The Inheritance of Livershunt in Irish Wolfhounds

By Maura Lyons, PhD

As this is an article about inheritance I have included the Glossary of Terms at the beginning to help readers understand the genetics terminology used throughout.

Glossary of Terms

Gene	A piece of DNA that provides the 'recipe' for an enzyme or a protein.
Gene locus	The position of a gene on a chromosome.
Allele	A version of a gene inherited from one of the parents. A gene may have many different alleles, but only 2 will occupy the gene locus, one from the dam and one from the sire.
Gamete	A sex cell; an egg from a female and a sperm from a male. Each sex cell has one complete set of chromosomes. During fertilisation, the egg and sperm fuse to create an embryo with a pair of each chromosomes, one from the father/sperm, one from the mother/egg.
Chromosome	Genes are arranged on chromosomes. Dogs have 39 pairs of chromosomes. One of each pair is inherited from the sire and one from the dam.
Autosome	All the chromosomes which are not sex chromosomes, therefore they will not show sex-linked differences.
Recessive	An organism needs two recessive alleles for a trait to be expressed. With recessive characteristics, an organism can be a 'silent' carrier if it is heterozygous at that locus.
Dominant	A dominant allele is one which will mask the expression of a recessive allele. So an organism only needs one dominant allele for the trait to be expressed.
Heterozygous	When the two alleles an organism carries differ from each other.
Homozygous	A gene where the two alleles an organism carries are both the same.
Genotype	The specific alleles which are carried by an organism.
Phenotype	The physical characteristic that is visible when a genotype is expressed.
Expressed trait	The characteristic that the gene codes for.
Healthy/Clear	When referring to an individual, we can say they are healthy or clear if they are not affected by the trait in question. The genotype would usually be homozygous for this gene.
Carrier	An individual that does not show symptoms of the disease or trait but has the ability to produce affected offspring. These individuals are heterozygous. We only get carriers when we are referring to a recessive condition.
Affected	An individual that is affected by the disease or trait in question.
Hardy-Weinberg Equilibrium	The Hardy–Weinberg equilibrium states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences. Because one or more of these influences are typically present in real populations, the Hardy–Weinberg principle describes an ideal condition against which the effects of these influences can be analysed.
Linkage Disequilibrium	When 2 genes are located closely together on the chromosome, the 2 alleles from a parent may be more likely to be inherited together, so that the inheritance of a specific genotype may be more likely.
Punnett Square	A diagram that is used to predict an outcome of a particular cross or breeding experiment.

Introduction

Livershunt, or intrahepatic portosystemic shunt, is a hereditary condition in Irish Wolfhounds. Essentially, it occurs when the ductus venosus fails to close shortly after birth. This means that the pup's blood is not filtered through the liver but deposited directly back into the main bloodstream. Since the liver has not detoxified the blood, a build up of toxins occurs and affects the pup's other organs. Whilst many papers show that this is a condition that is inherited in wolfhounds it has not been easy to decipher the exact mode of inheritance. In 2009, however, a Dutch team conducted experiments and proposed a mode of inheritance which includes 3 mutated alleles being present over 2 genes. In this article, I will try to explain what this means and how we can use this information to minimise our risk of having affected puppies. I will show how to use genetic tools to calculate the likely risk associated with a proposed mating. These calculations will only be accurate and therefore useful, if the breeder has a good knowledge of where affected puppies and known producers occur within the pedigree. This information and knowledge of previous shunt test results are crucial to avoid having affected litters.

Dutch Research

In a paper, I came across some time ago (*FG van Steenbeek et al. 2009. Evidence of Inheritance of Intrahepatic Portosystemic Shunts* (IHPSS or livershunt) *in Irish Wolfhounds. J Vet Intern Med 23(4):950-2*) the mode of inheritance of IHPSS in Irish Wolfhounds was proposed to be *digenic* (two genes) *triallelic* (three alleles). This proposition was based on test matings between 3 affected siblings, one male and his 2 sisters. Each sister had a single litter. The first litter of 5 puppies consisted entirely of livershunt affected puppies. The second litter of 11 puppies contained 5 affected and 6 clinically normal puppies. Livershunt was previously thought to be inherited via a simple autosomal recessive mode, this simple experiment has proved this is not the case. If livershunt was passed on as a simple monogenic recessive trait, then all puppies in both litters would be affected.

The digenic triallelic mode of inheritance means that affected individuals must possess 3 mutant alleles over 2 gene loci. The authors proposed that this meant affected individuals must possess at one gene locus one copy of a mutant allele displaying dominant characteristics, and 2 copies of the mutant allele at a second gene locus displaying recessive characteristics.

