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Haplotype sharing excludes canine orthologous *Filaggrin* locus in atopy in West Highland White Terriers

J. Barros Roque*, C. A. O'Leary*, M. Kyaw-Tanner*, M. Latter*, K. Mason[†], M Shipstone[‡], L Vogelnest[§] and D. L. Duffy[¶]

*School of Veterinary Science, The University of Queensland, St Lucia Queensland 4072, Australia. [†]Dermcare, 22 Aranda St, Springwood, Queensland 4127, Australia. [‡]Veterinary Specialist Services, 263 Appleby Rd, Stafford Heights, Queensland 4053, Australia. [§]The University of Sydney, University Veterinary Teaching Hospital, Camden New South Wales 2570, Australia. [¶]Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Herston Queensland 4029, Australia

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Source/description: Atopic dermatitis (atopy) is an inherited, allergic skin disorder of dogs[1] and humans,[2] and is common in West Highland White Terriers (WHWT).[3] Null mutations in *Filaggrin* (*FLG*) occur in 18–48% of humans with atopic dermatitis, compared with 7% of the general population.[4] *FLG* encodes a protein involved in epidermal barrier maintainance,[4] with impaired epidermal function contributing to the development of canine atopic dermatitis[5] by enhancing antigen presentation to the immune system.[6] The role of *flg* in atopy in WHWT was examined.

Methods: The gDNA was extracted from EDTA blood from 49 WHWT with atopy and 30 without atopy (Table S1) with a QIAamp Blood Midi Kit (Qiagen GmbH, Hilden, Germany), saline extraction,[7] or phenol extraction.[8] Canine *flg* was localized using BLASTN (http://www.ensembl.org) to CFA17 at 64.296–64.305 Mbp (genome build CANFAM2.0 May 2006). Fifteen genetic markers nearby were identified (Tables S2 & S3). Primers were supplied by GeneWorks (Adelaide, Australia). M13-labelling was used (Tables S4–S6),[9] and genotyping was performed on a 3130xl Genetic Analyzer (Hitachi/Applied Biosystems, Foster City, CA, USA), using POP 7 Polymer, size standard GENESCAN 500 Liz or GENESCAN 1200 Liz (ABI Biosystems, Foster City, CA, USA). Analysis was carried out using GENEMAPPER.

Preliminary pedigree analyses were carried out using Sib-pair,[10] including testing for Mendelian errors. A segregation analysis was carried out using MENDEL 9.0,[11] RELPAIR[12] confirmed the recorded pedigree information for first degree relationships from the marker data. Haplotypes were constructed using SIMWALK2,[13] and used for association and non-parametric linkage analysis in Sib-pair. Association analysis was carried out using a simulation-based method. Multipoint non-parametric and parametric linkage analyses were performed using SIMWALK2 and SUPERLINK,[14] in the latter maximizing over fully penetrant dominant and recessive models, assuming 50% and 90% allele frequencies in founders. The markers were ordered using positions in the canine sequence and confirmed using Mendel.

Results: As a result of complex ascertainment, the dataset was not very informative for segregation analysis of atopy. Observed inheritance patterns were equally consistent with a common fully penetrant dominant (risk allele frequency $P_D = 0.5$, model log likelihood LL = −19.28) or recessive ($P_D = 0.9$, LL = −18.91) major locus.

The observed intermarker recombination was consistent with the chosen genetic marker map (Table S2). There were no observed recombinants among the fine-mapping markers genotyped close to *flg* (two-point lod scores: M1–M4, maximum lod score $Z_{max} = 5.72$ at recombination fraction $c = 0$; M4–M8, $Z_{max} = 4.02$ at $c = 0$). Haplotypes were constructed (Table S7). The haplotype frequencies did not differ significantly between affected and unaffected animals ($P = 0.29$). Non-parametric linkage analysis failed to demonstrate linkage of the microsatellite marker haplotypes to atopy (affecteds-only analysis $P = 0.95$; general pairs method $P = 0.45$). On parametric linkage analysis under the dominant and recessive models, the LOD score for atopy to the haplotype (at $c = 0$) was LOD = −0.4.

Comments: This study excludes a large causative role for the canine *flg* orthologue in atopy in WHWT. We had expected that atopic disease in WHWT was likely to be monogenic, or to perhaps oligogenic, due either to a founder mutation or hitchhiking with a breeder-selected trait. Under either of these hypotheses and high trait prevalence, we would expect the gene to be highly penetrant and relatively common, thus displaying co-segregation of flanking marker haplotypes with disease. If such a haplotype was measured, our association analysis would have 100% power to detect its effect if fully penetrant, and 55% power if the penetrance was 50%. We therefore cannot exclude a role for mutations with small effect sizes.

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Correspondence: C. O'Leary (c.oleary@uq.edu.au)

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Supporting information

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Table S1 Diagnostic criteria for selection of dogs in this study.

Table S2 Information on 8 published genetic markers used in the study.

Table S3 Microsatellite details and amplification primer sequences for three newly identified microsatellites (M1, M4, M5) using Tandom Repeats Finder http://research.nhgri.nih.gov/dog_genome/ and four from http://www.vgl.ucdavis.edu/dogset/ (M8, M9, M11, M12) used in this study.

Table S4 Amplification conditions and PCR components in a 10-μl reaction volume using Multiplex PCR Kit (Qiagen GmbH, Germany).

Table S5 Thermocycling protocol for microsatellites *FH3369*, *PEZ8*, *FH3995*, *REN240A05* (multiplex 3) and *FH3047*, *REN294E18*, *FH4023* and *FH2869* (multiplex 4).

Table S6 Thermocycling Protocol for *M1*, *M4* and *M5* (multiplex 1), *M8*, *M9*, *M11* and *M12* (multiplex 2).

Table S7 Haplotypes constructed using microsatellite markers (*M12-M11-M9-M1-M4-M8*) in the region of the canine *flg* orthologue.

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