An overview of the current genetic and phenotypical selection strategies to reduce the prevalence of feline hypertrophic cardiomyopathy

Een overzicht van de huidige genetische en fenotypische selectiestrategieën tegen hypertrofe cardiomyopathie bij de kat

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a common and potentially lethal heart disease in cats. To reduce its prevalence, breeding cats are frequently screened on the basis of their phenotype or genotype. Although echocardiography is the most reliable phenotypical method, its efficacy is limited by the incomplete penetrance of HCM and by difficulties in distinguishing primary HCM from other causes of left ventricular hypertrophy. On the other hand, genetic testing is hampered by the genetic heterogeneity of the disease. Genetic tests are currently only available for Maine Coons and Ragdolls. Because of the high prevalence of HCM, stringent selection may have a negative impact on the genetic diversity of a breed. A more optimal selection would therefore be a slow and careful exclusion of phenotypically and/or genetically positive cats.

SAMENVATTING

Hypertrofe cardiomyopathie (HCM) is een veel voorkomende en potentieel dodelijke hartziekte bij katten. Fokkatten worden vaak gescreend voor deze ziekte, zowel op basis van hun fenotype als van hun genotype, om de prevalentie van deze aandoening te verminderen. Echocardiografie is de meest betrouwbare fenotypische techniek, maar de effectiviteit ervan wordt beperkt door de onvolledige penetrantie van HCM en het moeilijke onderscheid tussen primaire HCM en secundaire hypertrofie. Daar tegenover staat dat het gebruik van genetische testen wordt beperkt door de genetische heterogeniteit van de ziekte. Momenteel zijn genetische testen enkel beschikbaar voor de maine coon en de ragdoll. Vanwege de hoge prevalentie van HCM kan strenge selectie een negatief effect hebben op de genetische diversiteit van een ras. Geleidelijke en verstandige uitsluiting van positieve katten op basis van zowel fenotypische als genetische testen lijkt daarom een optimalere methode van selectie.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats, affecting almost 15% of the general population (Payne et al., 2015b). There is a strong suspicion that feline HCM, like human HCM, is inherited as a Mendelian disorder (Maron and Fox, 2015). The identification of causative variants in the Maine Coon and Ragdoll breeds has led to the development of genetic tests for these variants. Together with polycystic kidney disease, HCM is the feline disease, for which genetic testing is most often requested (Lyons, 2016). On the other hand, it has become common to screen breeding cats for HCM

phenotype by means of echocardiography (Häggström et al., 2015). Both methods have their advantages, but also serious limitations. Furthermore, considerable controversy exists around the genetic tests available for feline HCM, and breeders are not always aware that these tests are only appropriate in specific breeds (Lyons, 2012; Lyons et al., 2016). In this review, a general overview is provided of why genetic diseases occur that often in companion animals. Secondly, the strategies for reducing the prevalence of HCM in purebred cats and the role of genetic testing and echocardiography are discussed.

GENETIC DISEASE IN COMPANION ANIMALS

Unlike human populations, populations of domestic animals generally have a low level of genetic diversity. They descent from relatively small populations that were domesticated and, more importantly, have been divided into small, genetically isolated subpopulations by pedigree breeding (Jolly et al., 2016). Even in large breeds, few animals are used for breeding, making these breeds somewhat similar to endangered species (Traas et al., 2006).

At the moment of their creation or at some point in their history, many breeds had a small number of reproducing animals (a population bottleneck) or a particularly popular sire with many descendants. Rare variants that were present in this small group or individual animal have then been passed on to many animals within that breed. The spread of these initially rare variants in a population is called the founder effect (Jolly et al., 2016). When such a variant causes disease, that particular disease can become highly prevalent in that breed.

Breeding in a closed population with low genetic variability can not only cause the spread of genetic diseases in that breed, but also complicate elimination of such diseases. If the disease-causing variant is very common, abruptly excluding all animals that carry it from breeding, may have severe consequences. The genetic diversity of the breed may decrease further, variants encoding desirable traits may be lost and other disease-causing variants may increase in frequency (Traas et al., 2006). In the case of highly prevalent disease, it may therefore be wise to use some animals carrying a deleterious variant for breeding. In case of autosomal recessive disease, carriers and even affected animals can be safely bred with an animal that is confirmed to be homozygous wild type by a DNA-test (Mellersh, 2012). If the disease is inherited in an autosomal dominant fashion, breeding heterozygotes to homozygous wild type animals generates offspring at risk for the disease. Nevertheless, such a way of breeding may be necessary to maintain genetic diversity in a breed, in which that disease has a high prevalence. The carriers can then be replaced by animals from the next generation that are negative for this variant (Traas et al., 2006; Lyons, 2016).

