Microphthalmia in SCWT: Information to Help You Make Breeding Decisions

*The information in italics below specifically concerns microphthalmia in Soft Coated Wheaten Terriers (SCWT) and is summarized from an impressive collaborative publication (Kaukonen et al. 2018 Cell Reports 23(9):2643-2652) from the laboratories of Dr. Hannes Lohi (Department of Veterinary Biosciences, University of Helsinki, Finland) and Dr. Tom Glaser (School of Medicine, University of California, Davis).

What is microphthalmia? The term microphthalmia (Greek for small eye) refers to a condition in which one or both eyes are smaller than normal. In dogs, small eyes are seen that appear to be sunken into the eye socket. The third eyelid may be more visible than usual because the eye isn't large enough to hold it in place. Individuals with microphthalmia (humans and dogs, as well as other species) typically have various structural malformations of the eye globe (the medical term for eyeball). The lens and the cornea may be cloudy or opaque and the anterior chamber (front of the eye globe) as well as the retina (inside interior surface of the back of the eye globe) may have structural defects. Affected dogs are often clumsy, may have difficulty with coordination, behavior abnormalities, somnolence and partial or complete vision loss. Microphthalmia develops before birth, and therefore is referred to as a congenital disease. It can occur alone or as part of a syndrome (a disease that affects multiple different organs).

What research in SCWT lead to the DNA test? (This is description of the research. If you are not as interested in the actual experiments, skip to the "Considerations for genetic testing" below.)

**The research into microphthalmia in SCWT began in 2011 when unusually small eyes were observed in three puppies from a litter of six. Three additional litters containing puppies with small eyes were eventually identified. These litters were born to three different dams and four different sires in three countries in Europe. All parents were related within an extended pedigree. In addition to microphthalmia, these puppies had gaps and incomplete development of the critical tissue layers in the back of the inside of the eye globe that are responsible for vision (referred to in the paper as choroidal hypoplasia and retinal coloboma). Eye exams (before 10 weeks of age), revealed that affected dogs had microphthalmia of both eyes in addition to eye malformations, except for one puppy that had only the eye malformations but not microphthalmia.*

A whole genome association study (GWAS, a standard genetic approach analyzing thousands of DNA variations across all chromosomes) was performed, using DNA from 17 affected and 12 normal puppies (confirmed with eye exams). This study identified a specific region on dog chromosome 28 where the gene involved in this disease was located. The next step was to sequence the genome. (of an affected dog and compare it to the genome sequences of 340 dogs of multiple breeds. (Genome refers to all the DNA that makes up the chromosomes.) This was the most efficient method to find the specific DNA change in the region on chromosome 28 that was both unique to the SCWT with microphthalmia and that also caused a change in a protein involved in eye development. This step was successful and identified a small deletion in a gene, called RBP4, that had both copies (homozygous) of the version of the gene with the deletion variant. (We refer to this version as allele "2" or RBP2del and the normal version of the RBP4 gene as allele "1" RBP4+ (allele is the term geneticists use for the alternate versions of a gene that differ slightly in sequence). Therefore, 2-2 represents the genetic composition of dogs that have the RPB4del deletion allele for both copies of the RBP4 gene, dogs with one copy of the

RBP4del allele are designated as 1-2, and dogs with both copies of the normal RBP4+ allele are designated 1-1.)

The next step was to examine the genetic composition of all the available dogs in the extended pedigree to determine the mode of inheritance. As expected, all 12 SCWT cases were homozygous for the RBP4del variant (2-2) and all clinically evaluated normal controls were either heterozygous (1-2) or had only the normal RBP4+ version of the gene (1-1). However, and surprisingly, the ALL three dams who produced the four litters (all three dams were normal by eye exam) were 2-2 (both copies of the RBP4del variant). Importantly, these normal 2-2 dams had mothers that were 1-2. This suggested that this was a mode of inheritance with some characteristics of a recessive mode of inherence but that had a maternal effect.

To study this unusual pattern of inheritance, the Lohi laboratory examined additional DNA samples from almost 250 SCWT, and found eight additional 2-2 dogs, homozygous for the RBP4del allele. Three of these dogs had normal eye exams, four had normal general health exams with no apparent microphthalmia, and one had defective development of the retinal area of the back of the eye (chorioretinal hypoplasia). The dams of six of the seven normal dogs were all 1-2 (DNA from the seventh dam was not available), while the dam of the dog with chorioretinal hypoplasia was 2-2. So, based on this data, microphthalmia is express only in 2-2 pups whose mother was also 2-2. (There is the caveat that less than 100% of this type of pup had microphthalmia, although all pups had some sort of microphthalmia related eye defect.) They also performed very thorough examinations of the eyes of a number of clinically normal heterozygous carrier dogs (1-2) to determine if 1-2 dogs are at risk for vision loss. Of the 46 carriers that had eye exams, 37 of their mothers had DNA analyzed, with approximately equal numbers of 1-1, 1-2, and 2-2 mothers. None of the carriers had microphthalmia, and only two had chorioretinal hypoplasia and these were two were born to 2-2 mothers and were littermates of affected pups. Among the 248 dogs that were analyzed, the frequency of the RBP4 deletion variant was 14%.

The collaborating laboratories went on to perform elegant biochemical experiments to understand the molecular effects of the deletion in the RBP4 gene, particularly in utero and with respect to vitamin A metabolism.

