

## Risk factors for acquired myasthenia gravis in dogs: 1,154 cases (1991-1995)

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**Objective**—To determine frequency of initial clinical signs and risk factors for acquired myasthenia gravis (MG) in dogs.

**Design**—Retrospective study.

**Sample Population**—1,154 dogs residing within the United States from 1991 to 1995 with a confirmed diagnosis of acquired MG and 7,176 dogs with other neuromuscular disorders, including generalized weakness, megaesophagus, and dysphagia (control group).

**Procedure**—Records were retrieved from a database containing results of serum samples tested for acetylcholine receptor antibodies. Signalment, breed, age, state of origin, and month of onset of clinical signs were obtained. An antibody titer > 0.6 nmol/L was diagnostic for acquired MG. Unconditional logistic regression was used for statistical analysis.

**Results**—In comparison with mixed-breed dogs, dogs with the highest risk of acquired MG were Akitas, terrier group, Scottish Terriers, German Shorthaired Pointers, and Chihuahuas. Rottweilers, Doberman Pinschers, Dalmatians, and Jack Russell Terriers had low relative risks. Sexually intact males and dogs less than 1 year old had some protection from risk. Generalized weakness with megaesophagus and megaesophagus alone were the most common initial clinical signs.

**Clinical Implications**—Breed predispositions for acquired MG were demonstrated. Age and sex were contributing factors. Although most dogs had generalized clinical signs, a substantial proportion of dogs had focal signs. (*J Am Vet Med Assoc* 1997;211:1428-1431)

be focal (limited to pharyngeal, esophageal, or facial musculature)<sup>8</sup> or acute and fulminating (generalized collapse).<sup>9</sup> Acquired MG may be found associated with other immune-mediated disorders including hypothyroidism<sup>10</sup> and as part of a paraneoplastic syndrome associated with thymoma and other neoplasms.<sup>11-13</sup> Although this disorder has recently been described as uncommon in dogs,<sup>14</sup> acquired MG may be the most common neuromuscular disease that can be definitively diagnosed in this species.

During 1991 to 1995, 8,330 canine serum samples were submitted by veterinarians throughout the United States to the Comparative Neuromuscular Laboratory at the University of California-San Diego and tested for AChR antibodies by immunoprecipitation radioimmunoassay. Dogs tested had various forms of muscle weakness, exercise intolerance, or acquired megaesophagus, and 1,154 of these dogs had AChR antibody titers > 0.6 nmol/L. Serum AChR antibody titers > 0.6 nmol/L have been previously established to be diagnostic of acquired MG.<sup>6</sup> The database generated from this large population of dogs was used to determine the most common clinical signs of, and relative risks associated with, acquired MG relative to breed, sex, age, state of origin, and month of clinical referral.

### Criteria for Selection of Cases

Dogs included in this study resided in the United States during 1991 to 1995 and had variable clinical signs associated with generalized neuromuscular weakness or focal muscle weakness, including esophageal dilatation or hypomotility and pharyngeal, laryngeal, or facial muscle paresis. After quantification of AChR antibody titers, dogs were categorized as having confirmed MG or having other causes of neuromuscular weakness (controls).

### Procedures

Physical and neurologic examinations were performed and described by numerous veterinarians who submitted serum samples to the laboratory. Diagnosis of MG was confirmed by immunoprecipitation radioimmunoassay as previously described.<sup>15</sup> Briefly, AChR, used as the antigen, was solubilized from muscle of near-term canine fetuses in 2% nonionic detergent buffer.<sup>6</sup> Concentration of AChR was determined and expressed as moles of <sup>125</sup>I-labeled  $\alpha$ -bungarotoxin binding sites per liter. Aliquots of serum from dogs suspected of having MG were incubated overnight at 4 C (39.5 F) with labeled muscle extract. Labeled AChR-anti-AChR complexes were precipitated, and titers were expressed as moles of <sup>125</sup>I-labeled  $\alpha$ -bungarotoxin binding sites per liter of serum. The upper limit of the reference range had been previously established at 0.6 nmol/L.<sup>6</sup>

### Statistical Analysis

Proportionate changes in the risk for acquired MG by breed, state, month, and age were evaluated, using unconditional logistic regression.<sup>16</sup> German Shepherd Dogs and mixed-breed dogs were used separately as a refer-