CLINICAL ASPECTS OF FELINE HCM

Most of the cats affected by HCM are male domestic shorthairs (Fox et al., 2018). It is not known what causes the high prevalence of HCM in domestic shorthairs, in which selective breeding is uncommon. If these cases are genetic, their causative variants may have become widespread because the late onset after breeding results in limited natural selection against them (Payne et al., 2015b). A breed predisposition has been suggested for several breeds, but these do not seem to be at an increased risk compared to domestic shorthairs (Côté et al., 2011a; Granström et al., 2011; Longeri. et al., 2013). A remarkably high prevalence or severe clinical course has however been reported in the Sphynx (Silverman et al., 2012), Ragdoll (Lefbom et al., 2001; Payne et al., 2010) and Maine Coon (Trehiou-Sechi et al., 2012), indicating that these breeds may indeed be predisposed for HCM.

HCM is characterized by concentric hypertrophy of the left ventricle that cannot be attributed to another underlying disease. The hypertrophied ventricle initially has a normal systolic function, but its increased stiffness, delayed relaxation and narrower lumen impair the filling during diastole. As a result of this diastolic dysfunction, pressure in the left atrium may rise, leading to atrial dilatation with a risk of left-sided congestive heart failure or thrombus formation (Côté et al., 2011a). The electrophysiological conduction system of the heart can also be disturbed in HCM, making the heart prone to arrhythmias (Weissler-Snir et al., 2014; Bartoszuk et al., 2019).

Cats affected by HCM are born with a normal heart and only develop hypertrophy later in life (Kittleson et al., 1999). The age of onset and the progression of the hypertrophy differ widely between patients. Considerable hypertrophy can develop without clinical signs, as long as the cardiac function is adequate. In many mildly affected cats, the disease remains in this occult stage, leaving them free of symptoms until they ultimately die from other causes (Trehiou-Sechi et al., 2012). If the disease progresses to a clinical stage, asymptomatic cats may suddenly develop life-threatening complications, such as cardiogenic pulmonary edema, pleural effusion or arterial thromboembolism. In some cases, sudden death, likely caused by ventricular fibrillation, is the only clinical sign of the disease (Payne et al., 2015a). The incidence of lethal cardiovascular events in asymptomatic cats diagnosed with HCM is 6.3% per year (Fox et al., 2018).

No treatment is currently available to halt the progression of ventricular hypertrophy nor to prevent congestive heart failure or sudden cardiac death in cats, but prevention of arterial thromboembolism in high-risk cats is possible with clopidogrel (Hogan et al., 2015; Luis Fuentes and Wilkie, 2017). Treatment of arterial thromboembolism is often unsuccessful, while cats with congestive heart failure can have a good quality of life with combination therapy, usually a diuretic, platelet-inhibitor, ACE-inhibitor and some-

times pimobendan (Reina-Doreste et al., 2014; Hogan and Brainard, 2015).

DETECTING HCM

During the asymptomatic or "occult" stage of HCM, affected cats appear normal to the owner or breeder. Auscultation of these patients may reveal a heart murmur, gallop sound or arrhythmia. None of these findings is specific for HCM, and many affected cats show no abnormalities on auscultation (Payne et al., 2015b). Innocent heart murmurs are also very common in healthy cats and can be difficult to distinguish from pathological murmurs (Côté et al., 2015). Similarly, thoracic radiography and ECG may show nonspecific abnormalities, but are often unremarkable in cats with occult HCM (Ferasin et al., 2003). Cardiac biomarkers, such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI), are an attractive screening tool because of their low cost and easy use. However, there is some overlap in the concentrations of such biomarkers between healthy cats and cats with asymptomatic HCM (Fox et al., 2011; Hertzsch et al., 2019). Furthermore, increased levels of cardiac biomarkers can also be caused by other diseases, as has been shown for chronic kidney disease (Porciello et al., 2008), hyperthyroidism (Sangster et al., 2014) and hypertension (Bijsmans et al., 2017) in cats.

