

Genome-wide association analysis of canine atopic dermatitis and identification of disease related SNPs

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Abstract In humans, genome-wide association studies (GWAS) have been shown to be an effective and thorough approach for identifying polymorphisms associated with disease phenotypes. Here, we describe the first study to perform a genome-wide association study in canine atopic dermatitis (cAD) using the Illumina Canine SNP20 array, containing 22,362 single-nucleotide polymorphisms (SNPs). The aim of the study was to identify SNPs associated with cAD using affected and unaffected Golden Retrievers. Further validation studies were performed for potentially associated SNPs using Sequenom genotyping of larger numbers of cases and controls across eight breeds (Boxer, German Shepherd Dog, Labrador, Golden Retriever, Shiba Inu, Shih Tzu, Pit Bull, and West Highland White Terriers). Using meta-analysis, two SNPs were associated with cAD in all breeds tested. RS22114085 was identified as a susceptibility locus ($p=0.00014$, odds ratio=2) and RS23472497 as

a protective locus ($p=0.0015$, odds ratio=0.6). Both of these SNPs were located in intergenic regions, and their effects have been demonstrated to be independent of each other, highlighting that further fine mapping and resequencing is required of these areas. Further, 12 SNPs were validated by Sequenom genotyping as associated with cAD, but these were not associated with all breeds. This study suggests that GWAS will be a useful approach for identifying genetic risk factors for cAD. Given the clinical heterogeneity within this condition and the likelihood that the relative genetic effect sizes are small, greater sample sizes and further studies will be required.

Keywords Canine · Atopic dermatitis · Polymorphism · Genome-wide association study · SNP

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Introduction

Canine atopic dermatitis (cAD) is a common and often severe pruritic, inflammatory skin disease that is considered to be a naturally occurring, spontaneous model of human atopic dermatitis (eczema). The pathogenesis of the disease in both humans and dogs is strongly associated with immunological hyper-reactivity, although skin barrier function and microbial colonisation and infection are also thought to have a role (Olivry and Hill 2001; Vickery 2007). The prevalence of atopic dermatitis (AD) over the last 3 decades has increased in both dogs (Hillier and Griffin 2001) and humans (Williams 1992), two- to threefold in industrialised countries, (Williams 1992) suggesting a common environmental and/or genetic components in both species.

Both human and canine AD are thought to have a complex genetic basis, unlike most murine models of AD which are associated with single-gene defects. Being a complex disease

means that multiple genes and environmental factors are likely to contribute to disease pathogenesis. Therefore, unlike other animal models, dogs are of particular interest as they both share the same environment as humans and have similar spontaneous pathological disease features (Marsella and Girolomoni 2009; Marsella and Olivry 2003). Canine AD is therefore a good model and homologue for the counterpart human condition.

It has been clearly demonstrated that human AD is a complex disease with several genetic factors contributing to the pathology observed (Cookson 2004; Hoffjan and Epplen 2005). Genes involved in skin barrier formation, and the adaptive and innate immune systems have all been implicated in the aetiopathogenesis of human AD. However, not all the factors contributing to AD pathogenesis have been identified.

The strongest association found in human AD is with two independent losses of function variants in filaggrin, R501X, and 2282del4 (Smith 2006). Filaggrin is an epidermal barrier protein that contributes to barrier formation by causing the cells to compact into squames by aggregating to the keratin cytoskeleton (Rawlings and Harding 2004; Rawlings and Matts 2005). It is therefore likely that these loss of function variants are associated with poor skin barrier function.

However, the innate immune response has also been implicated in human AD through pattern recognition receptors (PRRs; Janeway and Medzhitov 2002). PRR activation leads to the production of chemokines, cytokines, and recruitment of immune cells. It also determines the extent of the adaptive immune response (Hammad and Lambrecht 2008). Single nucleotide-polymorphisms (SNPs) in Toll-like Receptor 2 (TLR)2, a PRR, has been associated with certain extreme cases of AD (Ahmad-Nejad et al. 2004), although this has not been confirmed by other studies (Akdis et al. 2006). Also, TLR9 has shown to be associated with intrinsic AD (i.e., the non-allergen mediated form of the disease) cases (Novak et al. 2007). The adaptive immune system has also been implicated in AD with the Th2 cytokine cluster genes, IL-3, IL-4, IL-5, IL-13, and granulocyte-macrophage colony stimulating factor, all associated with AD (Morar et al. 2006; Oiso et al. 2000; Rafatpanah et al. 2003; Tsunemi et al. 2002).

These associations demonstrate the complex involvement of multiple loci in human AD. Based on the similar pathology seen between canine and human AD, it is thought that similar associations would be seen in genetic studies of cAD, but no association studies to investigate this have yet been reported.

Strong evidence exists for a genetic contribution to cAD from clinical observations of breed associations. Breeds predisposed to cAD include West Highland White Terriers (WHWT) and boxers (Schick and Fadok 1986; Sousa and

Marsella 2001; Vollset 1985). In contrast, sighthounds (e.g., greyhounds and related breeds) are rarely affected suggesting a genetic component to cAD resistance (Tarpataki et al. 2006). Further evidence for a genetic component in cAD comes from pedigree analysis. A study of British guide dogs, for example, demonstrated an heritability co-efficient of 0.47 (Shaw et al. 2004). Despite this, no genetic association or linkage studies have been done and molecular studies have been limited to cytokine profiling and gene expression experiments (Merryman-Simpson et al. 2008; Nuttall et al. 2002). These have demonstrated similar findings to those reported in human AD.

