# Genetic insights in canine degenerative myelopathy

*Genetische inzichten in degeneratieve myelopathie bij honden*

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## ABSTRACT

**Canine degenerative myelopathy (DM) is a late-onset, progressive, neurodegenerative disorder with a fatal outcome, occurring in a vast number of dog breeds. Most dogs are at least eight years of age when they begin to show clinical signs, starting with general proprioceptive ataxia in the hind limbs and upper motor neuron paraparesis, evolving to lower motor neuron tetraplegia and brain stem signs. A definitive diagnosis can only be made postmortem by the histopathological observation of neuronal degradation and demyelination of the spinal cord. Most DM-affected dogs are homozygous for one of the known superoxide dismutase 1 gene (*SOD1*) mutations (ENSCAFT00000065394.1:c.82G>A, first described as NM_001003035.1:c.118G>A). A second mutation (NM_001003035.1:c.52A>T) in the same gene has been found but occurs only in Bernese mountain dogs. Not every homozygous dog develops the disease; this indicates that the disease is incompletely penetrant and that modifier loci might be present. In this review, the authors aim to give an overview of the disease progression and the current genetic knowledge of DM, which is of paramount importance for the correct diagnosis and to help reduce the disease incidence.**

## SAMENVATTING

Degeneratieve myelopathie bij honden is een progressieve neurodegeneratieve aandoening met een fatale afloop die voorkomt bij een groot aantal rassen. De meeste honden zijn minstens acht jaar oud wanneer ze klinische symptomen beginnen te vertonen, startend met paraparese en proprioceptieve ataxie in de achterpoten als gevolg van aantasting van de bovenste motorneuronen, waarbij de spinale reflexen nog intact zijn. Dit evolueert naar tetraplegie met verzwakte/afwezige spinale reflexen en het ontstaan van symptomen als gevolg van aantasting van de hersenstam. Een definitieve diagnose kan slechts post mortem worden gesteld door vaststelling van neuronale degradatie en demyelinisatie van het ruggenmerg via histopathologie. De meeste getroffen honden zijn homozygoot voor een van de gekende mutaties (ENSCAFT00000065394.1:c.82G>A, eerder beschreven als NM_001003035.1:c.118G>A) in het superoxide dismutase 1 gen (*SOD1*). Een tweede mutatie is bekend (NM_001003035.1:c.52A>T), maar komt enkel voor bij Berner sennenhonden. Aangezien niet elke homozygote hond de ziekte ontwikkelt, betekent dit dat de ziekte onvolledig penetrant is en dat er bovendien eventueel ziekte-modificerende loci aanwezig zijn. In dit artikel wordt beoogd een overzicht te geven van de ziekteprogressie en de huidige genetische kennis van degeneratieve myelopathie aangezien dit het startpunt is voor een correcte diagnose van de aandoening en het verminderen van de incidentie.

## INTRODUCTION

Canine degenerative myelopathy (DM) is a late-onset, neurodegenerative disorder with a fatal outcome, occurring in a vast number of dog breeds, with no sex predilection. Whereas the overall prevalence of DM, solely based on clinical signs and characteristics, in dogs is 0.19% (Coates et al., 2007; Wininger et al., 2011), these numbers are strongly breed-dependent. As such, this disease is far more common in dogs than in its human counterpart, amyotrophic lateral sclerosis (ALS), that occurs only in four to six individuals per 100,000, of which 10% of patients have a familial history (Tao and Wu, 2017). While a genetic diagnosis can be made as soon as a DNA sample can be obtained, which is basically at birth, the mean age-of-onset of clinical symptoms and diagnosis is nine years for larger dog breeds (Kathmann et al., 2006). However, among genetically affected dogs, the age-of-onset of clinical symptoms is highly variable and, as recently discovered, at least partially influenced by genetic modifiers (Ivansson et al., 2016). Because DM is notoriously difficult to diagnose, the progression of the disease and the normal clinical work-up will be discussed first. This is followed by an overview of the current genetic and molecular understanding of this disease and practical breeding advice.