Echocardiography is considered the gold standard for diagnosing HCM in cats. The main criterion is the thickness of the left ventricular free wall and interventricular septum. In general, an end-diastolic thickness < 5.5 mm at the level of the papillary muscles is considered normal, a thickness > 6.0 mm is diagnostic for HCM and measurements in between can be classified as equivocal (Côté et al., 2011a). If wall measurements are within the reference ranges, abnormalities of the papillary muscles or mitral valve might still lead to a classification as equivocal (Häggström et al., 2015). A general cut-off for wall thickness does not take into account that the normal values may differ between breeds, as they are for example lower in Sphynxes (Chetboul et al., 2012) than in Bengals (Scansen et al., 2015), nor does it take into account that these values increase with body weight (Häggström et al., 2016). Another difficulty is that in affected cats, the hypertrophy is not always generalized, but may also be confined to certain left ventricular segments or the papillary muscles (Fox, 2003). Finally, multiple methods of imaging and measuring are applied in practice. Altogether, these factors allow for considerable variation in classification, especially in equivocal cases. In one study on apparently healthy shelter cats, the prevalence of left ventricular hypertrophy ranged from 12% to 51% depending on the chosen cut-off (6.0 mm or 5.5 mm, respectively) and scanning mode (M-mode or 2D B-mode, respectively) (Wagner et al., 2010).

Similar to feline HCM, the diagnosis of the human HCM phenotype is based on the end-diastolic thickness of the myocardial wall, which may be assessed by echocardiography, CT or MRI. The cut-off recommended by the European Society of Cardiology is 15 mm in adults, 13 mm in first-degree relatives of diagnosed patients and two standard deviations above the population mean in children (Elliott et al., 2014).

Underlying diseases may cause left ventricular hypertrophy due to hormonal influences as in hyperthyroidism, acromegaly and hyperaldosteronism, or due to abnormal loading conditions as in hypertension and aortic stenosis (Stowasser et al., 2005; Côté et al., 2011a). HCM can also be mimicked by wall thickening due to edema or infiltrative diseases, such as myocarditis or myocardial lymphoma. Finally, hypovolemic cats may display pseudohypertrophy due to the reduced size of the left ventricular chamber (Côté et al., 2011a). A certain diagnosis of HCM requires the exclusion of these diseases, which may be relatively simple (e.g. measurement of blood pressure or total thyroxine level), but can also be complicated (e.g. in case of infiltrative disease). However, these differential diagnoses are either rare or less likely in cats of breeding age (Häggström et al., 2015). In some cases, mainly young cats with congestive heart failure after an antecedent stressful event, concentric left ventricular hypertrophy may spontaneously regress, a condition named transient myocardial thickening (TMT) (Novo Matos et al., 2018). At first presentation, it is not possible to distinguish TMT from early-onset HCM (Novo Matos et al., 2018).

In conclusion, despite being considered the gold standard, echocardiographic diagnosis of HCM in cats is not straightforward. It is a diagnosis of 'exclusion' that requires thorough knowledge of other diseases causing left ventricular hypertrophy and expert echocardiography skills. Furthermore, there is a significant overlap in the echocardiographic phenotype between HCM and several other feline cardiomyopathies (ACVIM consensus statement on feline cardiomyopathy, in press).

GENETIC ASPECTS OF HUMAN AND FELINE HCM

Feline HCM shows remarkable similarities in morphology and clinical course to human HCM, and it is likely that these diseases also share genetic aspects (Maron and Fox, 2015; Freeman et al., 2017). HCM is the most common inherited heart disease in humans and 60% of the patients indeed have a clear family history of HCM. In nearly all familial cases, the disease is inherited in an autosomal dominant pattern. However, HCM generally does not show full penetrance. The penetrance is the probability that an individual with a disease-causing genotype displays the disease (Broeckx et al., 2017). Because the HCM phenotype is usually not detectable at birth and only

develops later in life, the penetrance is said to be age-dependent. The penetrance is also incomplete, as some people carrying a causative variant never develop the HCM phenotype (Marian and Braunwald, 2017). The penetrance seems to be higher in men than in women, but the male overrepresentation is less pronounced than in cats (Olivotto et al., 2005).

The molecular genetic basis of human HCM was first identified in a family with a variant in *MYH7*, the gene that encodes the ventricular myosin heavy chain protein. This was soon followed by many other variants in the same gene as well as in other genes (Marian and Braunwald, 2017). Today, more than 1.500 variants in over fifty genes have been linked to HCM, but for many variants and genes, the causality has been disputed (Walsh et al., 2017; Ingles et al., 2019). Nevertheless, a large number of variants have been proven to cause HCM. This situation, in which a disease is caused by many different variants, is called genetic heterogeneity. A few HCM-causing variants are common in a specific population, suggesting a founder effect, but most are restricted to a few families or a single family (Marian and Braunwald, 2017).

