PTPN22 polymorphisms may indicate a role for this gene in atopic dermatitis in West Highland white terriers

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Abstract

Background: Canine atopic dermatitis is an allergic inflammatory skin disease common in West Highland white terriers. A genome-wide association study for atopic dermatitis in a population of West Highland white terriers identified a 1.3 Mb area of association on CFA17 containing canine protein tyrosine phosphatase non-receptor type 22 (lymphoid) *PTPN22*. This gene is a potential candidate gene for canine atopic dermatitis as it encodes a lymphoid-specific signalling mediator that regulates T-cell and possibly B-cell activity.

Findings: Sequencing of *PTPN22* in three atopic and three non-atopic West Highland white terriers identified 18 polymorphisms, including five genetic variants with a bioinformatically predicted functional effect. An intronic polymorphic repeat sequence variant was excluded as the cause of the genome-wide association study peak signal, by large-scale genotyping in 72 West Highland white terriers (gene-dropping simulation method, \( P = 0.01 \)).

Conclusions: This study identified 18 genetic variants in *PTPN22* that might be associated with atopic dermatitis in West Highland white terriers. This preliminary data may direct further study on the role of *PTPN22* in this disease. Large scale genotyping and complementary genomic and proteomic assays would be required to assess this possibility.

Findings

Canine atopic dermatitis (AD) is an allergic inflammatory skin disease that is common in West Highland white terriers (WHWTs) [1]. Following a genome-wide association (GWAS) in a group of related WHWTs, we found a 1.3 Mb area on CFA 17 which was significantly associated with the disease [2]. Based on its biological functions, expression patterns and proximity to this area of association, *PTPN22* was selected as a candidate gene for AD in this population. This gene encodes a lymphoid tyrosine phosphatase (PTPN22), a signalling mediator that regulates generic and specialised immune functions in mammals [3]. Activation of T and B lymphocytes is a key event in the pathogenesis of atopic disease [4], and the disruption of these pathways could cause hyper-reactive pathogenic T-cell responses, as well as affect B-cell selection, maturation and function [5,6]. In humans and dogs, genetic variants in the gene *PTPN22* have been associated with auto-immune diseases [7-9]. In humans, these include psoriasis, a chronic immune-mediated inflammatory skin disease that shares susceptibility loci with human AD [10,11]. To date, no association has been found between *PTPN22* variants and atopic disease in humans [12].

The University of Queensland Animal and Human Ethics Committees, and the University of Sydney Animal Ethics Committee approved this study. Written consent was obtained from all participating dog owners.

Criteria used to classify dogs in the present study are described elsewhere [1]. Fourteen set of primers were designed with primer3 [13], to sequence a total of 12.6 Kb of *PTPN22* in 14 PCR products (Table 1). Amplification reactions used the HotStar HiFidelity PCR Kit (QIAGEN Pty Ltd, Doncaster, Vic, Australia) and 0.5 \( \mu \text{M} \) (PCR products 5 and 12), 1.5 \( \mu \text{M} \) (6 and 14) or 1 \( \mu \text{M} \) (remaining PCR products) of primers; at 55°C (PCR
Table 1 Primer sequences used to amplify and sequence 12.6 Kb of canine PTPN22 in three atopic and three non-atopic WHWTs

