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Myxomatous mitral valve disease in dogs: Does size matter?

Heidi G. Parker, PhD[a,*] and **Paul Kilroy-Glynn, BS**[a,b]

[a]Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892 [b]School of Biotechnology, Dublin City University, Dublin, Republic of Ireland

Abstract

Myxomatous mitral valve disease (MMVD) is the most commonly diagnosed cardiovascular disease in the dog accounting for more than 70% of all cardiovascular disease in dogs. As are most canine diseases with genetic underpinnings, risk of MMVD is greatly increased in a subset of breeds. What is uncommon is that the vast majority of the breeds at elevated risk for MMVD are small or toy breeds with average adult weights under 9 kg. These breeds appear to have little in common other than their diminutive size. In the following review we propose a number of mechanisms by which relatively unrelated small breeds may have developed a predisposition for chronic valvular disorders. Although factors such as age are key in the expression of MMVD, taking a comprehensive look at the commonalities, as well as the differences, between the susceptible breeds may assist in finding the causal variants responsible for MMVD and translating them to improved treatments for both dogs and humans.

Keywords

Canine genetics; degenerative valve disease; canine phenotype; dog breeds

Introduction

Myxomatous mitral valve disease (MMVD) is the most common congenital heart disease in dogs accounting for more than 70% of all canine heart disease[1–3]. The disease is chronic and progressive with initial signs, usually a heart murmur, developing after the age of six. Approximately 30% of dogs with MMVD progress to mitral regurgitation (MR) and eventually heart failure[3]. The incidence is particularly high in some breeds such as the Cavalier King Charles spaniel (CKCS) with as many as 90% developing MMVD by the age of 10 years[3]. Evidence from highly susceptible breeds such as the CKCS and dachshund shows a strong inherited component to the disease and suggests a polygenic mode of inheritance[4–6]. In fact, two loci have been recently associated with MMVD in the CKCS[7]. The breed specificity of MMVD incidence is not unusual for a genetic disease in dogs as the breed structure of the modern domestic dog can easily lend itself to sustaining a detrimental genetic mutation. Breeds are essentially closed populations, once a malicious mutation develops or is introduced it can readily expand throughout the population. As a result, the presence of an inherited disease in a small number of breeds is to be expected. What is

*Corresponding author. hgparker@mail.nih.gov (H. G. Parker).

unexpected in the case of MMVD is that the majority of affected breeds display an average adult weight of less than 9 kg[8–10]. Why is MMVD so much more prevalent in small breeds as opposed to large? What do all small dog breeds share that might generate the optimal conditions for MMVD to develop?

To approach the likely cause of the small dog – MMVD correlation we need first to consider what all small breeds have in common and assess how those commonalities could relate to heart disease. To best accomplish this we will break down the relationships into three sources of common ground: morphologic, genetic, and historic. Morphologic commonalities include overall body size but might also include skeletal shapes or components that are common in very small animals. Genetic similarities arise from the mutations that restrict growth. Aside from the genes under selection, MMVD-causing mutations may be carried with these genes through what is commonly referred to as “hitchhiking”. Historically, it is possible that all small dogs that are affected with MMVD share a unique common ancestor and have inherited a set of mutations from that common source that have increased susceptibility to MMVD. In this review we will assess each of these possibilities and how they may contribute to the overabundance of MMVD in small breed dogs.

Body Size and Heart Disease

According to the American Kennel Club standards, the dog breed average adult weight is 23 kg. Breeds averaging 9 kg and below are in the lowest 21% of average dog weights with approximately 43 breeds fitting the criterion. These breeds are also in the lowest 20% of dog heights with the tallest averaging 33 cm at the shoulder[11]. In 1985, Thrusfield and colleagues assessed the effect of breed and sex on valve disease in dogs by examining the case records of dogs treated at the University of Edinburgh. They identified 12 breeds with an increased incidence of heart valve incompetencies. Of these twelve, nine had average adult weights of 9 kg or less and 10 had average adult weights less than 14 kg. Recently, Fleming *et al.* examined 74,556 entries in the Veterinary Medical Database, a compilation of cases from 27 veterinary teaching hospitals in North America, for cause of death by breed[10]. A major cause of death is considered one that affects more than 10% of the breed. Based on this criterion they found that nearly 75% of breeds with average body weight of less than 9 kg report cardiovascular issues as a major cause of death compared to only 25% of breeds with average weights over 9 kg. Can there be a link between being small and developing heart disease?