As a reminder of simple Mendelian genetics...

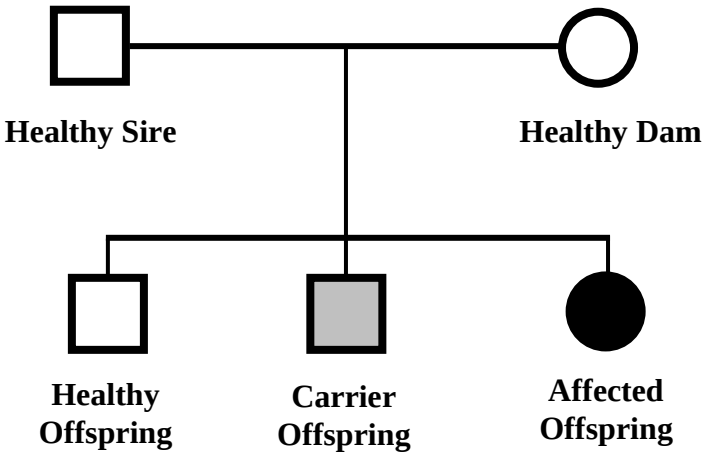
Dog genomes are diploid, meaning they carry two copies (alleles) of each gene, one allele inherited from the mother and one inherited from the father.

Depending on the mechanism of expression, alleles may be thought of as dominant or recessive. A dominant allele is one which masks the expression of the other allele at the same locus, so only one allele, the dominant one, needs to be present for a trait to be seen. A recessive allele (the one that is usually masked by its dominant partner) needs to be present in 2 copies to be expressed.

So for livershunt to be expressed, one dominant allele needs to be present at Locus A, and 2 recessive alleles need to be present at Locus B. This means that the dominant allele may be contributed by only one parent but the recessive allele must be inherited from both parents.

So, we have a range of possible outcomes when we mate apparently healthy dogs.

Figure 1. Possible Outcome of any Mating



Using a Punnett Square we can show the range of possible alleles which any sire and dam can pass on to their offspring and the proportion of genotypes amongst the offspring. (Dominant alleles will be represented by a capital letter and recessive by a lower case letter). For this Punnett Square we have assumed that both Sire and Dam are heterozygous (Aa) at the A gene locus.

Figure 2 Single-Locus Punnett Square

Genotype of Sire = Aa	Gamete 1 = A	Gamete 2 = a
Genotype of Dam = Aa		
Gamete 1 = A	Offspring 1 = AA (25%)	Offspring 2 = Aa (25%)
Gamete 2 = a	Offspring 3 = aA (25%)	Offspring 4 = aa (25%)

Consider the Punnett square above...
The sire can only contribute one allele to his offspring, either 'A' (dominant expression) or 'a' (recessive expression). Similarly the dam can only contribute one allele to each offspring. In this example the sire and dam are both heterozygous, that is they each carry a dominant allele 'A' and a recessive allele 'a'. Homozygous is the genotype where both alleles are identical. If heterozygous parents are mated there is the potential to produce all possible genotypes in the proportion; homozygous dominant AA = 25%, heterozygous Aa or aA = 50%, homozygous recessive aa = 25%. Remember though, that these proportions are only probabilities, a mathematically ideal scenario, nature seldom complies. Imagine if you have a litter of 7 puppies, how would you work out how many of each genotype you have?

What this Punnett Square does show is that the genotype of the sire and dam, which they inherited from their parents, will determine the likelihood of any given genotype in the offspring.

The possible genotypes for each of the 2 gene loci involved in IHPSS is the same (assuming there are only 2 alleles within the population) but whether or not they are expressed depends on which alleles, dominant or recessive, are carried at each locus.

The proportion of *phenotypes* expressed (the physical characteristic encoded by the gene) may be different depending on the mechanism of expression.

Dominant Trait
If the dominant allele is expressed in the phenotype, then the proportion is; affected = 75%, unaffected 25%. Remember we only need one copy of the dominant allele so AA and Aa and aA all show the trait. For Locus A only the recessive homozygous condition aa does not show the trait.

Recessive Trait
For a recessive trait to be expressed we need the homozygous genotype aa, so in this case affected individuals would be 25% of the population whilst unaffected individuals would be 75%. But with recessive traits we can get carriers which do not show the trait but have the ability to pass it on to their offspring. These are the heterozygous genotypes, some 50% of the population, Aa and aA.

