# Genetics and genomics of myxomatous mitral valve disease in dogs 

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## Declaration

I declare that this thesis is all my own work and has not been previously accepted for an award of another degree or diploma at any institution of tertiary education. The content in this thesis is either the product of my original research and analysis of data or, where it has been derived from the unpublished or published work of others, it has been acknowledged in the text and reference lists included within each chapter. All assistance received throughout the preparation of this thesis, and any sources relied on herein, have been acknowledged and referenced accordingly.

Mitchell John O’Brien
30 June 2021

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## Manuscripts and conference proceedings

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Claire Wade
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## Abbreviations

| ACVIM | American College of Veterinary Internal Medicine |
| :--- | :--- |
| Ao | Aortic root |
| bp | Base pair |
| BWA | Burrows-Wheeler Alignment |
| CFA | Canis lupus familiaris |
| CHF | Congestive heart failure |
| Cl | Confidence Interval |
| CKCS | Cavalier King Charles Spaniel |
| ECM | Extracellular matrix |
| EMMAX | Efficient Mixed-Model Association eXpedited |
| FROH | Inbreeding coefficients |
| FS | Fishers exact strand bias |
| FTA | Flinders Technology Associates |
| GATK | Genome Analysis Toolkit |
| GLM | Generalised linear model |
| GO | Gene Ontology |
| GPCR | G-protein-coupled receptor |
| GWAS | Genome-wide association studies |
| Het | Heterozygous |
| HKLLS | Hennekam lymphangiectasia-lymphedema syndrome |
| Hom | Homozygous |
| IBD | Identity by descent |
| IQR | Interquartile range |
| Kb | Kilobases |
| KC | The Kennel Club |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| kg | Kilogram |
| LA | Left atrium |
| LA/Ao | Left atrium to aortic root ratio |
| LD | Linkage disequilibrium |
| LQ | Lower quartile |
| LVDd | Left ventricular end diastolic diameter |
| LVIDdn | Left ventricular end diastolic dimension, normalised for body weight |
| MAF | Minor allele frequency <br> Megabases |


| MDS | Multidimensional scaling |
| :--- | :--- |
| MMVD | Myxomatous mitral valve disease |
| MQ | Mapping quality |
| MR | Mitral regurgitation |
| Mut | Mutant |
| MV | Multivariate |
| NCBI | National Center for Biotechnology Information |
| OR | Odds ratio |
| QD | Quality by depth |
| QQ plot | Quantile-quantile |
| QUAL | Quality |
| ROH | Runs of homozygosity |
| SD | Standard deviation |
| SIFT | Sort Intolerant From Tolerant |
| SNV | Single nucleotide variant |
| SRA | Sequence Read Archive |
| TGF- $\beta$ | Transforming growth factor Beta |
| UCSC | University of California Santa Cruz |
| UQ | Upper quartile |
| UTR | Untranslated region |
| UV | Univariate |
| VAI | Variant annotation integrator |
| VCF | Variant call format |
| VEP | Variant Effect predictor |
| WGt | Variant quality score recalibration |
| WGS | Wilde genome sequencing |
| MA | Ma |

## Chapter 1 Introduction

### 1.1 Synopsis

Myxomatous mitral valve disease (MMVD) is a degenerative disease resulting in valvular incompetency that can affect dogs of all breeds. As disease advances, the heart shows signs of left atrial enlargement and volume overload. In the most severe cases, MMVD may culminate in congestive heart failure (CHF). In dogs, the incidence of disease increases with age and is the most frequent cause of cardiovascular morbidity and mortality. Breed specific predisposition, age of onset and rate of progression support a genetic basis to MMVD. Despite an extensive knowledge of MMVD pathology, there remains a significant knowledge gap in the genetic mechanisms contributing to disease. In this chapter, I present a published literature review that highlights the genomic landscape of MMVD research. I briefly describe the clinical nature of MMVD and discuss physiological mechanisms that contribute to disease development. I then describe some of the consistent and contradictory outcomes of MMVD genetic research with a focus on gene profiling and comparative genomic studies. In this review, I highlight some of the successes and pitfalls in MMVD genomic research to date and suggest possible genetic approaches for identifying MMVD genetic risk factors. A major difficulty in comparing and understanding the outcomes of genetic research to date is significant variability in modelling the disease. Throughout this research, I emphasise the importance and benefit of phenotyping MMVD using standardised repeatable measures, particularly echocardiographic measures. The review covers research across all breeds affected by MMVD but highlights the Cavalier King Charles Spaniel (CKCS) as a robust resource in the genetic investigation of MMVD.

# Genetics of canine myxomatous mitral valve disease 

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## Summary


#### Abstract

Myxomatous mitral valve disease (MMVD) is the most common heart disease and cause of cardiac death in domestic dogs. MMVD is characterised by slow progressive myxomatous degeneration from the tips of the mitral valves onwards with subsequent mitral valve regurgitation, and left atrial and ventricular dilatation. Although the disease usually has a long asymptomatic period, in dogs with severe disease, mortality is typically secondary to left-sided congestive heart failure. Although it is not uncommon for dogs to survive long enough in the asymptomatic period to die from unrelated causes; a proportion of dogs rapidly advance into congestive heart failure. Heightened prevalence in certain breeds, such as the Cavalier King Charles Spaniel, has indicated that MMVD is under a genetic influence. The genetic characterisation of the factors that underlie the difference in progression of disease is of strong interest to those concerned with dog longevity and welfare. Advanced genomic technologies have the potential to provide information that may impact treatment, prevalence, or severity of MMVD through the elucidation of pathogenic mechanisms and the detection of predisposing genetic loci of major effect. Here we describe briefly the clinical nature of the disorder and consider the physiological mechanisms that might impact its occurrence in the domestic dog. Using results from comparative genomics we suggest possible genetic approaches for identifying genetic risk factors within breeds. The Cavalier King Charles Spaniel breed represents a robust resource for uncovering the genetic basis of MMVD.