In fact, height has been linked to a variety of heart diseases in human studies. In 1951, a study in the Journal of the American Medical Association examined the physical characteristics of 100 individuals with myocardial infarction prior to 40 years of age and found that they averaged 5 cm shorter than a similar control group[12]. Another study looking at a cohort of 7735 men in the United Kingdom in 1998 also showed a significant relationship between height and coronary heart disease[13]. In 2010 a meta-analysis was performed on a combined dataset of >3 million individuals. In this combined group the risk of cardiovascular death in short men and women was 50% higher than in tall men and women[14]. The short group was 5% smaller than the tall group in this analysis. In dogs the small breeds are 30% shorter at the shoulder than the average breed (<33 cm compared to 48 cm). If the correlation of height and heart disease observed in humans is true in dogs, it may be possible to attribute an overall increase in heart disease in small breeds to their size alone.

Though height has been associated with heart disease in general, the association does not account for the prevalence of valve disease compared to other forms of heart disease in the small breeds. Perhaps there are morphologic features common to the small breeds other than body weight or height. In 1981, Schutte *et al.* found that height and diameter of the chest

was reduced in individuals with mitral valve prolapsed (MVP), the human disease that shares some of the components of MMVD, compared to unaffected individuals of the same overall body size[15]. Similar correlations have been made between MVP and genetic disorders with specific upper-body phenotypes such as scoliosis, Marfan's syndrome, straight-back syndrome and funnel chest which may suggest that aspects of cardiac function are controlled by many of the same genes and pathways that shape the thorax. Alternately the shape of the chest may be directly responsible for the valve malformations. Raggi *et al.* measured the chest diameter, angulation of the mitral valve ring, and contact between the myocardium and the chest wall in over 150 MVP patients and concluded that the heart valve malformation is due to entrapment in the chest cavity[16]. In effect, disproportionate growth of the heart and chest cavity can cause distortion of the valve and lead to prolapse.

Unsynchronized morphological regulation has been observed in Insulin-like growth factor 1 (*IGF1*) negative mice. The body size of homozygous mutant mice is reduced to a third of the wild-type mice while the size of the heart is reduced by less than half [17]. *IGF1* also controls size variation in dogs and humans[18–20]. Given the extreme reduction in skeletal size in the smallest dog breeds, it would be interesting to measure the organ size and volume of the thorax in small and large dogs to see if the decrease is proportional or if the heart is being crowded in the ever decreasing chest cavities of small dogs.

Genetics of Body Size

It is possible that the shape and size of the small breed dogs is contributing directly to heart disease by restricting proper growth. Many centuries of selection for smaller size have resulted in a current range of body sizes in dogs that spans two degrees of magnitude with teacup sized toy breeds reaching an adult weight of less than 1 kg while a full-grown male mastiff can easily top 90 kg[21]. Is it not equally as possible that the same genes involved in skeletal growth are active in the developing heart? Multiple studies have been released that address the genetics of size variation in dogs from different viewpoints. In 2002, Chase *et al.* published a linkage study looking at the morphology of the Portuguese water dog (PWD)[22]. The PWD can range in size from 16 to 27 kg and 43 to 58 inches at the shoulder, allowing for segregation of a number of genes affecting overall body size. The authors obtained five different radiographs of >300 PWD and measured >70 aspects of skeletal shape and size. From these they calculated nine principal components (PCs), the first of which encompasses overall body size and accounts for ~45% of all variation in the breed. This PC is linked to two loci in the genome: one on canine chromosome 15 (CFA15) and a second on CFA[37]. The site on CFA15 is in the vicinity of the *IGF1* gene which had been shown to affect body size in both mice and humans[19,20]. To further characterize the locus, Sutter and colleagues compared single-nucleotide polymorphism (SNP) allele frequencies across the region in small and large breeds of dog and found a selective sweep spanning the *IGF1* gene in all small dogs. In fact, a single haplotype that was identical-by-descent was found in 93% of all dogs from breeds with adult weights averaging less than 10 kg. This study revealed two important facts about dogs: one, genes that affect major morphologic traits will be shared across multiple breeds, and two, all small dog breeds share at least one common ancestor that contributed the *IGF1* allele.

This finding is particularly intriguing when we consider that the *IGF1* gene has been implicated in cardiac development as well as body size[23,24]. While a loss of *IGF1* expression is related to decreased body size, over expression of *IGF1* leads to increased heart size by increasing the size of the cardiac myocytes. This response is under consideration as a means to treat heart failure by reducing the risk of myocardial ischemia[25,26]. As discussed earlier, *IGF1* affects both skeletal growth and heart size, though the effects are not proportionate according to studies performed in mice[17]. Since it has been

shown that *IGF1* is a major contributor to reduction in body size in dogs, if the heart is not shrinking at the same scale in small dogs, this mutation alone could be responsible for over-crowding leading to the valve malformations[16]. In addition, *IGF1* has a direct effect on heart growth which could lead to malformations if regulated improperly as would be expected under selection for small size.

