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The dog as a genetic model for immunoglobulin A (IgA) deficiency: Identification of several breeds with low serum IgA concentrations

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ABSTRACT

Immunoglobulin A (IgA) serves as the basis of the secretory immune system by protecting the lining of mucosal sites from pathogens. In both humans and dogs, IgA deficiency (IgAD) is associated with recurrent infections of mucosal sites and immune-mediated diseases. Low concentrations of serum IgA have previously been reported to occur in a number of dog breeds but no generally accepted cut-off value has been established for canine IgAD. The current study represents the largest screening to date of IgA in dogs in terms of both number of dogs ($n = 1267$) and number of breeds studied ($n = 22$). Serum IgA concentrations were quantified by using capture ELISA and were found to vary widely between breeds. We also found IgA to be positively correlated with age ($p < 0.0001$). Apart from the two breeds previously reported as predisposed to low IgA (Shar-Pei and German shepherd), we identified six additional breeds in which $\geq 10\%$ of all tested dogs had very low (< 0.07 g/l) IgA concentrations (Hovawart, Norwegian elkhound, Nova Scotia duck tolling retriever, Bullterrier, Golden retriever and Labrador retriever). In addition, we discovered low IgA concentrations to be significantly associated with canine atopic dermatitis (CAD, $p < 0.0001$) and pancreatic acinar atrophy (PAA, $p = 0.04$) in German shepherds.

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Abbreviations: IgAD, IgA deficiency; CAD, canine atopic dermatitis; SPAID, Shar-Pei autoinflammatory disease; AD, Addison's disease; PAA, pancreatic acinar atrophy; LT, lymphocytic thyroiditis; DM, diabetes mellitus; SRMA, steroid responsive meningitis arteritis; TBST, tris-buffered saline and Tween; PBST, phosphate-buffered saline with Tween; PNPP, Para-Nitrophenylphosphate; NSDTR, Nova Scotia duck tolling retriever.

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1. Introduction

IgA is the predominant antibody at mucosal sites and serves as the basis of the secretory immune system. IgA protects the linings of the gastrointestinal-, respiratory and genitourinary tracts by neutralizing antigens and prevents adherence of bacteria (reviewed in Macpherson et al., 2008). A selective deficiency of IgA (IgAD) is the most common primary immunodeficiency in man. It is associated with an increased risk of recurrent infections at mucosal sites and an increased predisposition for autoimmune diseases, allergy and gastrointestinal infections (Cunningham-Rundles, 2001; Jacob et al., 2008; Yel, 2010). In man, IgAD is defined by a serum IgA concentration <0.07 g/l, together with normal levels of IgG and IgM in individuals older than 4 years (Al-Herz et al., 2011). Unlike many other immunodeficiency disorders, the absence of an animal model that fully resembles human IgAD has limited the knowledge about the pathophysiological mechanisms involved. In selected dog breeds, including the German shepherd (Whitbread et al., 1984), Chinese Shar-Pei (Moroff et al., 1986; Rivas et al., 1995) and specific populations of Beagle (Felsburg et al., 1985) low IgA concentrations or even overt deficiency have been observed in some animals. Low serum IgA concentrations in dogs have also been reported to clinically resemble human IgAD in terms of an association to recurrent infections (Felsburg et al., 1985; Moroff et al., 1986; Willard et al., 1994) and immune mediated disease (Day, 1996).

In dogs, quantification of the five immunoglobulins is one of the most common assessments of immune function. Hence the literature on IgA concentrations in dogs is extensive. Despite this attention, the normal range of serum IgA as well as a generally accepted cut-off value for IgA deficiency has not been established yet. In the present study, we performed an extensive screen of serum IgA concentrations in dogs, using 1267 dogs from 22 different breeds. We aimed to define the normal range of serum IgA in dogs and to identify breeds with a high prevalence of low IgA concentrations to serve as a basis for future gene mapping studies. In addition, we investigated the possible association to immune mediated diseases including allergy, autoimmune- and autoinflammatory disease.

2. Materials and methods

2.1. Samples

Blood samples were collected with the owners' written consent in collaboration with veterinarians in the United States, Sweden and Switzerland from privately owned purebred dogs aged between 0.5 and 17 years. Ethical approval was granted by the Swedish Animal Ethical Committee (no.C62/10), the Swedish animal Welfare Agency (no. 31-1711/10) and The Broad Institute: Lindblad-Toh 0910-074-13 and the canton of Bern: Tosso Leeb 23/10. Serum was isolated from blood samples after centrifugation and stored at -20°C until used. A smaller proportion of the samples underwent one additional thaw/freezing cycle

as they were used for other purposes prior to this study. However, considering the high stability of serum IgA, this is not expected to have influenced the results (Janzi et al., 2005).