Keywords congestive heart failure, dog, endocardiosis, genetics, heart, mitral valve, myxomatous mitral valve disease

## Myxomatous mitral valve disease (OMIA 000654-9615)

Myxomatous mitral valve disease (MMVD), also known as mitral valve disease, degenerative mitral valve disease, endocardiosis, and chronic valvular disease, is the result of intra-valvular degenerative processes. MMVD is the most common pathophysiological cause of congestive heart failure (CHF) and cardiac morbidity in dogs (Olsen et al. 1999; Serres et al. 2007; Atkins et al. 2009; Keene et al. 2019). The disease is characterised by the progressive myxomatous degeneration of atrioventricular valves, particularly the mitral valve (MV) apparatus (Thrusfield et al. 1985; Serfass et al. 2006; Serres et al. 2007; Olsen et al.

[^0]2010). Mild MMVD is characterised by the disorganisation of valvular structural components as well as the weakening and elongation of the chordae tendineae (Whitney \& Whitney 1974; Jacobs et al. 1995; Olsen et al. 2010). Disruption in valvular structure causes abnormal coaptation of the MV leaflets during ventricular systole, which permits the backflow of a percentage of the left ventricular (LV) stroke volume backwards into the left atrium (LA), an anomaly called mitral regurgitation (MR). On a microscopic level, as MMVD increases in severity, valvular tissue shows thickening of the spongiosa layer of the valve, altered collagen, glycosaminoglycan infiltration, as well as disruption of valvular interstitial cells (VICs) and valvular endothelial cells (Whitney \& Whitney 1974; Rabkin et al. 2001; Black et al. 2005; Hadian et al. 2007; Disatian et al. 2008; Hadian et al. 2010; Olsen et al. 2010). In later stages, secondary fibrosis can lead to contraction of the leaflets, resulting in substantial worsening of MR and left-sided eccentric hypertrophy. The left-sided cardiac dilatation exaggerates the abnormality in valve apposition leading to
secondary MR, and ultimately left-sided CHF (Olsen et al. 2010; Lord et al. 2011).

Myxomatous MV disease is a progressive disorder that is more prevalent with age and may occur in elderly animals of all breeds. Early onset is common in some breeds including the Cavalier King Charles Spaniel (CKCS) and Dachshund in which MMVD is commonly diagnosed before 6 and 10 years of age respectively (Häggström et al. 1992; Pedersen et al. 1999b; Egenvall et al. 2006; Serres et al. 2007). This higher breed risk is evidence for an inherited component of the condition. Within breeds, the prevalence, progression and mortality rates of MMVD are considered to be higher and more severe in male dogs with a lower age of onset (Thrusfield et al. 1985; Egenvall et al. 2006; Serfass et al. 2006; Serres et al. 2007). Although some dogs have a rapid and early onset of the disease, many can long lives free from clinical signs (Kvart et al. 2002; Atkins et al. 2007; Borgarelli et al. 2008; Meurs et al. 2019). Many dogs that are affected with milder forms of MMVD die from unrelated illnesses before developing CHF (Serfass et al. 2006). Conversely, it is not uncommon for severely affected dogs to be euthanised or die of CHF much before their expected natural lifespan (Borgarelli et al. 2008). Inherited components that impact the pathogenicity and severity of disease remain unclear.

## Phenotyping canine MMVD for genetic evaluation

There are several classification systems that categorise the severity of MMVD in dogs. Earlier systems, such as the International Small Animal Cardiac Health Council scheme, categorised dogs into a four-class system (Class I-IV) based on the clinical symptoms exhibited by the dog. Since then, a more stringent system has been developed by the American College of Veterinary Internal Medicine Specialty of Cardiology consensus panel (Atkins et al. 2009; Keene et al. 2019). Classification frameworks are important for clinically comparing patients, directing therapeutic decisions, and for the consistent stratification of individuals used as subjects in MMVD research. Early stages of the disease may be characterised by the presence of a soft apical systolic murmur via auscultation. But murmurs may escape detection and occur in synchrony with every heartbeat or else intermittently (Häggström et al. 1995; Pedersen et al. 1999b). Alternatively, stress and physical activity can generate heart murmurs even in healthy animals and can increase the apparent intensity of the murmur in patients with mild murmurs (Pedersen et al. 1999b). For this reason, echocardiography has become the ideal additional diagnostic tool for evaluating and monitoring the presence and severity of MMVD (Atkins et al. 2009; Keene et al. 2019). Echocardiography allows for the comprehensive and simultaneous visualisation of valve morphology, valve leakage, and secondary heart enlargement, making it possible to evaluate the progression and severity of the disease
(Hansson et al. 2002; Atkins et al. 2009; Bonagura \& Schober 2009; Keene et al. 2019). LA enlargement has been demonstrated to be positively correlated with the advancement of MMVD (Atkins et al. 2009; Keene et al. 2019). A measurement that is considered to be a significant prognostic variable is a comparison between the diameter of the LA with that of the aorta, termed the ratio of the LA to the aortic root (LA:Ao; Borgarelli et al. 2008; Tidholm et al. 2015; Caivano et al. 2018). Similarly, the LV internal diameter in diastole is a good indicator for LV hypertrophy, although the measurement should be normalised for body weight (Cornell et al. 2004; Moonarmart et al. 2010; Hezzell et al. 2012; Boswood et al. 2016; Boswood et al. 2018). LA: Ao along with the normalised left ventricular internal diameter in diastole (LVIDdn) are measurements commonly used as prognostic variables for MMVD (Gordon et al. 2017; Boswood 2018; Boswood et al. 2018; Summerfield 2018).