Following the *IGF1* findings, two additional studies were released looking at the genetics of body size across multiple breeds. In 2008 Jones *et al.* completed a low density SNP genome-wide association study (GWAS) in 148 dog breeds to look for major morphological control regions[27]. By assigning traits based on breed standards and photographs, the authors identified eight loci associated with height, weight, or a combination of the two. Five of these loci contained strong candidate genes for size such as *IGF1*, high mobility group AT-hook 2 (*HMGA2*), and insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*) (Table 1). Four out of five of the candidate genes identified for size control also affect heart development. For instance, Monzen *et al.* showed that *HMGA2* affects cardiomyocyte differentiation and that a morpholino mediated knock down of the gene leads to improper heart formation in frog embryos[28]. Perhaps the most interesting gene in this group is SMAD family member 2 (*SMAD2*) which interacts directly with transforming growth factor beta (*TGFβ*)[29]. Recent studies show that *TGFβ2*, working through *SMAD2/3*, is required to achieve mature valve structure[30]. In addition, an increase in *TGFβ* signaling, identified by the correlated increase in *SMAD2* expression, contributes to mitral valve degeneration in a mouse model of Marfan's syndrome in which the mitral valves show increased leaflet length and thickness and folding conformation[31]. Given this correlation, a dog with altered *SMAD2* expression due to selection for its growth retarding properties may also experience problems with cardiac valve development.

More recently a large SNP dataset of ~50,000 markers was used in a multi-breed GWAS to identify genetic causes of morphologic traits. Boyko *et al.* examined 900 dogs from 80 diverse breeds to identify loci for size and shape[32]. They found six loci strongly associated with body size, two on the X chromosome and four on autosomes. Three of the four autosomal loci had been identified in at least one of the two earlier studies. The fourth on CFA4 was near the candidate gene stanniocalcin 2 (*STC2*) which has been shown to inhibit growth in mice[33]. Four of the six size associated loci were among the top six regions of the genome that displayed significantly increased fixation indices across the breeds. This indicates that size loci are under strong selection, corroborating the earlier assumption that genes affecting morphologic traits are largely shared across breeds.

Linkage Disequilibrium and Hitchhiking Genes

While the same genes can affect both body size and heart development, the act of selecting for particular traits can influence disease susceptibility by altering the genome architecture. Early proponents of mapping traits in dogs have assumed that the linkage disequilibrium (LD) found on dog chromosomes would be extensive due to the non-random aspects of dog husbandry. Beginning with the development of the breed clubs and competitive dog shows in the early 1800s, dog breeders have been required to contain their breeding program to only those dogs that have been officially recognized as members of the breed. The inevitable inbreeding that follows leads to an overall loss of heterozygosity (reviewed in Ostrander et al. 2000[34],and Parker et al. 2010 [35]). The heterogeneity within a breed is further lowered by the use of popular sires. Dogs that have performed well in competitions are selected to father large numbers of litters causing an over-representation of one set of alleles in later generations. Within dog breeds, LD is measured at 10–100 times the average length found in humans and varies depending on the breed and its demographic history[36–38]. Therefore,

large stretches of each chromosome will remain unbroken from generation to generation within each breed.

In addition to the high levels of LD, canine chromosomes contain large tracts of homozygosity likely the result of selective pressures. When the draft sequence of the Boxer was completed in 2005, it was estimated that >60% of the genome lay within homozygous tracts that averaged over 6Mb in length[37]. Analyses of SNP genotyping data from at least 10 dogs each of 60 breeds found that individuals from all breeds showed 10–50 regions of homozygosity spanning more than 10 Mb[32]. In the majority of breeds, all individuals showed overlapping homozygous regions of at least 1 million bases. These stretches of homozygosity are likely the result of selection for traits within the breeds. When a trait is fixed within a breed a region of homozygosity or reduced heterozygosity is created around the selected mutation which is referred to as a selective sweep. This region can extend over 1 Mb within a breed depending on the age of the mutation under selection[18,39]. This means that any mutation within a gene or regulatory element within a million bases surrounding a selected mutation may become fixed within the population along with the desired trait and at the very least will increase in frequency in response to the selective pressures. Assuming that all small breeds are under selection for mutations within the seven regions that have been identified as size controlling loci in multiple GWAS, there is the potential for seven Mb of DNA to be shared by all of the breeds simply because they are all small in stature. These seven million bases contain, on average, 70 genes any one of which could contribute to an increased risk of heart defects in the breeds carrying them.