The study population consisted of 1267 dogs from 22 different breeds, both healthy and diseased (Supplementary Table 1). Diseases with a significant number of cases represented were: canine atopic dermatitis (CAD, $n=289$), Shar-Pei autoinflammatory disease (SPAID, $n=84$), Addison's disease (AD, $n=58$), pancreatic acinar atrophy (PAA, $n=36$), lymphocytic thyroiditis (LT, $n=13$), diabetes mellitus (DM, $n=10$), and steroid responsive meningitis arteritis (SRMA, $n=9$). The breakdown of the total population, diagnostic criteria and the corresponding phenotypic data is presented in Supplementary Table 2. Due to low age (<1 year), 20 dogs were removed from all analyses (except the test of a correlation between IgA and age): Shar-Pei ($n=5$), Nova Scotia duck tolling retriever ($n=3$), Bullterrier ($n=3$), Golden retriever ($n=4$), Labrador retriever ($n=4$) and German shepherd ($n=1$).

2.2. Canine IgA ELISA

Serum immunoglobulin concentrations were measured by capture enzyme-linked immunosorbent assay (ELISA). All samples were run at least twice in duplicates. Samples showing strong variation ($\text{CV} > 15\%$) between the duplicates were rerun and potential outliers were subsequently excluded ($n=2$). The protocol has previously been used to screen dog serum samples for IgA deficiency in our laboratory (Tengvall et al., 2013; Frankowiak et al., 2013). In short, serum samples were measured for their serum IgA concentration using polyclonal goat anti-dog IgA antibodies (AbD Serotec, Oxford, UK) and alkaline phosphatase-conjugated polyclonal goat anti-dog IgA (Bethyl Laboratories, TX, USA). The antibodies were diluted 1:2,000 with carbonate-bicarbonate buffer (0.05 M, pH 9.6) or Tris-buffered saline and Tween (TBST) respectively. Samples were diluted 1:25,000, 1:50,000 and 1:100,000 with phosphate-buffered saline with Tween (PBST). Para-Nitrophenylphosphate (PNPP) was used as a substrate. Serum samples from dogs, kindly provided by Professor M.J. Day, Bristol University (England), with previously determined IgA concentrations were used as controls for each individual measurement.

2.3. Statistics

All descriptive statistics were calculated using routine procedures and software (GraphPad Prism 6.0, La Jolla, CA, USA). Normal distribution was tested by D'Agostino-Pearson omnibus K2 test and the effect of age on IgA concentration was assessed by Spearman correlation test. Comparisons of IgA concentrations between different groups of animals (females/males, castrated/non-castrated, case/control for a certain disease phenotype) was assessed by the Mann-Whitney test and plotted following log transformation of the values. Case-control tests of disease phenotypes were performed within breeds and significance levels were considered if $p < 0.05$.

3. Results and discussion

IgA concentrations ranged from 0.01 to 3.0 g/l and deviated significantly from a Gaussian distribution (Supplementary Fig. 1). The median IgA concentration in the entire study population was 0.18 g/l, the mean 0.27 g/l and the lower 95% CI of mean 0.26 g/l (Table 1). Previous cut-offs for IgA deficiency in dogs have been established from the 95% CI of the mean in one-breed studies (0.18 g/l in Felsburg et al., 1985 and 0.15 g/l in Rivas et al., 1995; Day, 1999). In our IgA screen of multiple dog breeds, we report a higher 95% CI of the mean, more similar to what earlier has been observed in a population of crossbred dogs (0.22 g/l, Day and Penhale, 1988). The production of IgA is known to be age-dependent and this was also reflected in our study population where IgA concentrations were positively correlated to age ($p < 0.0001$, Supplementary Fig. 2). In dogs, IgA production is assumed to stabilize at around one year of age (Reynolds and Johnson, 1970; Felsburg et al., 1985; Glickman et al., 1988). Therefore, 20 dogs younger than one year were excluded in the subsequent analyses. In humans, serum IgA concentrations are recognized to be higher in males than in females (Weber-Mzell et al., 2004; Gonzalez-Quintela et al., 2008), whilst in dogs the literature is contradictory (Glickman et al., 1988; Schreiber et al., 1992; Griot-Wenk et al., 1999). In our study, no sex-dependency could be detected for serum IgA concentrations and no difference was noted between dogs castrated or not (Supplementary Fig. 3a and b).