In humans, GWAS have been shown to be an effective approach in identifying gene polymorphisms associated with disease phenotypes. GWAS approaches use simultaneous high density SNP genotyping and aim to provide coverage across the complete genome. This hypothesis-free approach allows many novel disease susceptibility loci to be discovered in a single experiment. Given the chance of false positive discovery through multiple testing, these studies should be repeated to validate the findings.

For example, the Wellcome Trust Case Control Consortium have recently had great success in identifying novel SNPs in bipolar disorder, coronary heart disease, Rheumatoid arthritis, and type 1 and 2 diabetes (The Wellcome Trust Case Control Consortium 2007). Moreover, GWAS was recently used to investigate human AD in approximately 1,000 cases and 1,000 controls, and an association was found with an intergenic SNP on chromosome 11; however, validation in approximately 3,000 cases and 4,000 controls was required to confirm this association (Esparza-Gordillo et al. 2009). This demonstrates that human disease studies have to be performed on large numbers of cases and controls (usually multiple thousands) to achieve sufficient statistical power to detect small genetic risk factors (≤ 1.5). In recent human AD study, the chance of identifying a risk factor of 1.5 was 84%, but this dropped to 12% when identifying a risk factor of 1.2 (Esparza-Gordillo et al. 2009). The most recent SNP arrays used in human studies are of greater density (up to 1.6×10^6 SNPs), which is needed to detect association effects in humans where linkage disequilibrium (LD) across the genome is weak (10–100 kb (Andersson 2009; Reich et al. 2001)).

In contrast to humans, domestic dog breeds are of recent origin and represent highly inbred genetic isolates. Consequently, LD extends over long distances (0.8–5 Mb) (Lindblad-Toh et al. 2005; Sutter et al. 2004). This is highly advantageous in GWAS as fewer genetic markers and smaller sample sizes are required to find disease associations in dogs (Starkey et al. 2005; Sutter et al. 2004). For example, to achieve complete coverage of the dog genome, only 5,000 to 30,000 SNP markers are required (Andersson 2009), compared to an estimated

200,000 to 500,000 SNP markers in humans (Starkey et al. 2005), making GWAS in dogs both cheaper and easier to conduct (Starkey et al. 2005; Sutter et al. 2004).

High density SNP arrays for GWAS in dogs are now available and are being used to analyse both single-gene and complex canine diseases (Karlsson et al. 2007; Drögemüller et al. 2008; Wiik et al. 2008; Awano et al. 2009). We are the first to apply GWAS technology to analyse cAD in Golden Retrievers and then re-examine these findings in eight dog breeds.

Methods

The first stage of this study focused on genotyping atopic and healthy control Golden Retrievers on the canine GWAS array to identify potentially associated disease loci in cAD. The results from the GWAS were then used to inform SNP selection for the second stage of this study which comprised a genotyping validation study on a large number of cases and controls across eight breeds using Sequenom-based typing.

Sample selection

The initial GWAS included 48 Golden Retrievers ranging from 1 to 14 years with a mean of 7 years. There were 25 (14 female, 11 male) with atopic dermatitis and 23 healthy controls (nine female, 14 male).

Six hundred and fifty-nine DNA samples were included in the validation study (41 of these were samples included on the GWAS array to check genotyping concordance). Eight breeds were included in this part of the study. The breakdown of the breeds, geographic regions, and disease status are summarised in Table 1.

Residual blood samples excess to diagnostic requirements were made available from UK, Japanese, and US veterinary clinics for both atopic dogs and healthy controls.

The diagnosis of cAD was made by specialists in veterinary dermatology and was based on compatible history, clinical signs, and exclusion of other causes of pruritus (DeBoer and Hillier 2001). Additional control DNA samples for the validation study were obtained through the UK DNA Archive for Companion Animals (http://www.liv.ac.uk/dna_archive_for_companion_animals/, last accessed 01/07/09).

DNA was extracted from ethylenediamine/tetraacetic acid (EDTA) blood samples using Qiagen Qiaamp DNA blood midi kits (Crawley, UK) as previously described (Clements et al. 2007). The DNA was measured using a Nanodrop® spectrophotometer ND-1000 (Thermo scientific, Delaware, USA) and normalised to 50 ng/μl in 10×TE (100 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0).

GWAS array

The Illumina Canine SNP20 (San Diego, California, USA) was used for this study; it includes 22,362 canine SNPs from the CanFam 2.0 assembly which is based on the boxer dog with the partial sequence of a standard poodle (genome coverage of 1.5 times) and 100,000 sequence reads from nine different breeds. The SNPs used were highly polymorphic meaning they are viable for use in breeds other than the poodle and Boxer (Karlsson and Lindblad-Toh 2008). The SNP array analysis of the 48 Golden Retriever samples was performed by the Barts and the London Genome Centre according to the manufacturers' instructions using Illumina's software to call the data.