Definitive evidence for a role in HCM has been provided for eight sarcomeric genes (*ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3* and *TNNT2*) (Walsh et al., 2017; Ingles et al., 2019). In 50% of the patients in whom a causative variant is identified, this variant lies in *MYBPC3*, that codes for the myosin-binding protein C, or *MYH7*, making these two genes the most important in human HCM (Marian and Braunwald, 2017).

The identification of a causative variant in a patient allows the screening of family members for this variant. This has led to the identification of genotype-positive - phenotype-negative patients, who carry a causative variant without displaying ventricular hypertrophy, but who are nevertheless considered at risk of sudden death (Maron et al., 2011). On the other hand, no causative variant is found in a large number of patients who display the HCM phenotype (Marian and Braunwald, 2017). This shows that the phenotype and genotype of HCM, and therefore the diagnostic test based on either of them, do not fully overlap. As in cats, human HCM shows extensive variation in the progression and outcome of the disease. There is no clear relationship between the causative variant and the clinical manifestation of the disease: the same variant may lead to sudden cardiac death at young age in one patient, but cause no clinical signs at all in another patient (Landstrom and Ackerman, 2010). Although the cause of the disease is a deleterious sarcomeric variant, variants in other genes, such as genes of the renin-angiotensin-aldosterone system, as well as epigenetic and environmental factors seem to strongly modify the phenotype of a patient (Sabater-Molina et al., 2018).

HCM has also been described as a familial disease in cats, although the documentation is far more limited in cats than in humans. Familial occurrences of HCM have been reported in American Shorthairs (Meurs et al., 1997), British Shorthairs (Putcuyps et al., 2003), Maine Coons (Kittleson et al., 1999), Norwegian Forest Cats (März et al., 2015), Persians (Martin et al., 1994) and Sphynxes (Chetboul et al., 2012; Silverman et al., 2012), as well as Domestic Shorthairs (Baty et al., 2001; Krauss et al., 1999; Nakagawa et al., 2002). Although pedigree data is often limited, these reports are compatible with an autosomal pattern of inheritance. In some cases, it has been suggested that the penetrance is incomplete (Chetboul et al., 2012; März et al., 2015).

HCM-CAUSING VARIANTS IN CATS

The first HCM-causing variant identified in cats was *MYBPC3* c.91G>C, a change from guanine to cytosine at the 91th coding position of the *MYBPC3* gene (Meurs et al., 2005; reference sequence: XM_019812396.1). This variant was first found in a research colony of Maine Coons where HCM seemed to segregate according to an autosomal dominant pattern (Kittleson et al., 1999). The variant then turned out to be present in over 30% Maine Coons around the world (Fries et al., 2008), but to be extremely rare in other breeds (Mary et al., 2010; Longeri et al., 2013).

In the colony where the variant was first found, HCM developed in all homo- and heterozygotes, though more severe in the homozygotes (Meurs et al., 2005). However, in subsequent studies in client-owned Maine Coons, it has been found that many cats carrying the variant are phenotypically normal, leading to doubts about the clinical significance of this variant or about its proposed mode of inheritance (Wess et al., 2010; Longeri et al., 2013).

To provide more evidence for the mode of inheritance and optimal breeding advice, a meta-analysis of five studies has been performed (Mary et al., 2010; Wess et al., 2010; Godiksen et al., 2011; Longeri et al., 2013; Pellegrino et al., 2017). In this meta-analysis, the penetrance of HCM was found to be 0.04 in homozygous wild type Maine Coon cats. The penetrance in heterozygous cats is very similar (0.07; $P = .23$), while it is much higher (0.54; $P < .001$) in homozygous variant cats. These studies therefore do not confidently show a clinical effect of the variant in heterozygotes and suggest that if there is such an effect, it is likely to be small. An important limitation of these studies is that most investigated cats were young females, which may not yet have developed the HCM phenotype. Although the actual penetrance would probably have been higher overall if these cats had been older, the ages of the different genotype groups in the studies were comparable. As such, it is unlikely that young age can explain why the penetrance in heterozygotes is lower than the penetrance in homozygous variant cats and similar to the penetrance in

homozygous wild type cats. The presence of HCM in wild type cats implies that HCM in Maine coons can also arise due to other causes, either genetic or environmental (Longeri et al., 2013).

A second variant in the same gene in Maine Coons, *MYBPC3* c.220G>A, has also been proposed to be associated with HCM in an abstract by Nyberg et al. (2007). This variant is not specific for Maine Coons, as it has been found in many other breeds as well as in Domestic Shorthairs (Longeri et al., 2013). However, in subsequent studies, no convincing evidence has been found that this variant causes HCM in cats (Wess et al., 2010; Longeri et al., 2013).