We found IgA concentrations to vary widely amongst breeds (Fig. 1, Table 1). This pronounced difference may explain the lack of an established normal range of IgA and an accepted cut-off value for IgA deficiency in dogs. The 95% CI of the means of each breed, that would be used to define the cut-off for abnormally low IgA concentrations in a population, ranged from 0.05 to 0.73 g/l. In ten of the breeds, we observed a high percentage ($>10\%$) of individuals with low IgA concentrations (Table 1, Supplementary Fig. 4) when applying the human cut-off for IgA. This cut-off value is much lower than the suggested canine definitions of IgAD (Felsburg et al., 1985; Rivas et al., 1995; Day, 1999), and thus we may even underestimate the frequency of IgA deficiency in the current study. On the other hand, we may overestimate the number of breeds predisposed to IgA deficiency as the sample set does not represent a random collection of dogs from each breed but is slightly biased to include more dogs diagnosed with different diseases. However, it is not clear whether the link between immune mediated disease and low IgA is causative (primary) or secondary to the disease.

Shar-Pei stands out as the most high-risk breed for low IgA concentrations with the lowest median IgA concentration (0.08 g/l) and 45% 'IgA deficient' individuals. Other breeds with $\geq 10\%$ of dogs with low IgA are Hovawart, Norwegian elkhound, Nova Scotia duck tolling retriever (NSDTR), Belgian shepherd, American Staffordshire terrier, Bullterrier, German shepherd, Golden retriever, Labrador retriever and Staffordshire bullterrier. For Belgian shepherd, American Staffordshire terrier and Staffordshire bullterrier, 'age at sampling' was unknown and thus, no further conclusions can be drawn regarding IgA deficiency

in these three breeds as it could potentially reflect a large proportion of young dogs. The other seven breeds were represented by adult individuals and can be added to the list below Shar-Pei of breeds of high risk of IgA deficiency. However, as IgG and IgM were not evaluated in this study, it is not clear whether the immunodeficiency observed in these breeds is of selective or common nature and supplementary studies are required to understand the immunodeficiency in each specific breed.

Also, it is worth noticing that few individuals represented some of the breeds identified to be at high risk of IgA deficiency, and an extended study with more dogs from these breeds would be beneficial to confirm the result. The genome structure in dog breeds (characterized by strong linkage disequilibrium and long haplotype blocks), allows mapping of disease-causing genes in case-control association studies using fewer markers and individuals compared to similar studies in humans (reviewed in Karlsson and Lindblad-Toh, 2008). To our knowledge, no attempts have been made to map genes and mutations underlying IgA deficiency in dogs. In this report, we suggest several breeds to be predisposed to IgA deficiency and any of these could theoretically serve as a genetic disease model for human IgAD.

Interestingly, the German shepherd breed which has been reported several times to be a high-risk breed for IgA deficiency (Whitbread et al., 1984; Batt et al., 1991), was not amongst those with the lowest IgA concentrations in our study. In German shepherd, IgA concentration was significantly lower in dogs diagnosed with canine atopic dermatitis (CAD, $p < 0.0001$) and pancreatic acinar atrophy (PAA, $p = 0.04$) (Supplementary Table 1). The association between CAD and low IgA concentrations has been described in one of our earlier studies (Tengvall et al., 2013), but the correlation to PAA has not previously been observed. CAD also occurred in a high frequency in Golden retriever and Labrador retriever in our study population, but no association with low IgA concentrations was detected in these breeds. The German shepherd is genetically predisposed to multiple immune mediated diseases and clearly has a defective mucosal immune response (Whitbread et al., 1984; Batt et al., 1991; Littler et al., 2006; Maeda et al., 2013). No other significant correlations were observed between dogs affected versus unaffected by various immune mediated diseases (Supplementary Table 1). However, the number of dogs in each breed diagnosed with SRMA, LT, Addison' disease or DM in our study population was fairly small and may not be sufficient to identify such correlations.

Most purebred dog breeds show a very high susceptibility to one or more genetic diseases due to founder effects, breeding practices and reproductive isolation (Ostrander et al., 2000; Karlsson and Lindblad-Toh, 2008). IgAD appears to be one of these diseases and shared by multiple breeds. In a previous study, we have reported low IgA concentrations in wolves (*Canis lupus*), the wild ancestor of the domestic dog (Frankowiack et al., 2013). In that study, we used the Scandinavian wolf population, which is known to be highly inbred with very low levels of genetic variation (Ellegren, 1999; Hagenblad et al., 2009). This suggests that

Table 1

IgA concentrations in 1247 dogs representing 22 breeds (reported in g/l).