Excessive fatigability and muscular weakness in acquired myasthenia gravis (MG) are caused by altered neuromuscular transmission resulting from autoantibodies against nicotinic acetylcholine receptors (AChR) at the neuromuscular junction. Cross-linking by antibody and an increased rate of internalization result in a net loss of AChR.<sup>1</sup> Diagnosis of MG in dogs is confirmed by demonstration of circulating AChR antibodies using <sup>125</sup>I-labeled  $\alpha$ -bungarotoxin bound to native AChR in a radioimmunoassay. This assay is objective, quantitative, and specific, proving an autoimmune response to AChR as opposed to another component of the neuromuscular junction.<sup>1</sup> Several reports<sup>2-7</sup> describe acquired MG in dogs. In addition to the generalized "classic" sign of exercise-associated weakness, clinical manifestations of MG may

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The authors thank Dr. Jon Lindstrom for donating iodinated bungarotoxin.

ence breed for comparison; California was used as a reference state for comparison. Results are given as odds ratios (OR) and 95% confidence intervals (95% CI). Because MG incidence was < 5% within all levels of covariates, OR was used to estimate relative risk.

## Results

During 1991 to 1995, acquired MG was diagnosed in 1,154 dogs with AChR antibody titers

Table 1—Clinical signs in 1,032 dogs with acquired myasthenia gravis (MG)

Clinical sign	MG					
	Focal			Generalized		
	No. of dogs	Percentage of total	Clinical sign	No. of dogs	Percentage of total	
M	367	31.8	G, M	440	38.1	
M, P	7	<1	G, M, P	7	<1	
M, LP, B	3	<1	G, M, B	34	2.9	
M, P, B	3	<1	G, M, LP	3	<1	
M, LP	6	<1	G	77	6.7	
M, B	14	1.2	G, LP	1	<1	
M, CMM	16	1.3	G, B	5	<1	
P	13	1.1	G, M, CMM	15	1.3	
P, LP	2	<1	G, M, B, CMM	2	<1	
MISC	7	<1	MISC	10	<1	

M = megaesophagus; G = generalized weakness; P = pharyngeal weakness; LP = laryngeal paralysis; B = poor or no blink response; CMM = cranial mediastinal mass; MISC = poor esophageal motility (n = 3), chronic pneumonia (1), cranial mediastinal mass only (2), extraocular muscle weakness only (1); generalized weakness and third degree heart block (3).

> 0.6 nmol/L. Clinical signs consistent with generalized MG were described in 57% (587 dogs), and localized weakness without clinically detectable limb muscle weakness was described in 43% (438 dogs; Table 1). Among dogs with generalized MG, 13.1% (77/587) did not have clinical signs of esophageal or pharyngeal dysfunction. Pharyngeal weakness alone was described in 1.1% (7/438) of dogs with focal MG. Laryngeal paralysis without esophageal or pharyngeal weakness was not described. An anterior mediastinal mass was found in 3.4% (35/1,025) of the dogs.

Relative risk determined by breed—Information on breed was available for 1,147 dogs with MG and 6,596 control dogs. Relative risk for acquired MG was calculated for 61 breeds. Using mixed-breed dogs as a reference group for comparison, several breeds were at a higher relative risk. Dogs with the highest relative risk of MG were Akitas, terrier group, Scottish Terriers, German Shorthaired Pointers, and Chihuahuas (Table 2). Although these breeds were at higher risk, they accounted for only 7.5% of the total morbidity. Rottweilers, Doberman Pinschers, Dalmatians, and Jack Russell Terriers had lower relative risks. Using German Shepherd Dogs as a reference group for comparison, only Akitas (OR = 2.10; 95% CI, 1.25 to 3.50) and the terrier group (OR = 1.96; 95% CI, 1.01 to 3.80) were at higher risk. Dogs accounting for highest total morbidity (49.9%) included German Shepherd Dogs,