An HCM-causing variant in Ragdolls, the *MYBPC3* c.2455C>T variant, has been identified by sequencing the *MYBPC3* gene in client-owned affected cats (Meurs et al., 2007). Again, the variant was found in approximately 30% of the Ragdolls, but not in other breeds (Longeri et al., 2013; Casamian-Sorrosal et al., 2014). Fewer studies are available on this variant than on the *MYBPC3* c.91C>G variant, but cats carrying this variant have an increased wall thickness than wild type cats (Borgeat et al., 2015). In a small study population, the overall penetrance for homo- and heterozygotes was 0.33 (Borgeat et al., 2015). In a survey filled out by Ragdoll owners, a high risk of cardiac death was found in cats homozygous for the variant, while the risk in heterozygotes was not different from the risk in wild type cats (Borgeat et al., 2014). The same variant has also been identified as a cause of cardiomyopathy in humans (Ripoll Vera et al., 2010). Some Ragdolls diagnosed with HCM are negative for the *MYBPC3* c.2455C>T variant, again indicating that there are also other causes of HCM in this breed (Borgeat et al., 2015).

A variant in *MYH7*, c.5647G>A, has recently been identified as the cause of HCM in a Domestic Shorthair (Schipper et al., 2019; reference sequence: XM_006932746.4). The same variant has been identified in a HCM-affected human patient (Taisjharghi et al., 2007) and a homologous variant in *MYH6* is known to cause cardiac disease in humans (Bowles et al., 2015; Preuss et al., 2016). The variant affects an important functional domain of the protein and has been shown to have a damaging effect in functional studies (Sohn et al., 1997; Viswanathan et al., 2017). In the study that identified this variant, the variant was absent in two hundred other cats, of which most were considered HCM-negative. Although very little family information was available, the cat affected by the variant was a heterozygote and an autosomal dominant pattern of inheritance seemed most likely (Schipper et al., 2019).

The high prevalence of the *MYBPC3* c.91G>C and c.2455C>T variants within the Maine Coon and Ragdoll breeds, together with their absence in other breeds, suggests that these variants have been spread by the founder effect. However, the finding of variant-negative cats with HCM makes it likely that there are more causative variants in these breeds and that feline HCM, like human HCM, knows considerable genetic heterogeneity.

SCREENING BREEDING ANIMALS FOR DISEASE-CAUSING VARIANTS

The high prevalence of genetic diseases in purebred animals has led to the development of screening programs for breeding animals. These programs primarily aim to reduce the prevalence of genetic disease by preventing matings that may produce offspring at risk (Meyers-Wallen, 2003). Concerns about animal welfare, self-interest of breeders and owners and public pressure to increase the health of purebred animals may all be motivations to participate in screening programs (Mellersh, 2012; Jolly et al., 2016). Prioritization of genetic diseases for screening can be based on the prevalence and severity of the diseases (Collins et al., 2011). Screening programs depend on diagnostic tests that identify animals that can transmit the disease. Ideally, a diagnostic test is cheap, minimally invasive and requires minimal time and expertise to perform (Drobatz, 2009). The test can either be a phenotypical or a genetic test.

Phenotypical tests select breeding animals on the basis of clinical features. They have the advantage that no knowledge of the underlying genetic cause is required. However, they cannot detect heterozygous carriers of recessive alleles or animals that are genetically at risk but do not display the disease due to incomplete penetrance. Another possible problem is a late age of onset, in which case affected animals may already have offspring at the time of diagnosis (Mellersh, 2012).

Genetic tests require DNA, often isolated from EDTA blood. By methods such as PCR, Sanger sequencing or restriction enzyme fragment length polymorphism, the disease-causing variant can be directly detected (Jolly et al., 2016). This circumvents the problem of incomplete penetrance; however, these tests are only possible when the disease-causing variant is known. Furthermore, there should be convincing evidence that this variant indeed causes the disease. Selection against a common variant that has no clinical significance can needlessly reduce the genetic variability of a breed (Lyons et al., 2016).

Genetic screening against a widespread causative variant, as in cases of a founder effect, can be very effective in reducing the prevalence of a disease. In cases of genetic heterogeneity, selection against a few uncommon causative variants amongst many variants has a far lower impact (Meyers-Wallen, 2003).