Breed	Number of ind.	IgA min	25% percentile	Median IgA	75% percentile	IgA max	Mean IgA (SD)	Lower 95% CI of the mean	IgA <0.07
Total population	1247 ^a	0.01	0.1	0.18	0.33	2.99	0.27 (0.28)	0.26	15%
Shar-Pei	157	0.01	0.05	0.08	0.15	0.56	0.12 (0.97)	0.1	45%
Hovawart	19	0.04	0.05	0.09	0.28	0.59	0.18 (0.16)	0.1	32%
Norwegian elkhund	14	0.05	0.08	0.14	0.2	1.26	0.23 (0.31)	0.05	21%
Nova Scotia duck tolling retriever	11	0.02	0.06	0.13	0.44	0.77	0.24 (0.23)	0.1	20%
Belgian shepherd	10	0.02	0.12	0.18	0.32	0.85	0.26 (0.24)	0.08	20%
Bullterrier	14	0.02	0.08	0.19	0.28	0.37	0.18 (0.11)	0.12	15%
American Staffordshire bullterrier	13	0.04	0.09	0.22	0.32	0.53	0.22 (0.13)	0.13	15%
German shepherd	319	0.02	0.12	0.16	0.27	1.34	0.21 (0.16)	0.19	14%
Golden retriever	168	0.03	0.1	0.18	0.34	1.12	0.26 (0.21)	0.22	13%
Labrador retriever	141	0.03	0.1	0.19	0.32	1.98	0.28 (0.3)	0.23	12%
Staffordshire bullterrier	20	0.04	0.11	0.23	0.35	0.58	0.24 (0.15)	0.17	10%
Bearded collie	49	0.04	0.12	0.15	0.23	0.61	0.19 (0.13)	0.15	6%
Tibetan spaniel	16	0.06	0.13	0.23	0.33	1.33	0.33 (0.33)	0.15	6%
Rottweiler	20	0.05	0.14	0.21	0.31	0.95	0.27 (0.20)	0.17	5%
Giant schnauzer	20	0.05	0.27	0.4	0.65	0.91	0.43 (0.24)	0.32	5%
Standard Poodle	137	0.03	0.17	0.29	0.49	2.6	0.41 (0.38)	0.36	3%
Boxer	20	0.14	0.19	0.25	0.47	0.66	0.33 (0.16)	0.25	0%
Border collie	20	0.08	0.15	0.37	0.49	0.92	0.37 (0.24)	0.25	0%
Jämthund	19	0.07	0.21	0.37	0.67	1.8	0.56 (0.48)	0.32	0%
Berner sennen	21	0.09	0.28	0.45	0.65	0.96	0.48 (0.25)	0.36	0%
Samoyed	19	0.17	0.31	0.75	1.15	3.0	0.89 (0.81)	0.5	0%
Leonberger	20	0.41	0.65	0.85	1.1	1.8	0.89 (0.32)	0.73	0%

^a 20 dogs removed from the total 1267 due to low age (<one year).

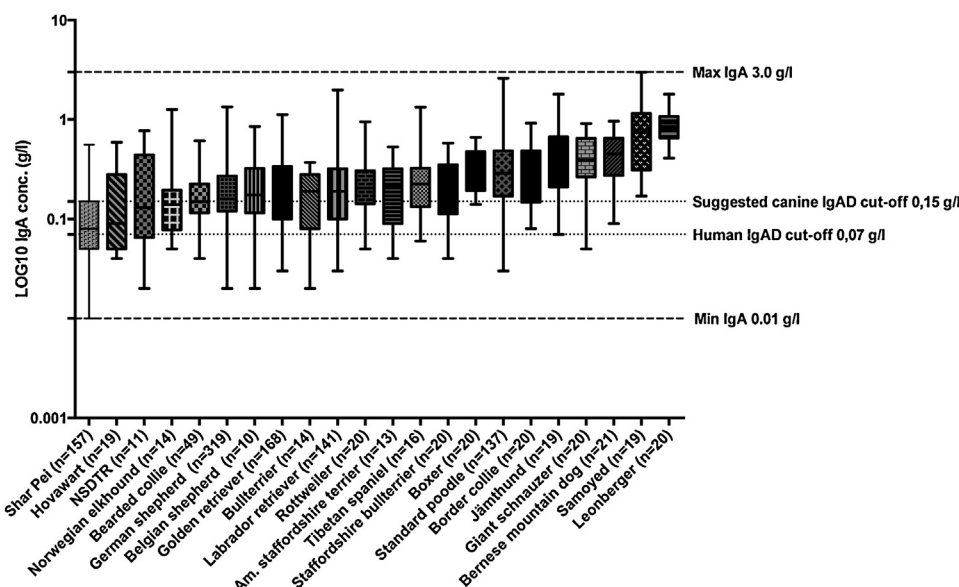


Fig. 1. IgA concentrations are plotted with a log transformation of the values and sorted based on median values. In the total population, IgA ranged between 0.01 and 3.0 g/l but varies widely among the 22 breeds in the study. The dotted lines represent the human cut-off value for IgAD (0.07 g/l) and a canine cut-off (0.15 g/l), suggested twice by others (Rivas et al., 1995; Day, 1999).

the risk allele(s) predisposing to IgAD may have been transferred from wolves to dogs during domestication. As there is no obvious relatedness between the breeds identified to frequently have low IgA concentrations in this study (see Supplementary Fig. 5), it is more likely that IgA deficiency is yet another example of an enrichment of risk alleles for diseases within dog breeds.

