5'-triphosphate and lipopolysaccharide induce interleukin-1beta release in canine blood. *Veterinary Immunology and Immunopathology* **157**, 105–10.
- Stevenson R.O., Taylor R.M., Wiley J.S. & Sluyter R. (2009) The P2X7 receptor mediates the uptake of organic cations in canine erythrocytes and mononuclear leukocytes: comparison to equivalent human cell types. *Purinergic Signalling* **5**, 385–94.
- Syberg S., Schwarz P., Petersen S., Steinberg T.H., Jensen J.E., Teilmann J. & Jorgensen N.R. (2012) Association between P2X7 Receptor Polymorphisms and Bone Status in Mice. *Journal of Osteoporosis* **2012**, 637986.
- Truve K., Dickinson P., Xiong A. *et al.* (2016) Utilizing the dog genome in the search for novel candidate genes involved in glioma development-genome wide association mapping followed by targeted massive parallel sequencing identifies a strongly associated locus. *PLoS Genetics* **12**, e1006000.
- vonHoldt B.M., Pollinger J.P., Lohmueller K.E. *et al.* (2010) Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. *Nature* **464**, 898–902.
- Zuccarini M., Giuliani P., Buccella S. *et al.* (2017) Modulation of the TGF-beta1-induced epithelial to mesenchymal transition (EMT) mediated by P1 and P2 purine receptors in MDCK cells. *Purinergic Signalling* **13**, 429–42.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Distribution of *P2RX7* gene missense variants in a random sample of dogs.

Table S2 Primers used to amplify and sequence missense variants of the canine *P2RX7* gene.

Table S3 Prevalence and allele frequency of the rs851148233 (p.Arg270Cys) and rs850760787 (p.Arg365Gln) variants in dogs of Cocker Spaniel and Labrador Retriever pedigree respectively.