GWAS data were analysed using the freely available whole-genome association analysis toolset PLINK (Purcell et al. 2007). All data were subject to quality control procedures; only SNPs which conformed to Hardy-Weinberg expectations (HWE) exhibited 90% genotyping success and had a minor allele frequency ≥ 0.01 were included in subsequent analysis. Two hundred and twenty-nine SNPs were excluded on this basis. To compare atopic dogs and

Table 1 Samples for genotyping validation study

Breed	Case				Control				Grand total
	UK	USA	JAPAN	Total	UK	USA	JAPAN	Total	
Boxer	11	-	-	11	24	-	-	24	35
GSD	10	9	-	19	23	15	-	38	57
Labrador	23	33	8	64	75	43	11	129	193
G. Retriever	4	23	13	40	28	21	17	66	106
Shiba Inu	-	-	23	23	-	-	33	33	56
Shih Tzu	2	6	39	47	12	2	48	62	109
Pit Bull	-	20	-	20	-	17	-	17	37
WHWT	14	4	-	18	34	14	-	48	66
Total				242				417	659

healthy controls, the Fisher's exact test was performed and the Benjamini and Hochberg correction/false discovery rate was applied (Benjamini and Hochberg 1995). Significance values from this were used to generate a whole-genome association plot of significance using the statistical package R (<http://www.r-project.org/>). This displays the results for each chromosome as a log of the p value. A Q-Q plot to show the difference between observed and expected results was also generated. Using a cut off of 0.05 HWE and a 95% confidence interval, the top 40 SNPs were selected for validation.

Validation study

Forty SNPs were selected for validation as described above. Probe and primer sequences for Sequenom-based genotyping are listed in supplementary file 1.

Polymerase chain reaction assays were all performed in 384-well plate format. A 5 μ l reaction volume was used per well, and this consisted of 0.1 μ l hot start Taq (5 U/ μ l, Qiagen, Crawley, UK), 0.5 μ l each of 500 nM forward and reverse primers (Metabion, Germany), 0.325 μ l MgCl (25 mM, Qiagen, Crawley, UK), 0.1 μ l dNTPs (25 mM), 0.625 μ l $\times 10$ buffer (Qiagen, Crawley, UK) 0.35 μ l distilled water, and 2.5 μ l of DNA at 10 ng/ μ l. Amplification cycles comprised 35 cycles at 94°C for 15 min, 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min with a final elongation phase of 72°C for 3 min.

Shrimp Alkaline Phosphatase clean, the iPLEX reaction, and the resin clean were all performed as stated by Sequenom (San Diego, CA, USA).

Spectro chip® arrays were spotted using a nanodispenser, according to the manufacturers' instructions (Sequenom, San Diego, CA, USA). A Sequenom MALDI-TOF spectrophotometer was used to read the array.

The SpectroAquire and MassARRAY Typer Software packages (Sequenom, San Diego, CA, USA) were used for interpretation and Typer analyser V3.4.0.18 was used to review and analyse all data.

PLINK (Purcell et al. 2007) was used for all the downstream analysis, and validation data were subject to the same quality control procedures as described for GWAS. On this basis, six of the 40 SNPs tested were excluded (rs23567144, rs22020166, rs22155657, rs9081246, rs22959786, and rs23122280). A variety of association tests were undertaken (allelic association, logistic regression, and genotypic model), using PLINK, on all breeds separately. Meta-analysis (Cochran-Mantel-Haenszel test for $2 \times 2 \times K$ stratified tables) was also undertaken to analyse these data as a whole, taking into account the variables between them (e.g., breed and geographic region, as defined from supplied phenotype information). This eliminates false positives due to population substructure (Tian et al. 2008). The p values were corrected for multiple testing by using the number of independent loci (38 in this study).