Given the strong evidence of an inherited component of MMVD, genetic studies have the potential to highlight genes and pathways pivotal in understanding disease pathogenicity. However, given the quantitative and progressive nature of MMVD, isolating convincing controls is difficult. Furthermore, with a prevalence of up to $100 \%$ in some breeds, it is possible that major predisposing genetic factors for MMVD are fixed in certain breeds (Beardow \& Buchanan 1993; Swenson et al. 1996; Chetboul et al. 2004; Serfass et al. 2006; Mattin et al. 2015). This makes the accurate phenotyping of the disease and the identification of causative genes challenging. In the case of MMVD, which can negatively impact dog longevity and welfare, it makes sense to amend the focus of genetic research from identifying the genetic causes of disease to identifying genetic factors that impact the speed of progression or severity of the disease. Genetic pathways that stimulate progression of MMVD to the point of CHF are promising targets for pharmaceutical intervention. More importantly, identification of factors pushing dogs toward cardiomegaly and CHF may enable the selection of breeding stock to increase the proportion of animals that can live long asymptomatic lives (Borgarelli et al. 2008). Repeatable, objective prognostic variables, such as LA:Ao, are expected to yield more reliable associations with the underlying genes than categorical measures such as the presence and severity of murmurs.

## Canine breed predisposition to MMVD

Over 300 breeds of domestic dog are recognised by the Federation Cynologique Internationale (http://www.fci.be/) and as a result of the historical popularity of certain breeding recommendations and show standards, many dog breeds represent genetically isolated groups (Parker et al. 2010; Parker \& Kilroy-Glynn 2012). Founder effects associated with breed selection have resulted in genetically distinct breeds that exhibit a low within population variance and heterozygosity and extensive linkage
disequilibrium within populations (Sutter et al. 2004; Lindblad-Toh et al. 2005). As such, breed specific predispositions to inherited diseases are not uncommon and can be useful in uncovering the genetic basis of disease. Heredity has been shown to play an important role in the development of MMVD. Evidence for this is a predisposition to early onset of the disease in certain breeds; and an observed similarity of the existence, severity, and age of onset of MMVD in parents and offspring (Swenson et al. 1996; Olsen et al. 1999; Lewis et al. 2011; Garncarz et al. 2013; Summers et al. 2015). The disease is described as having an age-related penetrance with polygenic inheritance (Olsen et al. 1999). MMVD is most commonly diagnosed in small to medium sized dogs but can also occur in large breeds (Thrusfield et al. 1985; Borgarelli et al. 2004; Serfass et al. 2006). The most affected dog breeds include, but are not limited to, Chihuahuas, Cocker spaniels, Dachshunds, Poodles, Whippets, and CKCS (Serfass et al. 2006; Fleming et al. 2011; Mattin et al. 2015). Although the exact reason for the development of MMVD in each cohort is unknown, it is clear that MMVD manifests as a profound welfare problem in some breeds.

The CKCS has been identified as a breed with a major welfare issue relating to MMVD. As a breed the CKCS dates back as far as the $16^{\text {th }}$ century, named as such for being the prized breed of a young King Charles II. Throughout its existence the breed has undergone multiple changes in the preferred facial conformation (Knowler et al. 2019). The modern CKCS was established in the 1920's from a founder of King Charles Spaniels, with a similar appearance but predominantly shorter snout. Since then, the CKCS has become one of the most popular toy breeds (Shariflou et al. 2011). Notably, the popularity of the CKCS along with its relatively recent establishment, means the CKCS is a genetically homogenous breed (Mellanby et al. 2013; Dreger et al. 2016). The incidence of MMVD is significantly higher in the CKCS than other breeds with a prevalence in the breed of up to $100 \%$ by the age of 11 years (Swift et al. 2017). CKCS also have a higher risk of disease progression (Mattin et al. 2019), as shown by elevated within-breed mortality resulting from cardiac disorders. Due to the high prevalence of MMVD in the CKCS, the breed represents one of the most studied dog breeds with the disorder (Tarnow et al. 2004; Eriksson et al. 2014; Reimann et al. 2014; Cremer et al. 2015; Lu et al. 2016; Menciotti et al. 2018). Both the presence and severity of cardiac murmurs that arise secondary to MMVD show significant heritability in the CKCS (Lewis et al. 2011). In theory, selective breeding against MMVD is possible, although previous schemes aimed at controlling the problem have had varying success (Lundin et al. 2010; Birkegård et al. 2016). Furthermore, such programs should proceed with caution as it is possible that strong selection against one disorder trait, without consideration of other selection criteria, may increase the risk of other genetic conditions, unless concurrently
managed. For example, it has been suggested that selection against heart disease in the CKCS resulted in an increased prevalence of otherwise rare disorders such as the neurological syndrome syringomyelia (Rusbridge \& Knowler 2004; Rusbridge 2005). Regardless of the efficiency in reducing the incidence of complex disorders through careful breeding, there remains the issue of improving treatment of extant animals that either exhibit the disease or that have a genetic predisposition. The application of advanced genomic technologies has potential to provide information that may impact treatment, prevalence, or severity of MMVD through the elucidation of pathogenic mechanisms and the detection of predisposing genetic loci of major effect. Based on the high prevalence of MMVD in the CKCS, the breed represents a promising resource for understanding the genetic basis of MMVD.

## MMVD genetics and genomics

Applications of advanced genetic and genomic technologies can improve our understanding of MMVD pathophysiology at both genomic and transcriptomic levels. The canine genome represents the first companion animal reference genome to be constructed (Lindblad-Toh et al. 2005; Hoeppner et al. 2014). The provision of the genome and its associated resources has dramatically aided the investigation of traits relevant to canine health.