An interesting case can be found in the region of the *STC2* gene which was identified as a candidate for small size[32]. *STC2* has not been associated with heart development to date but it is located less than 80 kb from the gene NK2 homeobox 5 (*NKX2–5*). Given their proximity, these genes are likely in high LD with each other and within the range of an average selective sweep[18,39,40]. *NKX2–5* works downstream of *TGFβ* and *SMAD2* in a signaling pathway that controls cardiac valve formation[41]. Mutations in *NKX2–5* have been associated with numerous congenital heart defects including mitral valve anomalies in humans[42]. A mutation affecting *NKX2–5* could easily be swept along with the *STC2* gene when it was under selective pressure at any point during the process of breeding small dogs.

Population Structure and Common Ancestors of Small Breeds

One final explanation for the over-representation or MMVD in small dogs could be common ancestry that is not directly related to size. If all small dogs stem from a common founder, MMVD may have been introduced at a very early stage of breed development and has reached relatively high rates due to reductions in the gene pools of most breeds after their development. A number of studies have shown that each breed carries a unique genetic pattern that is identifiable through analysis of markers scattered throughout the genome[32,43,44]. However, in addition to individual breeds, both microsatellite and SNP studies have grouped the breeds into clusters that largely correspond to specific morphologies, geographical origins, or historical occupations that are shared among the breeds in the cluster[43,44, 45]. The first of these groups is commonly referred to as the Ancient group. This group is comprised of breeds developed in Asia and Africa, and is distinct from the Modern breeds, which are largely of European origin. The Modern breeds are further divided into the Herding-Sighthound cluster, the Mastiff-Terrier cluster, the Mountain cluster, and the Hunting cluster based on microsatellites[44]. The Hunting cluster is the most diverse including not only gun dogs but also hounds, and a subset of terriers and working dogs. All toy breeds do not form a separate group but rather display a mixture of all five groups in different proportions.

An analysis of ~50,000 SNPs genotyped in 80 breeds was used to develop a dendrogram of the breeds built on sharing of 15-SNP haplotypes[45]. This analysis effectively separated the individual breed types from the microsatellite analysis while maintaining the primary breed clusters. Based on haplotype sharing, the majority of toy dogs seem to form one cluster within the Modern group with the closest breeds belonging to the Hunting cluster. This branching is not statistically significant but these toy breeds appear more similar to each other than to the larger breeds in the study. The SNP haplotype analysis may have been able to pick out the sharing of selected loci that was not evident in microsatellite analyses. Although a subset of the toy dogs seem to show a common ancestral line, all breeds with an average adult weight of 9 kg or less, and susceptible to MMVD, are not toys. Many of these breeds are terriers, small hounds and spaniels, which come from nearly all regions of the breed tree (Figure 1). All of the breeds that are susceptible to MMVD are part of the Modern cluster, which comprises four of the five primary breed clusters[44] and eight of the ten breed groups[45]. The microsatellite cluster analysis shows that Pekingese and the Shih Tzu gain a portion of their genome from the Asian cluster but they also are included in the Modern breed groups thus a common mutation is more likely to come from that portion of their heritage. Looking at the contribution of the five primary breed groups to the high risk breeds, the Hunting group is by far the most significant contributor with an average of 42% of their genomes coming from this ancestral cluster (Figure 2).

If we look individually at the at-risk breeds that are small but not considered toy dogs (i.e. the dachshund, Boston terrier, cocker spaniel), they have all gone through reductions in size during some period of their development. The Boston terrier was originally bred from English bulldogs and bull terriers, breeds that average 23 kg or more as adults where as the Boston averages only 9 kg with many members weighing less than 7 kg when full grown[11]. This indicates not only selection for size but outcrossing with much smaller breeds such as the pug.

The cocker spaniel is the smallest of the hunting spaniels and in the late 1700s averaged only 5–7 kg as an adult, placing it within the size range of today's toy breeds[46]. The average body size of dogs from this breed has increased since then to the current 11–14 kg. However, since the cocker was divided into two breeds, the English and American, the American underwent additional changes creating "cute" features that made it the most popular breed in the United States for 16 years[11,47]. These features include a rounded skull and large eyes similar to modern toy dogs, likely indicating outcrossing in addition to selection.