## DISEASE PROGRESSION

Similar to ALS in humans, DM is characterized by both upper (UMN) and lower motor neuron (LMN) atrophy and death (Boillée et al., 2006). Whereas UMNs extend from the cerebral cortex or brainstem and carry information down to the spinal cord, LMNs connect the spinal cord with the skeletal muscles and are responsible for movement. Degeneration of the UMN results in modest weakness and spasticity, whereas LMN degeneration triggers more disabling weakness. When LMNs degenerate, the skeletal muscles no longer obtain the impulses necessary for movement and therefore begin to atrophy as well (Kato et al., 2008). The UMNs are first affected, followed afterwards by the LMNs; this also explains the evolution of symptoms observed during the disease progression.

Asymmetric UMN paraparesis, pelvic limb general proprioceptive ataxia with a T3-L3 neuroanatomic localization and lack of spinal hyperesthesia are the initial signs and key clinical features of DM (Olsonet al., 1982; Kathmann et al., 2006; Coates and Wininger, 2010).

At this stage, spinal reflexes generally remain intact (Griffiths and Duncan, 1975; Coates and Wininger, 2010). Often, dogs progress to non-ambulatory paraparesis and are euthanized during this disease stage. If the dog is not euthanized, the initial signs will progress to LMN paraplegia and ascend to affect the thoracic limbs. Urinary and fecal incontinence usually only develop in the later disease stages when paraplegia is present (Table 1). LMN signs emerge as hyporeflexia of the patellar and withdrawal reflexes, flaccid paralysis, and widespread muscle atrophy beginning at the pelvic limbs as the dogs become non-ambulatory. Flaccid tetraplegia occurs in dogs with advanced disease. Brainstem signs include swallowing difficulties and the inability to bark (Kathmann et al., 2006; Coates and Wininger, 2010).

## DIAGNOSING DM CLINICALLY

The diagnosis of DM is challenging, because the clinical signs in older dogs can be mimicked by several other neurologic and orthopedic diseases, such as degenerative lumbosacral syndrome, intervertebral disc disease, spinal cord neoplasia and degenerative joint diseases, e.g. hip dysplasia (Olson et al., 1982). As such, unfortunately, a firm diagnosis of DM can only be made postmortem by a histopathological examination of the spinal cord. Diagnosis premortem is mainly based on the exclusion of other neurological and/or orthopedic disorders with similar features, on the progressive and characteristic deterioration of the patient and on a positive genetic test (Table 1).

Although DM is most common in the German Shepherd Dog (GSD), it is also frequently reported in the Pembroke Welsh Corgi (PWC), Boxer, Rhodesian Ridgeback and Chesapeake Bay Retriever (Table 2). There is no sex predilection. The age-of-onset of clinical signs varies, but the mean age is nine years for larger dog breeds (Table 2). The time between the disease onset and end-stage can take up to three years with a mean of six months for larger dog breeds (Coates et al., 2007).

At an early stage, a presumptive diagnosis is based on the exclusion of other diseases. Firstly, paw replacement tests for proprioceptive positioning are informative as these tests show neurological deficits and exclude orthopedic conditions from the differential diagnosis. Next, imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are used to rule out other spinal cord diseases (Kellett and Crocker, 2015). The imaging techniques are mainly used to exclude compressive spinal cord myelopathy. If MRI is unavailable, CT can also be performed. MRI is outstanding for imaging lesions of the brain, spinal cord and intervertebral discs, with a better soft tissue differentiation compared to CT.

Usually, in diagnosing DM, cerebrospinal fluid (CSF) analysis is done to rule out meningitis/myelitis as this should show a normal amount of total nucleated cell count and no protein abnormalities in DM-affected dogs. In the early stages of the disease, as in normal animals, no spontaneous muscle activity can be measured by electromyography (EMG) and axonal conduction rates remain normal. When dogs develop LMN signs, multifocal spontaneous muscle activity will appear on EMG and conduction studies will be

**Table 1. Overview of the sequence of clinical signs and diagnostic abnormalities encountered during disease progression. Degenerative myelopathy (DM) usually starts with upper motor neuron (UMN) pelvic limb (PL) paresis and general proprioceptive (GP) ataxia. When the disease progresses, lower motor neuron (LMN) weakness occurs and eventually also the thoracic limbs (TL) are affected. CSF: cerebrospinal fluid; EMG: electromyogram. Table adapted from Coates and Wininger (2010).**