4. Conclusion

The current study presents the hitherto largest screen of IgA in dogs in terms of number of breeds represented. IgA concentrations were found to vary widely between breeds, which explains the lack of a generally accepted diagnostic cut-off for IgA deficiency in dogs. We identified eight

breeds in which $\geq 10\%$ of all adult dogs were IgA deficient according to the human cut off value for IgAD. Apart from Shar-Pei and German shepherd, the other six breeds have not been described as IgA deficient before. We suggest this new knowledge to be taken into account when monitoring canine health in the veterinary practice. As dogs offer an advantageous comparative model for human disease, we have also identified six new potential genetic models of IgAD.

Conflict of interest statement

The authors declare that no competing interest exists.

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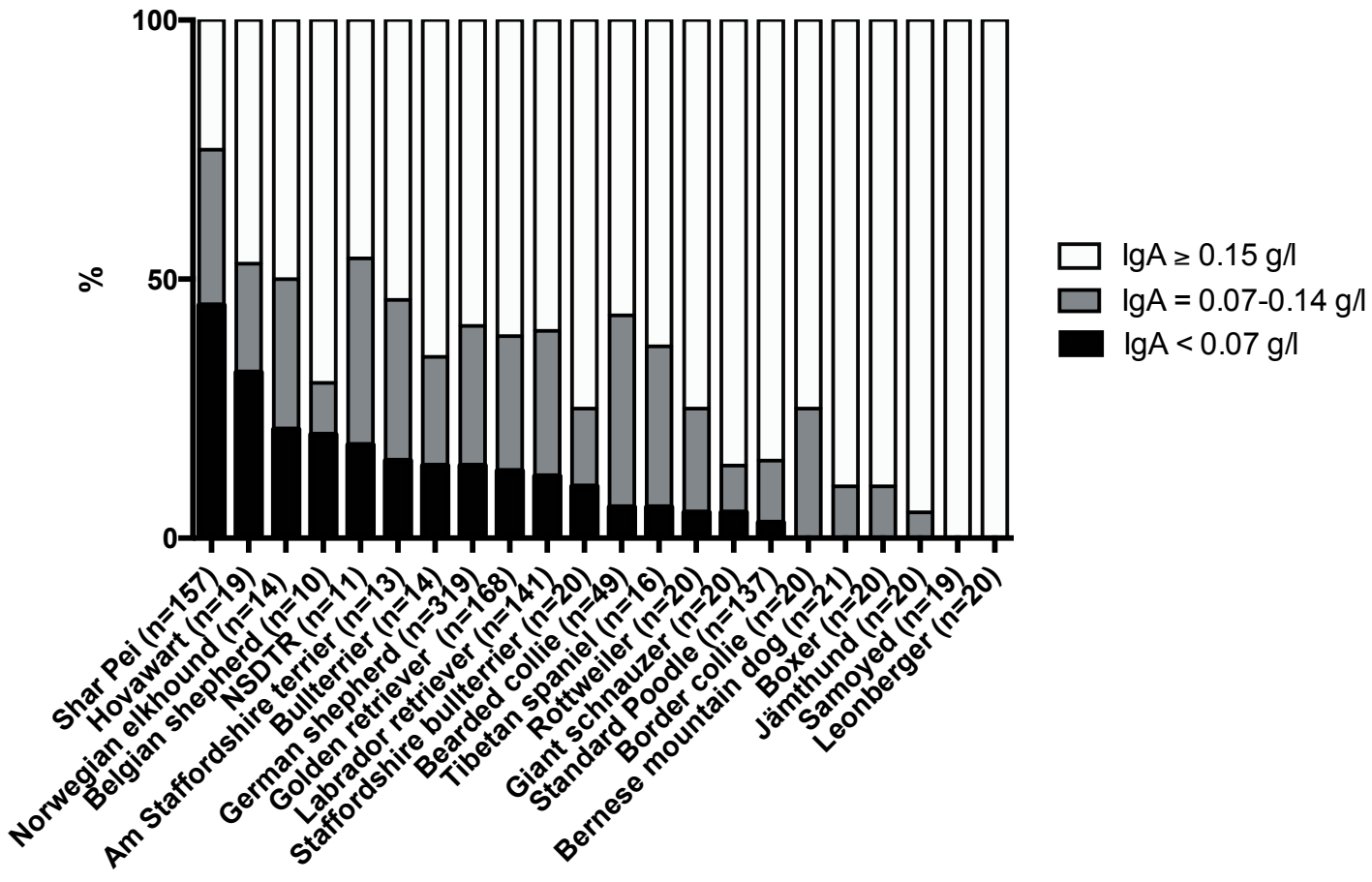
We thank all the dog owners and veterinarians for providing us with blood samples and clinical information of all the dogs. For support with sample collection we also thank Izabella Baranowska-Körberg, Matteo Biancho, Charlotta Lantz, Susanne Gustafsson, Tosso Leeb and Eliane Marti. We are also grateful to Professor M.J. Day, Bristol University for providing the canine IgA reference serum. This work was supported by the Swedish Medical Research Council (VR) (grant no. K2012-56X-05975). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