## MMVD gene and microRNA expression profiling

Consistent observations in gene expression between diseased and healthy individuals can highlight important aetiological pathways involved in MMVD pathogenesis and inform hypothesis-based studies. To date numerous genes and microRNAs have been identified as differentially expressed between healthy dogs and those either mildly or severely affected by MMVD (Oyama \& Chittur 2006; Moon et al. 2008; Aupperle et al. 2009; Lee et al. 2009; Nam et al. 2010; Moesgaard et al. 2014; Li et al. 2015a; Lu et al. 2015). Similar to gene expression studies, microRNA research has had limited overlap in transcripts expression, although shared functional pathways have been reported (Hulanicka et al. 2014; Li et al. 2015b; Yang et al. 2017; Jung \& Bohan 2018; Yang et al. 2018). To date a single microRNA study has used MV tissue, while the rest assessed circulating microRNAs.
Biological functions highlighted through expression studies are extensive and detailing genes and pathways implicated in MMVD in full is beyond the scope of this article. Some biologically relevant functions to consider when evaluating the role of heritable loci in disease pathogenesis include activation of quiescent cells, extracellular matrix (ECM) remodelling, cardiovascular development, cell signalling and movement, inflammatory/immune-response, cell senescence and apoptosis, endothelial function, and
calcium signalling (Oyama \& Chittur 2006; Zheng et al. 2009; Li et al. 2015b; Yang et al. 2017; Yang et al. 2018; Markby et al. 2020a; Markby et al. 2020b). Here we highlight some of the limitations to MMVD expression studies, briefly describe the driving hypothesis for MMVD development, and discuss how expression studies can benefit our interpretation of heritable loci.

## Barriers to gene expression profiling

As with any scientific approach, the reliability of gene expression research is conditional on the reproducibility of results. It is no surprise that the number of samples used in expression analyses can limit reproducibility and bias research outcomes (Tsai et al. 2005; Stretch et al. 2013; Schurch et al. 2016; Maleki et al. 2019). For reasons already mentioned, including varying age of onset and a spectrum of disease severity and classifications, MMVD is difficult to phenotype. Moreover, MV and LV tissue are difficult to acquire, particularly from healthy animals. For this reason, gene expression studies have predominantly examined elderly dogs with severe disease, euthanised due to CHF and compared it to young normal dogs. As such overlapping phenotypes using tissue-, age-, and breedmatched samples are rarely reported, and epigenetic changes are expected to be consequential rather than causative.

Variation can be observed in gene expression profiles across different cardiac tissue (Tabibiazar et al. 2003; Asp et al. 2011; Sun et al. 2013). Arguably, certain tissue types would benefit the assessment of specific phenotypes associated with MMVD, for example the use of LV tissue to assess epigenetic changes during cardiac remodelling. To date, most gene expression studies have utilised tissue from the MV. Alternatively, a single analysis has also been conducted on LV tissue (Zheng et al. 2009) and two studies have interpreted expression in both the MV and LV (Li et al. 2015a; Li et al. 2015b). Within cross-tissue studies, differentiation in gene expression is evident but the major gene categories implicated are similar (Li et al. 2015a; Li et al. 2015b). This alone is not enough to exclude the usefulness of phenotype driven, tissue specific studies and further research is necessary.

Constraints in sampling are frequently discussed within expression studies and have been explicitly highlighted in a few MMVD papers. A key example highlighting breed variation was presented in a recent study that found MV tissue from a multi-breed cohort had gene expression patterns more comparable to healthy valves than age and severity matched dogs of a specific breed, the CKCS (Markby et al. 2020a). Another contentious point for evaluating expression studies is variation in results when equally graded MV tissue is dissected into normal and diseased areas (Markby et al. 2020b). Generally whole tissue samples are collected for analysis, but the ratio of disease to healthy
constituents can influence outcomes. Despite these barriers, significant variation in select genes and pathways, such as those influencing ECM, have been observed across studies (Oyama \& Chittur 2006; Zheng et al. 2009; Li et al. 2015b; Yang et al. 2017; Yang et al. 2018; Markby et al. 2020a; Markby et al. 2020b).

As scientific endeavour moves towards the provision of open access materials, methodologies have been produced which allow for the meta-analysis of gene expression across varying tissue, sequencing, and genotyping platforms (Diego 2019; Toro-Domínguez et al. 2020; Yan \& Wong 2020). Given the difficulty in acquiring tissue for MMVD research, future studies would benefit from the use of metaanalyses to strengthen outcomes or validate findings. For such approaches to be applicable, consistency in the phenotyping and grading systems used in MMVD research is essential. Another justification for implementing the use of replicable measures when grading sample cohorts.

## Extracellular matrix remodelling of the MV

The ECM is a macromolecular network of proteins involved in the structural and functional integrity of tissue and organs including valves and myocardial muscle. The valvular ECM is acellular and is composed of collagens, proteoglycans/glycosaminoglycans, elastin, fibronectin, laminins, and several other glycoproteins (Theocharis et al. 2016). Beyond a role in scaffolding, the ECM may interact in various cellular processes including growth, differentiation, and haemostasis (Frantz et al. 2010; Theocharis et al. 2016). As previously mentioned, MMVD is characterised by the disorganisation of valvular structural components. Disease progression in MMVD is characterised by a build-up of excess proteoglycans and altered collagen in diseased valves (Black et al. 2005; Hadian et al. 2007; Hadian et al. 2010; Han et al. 2010). Although the mechanisms that lead to valve degeneration are poorly understood, there is building evidence to suggest that the activation of quiescent VICs and valvular endothelial cells into active myofibroblasts play an important role in ECM disorganisation (Corcoran et al. 2004; Disatian et al. 2008; Han et al. 2008; Han et al. 2013). Transforming growth factor- $\beta$ (TGF- $\beta$ ) and serotonergic signalling pathways represent two of the most frequently discussed and convincingly hypothesised effectors of ECM remodelling, with a possible role in VIC activation (Aupperle et al. 2008; Disatian \& Orton 2009; Zheng et al. 2009; Aupperle \& Disatian 2012; Orton et al. 2012; Markby et al. 2020b; Oyama et al. 2020). Some of the most recent evidence proposes eccentric ECM remodelling is a consequence of changes in TGF- $\beta$ signalling, which dominates other pathways, regardless of disease severity (Markby et al. 2020b). Changes in gene and protein expression that influence valvular ECM remodelling have been extensively reviewed (Aupperle \& Disatian 2012; Connell et al. 2012; Orton et al.