| UMN Paraparesis and GP ataxia | LMN Paraparesis to Paraplegia | LMN Paraplegia to TL Weakness | LMN Tetraplegia and Brain Stem Signs |
|---|---|---|---|
| • Progressive general proprioceptive ataxia<br>• Asymmetric and spastic paraparesis<br>• Postural reaction deficits in PL<br>• Intact spinal reflexes (Patellar reflex may be decreased)<br>• Lack of paraspinal hyperesthesia | • Mild to moderate loss of muscle mass in PL<br>• Reduced to absent spinal reflexes in PL<br>• Nonambulatory paraparesis to paraplegia<br>• Potential urinary and fecal incontinence | • Signs of TL weakness<br>• Flaccid paraplegia<br>• Absence of spinal reflexes in PL<br>• Severe loss of muscle mass in PL<br>• Urinary and fecal incontinence | • Flaccid tetraplegia<br>• Difficulty with swallowing and tongue movements<br>• Absence of spinal reflexes in all limbs<br>• Reduced to absent cutaneous trunci reflex<br>• Generalized and severe loss of muscle mass<br>• Urinary and fecal incontinence |
| **Diagnostics – EARLY**<br>• Normal neuroimaging<br>• Normal electrodiagnostic testing<br>• Normal CSF analysis<br>• Homozygosity for SOD1:c.82G>A or SOD1:c.52A>T | | **Diagnostics – LATER**<br>• EMG abnormalities<br>• Nerve conduction studies show temporal dispersion and slow velocities | |
| **Disease onset** | 6-12 months | 9-18 months | 14-24 months | **End stage (> 36 months)** |

**Table 2. Common breeds affected by degenerative myelopathy (DM), mean age-of-onset (in years), mean disease duration (in months) and mean age of death (in years) for every breed. Values obtained from Coates and Wininger (2010).**

| Breed | Age-of-onset (μ) | Disease duration (μ) | Age of death (μ) |
|---|---|---|---|
| German Shepherd Dog | 8.6 | 15.8 | 9.8 |
| Pembroke Welsh Corgi | 10.9 | 20.0 | 12.6 |
| Chesapeake Bay Retriever | 9.1 | 17.8 | 10.6 |
| Boxer | 9.3 | 11.2 | 10.3 |
| Rhodesian Ridgeback | 7.8 | 7.7 | 8.0 |

μ: mean

abnormal (Awano et al., 2009). Finally, genetic tests can also be performed to confirm whether the patient is at least genetically at risk for DM.

Aside from these routinely used diagnostic techniques, new molecular techniques are being developed. In a recent study on PWCs, a positive correlation between increases in the plasma levels of miR-26b and disease progression has been discovered (Nakata et al., 2019). MiR-26b is a microRNA (miRNA), i.e. a small non-protein coding RNA. The plasma miR-26b has been suggested to be a novel diagnostic biomarker for DM and the combination with clinical examination is anticipated to enhance premortem diagnostics.

## HISTOPATHOLOGY

As stated before, a definitive diagnosis of DM is based on the postmortem histopathological examination of the spinal cord. Pathognomonic lesions are axonal and myelin degeneration at any level of the spinal cord. The degeneration is however generally more pronounced in thoracic regions and also proportional with the severity of clinical signs, whereas cervical and lumbar regions show more modest degeneration (March et al., 2009) (Figure 1). Funiculi, which are small bundles of axons enclosed by perineurium located within the white matter of spinal cord, are all