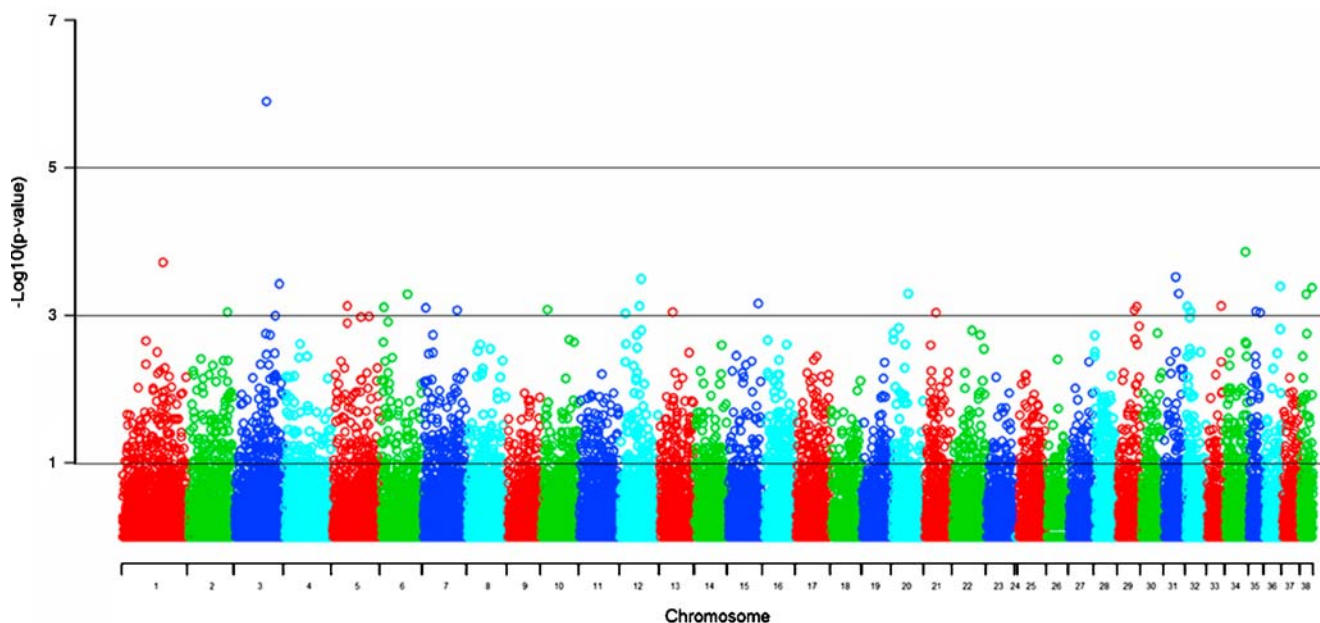


Fig. 1 Whole-genome association plot of significance for canine atopic dermatitis. A plot showing significance of association of all SNPs in the first stage GWAS Fisher's exact test with atopic and

healthy control phenotypes. SNPs are plotted on the x-axis according to their position on each chromosome against association with atopic dermatitis on the y-axis (shown as $\log_{10} p$ value)

Results

GWAS study

Figure 1 shows a genome-wide significance plot of logged p values for the GWAS analysis. One SNP was over the log5 threshold and 35 SNPs were over the log3 threshold, this means that these SNPs are significantly associated in cAD. The confidence intervals and odds ratios of these results were used to select the top 40 SNPs for the validation study, (Table 2). Some SNPs on the same chromosome were selected suggesting a haplotypic effect.

Validation study results

The 41 samples genotyped on both the GWAS and Sequenom arrays showed an average concordance of 99% for all SNPs genotyped.

Sequenom SNP data from all breeds were combined and analysed using meta-analysis to account for the differences between breeds and geographic region.

Table 3 shows the corrected p value significant SNPs from the meta-analysis analysis, and compares the results to the GWAS discovery study results and a combined meta-analysis of the GWAS discovery data and the validation study data. The full results from the meta-analysis are presented in supplementary data file 2.

Further to this, each breed was analysed by meta-analysis to account for differences in geographic region. The breeds were then split into separate geographic regions to perform allelic association, genotypic model, and logistic association tests (presented in supplementary file 3).

A conditional regression was undertaken on the two SNPs identified by meta-analysis as statistically significant in cAD. The p values were significant in the test indicating that the two SNPs are independent of each other (data not shown).

Discussion

Thirteen SNPs were found to be associated with cAD (supplementary files 2 and 3). Two of the 12 SNPs were associated with cAD in all eight breeds tested (Table 3). These two SNPs (rs22114085 and rs23472497, Table 3) are both intergenic within chromosome 10 and 29, respectively. A conditional regression analysis (data not shown) showed that these two SNPs are independent of each other; the odds ratios also indicate that one is a protective SNP and the other a susceptible SNP. The breeds were also analysed separately for associations, showing that rs22114085 was significantly associated with cAD in UK Labradors (see supplementary file 3). All Golden Retrievers showed a significant association with rs23472497, but individual

Table 2 Top 40 SNPs from GWAS analysis

SNP	Chromosome	Odds ratio	p value
rs22859255	2	4.4	0.0048
rs23545080	3	5.4	0.0019
rs23567144	3	0.2	0.0018
rs23602938	3	3.8	0.0047
rs24872415	3	9.5	1.27×10^{-06}
rs24194054	5	4.8	0.0011
rs24267550	5	0.2	0.0042
rs24318716	6	0.3	0.0076
rs24327271	6	0.2	0.0008
rs24332727	6	0.2	0.0012
rs24408651	7	0.2	0.0008
rs24482628	8	4.1	0.0025
rs22020166	10	3.8	0.0022
rs22114085	10	5.1	0.0008
rs22155657	11	5.9	0.0063
rs22181912	12	0.3	0.0028
rs22184220	12	4.3	0.0009
rs22185491	12	3.4	0.0045
rs22212359	12	7.2	0.0016
rs22212677	12	5.3	0.0049
rs24732893	12	0.2	0.0007
rs22328353	14	5.8	0.0025
rs22523565	16	3.3	0.0067
rs8649732	19	0.2	0.0044
rs22864357	20	4.1	0.0025
rs22913552	20	4.6	0.0005
rs22915894	20	0.2	0.0017
rs8806978	20	3.8	0.0021
rs9081246	20	0.2	0.0017
rs22959786	21	5.9	0.0009
rs22989468	21	0.2	0.0057
rs23026134	22	5.4	0.0019
rs23066224	22	7.2	0.0016
rs23122280	23	5.4	0.0019
rs23428471	28	3.6	0.0036
rs23478400	28	3.6	0.0036
rs23472497	29	0.2	0.0009
rs23690306	31	5.3	0.0003
rs23768387	32	0.2	0.0031
rs23828846	33	0.2	0.0007

breed associations with these two SNPs were not seen in the other breeds. This may have been due to the low sample sizes in some breeds affecting statistical power. These results suggest that the cAD associated SNPs, rs22114085, and rs23472497 may represent novel loci for further re-sequencing and fine mapping.

