

Assessment of cord1 PRA and the RPGRIP1 gene in the English Springer Spaniel for the ESSFTA

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The major form (cord1; cone rod dystrophy) of progressive retinal atrophy (PRA) in English Springer Spaniels was found to be controlled by a recessive mutation in the RPGRIP1 gene. The identified mutation (a 44 base pair insertion) was found while studying the same disease in Miniature Longhaired Dachshunds. At the announcement of the discovery and availability of a genetic test for the mutation in 2007, it was discussed that the mutation occurred at a high frequency in the ESS population, and that the majority of homozygous "at risk" dogs have normal retinal examinations - even at an advanced age.

To further understand the inheritance of cord1 PRA in the breed and its relationship to the identified mutation - and therefore to formulate an appropriate breed health strategy; the known parameters of the breed, the mutation, and population changes over time should be reviewed.

Clinical PRA in the breed

Based on ESS CERF eye examinations, and more recently OFA eye examinations by ACVO Boarded ophthalmologists, PRA is an infrequent diagnosis in the breed:

Time Period	PRA Diagnosis	Total#	ESS Eye Exams	Frequency of PRA
1991-1999	165	15,812		1.04%
2000-2009	231	20,017		1.15%
2010-2015	83	10,444		0.80%

(The genetic test was available in 2007 - therefore contributing to the later decline in afflicted dogs.)

Based on Dr. Kristina Narfstrom's work at Missouri (published in [Stem Cells Int.](#) 2012;2012:685901. doi: 10.1155/2012/685901) English Springer Spaniels with clinical cord1 PRA develop **clinical signs** of impaired vision on average between 6 to 9 years of age. **Ophthalmoscopic** (CERF/OFA exam) **changes** could be seen in some dogs as early as 1.5 to 2 years of age. However, Dr. Narfstrom documented that several homozygous "at risk" dogs had no ophthalmoscopic or electroretinographic changes of PRA even at an advanced age. Her conclusion was, *"It appears likely that additional factors are warranted for initiation of photoreceptor cell death such as additional loci involved as modifiers of the disease..."* (Loci=gene locations.)

Further study of the OFA Eye examinations since 2012 (which capture more individual dog data than the CERF exams) show that 295 ESS eye examinations occurred in dogs over 8 years of age. Of those, 23 exams resulted in a diagnosis of PRA (7.8%). (This does not necessarily represent the general frequency of PRA in elderly dogs, as the population of owners bringing in elderly dogs for OFA eye examinations is probably skewed towards homozygous "at risk" dogs.)

The OFA data also document several exams on identified homozygous "at risk" dogs over 10.5 years of age with normal retinal examinations. (As RPGRIP1 status was not a fill-in value on the exam forms, these data are based on ophthalmologist notes and no statistical data can be assigned.)

Testing for the RPGRIP1 mutation

Data was provided by the University of Missouri (Dr. Johnson's) genetic testing laboratory on 8,810 ESS tested for the RPGRIP1 mutation. The following results were extracted (full testing data are available):

Year	# Dogs tested	Homozygous "at risk"	Heterozygous carrier	Homozygous normal
2007	3,369 *	42.1%	38.0%	19.9%
2016-17**	667	24.3%	45.7%	30.0%

* Including dogs in the Mo. ESS DNA bank

** Through 8/9/17

These data show that breeders are using the genetic test to shift the population by diminishing the amount of homozygous "at risk" dogs, and increasing the amount of homozygous normal testing dogs. In addition, the mutant gene frequency for the breed population was 61.7% in 2007, and is 47.2% in the 2016-17 time period. This shows that breeders are not just preventing the production of homozygous "at risk" dogs with their mating choices, but are preferentially eliminating breeding dogs that have the RPGRIP1 mutation. Due to the high frequency of the mutation in the breed, this has caused a restriction of the breed gene pool and resultant loss of genetic diversity.

Estimates of the "penetrance" of the RPGRIP1 mutation for clinical PRA

There are no data that give us valid population statistics on the percentage of homozygous "at risk" dogs that will develop clinical PRA. However, using the above data, we can extrapolate what the highest and lowest percentages would be for clinical penetrance.

All eye examinations from 2000-2015 showed approximately 1% of ESS with changes consistent with PRA (0.8% to 1.15%). UMo testing from 2007-2017 showed 32.6% of ESS testing as homozygous "at risk". If these are taken to represent the same general population, then this would show the RPGRIP1 mutation to be 3.1% penetrant (the percent of "at risk" dogs showing clinical PRA). This is an **underestimation** of penetrance because eye examination data include many dogs that would be at a younger age than clinical diagnosis with an ophthalmoscopic exam.

OFA eye examinations of all ESS over the age of 8 examined since 2012 showed 7.8% clinically afflicted with PRA. During this time period, 22.9% of ESS tested by UMo were homozygous "at risk". If these are taken to represent the same general population, then this would show the RPGRIP1 mutation to be 34.0% penetrant. This is probably an **overestimation**, as both the percentage of examined dogs, as well as the tested mutation frequency in dogs represent different age groups that would alter the percentages.

That said, we can reasonably state that the "penetrance" of the RPGRIP1 mutation for causing clinical PRA lies somewhere between 3.1% and 34.0%. However, this is not really the proper use of the word "penetrance". Penetrance relates to the phenotypical (clinical) expression of the genotype due to other than the effects of other genes. It relates to an inherent issue of the genotype expressing itself. When a gene (or gene pair) is not expressed due to the effects of **other** genes, this is due to an epistatic (blocking) effect of complex (polygenic) inheritance. This means that if the "at risk" genotype is only expressed - let's say for example - 20% of the time, it is because only 20% of the population carries the additional genes necessary for clinical PRA. It is not a uniform 20% risk across the population, but risk clustered within families carrying the additional genes. This is an important concept, because we CANNOT state that "at risk" dogs have a xx% chance of developing clinical disease.