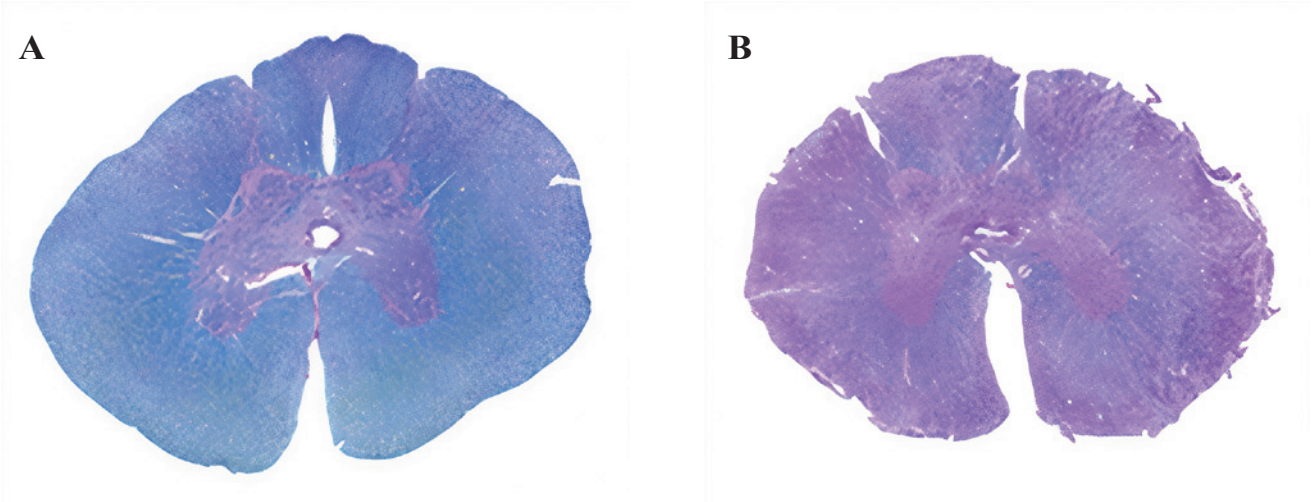


**Figure 1. Histopathological examination of the spinal cord. Luxol fast blue staining of a transversal section of a thoracic spinal cord segment of A. an unaffected dog and B. a dog affected by degenerative myelopathy (DM). The spinal cord of the DM-affected dog demonstrates more severe degeneration in the lateral and dorsal funiculi than the spinal cord of the unaffected dog. White matter degeneration is portrayed by distinct regions of pallor and the loss of blue color. Figure adapted from Kobatake et al. (2017).**

affected, but lesions are predominantly present in the dorsal fraction of the lateral funiculus (Griffiths and Duncan, 1975; Johnston et al., 2000). Affected dogs display distinct forms of axon cylinder vacuolization and loss, which is followed by astrogliosis (Johnston et al., 2001). While present in numerous breeds, DM has only been histopathologically confirmed in the GSD (Johnston et al., 2000), PWC (March et al., 2009), Boxer (Miller et al., 2009), Rhodesian Ridgeback (Awano et al., 2009), Chesapeake Bay Retriever, Siberian Husky (Bichsel et al., 1983) and the Miniature Poodle (Coates and Wininger, 2010), and breed-specific differences have been observed (Table 2). Affected GSDs demonstrate discontinuous, bilateral and asymmetric lesions. Findings on PWC were similar, but more severe and more widely spread, which might be a histopathological feature specific for small dog breeds (March et al., 2009). Knowledge of the effects of DM on brain tissue remains limited. In some studies, abnormalities have been found in certain parts of the brain using immunocytochemistry and electron microscopy, whereas in other studies, in which light microscopy was performed, abnormalities were not detected (Johnston et al., 2000; March et al., 2009).

## GENETIC ASPECTS OF DM

Due to the consistency of clinical signs and disease progression and the occurrence in certain but not all breeds, DM has been expected to have a genetic origin. The observation of familial segregation of DM in the PWC (Coates et al., 2007), the Siberian Husky and the Chesapeake Bay Retriever (Long et al., 2008) provided additional support for this hypothesis. Finally, in 2009, in a genome-wide association study, the first single nucleotide polymorphism was identified in the superoxide dismutase 1 *(SOD1)* gene (ENSCAFT00000065394.1:c.82G>A, first described as NM_001003035.1:c.118G>A) (rs853026434) responsible for DM (Awano et al., 2009). As mutations within this gene give rise to 2-10% of the familial cases of ALS (Majoor-Krakauer et al., 2003), this gene is a likely candidate for DM as well.

SOD1 is a highly conserved protein and is the major scavenger of cytoplasmatic superoxide radicals ($O_2^-$) and, as such, is important in processes associated with oxidative stress (Borchelt et al., 1994). Release of reactive oxygen species (ROS) contributes to a large extent to cell damage and death both through direct and indirect (e.g. apoptosis) signaling (Li et al., 2019). The SOD1 protein reduces and controls the malignant effects of ROS.

When compared to unaffected wild type control dogs, the mutation results in a transition from a glutamic acid to a lysine amino acid at position 28 (ENSCAFP00000013012.4:p.E28K, first described as p.E40K) within the folded protein. Despite the missense mutation, the diseased phenotype does not seem to be a consequence of loss of normal protein function (Sau et al., 2007; Sahin et al., 2017). The E28K substitution rather leads to a misfolded protein with reduced net negative charge, which makes it more susceptible to form aggregates due to reduced repulsion between individual proteins (Sandelin et al., 2007). It is exactly that aggregate formation that might be responsible for the observed neurodegeneration (Bruijn et al., 1998). In fact, it has recently been demonstrated that motor neurons and other cells actively take up SOD1 protein aggregates by endocytosis (Benkler et al., 2018). Furthermore, these SOD1 aggregates are also transported to neighboring cells within the spinal cord and this protein transfer is assisted by oligoden-

drocytes (Thomas et al., 2017). However, the exact pathophysiological effects of the SOD1 aggregates still need to be elucidated.