Table 3 Comparison of the validation study meta-analysis, GWAS analysis, and a combined analysis of validation and GWAS data

SNP	GWAS discovery study		Validation study—meta-analysis		Combined validation study and GWAS meta-analysis	
	Odds ratio	Corrected <i>p</i> value	Odds ratio	Corrected <i>p</i> value	Odds ratio	Corrected <i>p</i> value
RS22114085	5.1	0.00084	2.0	0.00014	2.0	4.8×10^{-05}
RS23472497	0.2	0.00086	0.6	0.0015	0.6	0.0011

An individual breed analyses was also undertaken, as it is unknown whether cAD has a common genetic cause in all breeds. However a lack of inter-breed correspondence between suggested diseases markers may also be due to a lack of statistical power in this study. The results of this analysis are presented in supplementary file 3. Thirteen SNPs were associated with cAD in various breeds, but as the majority of the associations found were intergenic (not within genes) only the intronic associations will be discussed here.

In all Golden Retrievers, two intronic SNPs (rs23602938 [PROM1] and rs22859255 [RAB3C]) were significantly associated with cAD. RAB3C is potentially relevant to the pathogenesis of cAD, as it codes for a protein transporter found on lipid anchors (Cheng et al. 2002) that may be important in skin barrier function. The importance of lipids and skin barrier function is thought to be important in the pathogenesis of both human and canine AD (Callard and Harper 2007; Hoffjan and Stemmler 2007; Olivry and Hill 2001; Piekutowska et al. 2008; Vickery 2007). Moreover, using the GWAS data, we have demonstrated that this cAD-associated SNP forms a significant haplotype with another intronic SNP from RAB3C (rs22859255 and rs22784610, *p* value=0.00039, data not shown). This finding, coupled with the validation of this SNP by Sequenom, indicates that this area is of potential importance in cAD.

In the Labrador meta-analysis, no significant associations were found, but when the analysis was split into geographic regions the UK Labradors and WHWTs showed associations with an intronic SNP within RAB7A, rs22915894, and rs8806978 which is downstream of RAB7A. RAB7A produces a protein which is a cellular component of the melanosome, containing melanin for skin pigmentation. The association with cutaneous metabolism suggests that RAB7A could be involved in cAD although the precise pathogenesis is unclear. These results may also suggest that there is a haplotypic effect and further investigation by fine mapping of this region is required. Interestingly, these SNPs were shown to be significant before multiple testing corrections in all breeds on the validation study meta-analysis (see supplementary file 3). This could suggest that SNPs in linkage in this region could be causative or have a haplotypic effect in other breeds.

Interestingly, rs24872415, an intronic SNP within SORCS2, which was the most significant SNP on the

GWAS analysis ($p=1.27 \times 10^{-6}$, odds ratio 9.55) was only associated with cAD in UK German Shepherd dogs in the validation study, and the level of significance was lower than as seen in the GWAS. This finding could therefore be a false positive, potentially caused by unrecognised population structure.

The validation of the GWAS results does indicate that the use of GWAS as a novel SNP discovery tool is valid in dogs. However, there are some weaknesses with this study. Firstly, the numbers used on the GWAS array were very small, especially for a complex disease such as cAD. It is possible that SNPs with smaller relative risk factors could have been missed and the lack of statistical power could mean that potentially important SNPs fell below the significance thresholds. The GWAS results may also have been affected by discreet population structure within the breed used, as the Q-Q plot shows a large difference between the observed and expected results. It would, therefore, be better in future studies to select samples for GWAS using ancestry informative markers to initially define the population structure, and then select grouped individuals for inclusion (Tian et al. 2008). This use of ancestry informative markers for the large-scale validation study would have also been beneficial to the analysis of the results.

It is important to note that while the GWAS array used is defined as having good coverage across the genome, the success of the study depends on whether the array includes the causative allele. In this array, there are large gaps in coding regions. Filaggrin, for example, has been demonstrated to be of great importance in human AD (Howell et al. 2007; McGrath 2008; Morar et al. 2007; Nomura et al. 2007; Weidinger et al. 2006), but was not covered by the GWAS array used.

It may also have been beneficial to use the same breed for the validation study to ensure a more direct comparison. The cAD phenotype is clinically heterogeneous (Griffin and DeBoer 2001), which may have caused problems in the across-breed comparisons. It is possible that cAD is associated with different loci in different breeds, as many of the associations found were only in one breed. However, the fact that the SNP is associated in one breed, but not another, doesn't necessarily mean the SNP isn't a risk factor for cAD just that the SNP may have different levels of

penetrance in different breeds (McCarthy and Hirschhorn 2008). There are examples of this in human GWAS where discovery of important SNPs was made in non-European populations first, and then demonstrated to be present in European populations at a different penetrance (Nalls et al. 2008; Unoki et al. 2008; Yasuda et al. 2008). The inclusion of multiple breeds is, nevertheless, supported by the observation that it can be beneficial to analyse multiple populations to increase statistical power, even if the effects of certain variations may differ in different populations (McCarthy and Hirschhorn 2008). Demonstrating the same association in different populations, furthermore, is advantageous to further study design and increases confidence in the results (Lindblad-Toh et al. 2005).