Data from the commercial Genoscooper/Mars DNA screening of multiplexed genetic markers across all mixed and purebred dogs shows that in all purebred dogs, 1.41% carry the RPGRIP1 mutation, and 3.66% of mixed-breed dogs carry the RPGRIP1 mutation; making it the second most common testable mutation in all dogs. It has not to date (to my knowledge, that of the researchers and a PubMed search)

caused clinical PRA in other than the MLD and ESS breeds. The gene frequencies of the RPGR1PI mutation are much higher in these two breeds. While there is/are additional gene(s) required for the expression of clinical cord1 PRA, the additional liability gene(s) may differ between the MLD and ESS breeds.

Questions regarding the RPGR1PI mutation

Some breeders question whether the RPGR1PI mutation is actually of any use. They see the high number of elderly "genetically affected" dogs that are not "afflicted" and question the validity of the mutation test. Research at Dr. Aquirre's lab (UPenn; Kuznetsova et. al. ; Invest Ophthalmol Vis Sci. 2012 Aug 15;53(9):5486-501. doi: 10.1167/iops.12-10178.) concluded that the 44 base pair insertion was not the molecular cause of retinal cell death, but instead another mutation in the RPGR1PI gene was the cause. This new information is inconsequential, because it still occurs in the same "critical interval" (location) that is being tested for with the current RPGR1PI gene test.

There is no question that ALL ESS clinically afflicted with the cord1 form of PRA test homozygous "at risk" with the RPGR1PI mutation test. Therefore the RPGR1PI test is definitely telling us something about the possibility of developing or NOT developing cord1 PRA. There are some PRA afflicted dogs in the breed that do not test homozygous "at risk", and the breed has been aware for some time that there are other unidentified PRA-causing mutation(s) present in the breed. This is a common occurrence in many breeds. However, the vast majority of PRA afflicted ESS have cord1 PRA.

What does this all mean for today's dogs?

It is obvious that the RPGR1PI mutation occurs at a high frequency in the ESS breed. In the 2016-2017 period 70% of ESS are carriers or homozygous "at risk" for the mutation. Breeders are removing English Spring Spaniels from breeding based solely on the results of RPGR1PI mutation testing. This is putting a significant drain on the breed's gene pool and loss of genetic diversity for reasons that do not have to do with the individual dog's health or ability to pass on disease.

Recommendation: Breeding decisions on dogs should NOT be based on the RPGR1PI mutation results unless they are closely related to clinically afflicted dogs.

The ideal way to utilize the RPGR1PI mutation test would be to determine the specific pedigree background of the ESS population that is at higher risk of carrying other liability genes necessary to cause clinical disease AND therefore those who should undergo selection based on the mutation test. The most objective way to do this would be to maintain an open health pedigree database that shows homozygous "at risk" dogs validated as clinically afflicted with cord1 PRA. Then relative risk pedigree analysis could determine the risk of any ESS to develop PRA, and those with significantly greater risk than the breed average should utilize the mutation test for breeding decisions.

The establishment of such a database requires the cooperation of breeders and owners to release information on verified cord1 PRA afflicted dogs. This is a significant undertaking through my experience with other breeds. The ESSFTA and the ESS Foundation need to consider whether going in this direction is right for the breed. The alternative to this would be to identify other liability genes that are affecting the expression of cord1 PRA and develop additional genetic tests run as a panel (including the RPGR1PI mutation). This is probably a more attainable and logical goal with the current state of molecular genetic research.

Without an open database of cordl afflicted dogs and a relative risk pedigree analysis database, breeders are left to determine **on their own** whether their breeding dogs are closely related to verified cordl PRA afflicted dogs.

Recommendation: The ESSFTA and the ESS Foundation should consider whether to establish a voluntary database of clinically verified cordl afflicted ESS that is freely available to its members, for the pedigree determination of cordl afflicted risk.

The search for other genes that are also responsible in concert with the RPGRIP1 mutation for causing clinical cordl PRA in the breed is an undertaking that ESSFTA and the ESS Foundation should be promoting and funding. Dr. Keiko Miyadera, when working with Dr. David Sargan at Cambridge identified a location (MAP9) on chromosome 15 in Miniature Longhaired Dachshunds that when homozygous in RPGRIP1 "at risk" dogs developed early onset cordl PRA (Mamm Genome. 2012 Feb;23(1-2):212-23. doi: 10.1007/s00335-011-9384-9.) Based on my discussions with the researchers the MAP9 mutation has not been found to date in ESS. It is possible that ESS have a different additional gene or genes responsible for cordl expression (than in MLD).

Ors. Miyadera is now in residence at the University of Pennsylvania with Dr. Aguirre and they recently published an article on the MLD (Sci Rep. 2017 Oct 9;7(1):12823. doi: 10.1038/s41598-017-13112-w.) that states, "Our results indicate that cordl is a multigenic disease in which mutations in neither RPGRIP1 nor MAP9 alone lead to visual deficits, and additional gene(s) contribute to cone-specific functional and morphologic defects."

Dr. Miyadera has contacted the ESSFTA/ESS Foundation with her willingness to pursue further molecular genetic research into cordl PRA in the ESS. There are other excellent researchers who may also wish to pursue this investigation.

Recommendation: The ESS Foundation should initiate a request for proposals (RFP) and should review submitted grant proposals to determine funding. This should probably be done through the CHF with their established system of administrative oversight and review.

Please let me know if you have any further questions on this topic.

Updated 24 March 2018