Table 2—Relative risk in various breeds of dogs for acquired MG

Breed	No. of cases	No. of controls	OR	95% CI	Breed	No. of cases	No. of controls	OR	95% CI
Akita	29	39	5.8	3.5-9.7	Labrador Retriever	103	668	1.2	0.9-1.6
Terrier group	16	23	5.4	2.8-10.5	English Setter	3	19	1.2	0.3-4.2
Scottish Terrier	19	31	4.8	2.6-8.7	Other purebreds	43	281	1.2	0.8-1.7
German Shorthaired Pointer	13	22	4.6	2.3-9.4	Cocker Spaniel	31	207	1.17	0.8-1.8
Chihuahua	9	16	4.4	1.9-10.1	Poodle	23	166	1.1	0.7-1.7
Fox Terrier (Wirehair)	11	25	3.4	1.7-7.1	Chow Chow	6	41	1.1	0.5-2.7
Australian Shepherd	19	45	3.3	1.9-5.8	Lhasa Apso	5	37	1.1	0.4-2.7
Giant Schnauzer	8	19	3.3	1.4-7.7	Mixed breed	129	1,005	1.0	NA
Vizsla	6	14	3.3	1.3-8.8	Basset Hound	6	48	1.0	0.4-2.3
German Shepherd Dog	159	448	2.8	2.1-3.6	Bullmastiff	7	54	1.0	0.4-2.3
Pit Bull	14	42	2.6	1.4-4.9	Miniature Schnauzer	14	110	1.0	0.6-1.8
Cairn Terrier	5	15	2.6	0.9-7.3	Bernese Mountain Dog	3	24	1.0	0.3-3.3
Dachshund	36	112	2.5	1.7-3.8	Chinese Shar-Pei	5	42	0.9	0.4-2.4
Shetland Sheepdog	25	82	2.4	1.5-3.9	Airedale Terrier	4	40	0.8	0.3-2.2
Siberian Husky	17	56	2.4	1.3-4.2	Brittany Spaniel	4	37	0.8	0.3-2.4
Collie	27	108	2.0	1.2-3.1	Pug	3	28	0.8	0.3-2.8
Newfoundland	17	65	2.0	1.2-3.6	Greyhound	3	31	0.8	0.2-2.5
Shih Tzu	10	40	2.0	1.0-4.0	Samoyed	3	28	0.8	0.3-2.8
Beagle	14	57	1.9	1.0-3.5	Welsh Corgi	4	39	0.8	0.3-2.3
Border Collie	6	25	1.9	0.8-4.6	Yorkshire Terrier	5	53	0.7	0.3-1.9
Keeshond	8	38	1.6	0.7-3.6	Pekingese	1	12	0.6	0.0-5.0
Maltese	5	35	1.6	0.6-4.1	Boston Terrier	2	29	0.5	0.1-2.3
Springer Spaniel	17	82	1.6	0.9-2.8	Doberman Pinscher	13	223	0.5	0.3-0.8
Golden Retriever	146	746	1.5	1.2-2.0	Rottweiler	15	265	0.4	0.3-0.8
Boxer	18	92	1.5	0.9-2.6	Bulldog	3	52	0.4	0.1-1.5
West Highland White Terrier	6	31	1.5	0.6-3.7	Great Dane	6	108	0.4	0.2-1.0
Pomeranian	7	36	1.5	0.7-3.5	Irish Setter	3	59	0.4	0.1-1.3
Chesapeake Bay Retriever	5	28	1.4	0.5-3.7	Weimaraner	2	39	0.4	0.0-1.7
Alaskan Malamute	10	62	1.3	0.6-2.5	Rhodesian Ridgeback	1	21	0.4	0.0-2.8
Old English Sheepdog	7	41	1.3	0.6-3.0	Bichon Frise	1	25	0.3	0.0-2.3
					Dalmatian	3	104	0.2	0.0-0.7
					Jack Russell Terrier	4	236	0.1	0.0-0.4
					Missing information	7	580	NA	NA

OR = odds ratio; CI = confidence interval; NA = not applicable.

Golden Retrievers, mixed-breed dogs, Labrador Retrievers, and Dachshunds.