The dachshund has lost ~4.5 kg of adult body weight since its development, and has undergone the creation of a miniature version, likely through outcrossing with miniature pinschers[48]. Other breeds have clearly created smaller versions over time, such as the miniature and toy poodles and the miniature schnauzer. These observations imply that the small susceptible breeds may have experienced recent introgression that has allowed the sharing of detrimental mutations responsible for MMVD susceptibility.

Conclusions

MMVD is the most common heart disorder found in dogs and is particularly evident in the smallest of breeds. We have considered a variety of mechanisms to explain the overrepresentation of small and toy breeds in the list of those most at risk for MMVD. One possibility is that smaller dogs have a larger heart to body size ratio than do larger dogs. This could be tested by measuring the dimensions of the heart and the over-all body size, including chest volume, on dogs of many sizes to determine if there is a significant difference in the ratio in the small breeds. Another option would be to look for small dog

breeds that are not at risk for MMVD. For instance, the Brussels griffon is not on any of the published lists of breeds at increased risk of MMVD despite having an average adult weight of only nine pounds. Do they get MMVD? And if not, is there any difference in their dimensions that would account for the lack of the disease? Yet another possibility is to examine MMVD in a breed that has members both above and below 9 kg, such as the Boston terrier. A correlation between physical dimensions and development of the disease within a breed would help determine if body size is a primary cause of MMVD.

Many genes that affect skeletal growth and development also affect cardiac development. In addition, genes can be found near the growth loci that are required for normal cardiac valve formation. Because all small breeds carry many of the same mutations that create small size they will also carry linked genes that could increase susceptibility to MMVD. Selective pressures on these genes will make it difficult to map them in the smallest breeds because they will probably be nearing fixation. However, because size is a quantitative trait, there should be mid- to large-size dogs that carry at least one small size mutation. If these size-altering mutations are causing MMVD then the disease should appear at some small rate in the mid-sized breeds that carry them. Such breeds may prove invaluable in the search for the causal variant.

Many of the at-risk breeds share common ancestry from an early small dog that has transmitted susceptibility genes to its descendants. As the source of MVDD risk, this transmission could be identified through admixture mapping of the at-risk breeds with a dense set of markers and a large dataset of breed haplotypes. It is formally possible that each breed has developed a unique mutation that has become prevalent due to restrictive breeding practices. However, given the high incidence of the disease combined with similarity among susceptible breeds, it is more likely that shared loci contribute to the disease. None of the mechanisms listed above are mutually exclusive, and it is likely that further investigation will reveal that a combination of these characteristics proves causative for MMVD.

A final trait that is shared between small dogs that might contribute to the development of MMVD is extended lifespan. It is often noted that small dogs live longer, on average, than large dogs[49–51]. MMVD is reported to be a disease of the aging heart, as described in detail by Connell et al. in this issue [52]. This has led to the speculation that small dogs are diagnosed more often merely because they live long enough for the disease to progress. While age is doubtless a contributor, lifespan is unlikely to be the sole reason for the increased appearance of MMVD in small dogs. For instance, symptoms of MMVD may not present until after the age of 9 years, but evidence of valvular dysfunction can be detected prior to 4-years of age[8,52]. This is well within the lifespan of any dog breed regardless of size[49,53]. Consider also that studies of cellular aging suggest that large dogs age quicker than small dogs[51,54]. If cellular aging and valvular aging are comparable, then the 10 year old heart of a small dog would be similar to the 6 year old heart of a giant dog and the disease would be diagnosed at an earlier age in the larger dog[54]. Finally, MMVD is diagnosed in large dog breeds such as the German shepherd and the Great Dane, a breed often considered to have one of the shortest predicted lifespans of all breeds[53,54]. Therefore, it would seem that lifespan alone cannot account for the presence of disease. Unfortunately, the data does not exist to effectively answer the question of size versus age. This would require a comprehensive database of prophylactic heart exams conducted on dogs of all breed sizes over a course of years to determine the true prevalence of the disease in each. Data from such an undertaking would be invaluable as it could also address questions regarding ages of onset of the disease and rates of progression in different breeds.

MMVD has a high cost for dogs and people alike. The disease worsens with age and can lead to congestive heart failure and mortality. The combination of genetic and morphologic

traits correlated with MMVD in dogs provides a promising tool for dissecting the elements of the disease. When applied to molecular studies of the disease, these correlations should enhance both basic and translational progress toward understanding the cause of MMVD and improving treatment. We await the results of MMVD studies in the coming years with much anticipation. Though we have proposed many possibilities within this review, only time and experimental evidence will reveal the means by which MMVD has arisen in small breeds.