#NOTE-the research did not include measurements of vision/ability to see. The clinical ophthalmology exams used in the research measured structural changes in the eye or other disease pathology but did not measure vision.

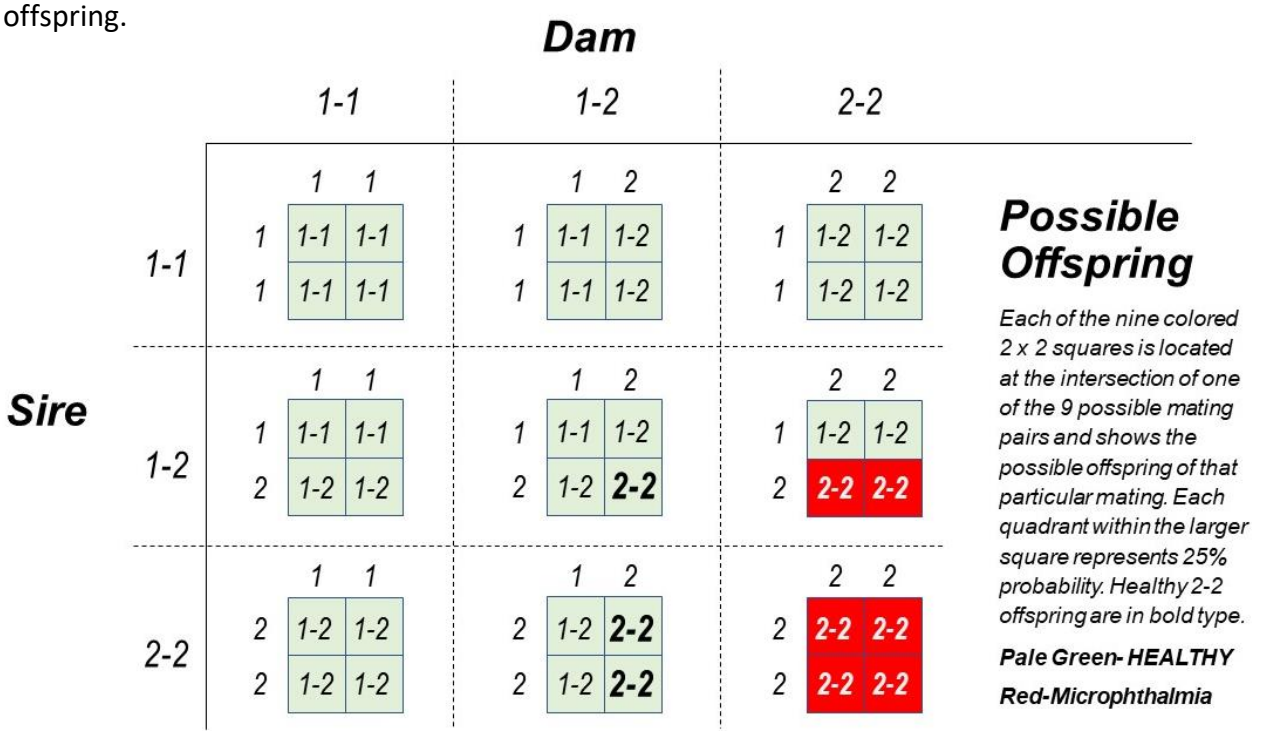
<u>Summary points and considerations for genetic testing</u>

- Identification of a small deletion in both copies of the RBP4 gene in dogs with microphthalmia provided the basis for a DNA-based genetic test.
- Microphthalmia is inherited in an unusual pattern [see the diagram below for specific mating outcomes]
  - Puppies are affected ONLY if:
    - they have two copies of the RBP4 deletion (2-2)
      AND
    - their dam also has both copies of the deletion version of the RBP4 gene

- this is possible only in matings between 2-2 dams bred to 1-2 or 2-2 sires

  - If the dam only has one copy of the gene with the deletion (1-2), all puppies are expected to be normal, regardless of their individual RBP4 gene configuration [1-1, 1-2, or 2-2[.
  - It is possible for both male and female puppies to be 2-2 but not be affected [matings 1-2 dams to either 1-2 or 2-2 sires]
  - Matings of 1-2 dams to 1-1, 1-2, or 2-2 sires can produce <u>healthy</u> 2-2 offspring. The female 2-2 offspring produced will go on to produce healthy offspring if bred to 1-1 sires but can produce offspring with microphthalmic if bred to 1-2 or 2-2 sires.

- Carrier offspring (1-2) of matings between 2-2 dams and 1-2 sires may be at risk for vision loss. (This mating is discouraged since it can produce 2-2 microphthalmic offspring)
- There are rare cases of 2-2 offspring of 2-2 dams that do not have microphthalmia (small eyes) but do have other eye defects.
- All breeding decisions should take into the account that recommendations are based on a study that included only 17 dogs affected with microphthalmia. Exceptions to the "rules" that this disease appears to follow may appear over time.
- The frequency of the RBP4 deletion allele in North America is not known. Of 20 random DNA samples stored and analyzed at PennGen, two were carriers (1-2). This strongly suggests that this disease-associated gene variant RBP4del is not restricted to European SCWT.
- Caution should be exercised to avoid losing variation in the breed gene pool while also avoiding microphthamic pups. Notice in the diagram, that no matter the gene configuration (genotype) of a sire or dam, there is a possible mating that can be performed that does not produce affected offspring.



Paula Henthorn, Ph.D.
Professor of Medical Genetics
University of Pennsylvania School of Veterinary Medicine