**Relative risk determined by age**—Information on age (adjusted for sex) was available for 1,137 dogs with MG and 6,743 control dogs. Using dogs less than 1 year old for comparison, dogs in the 1- to 15-years-old group (whose risk was empirically shown to be homogeneous) had a 35% increase in risk of MG (OR = 1.4; 95% CI, 0.95 to 1.9). Similarly, dogs in the more than 16-years-old group had a 3.58-fold increase in risk of MG, compared with dogs in the less than 1-year-old group (OR = 3.6; 95% CI, 1.5 to 8.5).

**Relative risk determined by sex**—Information on sex (adjusted for age) was available for 1,146 dogs with MG and 6,785 control dogs. Compared with sexually intact females, spayed females were at a higher risk (OR = 1.3; 95% CI, 1.1 to 1.5). Castrated males were at a higher risk (OR = 1.1) but this was not significant (95% CI, 0.9 to 1.3). There was some protection for sexually intact males (OR = 0.81; 95% CI, 0.66 to 1.0) relative to sexually intact females, spayed females, and neutered males.

**Relative risk determined by state of origin and month**—Information on state of origin was available for 1,088 dogs with MG and 7,094 control dogs. Using California as the reference state, New Mexico (OR = 2.43; 95% CI, 1.23 to 4.78) and Washington (OR = 1.80; 95% CI, 1.22 to 2.66) had higher risks; Florida (OR = 0.59; 95% CI, 0.43 to 0.81) was at a lower risk. Meaningful differences in risk by month of sample submission were not found.

## Discussion

Acquired MG is the best characterized autoimmune disease of the neuromuscular system and possibly the best characterized autoimmune disease in general.<sup>1</sup> Unequivocal evidence indicates that susceptibility to autoimmune disease is largely genetically determined, and the most clearly established genetic association with autoimmune disease predisposition is related to the major histocompatibility complex.<sup>17</sup> It has also been demonstrated that multiple genes contribute to the induction of pathogenic autoimmunity, and no single genetic abnormality is sufficient in itself to induce disease.<sup>17</sup> Genetic studies of these diseases have met with difficult problems because of the complexity of polygenic traits, considerable phenotypic and genetic heterogeneity, incomplete penetrance, and environmental factors. In the absence of thymoma in human beings with MG, it is clear that a genetic predisposition for the antibody-mediated autoimmune response to AChR is important, as reflected in human leukocyte antigen grouping in these patients<sup>17</sup> and in the increased frequency of other autoimmune diseases.<sup>18</sup> Age of onset and sex also have been shown to play a role in acquired MG in human beings.<sup>19,20</sup>

In MG in dogs, much is known about the variability of clinical signs and the propensity for the disease to mimic other neuropathic and myopathic disorders. Clinical signs may vary from focal disease involving extraocular, pharyngeal, laryngeal, or esophageal

muscles to generalized muscle weakness, stiff gait, or peracute collapse. It is clear from this study that acquired MG in dogs is not an uncommon disease and should be high on the differential diagnoses list for any dog with these clinical signs.

As shown in this study, an increased relative risk for acquired MG was found in several breeds of purebred dogs, compared with mixed-breed dogs. Because a clear genetic predisposition to autoimmune disease has been demonstrated in human beings, breed predispositions for acquired MG support a genetic basis in dogs. Although German Shepherd Dogs and Golden Retrievers did not have the highest relative risks, compared with mixed-breed dogs, they represented breeds with the highest absolute morbidity. This is, in part, consistent with the popularity of these breeds. Akitas had the highest relative risk and were ranked number 35 in popularity by the American Kennel Club in their list of top 50 breeds of dogs registered from Jan 1 through Dec 31, 1995. Rottweilers, ranked number 2 in popularity, had a lower relative risk for acquired MG, compared with mixed-breed dogs. Dogs less than 1 year old had an increased risk, and some hormonal protection for sexually intact males was found. Spaying and neutering increased the age-adjusted relative risk of MG. Significant ( $P > 0.05$ ) differences were not found in the month of onset of clinical signs, suggesting that seasonality does not play a role in onset of MG in dogs. Although some variability in risk was suggested for a few states, the importance of this finding is not known, because under the null hypothesis, we would expect approximately 2.5 states to have a 95% CI that did not cover the null value (ie, type-I error); thus, these findings were not unexpected.