Abbreviations

CKCS	Cavalier King Charles spaniel
GWAS	Genome-wide association study
LD	Linkage disequilibrium
MMVD	Myxomatous mitral valve disease
MR	Mitral regurgitation
MVP	Mitral valve prolapse
PC	Principal components
SNP	Single-nucleotide polymorphism

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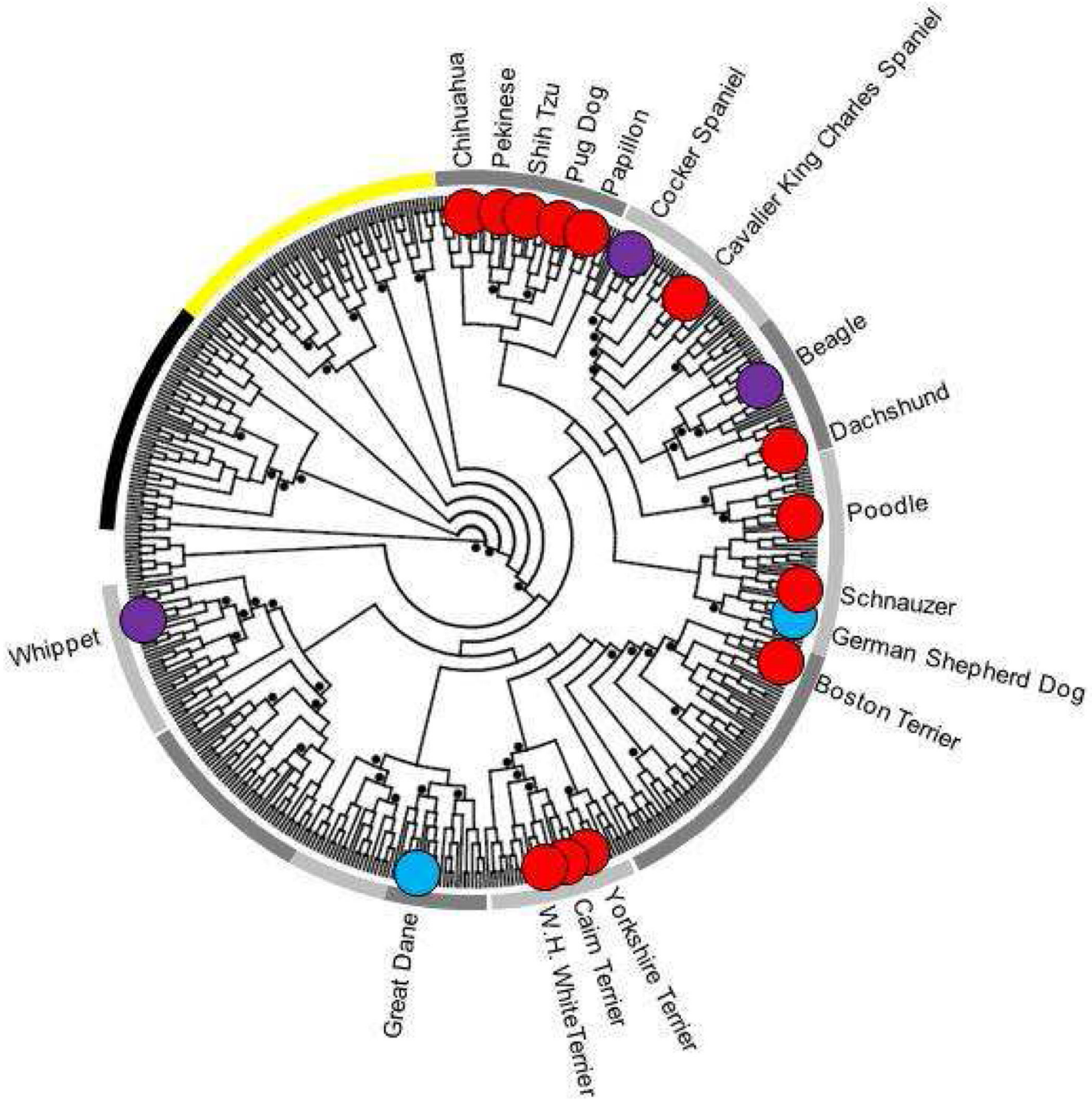

Figure 1.