The high prevalence, risk of agonizing or lethal complications and limited treatment options are reasons to give HCM high priority among feline genetic diseases. The age-dependent and incomplete penetrance can preclude (early) phenotypical detection of

breeding animals that carry a causative variant. Echocardiography, the preferred phenotypical test, requires a high level of expertise and even experts can have difficulties in distinguishing mildly affected animals from normal animals (Häggström et al., 2015). On the other hand, the genetic background of feline HCM seems to be a combination of both highly prevalent, breed-specific variants and rare variants that display significant heterogeneity. This heterogeneity will make genetic screening only partially effective.

Phenotypical selection could be complemented by pedigree analyses. The HCM status of close relatives can help to identify cats at high risk of carrying HCM-causing variants, whose use in breeding should be postponed until they are shown to be free of the HCM phenotype at mature age (Häggström et al., 2015). The use of pedigrees to obtain estimated breeding values, as advocated for hip dysplasia selection, seems less applicable, as HCM is considered a qualitative rather than a quantitative trait (Wilson et al., 2011).

The high prevalence and potentially severe consequences of HCM may also be seen as reasons for population-wide screening of all cats. However, such extensive efforts are only justified if a number of conditions are met, including the availability of cheap diagnostic tests and effective treatment (Shaw, 2017). The low availability of expert echocardiographers and the limited treatment options for feline HCM do not fulfill these criteria.

RECOMMENDATIONS FOR SCREENING IN BREEDS FOR WHICH NO GENETIC TEST IS AVAILABLE

There are no suitable genetic tests for other breeds than the Maine Coon and Ragdoll (Côté et al., 2011b). For these other breeds, phenotypical screening is the only available method of screening for HCM. This should be based on echocardiography performed by an experienced echocardiographer. Other methods (e.g. thoracic radiography alone) carry a high risk of false negatives, allowing the transmission of deleterious variants, or false positives, unnecessarily excluding healthy animals from breeding. Measurements of cardiac biomarkers with validated assays can be used to identify cats at an increased risk. However, this should always be followed by an echocardiography (in combination with ancillary diagnostic testing if necessary) to confirm or exclude the diagnosis of primary HCM. Recommended screening schemes consist of an annual examination by an experienced cardiologist during the period that the cat is used for breeding and a follow-up examination at the age of five to eight years (Häggström et al., 2015).

Phenotypical screening for HCM is currently performed in the Siberian, Norwegian Forest Cat, British Shorthair/Longhair, Bengal, Birman, Cornish and Davon Rex, Sphynx and Persian/Exotic in the PawPeds program (www.pawpeds.com), which is the largest