In addition to the SOD1:c.82G>A variant resulting in a E28K amino acid substitution, which is widely distributed in the overall dog population, another *SOD1* variant has been found, but solely in Bernese Mountain Dogs (BMDs) (NM_001003035.1:c.52A>T) (Wininger et al., 2011). In a study with 912 BMDs, an allele frequency of the SOD1:c.52A>T variant of 3.5% was found, which was considerably lower than the 38% frequency of the SOD1:c.82G>A allele in this breed (Zeng et al., 2014). Both the c.82G>A and c.52A>T mutations cause disease in a homozygous state, consistent with an autosomal recessive mode of inheritance. However, within the BMD, compound heterozygotes have also been found and 4 out of 24 of the dogs in the study by Zeng et al. (2014) actually developed clinical signs of DM.

## THE COMPLEX RELATION BETWEEN PHENOTYPE AND GENOTYPE

As DM is a disorder with a late onset, there can be a long lag in time between genetic diagnosis and the first symptoms. In addition, the age-of-onset seems to vary between breeds (Table 1). Amongst PWCs carrying two copies of the risk allele, some dogs develop DM fairly early (< 8 years), whereas others never demonstrate any signs (> 11 years) (Ivansson et al., 2016). This indicates that DM has incomplete penetrance, with penetrance being defined as how likely an individual is to present a specific physical trait, taking into account their genetic profile. The term complete penetrance indicates that every dog with a specific variant in a state that should result in disease, will develop the disease. In contrast, incomplete penetrance denotes that some dogs at risk might actually never demonstrate any clinical signs (Awano et al., 2009). Reduced penetrance has an impact on how genetic profiles should be interpreted and challenges geneticists to predict the probability that an individual dog will develop clinical symptoms. The overall prevalence of DM, solely based on clinical signs and not a positive genetic test, in Pembroke Welsch Corgi dogs and Cardigan Welsh Corgi dogs is 1.51% and 0.58%, respectively (Coates et al., 2007). However, the exact percentage of dogs, which are genetically at risk, that will become clinically affected is not yet determined. To obtain this information, prospective studies should be performed, where individuals are included in the study before the development of clinical signs and are followed up for years to collect data. One of the main drawbacks of this kind of study are the high expenses and that it is very time consuming.

In contrast to the PWCs, almost every Boxer, homozygous for the *SOD1* risk allele, develops DM (Ivansson et al., 2016). These breed-specific differences suggest that modifier loci might exist that influence disease risk. The identification of these genetic loci is expected to assist in the understanding of the DM etiology and can also be of clinical use for the prediction of disease progression in patients. In a recent study by Ivansson et al. using PWCs, a first modifier locus was found within the SP110 nuclear body protein gene (*SP110*), which is located on the 25$^{th}$ chromosome (cfa25). In the analysis, both affected and unaffected PWCs homozygous for the *SOD1* variant, were used to find potential modifier loci. A 'risk haplotype', found within the *SP110* was present in 40% of affected and merely 4% of unaffected dogs (Ivansson et al., 2016). This risk haplotype was generally present as a single copy, which implies that one copy is sufficient to have an influence on disease risk.

SP110 is part of the SP100/SP140 protein family of nuclear body proteins. These proteins are predominantly expressed in immune cells (Bloch et al., 2000). The SP110 protein affects fundamental cellular processes, such as transcription, apoptosis, senescence and reaction to DNA damage or infection (Lallemand-Breitenbach et al., 2010) and might also play a role in immune responses (Roscioli et al., 2006). This strengthens the hypothesis that neuroinflammation is an important feature of ALS (Appel et al., 2010). Future research should focus on elucidating the exact role of the *SP110* gene in DM, whereas the discovery of novel (and potentially even breed-specific) modifier loci might further clarify the variable age-of-onset.