Another weakness of this approach is that GWAS is limited to identifying common SNPs with small effects. It cannot identify rare SNPs with large effects, untyped SNPs, parent of origin effects, and other structural variations (with the exception of copy number variations), e.g., microsatellites, variable number tandem repeats, retro-element insertions, deletions, and duplications.

In conclusion, this is the first report of applying GWAS to cAD, which has been subsequently validated in a larger cohort. In total, 13 SNPs were shown to be associated with cAD, two of which were associated in all eight breeds tested. However, the methods for analysis of GWAS results in dogs still require some development to ensure population structure is adequately controlled for and to increase the likelihood of identifying low penetrance alleles.

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SNP (rs number)	Reverse Primer	Forward Primer	Probe Sequence
rs22020166	ACGTTGGATGGTCTTTCTTCTTAACCTGC	ACGTTGGATGGGAATCAAGAAAGGCTTTAC	taTTAACCTGCATACAAAATTGTTGCT
rs22114085	ACGTTGGATGAAGCTCCACGATGAACAGTC	ACGTTGGATGGACTGTACACGACCTTTACC	CAGCAGCACTTCCAGAATGTACC
rs22155657	ACGTTGGATGATGTTGAAGATTGAATGAC	ACGTTGGATGTATCTAAACCACCAGTTTC	TGTATATAAAGATTTCTCCTCAA
rs22181912	ACGTTGGATGTTCTGCAAGGATGGGCATTG	ACGTTGGATGTCCAGTTTTTCTATGTCCTG	AGAACATGTCCTTGTGG
rs22184220	ACGTTGGATGAGACCATAGCCAGCTTGCAC	ACGTTGGATGTGGCACAATCCCTTTGATGG	GAGTTAAAGGTATCTTCCATAGG
rs22185491	ACGTTGGATGGGGAATACAGCGGTCTTGA	ACGTTGGATGTTGTTCTCCTTCATCCTGGG	GGAATACAGCGGTCTTGACGGGTTG
rs22212359	ACGTTGGATGAATCCCTCTTTTCCCCTCTG	ACGTTGGATGGCACAAGATGGAAGATGGAG	CCCCTCTGAAATTCCCTTT
rs22212677	ACGTTGGATGTAACCTTCTGCTCAGAAATG	ACGTTGGATGTGTGGTTTTGTATTCAAGGG	AATTGAGAATAATCAGTGAAATAG
rs22328353	ACGTTGGATGACCTGCTACCAGCATTAAAG	ACGTTGGATGTTGCTTTCTCTCCCTTAG	GCTACCAGCATTAAAGCTTTAAC
rs22523565	ACGTTGGATGAGACTGGATCTCTTGCTGTG	ACGTTGGATGGAAAAGGAGCTGTGAGGATG	CTTCACAAACTCTTGCC
rs22859255	ACGTTGGATGGCATCTCAAATATTTCTGGG	ACGTTGGATGTCAGAGAAAGCCAAAAGCCG	TCTGGGTAAGAGGAACA
rs22864357	ACGTTGGATGAGATCTCGCACGTGTACTGG	ACGTTGGATGACTGAGCGACACCACTTCAC	CGTCCTCCACTACCAC
rs22913552	ACGTTGGATGTGAAACAAGATCACAAAGCC	ACGTTGGATGTGTCAGCACTGTGTGTGTTT	cCAAAGCCAAACTGAACAATAATC
rs22915894	ACGTTGGATGGCCACTATCGTGTGCTAC	ACGTTGGATGCTGAGAATGGCTTCACAGAG	TGTCTACAGAACCAGATTTTGAGAAGG