Figure 3. 2-Locus Punnett Square
Remember there are putatively two gene loci involved with IHPSS, so now we will look at a 2-locus Punnett Square. This Punnett Square shows the proportion of genotypes in the puppies when both parents are heterozygous at both gene loci. When the parent is heterozygous the number of possible gametes that can be passed to the offspring is four; AB, Ab, aB and ab. If we assume that A is the gene whose trait shows when the dominant form is expressed and b is expressed in the recessive condition, then we can also assign phenotypes to each of these genotypes.

Genotype of Sire = AaBb	A B	A b	a B	a b
Genotype of Dam = AaBb				
A B	AA BB	AA Bb	Aa BB	Aa Bb
A b	AA Bb	AA bb	Aa Bb	Aa bb
a B	Aa BB	Aa Bb	aa BB	aa Bb
a b	Aa Bb	Aa bb	aa Bb	aa bb

With 2 genes to consider, each having only 2 alleles, the number of different possible genotypes in the population is 9. In this Punnett Square each possible offspring genotype represents 6.25%, some of the genotypes are duplicated which gives us the following 9 genotypes in these proportions...

Genotype	Proportion of Litter (%)	Phenotype
AABB	6.25	Carrier of Dominant
AABb	12.5	Carrier of Dominant and Recessive
AAbb	6.25	Affected, IHPSS
AaBB	12.5	Carrier of Dominant
AaBb	25	Carrier of Dominant and Recessive
Aabb	12.5	Affected, IHPSS
aaBB	6.25	Clear
aaBb	12.5	Carrier of Recessive
aabb	6.25	Carrier of Recessive

(There are a number of assumptions used here - that the genes are autosomal and that they exhibit no linkage disequilibrium, and that there are only 2 possible alleles at each locus. Suffice to say I have used the simplest model for the purposes of demonstration. This does not affect the validity of any conclusions reached.)

In this scenario, 18.75% of the puppies are affected with IHPSS (shown in red), whilst only 6.25% are clear, (shown in green). The rest of the population, 75%, are all carriers of either the recessive mutant allele, the dominant mutant allele, or both (all shown in black).

So what does all this mean for livershunt in wolfhounds?

As far as we can tell, global breed prevalence for livershunt has been steady at about 3% for many years, with 18% of litters born including affected puppies (Lifespan and Disease Predispositions in The Irish Wolfhound : A Review, S.R.Urfer, C. Gaillard, A. Steiger). In my own, albeit limited, experience the number of IHPSS affected puppies in any given litter is usually small, 1 or 2 in the entire litter, whilst many litters are born with no affected puppies at all. This is not representative of the 18.75% affected dogs proposed above in the mathematically ideal scenario. Given what we have learnt so far it is natural to presume that the genotype represented in the highest number in the Irish Wolfhound population is aaBB, this genotype is 'clear' at both loci. And when an aaBB sire is mated to an aaBB dam, all the puppies have the genotype aaBB.

So, what genotypes are capable of producing IHPSS affected puppies? Remember both parents must contribute a copy of the recessive mutant allele, whilst only one may contribute the dominant mutant allele. Also remember that dogs affected with IHPSS are seldom bred from, so we will assume that only carrier genotypes are used in breeding. (Any genotype bred to a 'clear' gentotype will not produce IHPSS affected puppies, but may produce more carriers.)

We have 7 different scenarios representing all the possible combinations of genotypes that have the potential to produce affected puppies.

Scenario 1. Litter of puppies is 25% Affected, 75% Carriers

Genotype of Sire = AABb	AB	Ab
Genotype of Dam = AABb		
AB	**AABB**	**AABb**
Ab	**AABb**	**AAbb**

Scenario 2. Litter of puppies is 25% Affected, 75% Carriers

Genotype of Sire = AABb	AB	Ab
Genotype of Dam = AaBb		
AB	AABB	AABb
Ab	AABb	AAbb
aB	AaBB	AaBb
ab	AaBb	Aabb

Scenario 3. Litter of puppies is 25% Affected, 75% Carriers

Genotype of Sire = AABb	AB	Ab
Genotype of Dam = aaBb		
aB	AaBB	AaBb
ab	AaBb	Aabb

Scenario 4. Litter of puppies is 50% Affected, 50% Carriers

Genotype of Sire = AABb	AB	Ab
Genotype of Dam = aabb		
ab	AaBb	Aabb

Scenario 5. Litter of puppies is 18.75% Affected, 75% Carriers, 6.25% Clear

Genotype of Sire = AaBb	A B	A b	a B	a b
Genotype of Dam = AaBb				
A B	AA BB	AA Bb	Aa BB	Aa Bb
A b	AA Bb	AA bb	Aa Bb	Aa bb
a B	Aa BB	Aa Bb	aa BB	aa Bb
a b	Aa Bb	Aa bb	aa Bb	aa bb