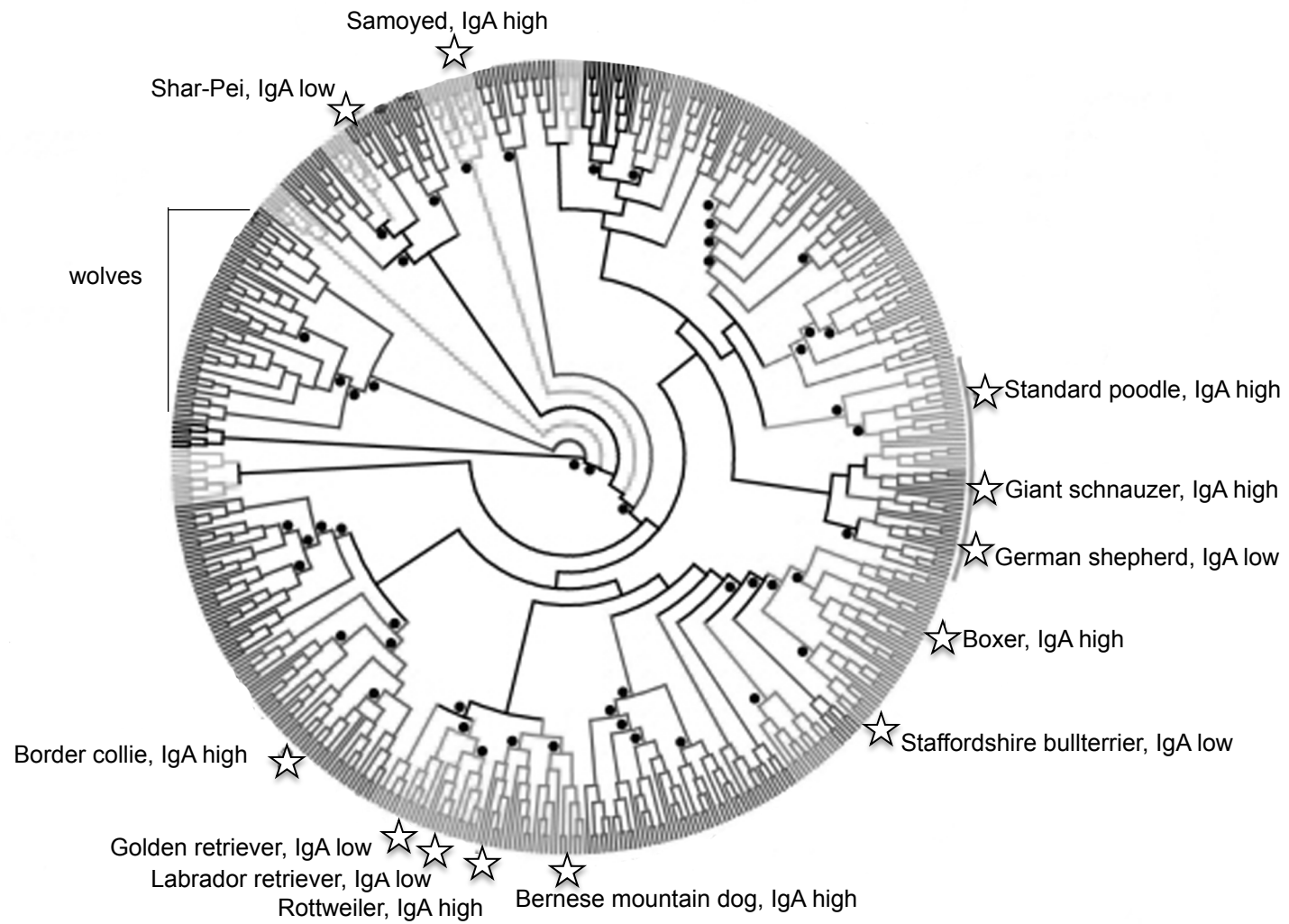
Appendix A. Supplementary data

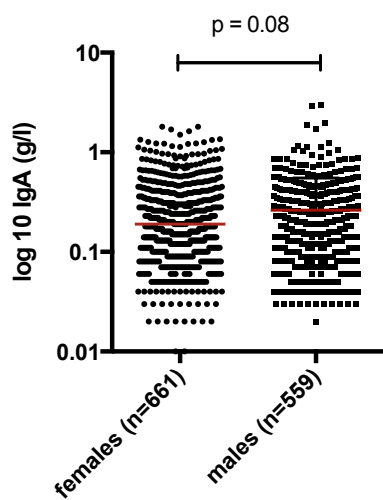
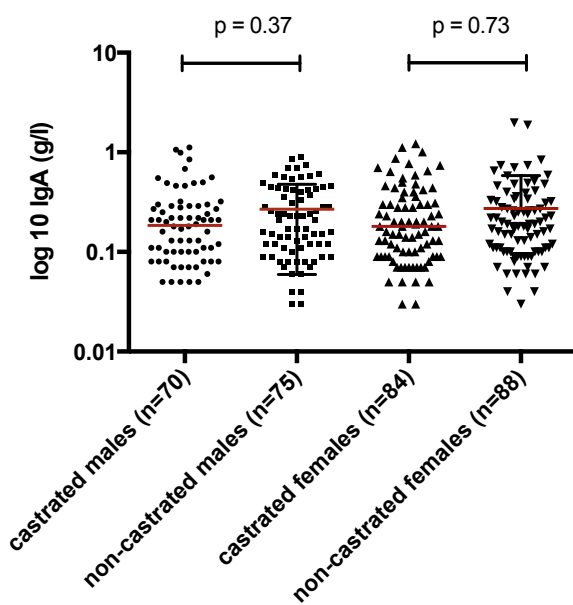
Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetimm.2014.05.010>.

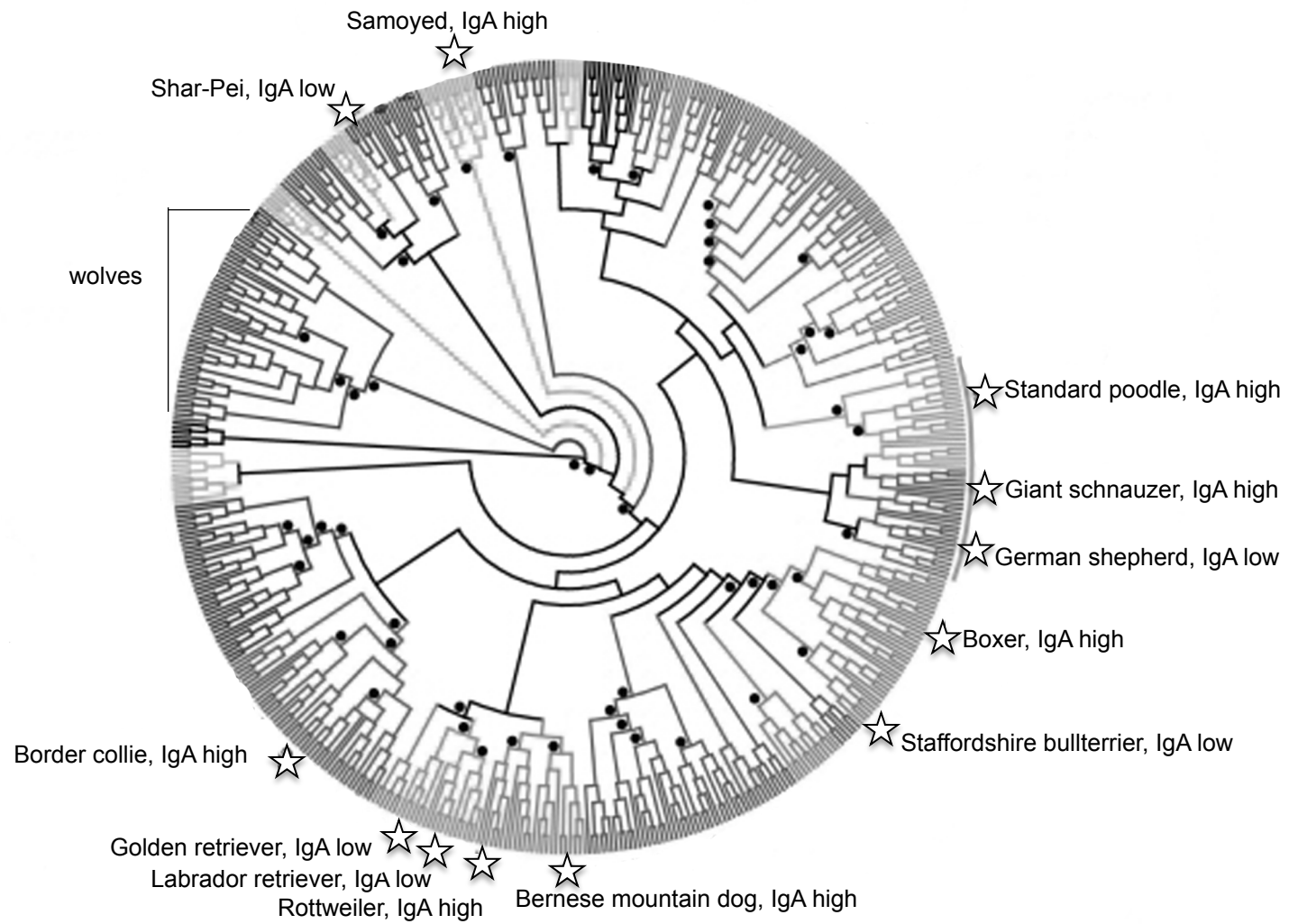
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A**B**