rs22959786	ACGTTGGATGGGATGCAGACCGTCTGGATT	ACGTTGGATGGTCAGCAGACAACACTTAAC	CCGTCTGGATTTCTTA
rs22989468	ACGTTGGATGAGGGAGTCACCTGCAAACCTG	ACGTTGGATGATGCTCACCTTGCGGAACCTC	GCAAATGCAGTTGTGATGCAGTCGTG
rs23026134	ACGTTGGATGAGATCCTGGTTTGGAAGGAG	ACGTTGGATGCTTGGTGAGATCTCCTATGG	GGAGCATTGAGTGAGTCTAT
rs23066224	ACGTTGGATGGGGCTATTAGTGACCCAATG	ACGTTGGATGTCTGCAGTCAGATCAACTGT	CAGAAGTAGGATCATCTGCTC
rs23122280	ACGTTGGATGGATTTCACTCAGCCCCATAG	ACGTTGGATGAAACAGATGTCTCTAGTTGG	AGCCCCATAGAGCTTTCTTTAAAT
rs23428471	ACGTTGGATGCCACATGCTGATTACAAGCC	ACGTTGGATGGACCACTTTTGTCTAGTAAG	CTATTGGAGTTGTATTTATCGACAAAC
rs23472497	ACGTTGGATGGGCCAGAGGATGGGAAATTC	ACGTTGGATGATCTCTGTCACTGCACTAGG	ACCTGTCAAGGTCATGTC
rs23478400	ACGTTGGATGTCTGCCTAGAATCTTGTCAC	ACGTTGGATGTTGCCCCAGACATTTTTTG	gTACTTACATATGCATTTCCCA
rs23545080	ACGTTGGATGCTTCAACCTGAGTGAAGGC	ACGTTGGATGTGTGTTTCTGGAGTCCTACC	TGTGTTTGTGGGGTTA
rs23567144	ACGTTGGATGGATGAGCCCACACACAGAG	ACGTTGGATGTATCCCTGAAGCATCTGGGC	CCCACACACAGAGGCTGCTGGAATA
rs23602938	ACGTTGGATGAGAGGGTCCTGTTGATACAC	ACGTTGGATGGCCAGTAAGCTAGAAGTTCCG	CCCATACTCCTCAGGC
rs23690306	ACGTTGGATGTACTTCACCTGCAAGCTTGG	ACGTTGGATGGGTAGTTGAGGTAGATGAGG	AATAAGCCAATCCTCCTG
rs23768387	ACGTTGGATGTCTCACTTCTCAGTCAGTGC	ACGTTGGATGGAAGAAAGAGATGAGGAGAG	TCACTTCTCAGTCAGTGCTGTCTCATA
rs23828846	ACGTTGGATGACTGCAGACTTCTATCTGG	ACGTTGGATGAGGTCAATCAGACAGTGGTG	TCTGCCCTCGACAA
rs24194054	ACGTTGGATGCAAACAGATTGCTCCGTGTG	ACGTTGGATGGGTACAGAGACCAAGGAAAG	GTTGATGGAGAACAGCATGT
rs24267550	ACGTTGGATGTCAATTTGAGCCCGGACTTG	ACGTTGGATGTCAAGAATGGACCCTCTGAC	CGGGTAGATTCTCATAGTC
rs24318716	ACGTTGGATGGTTGAACAGGACACCTCATC	ACGTTGGATGACTGTTGGAGGAGGCAGGAG	gaATCGGGCCCATTTGTTTCTA
rs24327271	ACGTTGGATGAGAACTCACTCTCTGTGGAC	ACGTTGGATGTGCCAAGAACAAGCCCAAAC	TCTGTGACTAACTGAAA
rs24332727	ACGTTGGATGTGTGGAGAGATGGATGGAAC	ACGTTGGATGATCCTTGAGCAACAGCCTTC	GTGAGGCTGTGTGTCC
rs24408651	ACGTTGGATGTCCTCTACCAAATCTATGTG	ACGTTGGATGGCCAACAGTATGCTGGTTAG	CAAACTATGTGAATAGCCCT
rs24482628	ACGTTGGATGCAGGATGCAGATTTTCCAG	ACGTTGGATGACTTGGTGATTCTATGGAGG	TTCCAGTTTTCCCATAGCCTG
rs24732893	ACGTTGGATGTTCTTTGATTCTCTGGCGG	ACGTTGGATGTTGGTACCAGATCCAGCTAC	ggccGGCGAAGATCCCCAA
rs24872415	ACGTTGGATGTTGGCAAGCCTGACAAATGG	ACGTTGGATGTGTTGAGGAGACTTCACCTG	ACCCAAGTGCCCAACAA
rs8649732	ACGTTGGATGGCTATTCAACTTTGGTGCGG	ACGTTGGATGTGGTTGTTGTCACTAGTTGG	CCTCACAAATCGGCTTAACCT
rs8806978	ACGTTGGATGGATGTGACAAGCCTTTCTGC	ACGTTGGATGAGTCCGTGCTTTATCAAGCC	AGAGGCACCACATGTGCCAGTTA