Breeds at risk for MMVD trace back to an ancient common ancestor of the modern breeds. A neighbor joining tree based on 10-SNP haplotypes groups 80 dog breeds into approximately 10 breed clusters. The black arch indicates the position of wild canids on the tree and the yellow arch indicates the ancient breeds. All other branches comprise the modern breeds. These breeds are grouped in the following order clockwise from the top and indicated by alternating gray bands: toy, spaniel, scent hound, working, mastiff, terrier, mountain, retriever, herding, and sighthound. The positions of the MVDD at risk breeds are indicated by circles on the tree. Red circle=breed with an average adult weight less than 9

kg, purple circle=average adult weight between 9 and 14 kg, blue circle= average adult weight over 14 kg. The haplotype tree of breeds was originally published in Nature[45].

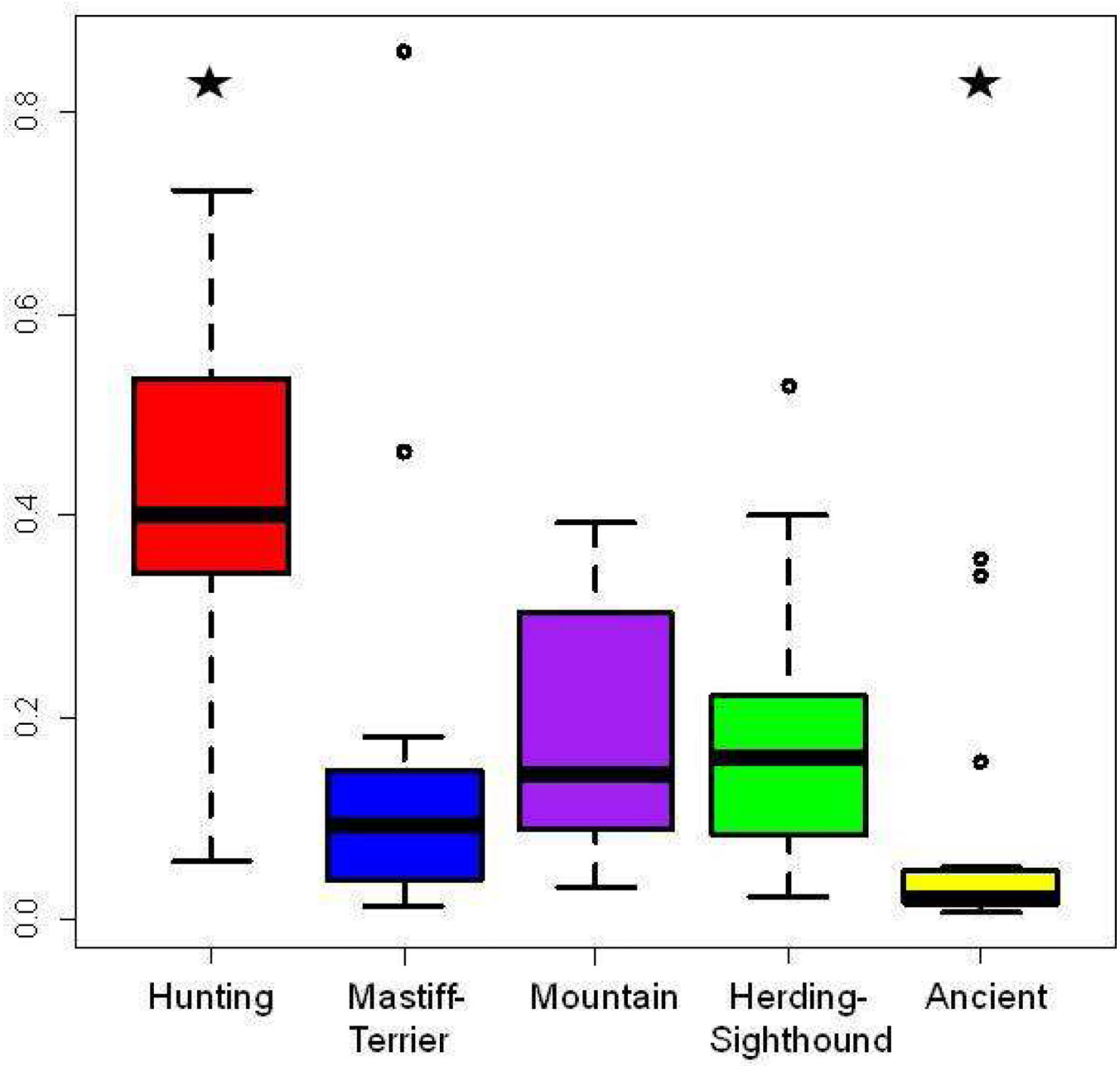

Figure 2.