| PCR product | Forward amplification primer | Reverse amplification primer | Internal forward sequencing primer | Internal reverse sequencing primer | Predicted gene region | Product size |
|-------------|-------------------------------|-------------------------------|-----------------------------------|-----------------------------------|-----------------------|-------------|
| 1           | CCTCATCGGTGCTCTTGTT          | GGTGTTGGGCTCGTCCTGCGT         | TGAAGTGGAAGGATCTCAAGGCG          | AGAAAGCACAAGGCAGGT             | 5'UTR, exon 1        | 1041        |
| 2           | GCCTCTGCTCGAATGGAGGAG       | TCTGCGCTTACCAAGGACACT         | -                                 | -                                | Exons 2,3             | 858         |
| 3           | CAAATAGAGTGGGGGGTGA         | CTACTGGGAAAAATGGGGAAT        | AGAAAGGGAAGGGAAGGAGCA           | TCTGTCCCTCTCCCTCCCTTCC         | Exons 4,5             | 863         |
| 4           | ACCACATGGTGACCTTGGGATAA     | AGATGGAAGGACATCAATGTC         | -                                 | -                                | Exons 6,7             | 1182        |
| 5           | CGTTGACCTGACCTCAAGCAAGC     | ACCACGCCTTCCACACCAG          | -                                 | -                                | Exons 8               | 1172        |
| 6           | TGCTCCTGGAGAGTGGGATG        | CAAGGCAAGGCAAGCATAGGAA       | AATCCACCCACACCAAAAAACCT         | AGCCCGTATTCTCCACTCC           | Exons 9,10            | 1267        |
| 7           | CCGAAATGAGGTAGGCTAAACC     | GCCCTGTACCTCCACCTTAT         | -                                 | -                                | Exons 11              | 483         |
| 8           | TGGAAACTCCACCTCTTGTAA       | TCTTGGAGGAAGGAAAGGAAAGAGA    | CAGAGTGAGGAGCACCAAAAAACCA       | CCAGCTCCCTGGTGTCTCCT           | Exons 12,13           | 1296        |
| 9           | GAAGCGAGCAAAACCTCTACA      | ACCCCACATCCCTCTACGCA         | GATCCACATTTGCTGTGCC          | TGGGCCCATTCTTACAGGT             | Exons 14,15           | 889         |
| 10          | GGGTAAAGGGATGCGTTTCA       | TGGGAAGCTATTAGGGGAAAC       | -                                 | -                                | Exons 16              | 332         |
| 11          | TGGAGCTCCAGTTTATGGTTCA     | CAGTCTGTTGTCCTCAATCGTCTCGT  | AAGTGGGACCTAAATGGAAAAAG       | CCTTTTCATTTAGGTGCCACT          | Exons 17,18           | 747         |
| 12          | GAGATGGAAGAAAAATAGAGCAAGG  | TTCTGATACAAAGACCCATAGCA     | -                                 | -                                | Exon 19               | 410         |
| 13          | TCTCCCTTACTGTGTTGCTTT      | TGGCTTTTGCTGCTAGCAT         | -                                 | -                                | Exon 20               | 92          |
| 14          | GCGTGAATCCAAAGGGTTGT      | TCCACAAATCCACTCGCTAGGG      | TCGCAAAATTCTGACCTGTG           | TGGAGATGGAAGGAAATTT          | Exon 21, 3'UTR        | 550         |
Table 2 PTPN22 sequence variants identified by sequencing genomic DNA from three atopic and three non-atopic WHWTs.