Although much is known about MG, 2 important aspects still remain a mystery: what initiates and sustains the autoimmune response to AChR and how does one specifically suppress the autoimmune response. Dogs may play an important role in the study of both of these aspects, because the disorder develops spontaneously in dogs, indicating that triggers may be similar to those initiating the disease in human beings, and dogs also have spontaneous remissions, indicating that their immune system is able to correct the imbalance that results in clinical disease. The role of the thymus is also unknown in MG in dogs.

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\*Triton X-100, Calbiochem, La Jolla, Calif.

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## References

1. Lindstrom J, Shelton D, Fujii Y. Myasthenia gravis. *Adv Immunol* 1988;42:233–284.
2. Palmer AC. Myasthenia in the dog. *Vet Rec* 1974;16:452–454.
3. Pflugfelder CM, Cardinet GH III, Lutz H, et al. Acquired canine myasthenia gravis: immunocytochemical localization of immune complexes at neuromuscular junctions. *Muscle Nerve* 1981;4:289–295.
4. Lennon VA, Lambert EH, Palmer AC, et al. Acquired and congenital myasthenia gravis in dogs: a study of 20 cases. In: Satoyoshi E, ed. *Myasthenia gravis: pathogenesis and treatment*. Tokyo: University of Tokyo Press, 1981;41–54.
5. Garlepp MJ, Kay PH, Farrow BR, et al. Autoimmunity in

spontaneous myasthenia gravis in dogs. *Clin Immunol Immunopathol* 1984;31:301-306.

6. Shelton GD, Cardinet GH III, Lindstrom JM. Canine and human myasthenia gravis autoantibodies recognize similar regions on the acetylcholine receptor. *Neurology* 1988;38:1417-1423.

7. Garlepp M, Farrow B, Kay P, et al. Antibodies to the acetylcholine receptor in myasthenic dogs. *Immunology* 1989;37:807-810.

8. Shelton GD, Willard MD, Cardinet GH III, et al. Acquired myasthenia gravis: selective involvement of esophageal, pharyngeal, and facial muscles. *J Vet Intern Med* 1990;4:281-284.

9. Dewey CW, Bailey CS, Shelton GD, et al. Clinical forms of acquired myasthenia gravis in dogs: 22 cases (1988-1994). *J Vet Intern Med* 1997;11:50-57.

10. Dewey CW, Shelton GD, Bailey CS, et al. Neuromuscular dysfunction in five dogs with acquired myasthenia gravis and presumptive hypothyroidism. *Prog Vet Neurol* 1995;6:117-123.

11. Aronsohn MG, Schunk KL, Carpenter JL, et al. Clinical and pathologic features of thymoma in 15 dogs. *J Am Vet Med Assoc* 1984;184:1355-1362.

12. Krotje LJ, Fix AS, Potthoff AD. Acquired myasthenia gravis and cholangiocellular carcinoma in a dog. *J Am Vet Med Assoc* 1990;197:488-490.

13. Klebanow ER. Thymoma and acquired myasthenia gravis

in the dog: a case report and review of 13 additional cases. *J Am Anim Hosp Assoc* 1992;28:63-69.

14. Braund KG. Peripheral nerve disorders. In: Ettinger SJ, Feldman EC, eds. *Textbook of veterinary internal medicine*. Philadelphia: WB Saunders Co, 1995;701-726.

15. Lindstrom J, Einarson B, Tzartos S. Production and assay of antibodies to acetylcholine receptors. *Methods Enzymol* 1981;74:432-460.

16. Breslow NE, Day NE. Statistical methods in cancer research. In: *The analysis of case-control studies*. Vol 1. New York: Oxford University Press, 1980;191-246.

17. Compston DAS, Vincent A, Newsom-Davis J, et al. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain* 1980;103:579-601.

18. Kerzin-Storarr L, Metcalf RA, Dyer PA, et al. Genetic factors in myasthenia gravis: a family study. *Neurology* 1988;38:38-42.

19. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody characteristics in myasthenia gravis. I. Patients with generalized myasthenia or disease restricted to ocular muscles. *Clin Exp Immunol* 1982;49:257-265.

20. Limburg PC, The TH, Hummel-Tappel E, et al. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part I. Relation to clinical parameters in 250 patients. *J Neurol Sci* 1983;58:357-370.