Scenario 6. Litter of puppies is 12.5% Affected, 75% Carriers, 12.5% Clear

Genotype of Sire = AaBb	A B	A b	a B	a b
Genotype of Dam = aaBb				
a B	Aa BB	Aa Bb	aa BB	aa Bb
a b	Aa Bb	Aa bb	aa Bb	aa bb

Scenario 7. Litter of puppies is 25% Affected, 75% Carriers

Genotype of Sire = AaBb	A B	A b	a B	a b
Genotype of Dam = aabb				
a b	Aa Bb	Aa bb	aa Bb	aa bb

Considering what we have deduced, it seems likely that scenario 6 is the most common mating of genotypes which produces IHPSS puppies. In this example, 1 in 8 pups would be affected with IHPSS. If we look closely at the genotypes of the parents involved (it does not matter which genotype is assigned to which parent) we can see that the Dam's genotype, aaBb, only differs by one allele from the (putative) most common wolfhound genotype, aaBB, and the Sire's genotype, AaBb, only by 2 alleles.

This is the minimum number of mutated alleles that must be mated in order to produce affected puppies. Please remember these figures represent statistical probabilities, real life often doesn't match the numbers expected exactly.

Now, knowing that, we can see that if a litter has produced an affected puppy, then 75% of the affected puppy's siblings are carriers of at least one of the mutated alleles, and only 1 in 8 of the litter can be considered to have a clear genotype.

So, can we do a similar deduction for the litter siblings of known producers?

Known producers are proven carriers, because they have already produced affected puppies but have shown no symptoms of IHPSS themselves, often having been subject to a bile-acid test when they themselves were puppies. So, if we assume that each known producer has come from one clear parent (aaBB, the most common wolfhound genotype) and one parent with the minimum number of mutations needed to produce a carrier of both the recessive and dominant mutation, or a carrier of only the recessive mutation, we get the following Punnett Squares. We know that at least one of the known producers has to carry the dominant mutation but it is almost impossible to decipher which parent it is.

Scenario 8. Litter of puppies is 25% Clear, 75% Carriers

Genotype of Sire = aaBB	aB
Genotype of Dam = AaBb	
AB	AaBB
Ab	AaBb
aB	aaBB
ab	aaBb

Scenario 9. Litter of puppies is 50% Clear, 50% Carriers

Genotype of Sire = aaBB	aB
Genotype of Dam = aaBb	
aB	aaBB
ab	aaBb

Again, it does not matter which genotype is assigned to which parent, the only unfortunate fact is that it is impossible to tell for certain which is the clear parent. Also remember that I have used the simplest model, it may be that neither parent has a 'clear' genotype, that is no mutant alleles. As can be seen, the litter siblings of known producers also carry at least a 50% chance of also being carriers.

Note, when I have spoken about carriers I have made no distinction between carriers of single mutant alleles whether dominant or recessive, or carriers of both traits. A carrier of a single dominant mutant allele without the recessive mutant allele, will never become a known producer – but may be the parent of a known producer, if mated to another carrier.

In conclusion, as a relatively new breed fancier, only 20 years under my belt, with aspirations to responsible and ethical breeding, and a scientific background, my natural leaning is more toward research than 'gut instinct'. I do believe in trying to demystify the area of genetics and inheritance and utilising that knowledge to make informed breeding decisions.

These are not spontaneous mutations occurring frequently and randomly within the population. It is likely that one founder had the dominant mutant allele and another different founder the recessive, from these small beginnings the two mutant alleles have silently spread through the population until reaching a point where both mutations appeared within a single individual to produce the effect known today as IHPSS.

I believe that responsible breeders will use this knowledge wisely. If you know that there are known producers or their siblings or parents in your line, then choose your mate very, very carefully. Do not just study your preferred mate's pedigree but also their siblings' pedigree, and enquire after the outcome of IHPSS tests of any previous litters that have been produced by siblings and parents siblings. If you are a breeder with a website, consider publishing every test result you get, whether the pup will be used for future breeding or not. This information will be invaluable to the Irish Wolfhound population. Perhaps in future, this information could be collated into a valuable database resource. By being thorough in our research and making good decisions based on the available knowledge we can minimise (but probably not entirely eliminate) the risk of producing IHPSS affected puppies in our litters, and therefore minimise the heartache associated with breeding an affected puppy.