2012; Markby et al. 2017a; Markby et al. 2017b; Oyama et al. 2020). Frequently discussed genes belong to the TGF$\beta$, bone morphogenic protein, serotonin, hyaluronic acid, and matrix metalloproteinase families (Table 1). Additionally, microRNAs that influence ECM remodelling including family members of let7, mir-30, mir-20, mir-17, mir-133, and mir-29 have shown differential expression in MMVD progression and CHF models (Hulanicka et al. 2014; Li et al. 2015b; Yang et al. 2017; Jung \& Bohan 2018; Yang et al. 2018). The given microRNAs are generally downregulated and considered to have a regulatory impact on TGF- $\beta$ signalling. Given a clear role of these pathways in response to valvular injury and disease progression, genes involved in upstream and downstream processes represent ideal candidates for a heritable component of MMVD.

Using gene expression studies to identify candidate genes and variants

Increasing evidence suggests that heritability of complex traits is influence by genetic variants modulating gene expression (Albert \& Kruglyak 2015; Cookson et al. 2009; Lee et al. 2018). Genes and microRNAs differentially expressed during advancing disease are key in understanding pathogenesis. Pathways highlighted by expressionbased research can help link genetic variation to phenotype. Publicly available databases such as KEGG (Kanehisa 2019), AmiGO (Carbon et al. 2009), and string (Szklarczyk et al. 2018) are valuable resources for categorising gene functions and interactions. Given the growing catalogue of genes and pathways implicated in MMVD such tools are useful to identify candidate genes and variants in heritable loci. Still, it cannot be presumed that variants in genes variably expressed have a direct influence on disease progression unless there is either validation of the gene effects through protein quantification studies using spectral analysis or the loci are individually validated through denovo mapping experiments. Similar to the dog, human MMVD counterparts show marked variation in TGF- $\beta$ signalling pathways and ECM remodelling (Aupperle \& Disatian 2012; Hulin et al. 2012; Thalji et al. 2015; Greenhouse et al. 2016). Heritable components have not been elucidated in human MMVD and disorders with analogous phenotypes. However, variants in genes from key functional pathways have been identified (Chou et al. 2004a; Chou et al. 2004b; Lardeux et al. 2011; Dugan et al. 2015; Durst et al. 2015), demonstrating a capacity for expression analysis to highlight candidates.

## Identification of major MMVD loci

The groundwork for successful complex trait mapping, through genome wide association (GWA) studies, was laid by those working with heart disease and diabetes in humans where many research groups collaborated and
Table 1 Key extracellular matrix remodeling gene/protein expression changes.
 Change: $\uparrow=$ increased, $\downarrow=$ decreased, $-=$ no change, $x=$ undetected. Method: $M A=$ microarray analysis, RT-qPCR = real-time polymerase chain reaction, IHC $=$ immunohistochemistry, RNA-Seq $=$ RNA sequencing. Tissue: $M V=$ mitral valve, $L V=$ left ventricle.
many thousands of human test subjects were included in the analysis (Lohmueller et al. 2003; Cupples et al. 2007; Zeggini et al. 2008; Voight et al. 2010; Strawbridge et al. 2011; Morris et al. 2012). Fortunately, given the unique history of canine domestication and breed development, linkage disequilibrium in the dog breed is extensive and lends itself to genetic mapping of heritable traits. In the dog, mapping of Mendelian traits requires approximately 10 times fewer markers and samples than in humans when conducted on a within-breed basis (Sutter et al. 2004; Lindblad-Toh et al. 2005; Gray et al. 2009). Nevertheless, the application of GWA to complex traits is far more challenging, even given the remarkable population structure of the dog. Still, it has been shown that many complex phenotypes in dog breeds are simply inherited (Jones et al. 2008; Cadieu et al. 2009; Boyko et al. 2010; Shearin et al. 2012; Hayward et al. 2016). This is the basis for complex GWA studies in dogs, which has identified loci and genes associated with obsessivecompulsive disorder, arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, systemic lupus erythematosus, and various cancers (Meurs et al. 2010; Wilbe et al. 2010; Mausberg et al. 2011; Meurs et al. 2012; Philipp et al. 2012; Karlsson et al. 2013; Tang et al. 2014; Tonomura et al. 2015; Hayward et al. 2016). Despite the complexity of MMVD, there remain hints of major gene involvement, indicated by the rapid reduction of disease prevalence in a regional population that was subjected to selection against a related phenotype (MV regurgitation; Birkegård et al. 2016). For this reason, GWA is a promising approach for improving our understanding of the genetic changes contributing to MMVD pathogenesis.