As previously stated, miR-26b has been found to be associated with disease progression and might be an interesting novel biomarker (Nakata et al., 2019). However, its exact role has not been elucidated yet. A pathway analysis proposed that miR-26b mediates transcription of the amyloid beta precursor protein; however, the biological role of the protein has not been defined yet. Both miR-26b and amyloid beta precursor protein are active, upstream of the SOD1 expression cascade and might indirectly mediate its expression (Nakata et al., 2019).

## GENETIC TESTING AND BREEDING ADVICE

Genetic testing can be used in the diagnostical work-up of patients suspected to have DM, but also as a screening tool to identify carriers and dogs at risk and to give breeding advice. Identifying carriers is important as they can pass on the allele to future generations, without developing clinical signs themselves. However, due to the late onset of disease, even dogs homozygous for the mutant allele, usually only develop symptoms after the breeding age. Fortunately, DNA tests are commercially available (Mellersh et al., 2012). It is however important to use the correct DNA-test, taking the breed into account. For every breed, the SOD1:c.82G>A should be genotyped as this mutation is widely spread in numerous breeds. In addition, solely for the BMD, the SOD1:c.52A>T should be analyzed as well. Overall, dogs homozy-

gous (or compound heterozygous) for the *SOD1* mutations, are at risk of developing DM.

In the context of diagnosis, it is crucial not to draw conclusions from clinical signs and DNA-testing alone, as concomitant diseases may be present. It is of paramount importance to rule out every other possible origin of the deterioration to avoid wrong diagnosis and as a consequence, choices concerning euthanasia. Furthermore, as DM is a disease with incomplete penetrance, carrying two copies of the variant allele does not necessarily result in developing the disease.

In the context of screening and breeding advice, two (at first sight conflicting) concepts have to be kept in mind. Firstly, as genetic diversity is dangerously low in several breeds, the general focus should be to improve this. Practically, this means excluding as little animals as possible from breeding. Secondly, the goal is to reduce the prevalence of (genetic) diseases, which is usually achieved by excluding animals from breeding.

Conventional breeding methods, responsible for creating the domestic dog as we know it, have resulted in a remarkable reduction of the genetic diversity (Lindblad-toh et al., 2005; Wijnrocx et al., 2016). This reduction is often due to strong selective breeding practices, such as inbreeding and the use of a popular sire (Calboli et al., 2008). Each dog is undoubtedly carrier of mutant alleles that have no significant effect on the carriers' health. This is exemplified by a recent study that showed that nearly two in five dogs are carrier of at least one copy of a 'diseased allele' when screened for 152 currently known genetic disease-associated variants (Donner et al., 2018). However, when present in homozygous conditions, dogs can become affected. Decreasing genetic diversity will result in a higher frequency of a mutant allele and thus, the amount of homozygotes in the population (Donner et al., 2018). For this reason, breeding programs should focus on the prevention of hereditary disorders by reducing the causative genetic burden but at the same time aim to enhance genetic diversity (Marsden et al., 2016). Luckily, this can be achieved for DM as it is an autosomal recessive disorder.

Due to the recessive nature, the frequency of the mutant allele, and as a consequence also the number of affected dogs, can be reduced in a reasonable timeframe. Rather than completely excluding every carrier or even affected dogs from the breeding program, specific genotypes should be combined in such a way that no diseased animals are born (Broeckx et al., 2013). In autosomal recessive conditions, carrier dogs should not be combined with another carrier or an affected dog (Figure 2). Both dogs homozygous and heterozygous for the mutant allele should only be combined with wild type 'healthy' dogs. This is made possible by the use of DNA-tests. There is however an important remark when it comes to using dogs homozygous for the mutant allele in breeding programs.