Breed	Disease phenotype	Nr. of affected	Nr. of unaffected	Median IgA (g/l) affected		Median IgA (g/l) unaffected	p-value
German shepherd	CAD	128	131	0.13	<	0.22	<0.0001
German shepherd	PAA	36	131	0.17	<	0.22	0.04
Standard Poodle	AD	45	67	0.23	<	0.34	0.054
NSDTR	SRMA	6	5	0.13	<	0.27	0.19
Shar-Pei	SPAID	84	67	0.08	<	0.1	0.29
Jämthund	DM	4	13	1.23	>	0.37	0.30
Golden retriever	CAD	82	85	0.17	<	0.19	0.33
Labrador retriever	CAD	67	74	0.17	<	0.20	0.36
Giant schnauzer	LT	6	14	0.37	<	0.45	0.41
Hovawart	LT	4	7	0.19	>	0.09	<i>too few</i>
Bearded collie	AD	13	36	0.15	>	0.16	0.56
Bullterrier	CAD	12	2	0.19	>	0.04	<i>too few</i>
Norwegian elkhund	DM	1	12	-	-	-	<i>too few</i>
Samoyed	DM	2	16	1.05	>	0.65	<i>too few</i>

* CAD = canine atopic dermatitis, PAA=pancreatic acinar atrophy, AD = Addisons disease, SRMA = steroid responsive meningitis arteritis, SPAID = Shar-Pei Autoinflammatory disease, DM = diabetes mellitus, LT = lymphocytic thyrioditis

Breed	Nr.of ind.	Information available				Diagnose *
		Age at sampling	Sex	Complete health status	Castration	
Total population	1267	1033	1220	812	318	-
German shepherd	320	283	319	309	NA	CAD (n=128), PAA (n=36)
Golden retriever	172	167	173	NA	173	CAD (n=82)
Labrador retriever	145	143	145	145	145	CAD (n=67)
Bullterrier	17	17	17	NA	NA	CAD (n=12)
Shar-Pei	162	154	162	156	NA	SPAID (n=84)
Standard Poodle	137	114	111	111	NA	AD (n=45)
Bearded collie	49	32	32	32	NA	AD (n=13)
Hovawart	19	17	16	19	NA	LT (n=7)
Giant schnauzer	20	20	20	20	NA	LT (n=6)
Jämthund	19	18	19	NA	NA	DM (n=7)
Samoyed	19	NA	19	NA	NA	DM (n=2)
Norwegian elkhund	14	14	14	NA	NA	DM (n=1)
Nova Scotia duck tolling retriever	14	14	14	NA	NA	SRMA (n=9)
Berner sennen	21	NA	21	NA	NA	NA
Rottweiler	20	NA	20	NA	NA	NA
Boxer	20	20	20	NA	NA	NA
Border collie	20	NA	20	NA	NA	NA
Leonberger	20	20	20	20	NA	NA
Staffordshire bullterrier	20	NA	20	NA	NA	NA
Tibetan spaniel	16	NA	15	NA	NA	NA
American Staffordshire bullterrier	13	NA	13	NA	NA	NA
Belgian shepherd	10	NA	10	NA	NA	NA

* CAD = canine atopic dermatitis (for diagnostic criteria, see Tengvall et al. 2013), PAA = pancreatic acinar atrophy (diagnosis based on TLI-test and clinical signs), SPAID = Shar-Pei Autoinflammatory disease (for diagnostic criteria, see Olsson et al., 2013) AD= Addisons disease (diagnosis based on ACTH test), LT = lymphocytic thyroiditis (for diagnostic criteria, see Ferm et al., 2009), DM= diabetes mellitus (diagnostic criteria was polyuria and polydipsia, a persistent fasting hyperglycaemia of more than 8 mmol/l and glycosuria), SRMA = steroid responsive meningitis arteritis (for diagnostic criteria, see Hamlin & Lilliehöök, 2013).