| Sequence variant identity | Position on CFA17 (bp)a,b | Predicted location in gene | Nucleotide in reference databaseb | Sequence of variant | Reference SNP identity | Predicted functional effectc | Variant risk scorec | Atopic dogs | Non-atopic dogs | Cross-species conservation of variant nucleotide sequenceb.d |
|---------------------------|---------------------------|----------------------------|----------------------------------|---------------------|------------------------|---------------------------|----------------|-------------|---------------|----------------------------------------------------------|
| 1                         | 54759173 UTR C T          | rs22597162                 | Transcription regulatory (score 86.5) | 1-3 C/C C/T C/C C/C C/C | Conserved in 10/10 |
| 2                         | 54759006 UTR A del        | New variant (dbSNP ss 315790492) | Transcription regulatory (score 87.7) | 1-3 del/del del/A del/A T/A del/A A/A | Conserved in 9/10 |
| 3                         | 54742593 Intrinsic A G    | rs22597162                 | NA                               | 0-2 G/G G/A G/G G/G G/G G/G Not conserved |
| 4                         | 54742027 Intrinsic A T    | rs22559551                 | NA                               | 0-2 T/T T/A T/A A/A T/A A/A Conserved in 6/10 |
| 5                         | 54739568 Intrinsic T C    | rs22559538                 | NA                               | No risk T/T C/T C/T C/C C/C Not conserved |
| 6                         | 54739315 Intrinsic A G    | New variant (dbSNP ss 315790493) | NA                              | 0-2 G/G G/G G/G A/A A/G A/A Not conserved |
| 7                         | 54738923 Intrinsic G del  | New variant (dbSNP ss 15790494) | NA                              | No risk del/del del/del del/del del/del del/del del/ del NA |
| 8                         | 54738927 Intrinsic - A    | New variant (dbSNP ss 315790495) | NA                              | No risk A/A A/A A/A A/A A/A NA |
| 9                         | 54734456 Intrinsic T C    | rs22559532                 | NA                               | 0-2 C/C C/T C/T C/C C/C C/C Not conserved |
| 10                        | 54734415 Intrinsic A G    | rs22559522                 | NA                               | No risk A/A A/G A/G G/G G/G G/G Conserved in 10/10 |
| 11                        | 54717953 Exonic G A      | New variant (dbSNP ss 315790496) | Synonymous Splicing regulatory (score 85.4) | 1-4 G/G G/G G/G A/A A/A A/A Conserved in 7/10 |
| 12                        | 54715779 Intrinsic T C    | rs22578128                 | NA                               | 0-2 C/C C/T C/T T/T T/T T/T Conserved in 2/10 |
| 13                        | 54709793 Intrinsic (spice site) 17-T repeat (wild) 22-T repeat (variant) | New variant (dbSNP ss 315790497) | Alternative splicing regulatory (score 3.39) | 3-4 variant/variant variant/wild variant/wild variant/wild variant/wild Conserved in 10/10 |
| 14                        | 54699432 UTR C T          | New variant (dbSNP ss 315790498) | NA                              | 0-2 C/C C/C C/C T/T T/T T/T Not conserved |
| 15                        | 54698793 UTR G T          | New variant (dbSNP ss 315790499) | NA                              | 1-3 T/T T/T T/T T/T T/T NA |
| 16                        | 54698788 UTR C T          | New variant (dbSNP ss 315790500) | Transcription regulatory (score 85.4) | 1-3 T/T T/T T/T C/C C/C C/C Conserved in 7/10 |
Table 2  **PTPN22** sequence variants identified by sequencing genomic DNA from three atopic and three non-atopic WHWTs. (Continued)

|   | S4698729 | UTR   | T     | C     | New variant (dbSNP ss315790501) | NA | 1-3 | C/C | C/C | C/C | C/C | C/C | C/C | NA |
|---|----------|-------|-------|-------|---------------------------------|----|-----|-----|-----|-----|-----|-----|-----|----|
| 17|          |       |       |       |                                 |    |     |     |     |     |     |     |     |    |
| 18|          |       |       |       |                                 |    |     |     |     |     |     |     |     |    |

Sequence variants with a predicted medium to high disease-associated functional effect, with strongly conserved sequence across 10 mammals (dog, human, pig, horse, mouse, rat, cattle, chimpanzee, gorilla and orangutan) and differential distribution between atopic and non-atopic dogs are underlined (Sequence variant identities 1, 2, 11, 13 and 16).

*reverse strand; *based on the 1.5× poodle genome (version 1) and the boxer 7.6× whole-genome sequences (CanFam2.0), accessed in March 2010 from http://www.ncbi.nlm.nih.gov and http://genome.ucsc.edu; *as predicted by FASTSNP [5]; disease-risk possibilities are 0 (no potential functional risk), 1 (very low risk), 2 (low risk), 3 (medium), 4 (high risk) and 5 (very high risk); FASTSNP provides a “risk score” for each SNP based on its putative biological function; *analyzed following genomic alignment of flanking regions containing the genetic variants in 10 possible species (dog, human, pig, horse, mouse, rat, cattle, chimpanzee, gorilla and orangutan); UTR: untranslated region (DNA); NA: not accessed; del: nucleotide deletion.
product 8), 57°C (3 and 14), 58°C (7, 10 and 13), 64°C (5) or 60°C (remaining products) annealing temperatures. PCR products were purified with MinElute PCR Purification Kit (QIAGEN Pty Ltd, Doncaster, Vic, Australia), and bi-directionally sequenced at the Australian Equine Genetics Research Centre using 0.5 μM (PCR product 3, 4, 5, 12, 14) or 1 μM (remaining PCR products) of forward and reverse amplification primers and 0.5 μM of internal sequencing primers (Table 1), and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Primers were supplied by GeneWorks (Hindmarsh, SA, Australia).