Distribution of proportion membership in five primary breed clusters by breeds at risk for MMVD. 132 breeds were clustered into five populations using 96 microsatellite markers as described by Parker et al.[44]. Twenty breeds that have been designated at risk were extracted and their genomic proportion in each breed cluster plotted. The stars at the top of the graph indicate significant differences in the distributions: comparing the contribution of Hunting to Mastiff, Mountain, Herding, and Ancient : P= 1.37×10^{-5}, 7.16×10^{-6}, 3.41×10^{-6}, and 4.56×10^{-10} respectively. The contribution of the Ancient group was also significantly lower than the Mountain and the Sighthound clusters with P=0.000655 and 0.003587 respectively. Breed groups are listed below the graph. The y-axis shows percent membership in each breed group.

Table 1

Breeds that may be at an increased risk for MMVD.

Breed	Height (cm)	Weight (kg)	AKC Group	Genetic Group	Ref.[a]
Chihuahua	15	2	Toy	Toy	1,5,6,7,9
Maltese	23	2	Toy	Toy[b]	1,5
Yorkshire terrier	15	3	Toy	Terrier	1
Poodle - Toy	25	4	Toy	working	1,6,7,9
Papillon	24	4	Toy	Toy	9
Pekingese	19	4	Toy	Toy	6,9
Miniature pinscher	28	5	Toy	Toy[b]	6,9
Bolognese	19	5	n.d.	n.d.	1
Dachshund	18	5 and 11[c]	Hound	Scenthound	1,2,3,6,9
Shih Tzu	24	6	Toy	Toy	1
Cairn Terrier	25	6	Terrier	Terrier	6,9
Miniature Schnauzer	33	6	Terrier	Terrier[b]	7
Bichon Frise	25	6	Toy	Toy	1
Cavalier King Charles Spaniel	32	7	Toy	Spaniel	1,4,6,7,8,9
Pug Dog	33	7	Toy	Toy	1
Miniature Poodle	32	7	Non-Sporting	Working	1,6,7,9
West Highland White Terrier	28	8	Terrier	Terrier	1
Fox terrier	38	8	Terrier	n.d.	5,7
Boston Terrier	41	9	Non-Sporting	Mastiff	7,9
Welsh terrier	38	9	Terrier	Mastiff[b]	1
Whippet	48	10	Hound	Sighthound	6,9
Bull terrier	31 and 53[c]	11 and 27[c]	Non-Sporting	Mastiff	9
American Cocker Spaniel	37	12	Sporting	Spaniel	1,2,3
Beagle	36	12	Hound	Scenthound	1,2,6,9
Standard Poodle	38	26	Non-Sporting	Working	6,9
German Shepherd Dog	64	37	Herding	Working	8,9[d]
Great Dane	81	66	Working	Mountain	9

[a] 1- Aupperle, 200955, 2-Buchanan, 199956, 3- Detweiler, 1965 1, 4- Egenvall, 200657, 5-Fleming, 201110, 6 – Thrusfield, 19859, 7- The Quest Trial58,59, 8-Inherited Diseases in Dogs (IDID) Database60, 9-Canine Inherited Disorders Database (CIDD)61.

[b] Breed group determined by microsatellite analysis.

[c] Sizes for miniature and standard versions of the breed.

[d] German Shepherds are underrepresented in MVDD studies by Buchanan and Thrusfield.

Table 2

Genes within loci linked to body size in the dog and their possible associations to heart development.

Chr	Mb[a]	Gene	Size GWAS[b]	Cardiac Involvement	L.G.[c] Cardiac Involvement	Ref.[d]
4	42.3	STC2	3		*NKX2–5* – valve formation defects	41
7	46.7	SMAD2	2,3	Valve remodeling and development		30
10	11.4	HMGA2	2,3	Cardiogenesis		28
15	37	SOCS2	2	Interacts with *IGF1*R, controls heart growth		62
15	44.2	*IGF1*	1,2,3	Controls heart growth		63
34	21.4	IGF2BP2	2		*SENP2* – embryonic heart defect	64
37	29.7	OBSL1	1		*CHPF* - valve development	65

A) Positions in Mb on CanFam2 build.

B) References for the canine body size GWAS studies: 1=Chase *et al*. 200222, 2=Jones *et al*. 200827, 3=Boyko *et al.* 201032.

C) L.G. = linked gene.

D) One representative reference relating each gene to heart development or disease.