rs9081246	ACGTTGGATGGACCCAAAGATATTCTGACC	ACGTTGGATGCCGCCCATGTCTCATGAATA	AAAGATATTCTGACCTAAAATACTATG
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GWAS validation on Sequenom: Meta analysis

CHR	SNP	A1	A2	BP	CHISQ	P	corrected p	OR	L95	U95
10	rs22114085	T	C	14087517	21.42	3.69E-06	0	1.98	1.47	2.65
29	rs23472497	A	G	34162404	16.88	3.99E-05	0	0.6	0.46	0.76
6	rs24332727	A	G	16258930	7.13	0.01	0.29	1.4	1.09	1.79
3	rs23545080	C	A	69975205	7.08	0.01	0.3	1.49	1.1	2.02
16	rs22523565	T	C	12801246	6.94	0.01	0.32	1.41	1.09	1.83
12	rs22181912	C	T	35696615	6.73	0.01	0.36	1.37	1.08	1.75
20	rs22915894	T	C	5771454	6.36	0.01	0.44	1.36	1.07	1.73
12	rs22184220	A	G	13111985	6.09	0.01	0.52	1.35	1.06	1.72
20	rs8806978	T	C	5779740	4.98	0.03	0.98	1.32	1.03	1.69
6	rs24327271	A	C	8499991	4.96	0.03	0.99	0.74	0.57	0.97
32	rs23768387	C	T	30365964	4.77	0.03	1.1	0.73	0.55	0.97
3	rs23602938	C	G	67125807	4.55	0.03	1.25	1.35	1.02	1.77
12	rs24732893	G	A	40204012	3.77	0.05	1.98	0.77	0.58	1.01
19	rs8649732	A	G	45495526	3.65	0.06	2.13	0.77	0.58	1.01
7	rs24408651	A	G	7829497	3.59	0.06	2.21	1.28	0.99	1.66
2	rs22859255	T	C	48522803	2.72	0.1	3.76	1.24	0.96	1.61
28	rs23478400	C	G	3406416	2.51	0.11	4.29	1.22	0.95	1.57
6	rs24318716	C	T	4224712	2.46	0.12	4.44	0.8	0.6	1.06
28	rs23428471	G	A	3366214	1.58	0.21	7.94	1.18	0.91	1.52
22	rs23026134	T	C	55676006	1.2	0.27	10.39	1.19	0.87	1.63
20	rs22913552	T	G	33818500	0.9	0.34	13.05	0.89	0.7	1.13
31	rs23690306	C	G	25600053	0.69	0.41	15.48	1.11	0.86	1.43
33	rs23828846	T	C	29024878	0.43	0.51	19.5	1.09	0.84	1.41
20	rs22864357	C	T	28025372	0.37	0.54	20.59	1.15	0.73	1.82
5	rs24194054	C	T	56199928	0.35	0.55	21.02	0.93	0.73	1.19
12	rs22212359	C	G	43043338	0.31	0.58	21.94	1.09	0.8	1.48
5	rs24267550	A	G	18815805	0.26	0.61	23.24	0.92	0.65	1.29
3	rs24872415	G	A	62441438	0.13	0.72	27.38	0.96	0.74	1.24
14	rs22328353	C	G	54644252	0.12	0.73	27.89	0.95	0.72	1.26
12	rs22185491	T	C	13370771	0.04	0.84	31.9	0.97	0.76	1.25
12	rs22212677	A	G	29078487	0.02	0.89	33.85	0.98	0.72	1.34
8	rs24482628	C	A	27285603	0.02	0.89	33.94	0.98	0.77	1.25
22	rs23066224	T	C	39938082	0.01	0.93	35.34	1.02	0.72	1.43

All corrected P value significant results from all statistical tests in the Sequenom GWAS validation study

Statistical test	Breed	CHR	SNP	SUB TEST	Odds ratio	P value	corrected p value	Lower 95%	Upper 95% CI
Meta-analysis	All Golden Retriever	2	RS228592	CLUSTERED BY LOCATIO	5,19	2,07E-05	0	2,36	11,41
Meta-analysis	All Golden Retriever	29	RS234724	CLUSTERED BY LOCATIO	0,32	0	0,03	0,16	0,64
Meta-analysis	All Golden Retriever	3	RS236029	CLUSTERED BY LOCATIO	3,93	0	0,02	1,79	8,65
Meta-analysis	All Golden Retriever	6	RS243272	CLUSTERED BY LOCATIO	0,23	0	0,01	0,1	0,52
Meta-analysis	All Golden Retriever	7	RS244086	CLUSTERED BY LOCATIO	0,3	0	0,02	0,15	0,61
Meta-analysis	All WHWT	20	RS229158	CLUSTERED BY LOCATIO	5,36	0	0,04	1,83	15,65
Meta-analysis	All WHWT	8	RS244826	CLUSTERED BY LOCATIO	8,08	0	0,03	2,2	29,7
Meta-analysis	All WHWT	20	RS880697	CLUSTERED BY LOCATIO	5,36	0	0,04	1,83	15,65
Allelic assoc	UK GSD	3	RS248724	ALLELIC	16,33	0	0,01	2,9	91,99
allelic assoc	UK Labrador	10	RS221140	ALLELIC	6,14	4,79E-06	0	2,68	14,07
genotypic mode	UK Labrador	10	RS221140	TREND		0	0,01		
logistic	UK Labrador	10	RS221140	ADD	3,68	0	0,02	1,76	7,71
allelic assoc	UK Labrador	20	RS229158	ALLELIC	4,42	4,12E-05	0	2,11	9,24
genotypic mode	UK Labrador	20	RS229158	TREND		0	0,01		
logistic	UK Labrador	20	RS229158	ADD	3,42	0	0,03	1,67	7
allelic assoc	UK Labrador	20	RS880697	ALLELIC	3,91	0	0,01	1,88	8,15
genotypic mode	UK Labrador	20	RS880697	TREND		0	0,03		
allelic assoc	uk whwt	5	RS241940	ALLELIC	7	0	0,03	2,08	23,52
allelic assoc	uk whwt	8	RS244826	ALLELIC	11	0	0,01	2,77	43,68
allelic assoc	USA Golden Retriever	16	RS225235	ALLELIC	5,41	0	0,02	2	14,66
allelic assoc	USA Golden Retriever	6	RS243272	ALLELIC	0,14	0	0,01	0,05	0,45
allelic assoc	USA GSD	12	RS221842	ALLELIC	9,5	0	0,03	2,32	38,88
allelic assoc	USA Labrador	22	RS230261	ALLELIC	0,13	0	0,01	0,04	0,44
genotypic mode	USA Labrador	22	RS230261	TREND		0	0,03		