Sequencing protocol was as recommended by the manufacturer, except annealing temperatures for PCR products 3, 9 and 11 were 50°C and 60°C for PCR products 4 and 5.

Sequence data were analyzed with ChromasPro v1.5 (Technylisium, Tewantin, Qld, Australia) and compared with the 1.5× poodle (version 1) and the boxer 7.6× whole-genome sequences (CanFam2.0). Among 18 variants identified [14], five variants showed a medium to high disease-associated risk as predicted by FASTSNP [15] and Mutation Taster [16]; three single-nucleotide polymorphisms (SNPs) in a predicted regulatory region of the gene, one synonymous SNP, and a variable sequence repeat in a predicted splice site (Table 2). These variants formed five different haplotypes (Table 3).

There were no recombinant events within this 12.6 Kb interval.

Variant sequence repeat c.2137-20 T(17_22) (Figure 1) has not been previously reported in dogs or other species and was bioinformatically predicted to have indirect structural effects on PTPN22. Comparable intronic repeat variations might interfere with normal gene expression [17-19] and have been associated with alternative splicing and disease in humans [20-23]. Thus, fluorescently labelled, amplified-fragment length genotyping of this variant was performed in 72 WHWTs, including 54 dogs from the GWAS. Primers and PCR conditions for amplification of PCR product 11 were used. Genotyping was performed on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analyzed using Genemapper (Applied Biosystems, Foster City, CA, USA). SIB-PAIR [24] showed no significant evidence for allelic association between this variant and the trait (gene-dropping simulation method, \( P = 0.01 \)). Large scale genotyping and complementary genomic and proteomic assays would be required to assess any potential effect of the remaining genetic variants in PTPN22.

**Availability of supporting data**

The data set supporting the results of this article is available in the National Center for Biotechnology Information Reference Assembly dbSNP repository, http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=O_LEARY_ATOPY.

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**Table 3 Haplotypes constructed using 18 genetic variants of PTPN22**

| Haplotype | Number of chromosomes |
|-----------|-----------------------|
|           | Atopic dogs | Non-atopic dogs |
| A         | C-del-G-T-T-del-A-C-A-C-C-variant\(^{5,7,11}\)-C-T-T-C-T | 4/6 | 0/6 |
| B         | T-A-A-C-C-G-del-A-T-G-C-T-wild\(^{12,14}\)-C-T-T-C-T | 2/6 | 0/6 |
| C         | C-A-G-C-C-A-del-A-C-G-T-wild\(^{15}\)-T-T-C-C-T | 0/6 | 4/6 |
| D         | C-A-G-C-C-A-del-A-C-G-T-wild\(^{15}\)-T-T-C-C-G | 0/6 | 1/6 |
| E         | C-del-G-T-T-G-del-A-C-A-C-T-variant\(^{5,7,11}\)-T-C-C-T | 0/6 | 1/6 |

*maximum-likelihood (Log likelihood = - 108.87) haplotype assignment for the dogs as predicted by Superlink [7]; \(^{5}\)22-T repeat allele; \(^{1}\)17-T repeat allele; del: nucleotide deletion

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**Figure 1 Relative location of the variant sequence repeat c.2137-20 T(17_22) in canine PTPN22.** Exons in the gene are marked in yellow, variants annotated in web-based databases are in green and the new intronic variant identified by sequencing in three atopic and three non-atopic WHWTs is highlighted in pink. Line numbering is relative to coordinate system.
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Authors’ contributions
JBR was responsible for all experimental procedures, analysis and interpretation of data, manuscript writing and editing; CAO conceived and coordinated the study, contributed to the experimental design and to manuscript editing; PG contributed to experimental procedures and analysis of data; LV, KM and MS were responsible for the diagnosis and recruitment of dogs. All authors contributed to the critical revision and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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