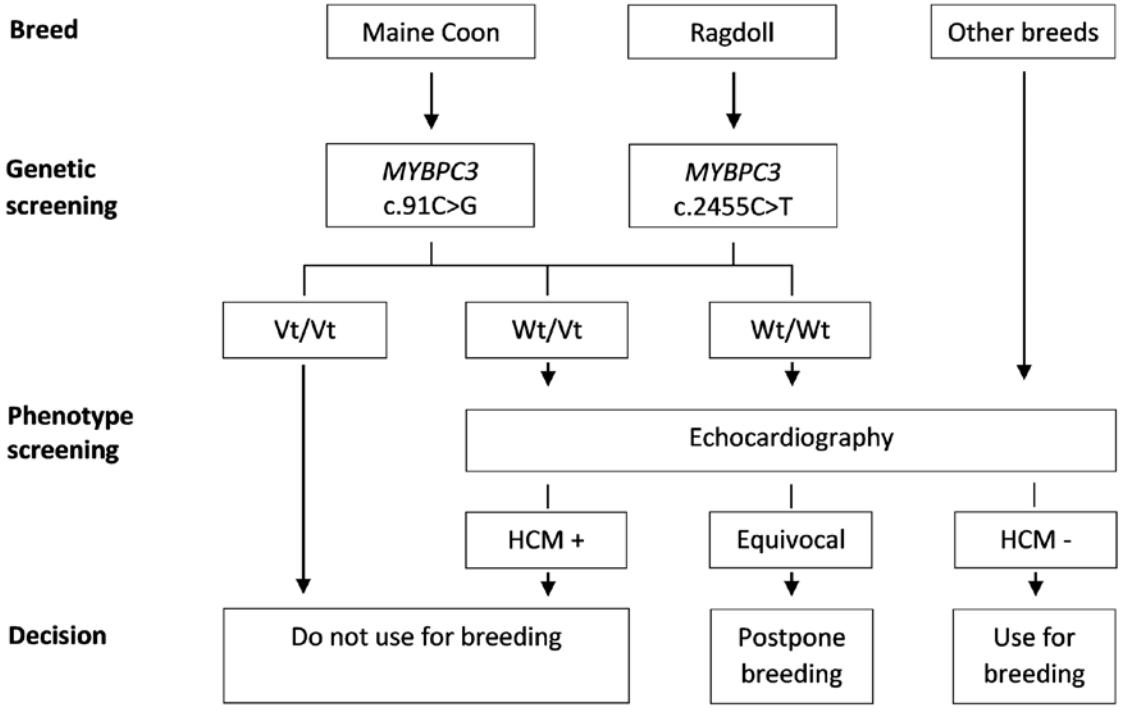

Figure 1. Recommendations for screening and breeding to reduce the prevalence of HCM in cats. Vt/Vt = homozygous for the disease-causing variant, Wt/Vt = heterozygous, Wt/Wt = homozygous wild type.

Table 1. Currently known HCM-causing variants in cats, the breed(s) in which they occur and their application in screening.

Variant	Breed	Recommended to use for screening?
MYBPC3 c.91C>G	Maine Coon	Yes, but only in Maine Coons
MYBPC3 c.220G>A	Multiple breeds	No, because the pathogenicity is not proven
MYBPC3 c.2455C>T	Ragdoll	Yes, but only in Ragdolls
MYH7 c.5647G>A	Domestic Shorthair	No, because the prevalence is low

international HCM screening program (Häggström et al., 2015). However, HCM also occurs in other breeds, of which some, such as the Chartreux and American Shorthair, have been described as predisposed (Côté et al., 2011b; Maron and Fox, 2015; Fox et al., 2018). Other breeds may therefore be added to this list.

RECOMMENDATIONS FOR SCREENING IN BREEDS FOR WHICH GENETIC TESTS ARE AVAILABLE

Genetic testing is possible for all four variants reported as causative for HCM, but this is not necessarily recommended. As there is no convincing evidence that the *MYBPC3* c.220G>A variant causes disease, there is a general consensus that screening for this variant is not recommended for any breed (Wess et al., 2010; Côté et al., 2011b; Longeri et al., 2013; Häggström et al., 2015; Lyons, 2015).

The incomplete penetrance of the *MYBPC3* c.91G>C variant has made some authors to advise against screening for this variant (Wess et al., 2010). However, as this variant increases the risk of HCM (especially in homozygotes), many authors recommend the genetic test in Maine Coons (Côté et al., 2011b; Longeri et al., 2013; Lyons , 2015). Less information is available on the *MYBPC3* c.2455C>T variant, but Borgeat et al. (2014) advise to take this variant into account in Ragdolls because of the increased risk of early cardiac death in homozygotes. The two variants have a high prevalence within the Maine Coon and Ragdoll breed, respectively, but they are virtually absent in other breeds (Longeri et al., 2013; Cassamian-Sorrosal et al., 2014). The authors therefore recommend to screen for the *MYBPC3* c.91G>C and c.2455C>T variants in Maine Coons and Ragdolls, respectively, but not in other breeds. As the *MYH7* c.5647G>A variant has only been found in a single Domestic Shorthair, the authors expect it to be very rare and do not recommend testing for this variant in any breed. The authors' recommendations for the use of genetic variants for screening against HCM are summarized in Table 1.

Because of the occurrence of HCM in Maine Coons and Ragdolls negative for these variants, it is possible that other genetic factors in these breeds also contribute to HCM. After selecting potential breeding cats with genetic tests, it is therefore recommended to also evaluate these cats by echocardiography in the same way as cats of other breeds (Häggström et al., 2015). The genetic tests allow selection against variants with low penetrance, which are unlikely to be detected by echocardiography, while the echocardiography may identify animals carrying disease-causing variants, for which no genetic test is available.

RECOMMENDATIONS FOR BREEDING

Assuming the proposed autosomal dominant pattern of inheritance, it is in theory not recommended to use Maine Coons or Ragdolls that are either heterozygous or homozygous for the *MYBPC3* c.91G>C or c.2455C>T variant for breeding. However, given the high prevalence of these variants and the apparently low penetrance in heterozygotes, it might be necessary to make exceptions to this rule (Mellersh, 2012). A rapid elimination of all cats carrying these variants from breeding may have undesirable consequences and therefore, a slow eradication is advised (Lyons, 2016). Given the strongly increased risk of HCM and cardiac death in homozygotes, the authors consider the prevention of mating that may produce homozygotes while maintaining genetic variation, as the main goal of selection on the basis of genotype.