Myxomatous MV disease is a complex trait and consequently a refined model and sizable cohort is implicit in identification of associated loci (Karlsson et al. 2007). Despite the usefulness of canine breeds in increasing the power and accuracy of GWA studies our knowledge of individual variants and loci associated with MMVD is lacking (Madsen et al. 2011; French et al. 2012; Stern et al. 2015; Lee et al. 2019). CKCS are implicitly affected by MMVD and present a strong candidate for genetic studies. However, mapping attempts using this breed have produced variable outcomes (Madsen et al. 2011; French et al. 2012). This is likely to be a result of association studies focusing the disease model on the development of MMVD using the presence and severity of MR to determine a case and control cohort. Using this model, a strong association of MMVD to markers on CFA13 and CFA14 was identified (Madsen et al. 2011). However, despite the strength of the association no follow-up research has been published, nor have the loci been validated (Madsen et al. 2011; French et al. 2012). A case-control GWA to determine development of MMVD, based on MR, is hampered when breeds such as CKCS nearly all present
with a murmur by the age of 10 years (Pedersen et al. 1999a; Swift et al. 2017)—meaning that producing a perfect within-cohort control group is near impossible. A further study accounting for the progressive nature, and age-related penetrance of the disease was conducted in Whippets (Stern et al. 2015). Utilising a quantitative phenotype, the analysis identified loci associated with MMVD on CFA2 and CFA15. To date, this GWA has produced the strongest signal in MMVD studies, highlighting the effectiveness of a quantitative approach in mapping MMVD (Stern et al. 2015).

Quantitative measurements, such as echocardiographic dimensions, are heritable in humans (Post et al. 1997; Bella et al. 2004; Jin et al. 2011), and can be considered distinct phenotypes. Such measurements are objective data and are particularly amendable for use in a continuous variable GWA study. It has been demonstrated that the use of empirical models with multiple quantitative trait measures can increase the statistical power and accuracy of locus detection despite the heterogeneity of measurements (Tin et al. 2013). Multi-trait analyses, conducted on cardiac variables, have highlighted trait-specific loci in human research (Vasan et al. 2007; Smith et al. 2010; Fox et al. 2013; Wild et al. 2017; Sáez et al. 2019). The successfulness of this approach may be transferrable to mapping loci in complex canine disease. Numerous quantitative variables, including circulating biomarkers, thoracic radiography and echocardiographic measures, have been shown to reliably detect consequences of MMVD, as well as predict disease severity and survival characteristics (Hansson et al. 2002; Cameli et al. 2011; Moesgaard et al. 2011; Ebisawa et al. 2013; Vieira et al. 2014; Baron Toaldo et al. 2018; Malcolm et al. 2018; Strohm et al. 2018). Well studied measurements like these are replicable and should be encouraged for use in MMVD mapping efforts. Quantitative variables, such as LA:Ao and LVIDdn, can be easily applied to continuous variable GWA studies and are expected to benefit MMVD modelling by removing the difficulty of phenotyping a perfect control cohort. Instead, all subjects are included on a disease severity spectrum. The approach is unlikely to identify MMVD causative genes but could highlight modifier genes and loci contributing to disease heterogeneity. Still, MMVD is a chronically progressive disease and given the prevalence of MMVD with increasing age, a refined model will also factor in an age-related penetrance of the disease by ageadjusting measurements and using a strict age criterion for the inclusion of samples, as has been done in the past (Stern et al. 2015). A multi-trait GWA approach was recently applied when studying MMVD in a population of purebred Maltese dogs (Lee et al. 2019). Here, researchers compared the sample cohort in both a quantitative and binary manner. While the paper did not successfully highlight associated loci, probably due to a small sample size, this method is optimal for studying complex disease
and should be applied to future MMVD GWA. Further modifications to the approach should include quantitative phenotypes, such as LA:Ao and LVIDdn, rather than using semi-quantitative approaches. Future genomic research could also attempt to identify loci influencing the rate of disease progression by repeating measures throughout a specific study period and mapping the rate of change in echocardiographic measures.

A clear distinction exists in the welfare of dogs affected with MMVD. Some develop severe forms of the disease, resulting in CHF, while others remain relatively asymptomatic (Borgarelli et al. 2008). To date, GWA studies have focused on phenotypes relating to the presence of MR and the age of onset (Madsen et al. 2011; French et al. 2012). From a canine welfare standpoint, it makes greater sense to amend the focus of GWA research to identify loci associated with late stage MMVD and the development of CHF. Adjusting the focus of mapping studies on phenotypes quantifying the degree of heart enlargement and CHF would facilitate the identification of candidate genes that impact disease morbidity and regulatory mechanisms promoting fatal outcomes. Additionally, the use of established quantitative measures should limit subjective phenotyping, improve the repeatability of experimental models and allow the combination of samples from multiple study cohorts.

## Selective sweep analyses

For traits that have been driven to fixation or near fixation in individual breeds, such as MMVD in the CKCS, identification of genes involved in disease development and early onset can be difficult. In situations where case/control data are difficult to obtain, a selective sweep analysis is warranted. Briefly, in species under selective pressure either through genetic drift or artificial selection, regions of homozygosity or reduced heterozygosity are created surrounding a desirable mutation, referred to as a selective sweep. Evidence suggests that artificial selection throughout domestication results in an accumulation of deleterious variation in genes situated in swept regions (Freedman et al. 2016; Marsden et al. 2016). Using bioinformatics approaches, the genome of individual breeds can be scanned for signatures of selective sweep and monitored for loci with candidate genes for a trait of interest. This approach has been used to identify genes and loci involved in morphology, behaviour, and disease (Sutter et al. 2007; Quilez et al. 2011; Vaysse et al. 2011; Arnott et al. 2015; Friedenberg et al. 2016; Sams \& Boyko 2019; Yang et al. 2019). It has been proposed that causative mutations for MMVD may have undergone selection alongside size-based haplotypes in dogs due to a higher predisposition in small breeds (Parker \& Kilroy-Glynn 2012). Presently, no studies exist that have used this approach to identify genes implicated with MMVD.