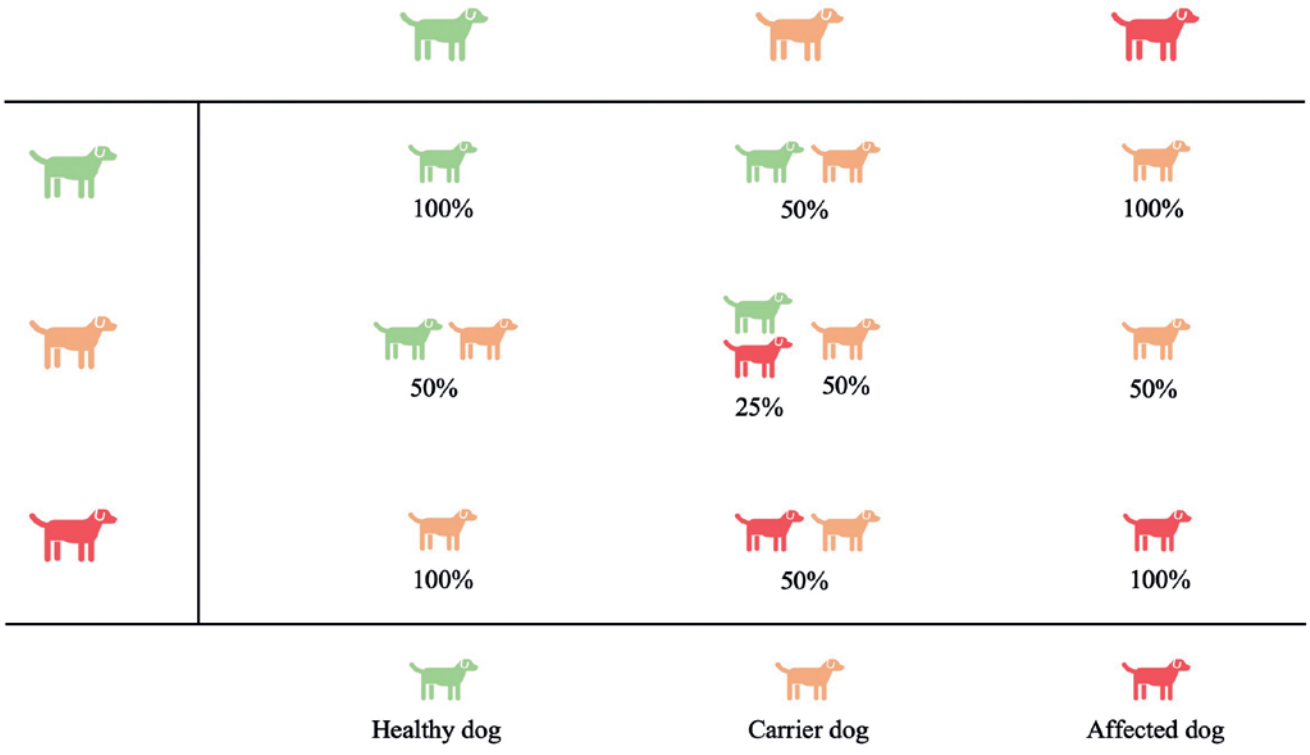


**Figure 2. The expected genotype frequencies and disease status in the progeny for all possible genotype combinations for phenotypes with an autosomal recessive mode of inheritance. Green dog: 'Healthy' dog for the disease of interest, carrying two copies of wild type allele; orange dog: 'Carrier' dog, having only one copy of the mutant allele; red dog: 'Affected' dog, homozygous for the disease variant, potentially displaying symptoms for the disease of interest.**

Dogs homozygous for the variant allele can be combined with healthy dogs, as offspring will be 100% carrier and thus, will not develop DM. However, it is of paramount importance to take the welfare of the animal into account. If the welfare has not been compromised at the time of breeding, there is in fact no problem in further use of the animal. If the animal is already starting to show symptoms and thus is unable to cope with pregnancy or breeding in general, it is strongly discouraged to continue using the animal for breeding purposes.

Next to carefully combining the correct genotypes for mating, it is important not to overuse a carrier or affected dog, as this might increase the frequency of the mutated allele in the population. This is however in line with the general recommendation to avoid the overuse of specific animals in breeding.

## CONCLUSION

Whereas the inheritance pattern of DM is simple, DM proves to be a phenotypically complex progressive disorder, similar to ALS in humans. It displays a characteristic pattern of clinical signs and is widespread in pedigree and mixed breed dogs. DM has a late onset, generally between eight and eleven years, and both sexes are equally affected.

The *SOD1* variant has been labeled as a major causative agent for developing DM. However, the inconsistent age-of-onset of clinical symptoms suggests that modifier loci might influence disease risk, as has recently been proven by the identification of the *SP110* risk haplotype. Detection and identification of these genetic loci are expected to assist in the interpretation and understanding of DM etiology. Due to the recessive autosomal nature of the *SOD1* mutation, the breeding advice is to carefully combine specific genotypes for breeding, rather than to completely exclude every carrier or even affected dogs from the breeding program as this would attribute to a further reduction of genetic diversity.

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