Côté et al. (2011b) advise to retain positive cats with desirable characteristics as breeding animals, but under three conditions: Firstly, they should be heterozygotes, as they pass on the variant to 50% of their offspring (compared to 100% for homozygous cats). Secondly, cats selected for breeding should only be mated to cats that are negative for this variant, to avoid homozygous kittens, which have the highest risk to develop HCM. Thirdly, they should be negative for the HCM phenotype on echocardiography. Testing kittens of these litters allows selection of negative animals for future breeding (Lyons, 2016). In this way, the prevalence of a disease-causing variant may be decreased slowly in a population without deleterious side effects (Côté et al., 2011b).

When screening on the basis of the phenotype, affected cats should be excluded from breeding. In principle, no distinction should be made between mild and severe cases in the context of breeding: the causative variant may be identical in these cases and the difference could be solely due to other genetic and/or environmental factors. As these modifying factors

might be different in the offspring, mildly affected cats may have severely affected offspring and vice versa (Lyons, 2016). When findings are equivocal, breeding can be postponed until more certainty about the diagnosis is obtained by a re-examination after six to twelve months (Häggström et al., 2015). An overview of recommendations for screening and breeding is provided in Figure 1.

The proportion of cats that are diagnosed with the HCM phenotype at breeding age is estimated relatively low at 3-5%, suggesting that eliminating all positive cats has a limited effect on genetic diversity (Häggström et al., 2015). Nevertheless, in some breeds where the prevalence of HCM seems high, such as the Sphynx, stringent selection might have negative consequences (Chetboul et al., 2012). In such cases, it is advisable to also consider mating affected animals to healthy animals. Cats that have mild and late-onset HCM could be selected, although this does not guarantee that these cats are not homozygous for a HCM-causing variant.

FUTURE PERSPECTIVES

The application of genetic tests for screening breeding cats against HCM is currently limited as only a few variants are known to cause this apparently heterogeneous disease. The utility of genetic testing could be much improved by the identification of new HCM-causing variants. The increased use of next-generation sequencing and genome-wide association techniques in cats may facilitate the search for such variants (Gandolfi et al., 2018; Ontiveros et al., 2018).

If the genetic background of HCM is as heterogeneous in cats as it is in humans, trying to identify and select against all causative variants is a futile effort. Nevertheless, identifying the most common causative variants in breeds where HCM is a major problem may provide a tool for careful elimination of these variants. This might already strongly reduce the prevalence of HCM in a breed. Furthermore, because homozygosity is most likely for a variant with a high frequency, the elimination of common variants will reduce the number of homozygotes, which generally have a worse prognosis than heterozygotes. In a study on eight DNA tests for canine recessive diseases, each test led to a decline in the number of affected dogs as well as a decline in the frequency of the causative allele, illustrating the effectiveness of genotypical selection (Lewis and Mellersh, 2019). Casamian-Sorrosal et al. (2014) found significantly fewer Maine Coons homozygous for the *MYBPC3* c.91G>C variant than expected under Hardy-Weinberg equilibrium, but it is not known whether this was the result of selective breeding.

As long as most genetic causes are unknown, echocardiography remains the principal screening method. The effectiveness of the current echocardiographic screening programs in reducing the prevalence of feline HCM is not known (Häggström et al., 2015). For dogs however, the effectiveness of cardiac screening programs has been analyzed. In a study by Birkegård et al. (2016), Cavalier King Charles Spaniels registered in the Danish kennel club, which requires that the parents are free from a relevant heart murmur on auscultation and mitral valve prolapse on echocardiography, had a lower risk of signs of degenerative mitral valve disease than dogs that were not registered. In a study of a compulsory Swedish scheme using auscultation alone, no effect was found, but this may be caused by the small number of dogs and the short duration of the study (Lundin and Kvart, 2010). In a large, long-term study in the UK, a very limited effect was found, probably because it relied on auscultation alone and/or because screening was not mandatory for registration and the compliance by the breeders was low (Swift et al., 2017). Although degenerative mitral valve disease is considered polygenic while HCM is monogenic, these results suggest that phenotypical selection against acquired heart disease can be effective, but only if the diagnostic test is adequate and the breeder compliance is high.

Because the efficacy of echocardiography is limited by the penetrance and that of genetic testing is limited by genetic heterogeneity, one method cannot fully replace the other. In an ideal situation, the tests are used complementary and breeders can combine the results to select the cats that are least likely to get offspring at risk of HCM.

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