## Mutation detection

Genetic mapping plays an important role in identifying chromosomal regions associated with a phenotype of interest. To supplement the limitations of mutation detection in mapping data, it is possible to use whole genome sequencing, positional candidate gene sequencing and exome sequencing as methods to explore the underlying genetic architecture in loci of interest. To date, candidate mutations in MMVD loci have not been published. Interestingly, the canine form of the disease is comparable with human valvular disorders (Pedersen et al. 2000; Aupperle \& Disatian 2012; Connell et al. 2012). Evaluation of genes implicated in the human form of the disease was conducted in the CKCS and Dachshund (Meurs et al. 2018). Although no causative mutations were detected, research of this type is warranted, and genes implicated in future human studies should be considered in MMVD research. Nevertheless, in heritable traits such as MMVD, mutation detection would benefit from being directed by GWA and sweep analyses.

## Conclusion

The clinical aspects of MMVD are well documented; however, the pathogenesis and aetiology of the disease is far from fully understood. Ongoing genomic research into MMVD exemplifies the difficulty in identifying heritable components of complex traits. While the condition shows an extraordinarily high level of heritability in certain breeds, the culmination of genetics research acts as convincing evidence that the disease is under a polygenic mode of inheritance. Dogs pre-disposed to MMVD, like the CKCS, present a robust resource and unique opportunity to expand on the genetic context of MMVD. Identification of signatures of artificial selection in dogs with a disproportionate prevalence of the MR and early onset disease is warranted. We recommend that future research into this trait maximise sample sizes and use vigorous phenotyping of sample cohorts, utilising echocardiographic measures for increased repeatability of experimental methods. The use of standardised repeatable measures, such as LA:Ao and LVIDdn, are key in phenotyping disease state and will be amendable to meta-analyses as the public repository of MMVD genetic data expands. Dysregulation of ECM remodelling via TGF$\beta$ and serotonin signalling remains the leading hypothesis for the development and progression of MMVD, although an underlying genetic cause is yet to be attributed to this feature. Genes and pathways consistently observed in transcriptomic research should help direct candidate gene identification and variant discovery of mapping projects. Where available, research papers will benefit from utilising as much open access data to strengthen and validate implicated variants and loci.

## Conflict of interest

Authors declare no conflict of interest.

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### 1.3 Aims of thesis

The broad aim of this thesis is to improve our understand of the genetic basis of MMVD in dogs (Canis lupus familiaris) by utilising the CKCS as a disease model. While the clinical aspects of MMVD are well documented, the genetic mechanisms that drive the development and progression of MMVD and increase the risk of congestive heart failure subsequent to MMVD are poorly understood. Ongoing genomic research into MMVD supports a polygenic mode of inheritance and demonstrates the difficulty of identifying disease risk variants in complex traits. Previous research has highlighted processes and pathways involved in disease development and advancement, but identification of loci and genetic variants that underly disease risk are limited and lack validation. Significant heterogeneity in the onset and rate of progression of MMVD makes the disease difficult to phenotype and can make replicating methods and validating results particularly onerous. Nevertheless, dogs predisposed to MMVD, like the CKCS, represent a valuable resource in the genetic investigation of MMVD.

In this thesis, genomic tools developed for the domestic dog were applied to a population of Australian CKCS with MMVD to improve our understanding of the genetic basis of the disease. The studies described apply bioinformatic tools and approaches to MMVD disease investigation.

Specifically, the aims of each research chapter are as follows:

## Chapter 2

- Refine phenotypes to assess the genomics of MMVD (used in chapters 3 and 4) by evaluating the strength of the relationship between frequently used prognostic variables and MMVD.


## Chapter 3

- Investigate the association of MMVD and candidate genes elected based on known pathways implicated in the development and progression of MMVD.


## Chapter 4

- Map the genetic basis of advancing MMVD and CHF in the CKCS through a genome wide association study, and
- Identify MMVD-associated risk haplotypes and variants.


## Chapter 5

- Identify signatures of selection and genomic regions near fixation in the CKCS through genome-wide characterisation of runs of homozygosity, and
- Identify candidate genes and risk variants that contribute to the heightened prevalence of MMVD in the CKCS.


# Chapter 2 Refinement of phenotypes to assess the genomics of myxomatous mitral valve disease 

### 2.1 Abstract

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in the domestic dog. It is characterised by the progressive degeneration of the mitral valve leaflets. When paired with eccentric cardiac remodelling, MMVD may lead to congestive heart failure (CHF) and premature mortality. Within-breeds the age of onset and rate of progression of MMVD varies such that dogs that present with milder forms of MMVD may eventually die from unrelated causes, while others develop severe CHF. The Cavalier King Charles Spaniel (CKCS) is a breed highly predisposed to MMVD that can experience cases of severe early onset. Genetic studies can highlight pathogenic pathways that are pivotal in understanding MMVD, however accurate phenotyping is difficult due to significant heterogeneity in MMVD progression. The disease is frequently classified by a disease severity score defined by American College of Veterinary Internal Medicine (ACVIM). Echocardiographic measures of left-sided heart enlargement are considered strong predictors of CHF. Measures of left atrium to aortic root ratio (LA/Ao) and weight normalised left ventricular end diastolic diameter (LVIDdn) are strongly recommended in the diagnosis and treatment of MMVD. The main objective of this research was to validate LA/Ao and LVIDdn as predictors of MMVD in an Australian population of CKCS, to facilitate accurate phenotyping of the disease. The outcomes of this research will then be utilised in mapping the genetic basis of severe forms of MMVD using samples within the same cohort.

Anthropometrics and heart condition data were collected from 240 Australian owned CKCS. LA/Ao and LVIDdn were calculated for each sample. Predictors of CHF were assessed using a binary logistic regression analysis. Variable data was then applied to the ACVIM classes using multivariate generalised linear model (GLM). Both logistic regression and GLM were performed in a backwards stepwise manner to assess the strength of predictive variables. Logistic regression analyses identified $\mathrm{LA} / \mathrm{Ao}(\mathrm{OR}=0.76 ; \mathrm{Cl} 1.75-2.078, \mathrm{P}<0.001$ ) and LVIDdn ( $O R=2.13$; $\mathrm{Cl} 1.78-2.67, \mathrm{P}<0.001$ ) as significant predictors for the development of CHF. Along with age ( $\mathrm{OR}=1.05, \mathrm{Cl}=1.04-1.07, \mathrm{P}<0.001$ ), $\mathrm{LA} / \mathrm{Ao}(\mathrm{OR}=2.37, \mathrm{Cl}=2.03-2.76, \mathrm{P}<0.001$ ), and LVIDdn ( $O R=1.80, C l=1.47-2.21, \mathrm{P}<0.001$ ) were also significant predictors of advancing MMVD. This research demonstrated the utility of variables, LA/Ao and LVIDdn, to predict the development of CHF and advancing MMVD in a cohort of CKCS. The use of individual echocardiographic and composite measurements to phenotype MMVD offers a great opportunity to accurately model disease severity. Repeatable and objective prognostic variables, like LA/Ao and LVIDdn, are expected to reduce error in phenotyping the disease state and aid in the accuracy of gene mapping studies. Similarly, the use of such measures should improve the reproducibility of phenotyping the disease in future research and aid in meta-analyses.

### 2.2 Introduction

Myxomatous mitral valve disease (MMVD; OMIA 000654-9615) is the most prevalent cardiovascular disease in dogs (Canis lupus familiaris) and frequent cause of congestive heart failure (CHF) ${ }^{2-4}$. The increased prevalence and severity of MMVD in certain breeds is evidence of a heritable component ${ }^{4-8}$. The Cavalier King Charles Spaniel (CKCS) represents the most dramatically affected breed. Almost all CKCS display signs of being affected with MMVD and many dogs demonstrate early onset with severe prognoses ${ }^{9-13}$. Genetic research can highlight heritable mechanisms driving disease pathogenicity. Given the historical process of breed development, the underlying genomic architecture of dogs is beneficial in mapping heritable traits ${ }^{14-16}$. However, the quality of trait phenotyping is fundamental to successful genetic research.

In dogs, MMVD is phenotypically heterogeneous and has a slow rate of progression permitting some individuals to live long asymptomatic lives that never develop into $\mathrm{CHF}^{17-19}$. Still, for dogs that do develop signs of CHF, the survival period is short, regardless of medical intervention ${ }^{18,20-22}$. Clinical assessment of dogs with MMVD involves grading the severity of disease as the disorder progresses. A staging system developed by the American College of Veterinary Internal Medicine (ACVIM) is frequently used to grade dogs from stage A, at-risk with no signs of MMVD, to end-stage D, dogs with evidence of CHF refractory to treatment ${ }^{1,23}$ (Figure 2.1). Stage B represents a preclinical period of MMVD and is characterised by varying degrees of progression ${ }^{18}$. During this period, evidence of cardiac remodelling might include increased measurements of left atrial and/or left ventricular size; measurements that are associated with the development of CHF and a decreased survival time ${ }^{24-27}$. Due to the variability of symptoms in the preclinical period of MMVD, detection of cardiac remodelling through echocardiography is considered a key diagnostic tool for monitoring the presence and severity of MMVD ${ }^{1,23}$.


Figure 2.1. Classification scheme developed by American College of Veterinary Internal Medicine (ACVIM) ${ }^{1}$. Staging system grades dog on a scale from healthy and predisposed to MMVD to dogs with refractory congestive heart failure. Stage B1 and B2 represent the preclinical period of MMVD where dogs can remain indefinitely or, through eccentric left sided cardiac remodelling, progress into stage C and D with evidence of left sided congestive heart failure. Figure is author's own work created with BioRender.com.

Two routinely used and recommended measures of left atrial and left ventricular size are the left atrium to aortic root ratio (LA/Ao) and left ventricular end diastolic dimension, normalised for body weight (LVIDdn) ${ }^{28-31}$. Increasing values for both measures are associated with reduced survival times in dogs with MMVD ${ }^{26,27,29}$. The diverse nature of disease progression can make the isolation of control samples difficult and to date, identification of MMVD genomic risk loci has been limited ${ }^{32-35}$. We contend that objective, repeatable and reliable prognostic markers like LA/Ao and LVIDdn, can benefit MMVD phenotyping and help in the identification of CHF risk loci.

Given the exceptionally high prevalence of MMVD among small dog breeds, particularly the CKCS, understanding the genetic basis of the disease is of strong interest. Identification of loci governing the contrast between dogs that develop CHF and those that live asymptomatically is key in improving the overall welfare of dogs implicitly affected by MMVD. This study highlights prominent variable measurements associated with MMVD disease progression. We test common measures of left-sided heart enlargement, LA/Ao and LVIDdn, as predictors of MMVD progression and propose that phenotyping by applying these variables may improve genetic risk prediction for MMVD in CKCS.

### 2.3 Methods and materials

### 2.3.1 Ethics

Recommendations from the Australian Code for the Care and Use of Animals for Scientific Purposes were adhered to throughout the process of the research described. Animal ethics approval was granted by the Animal Ethics Committee at the University of Sydney (approval numbers 2015/902 and 2018/1449).

### 2.3.2 Sample collection, Diagnosis and Classification of CHF

Anthropometric and heart condition data were collected by a qualified small animal cardiologist from 240 CKCS from both breeders and private owners across Australia. The diagnosis on MMVD was made at this time. Of the animals observed, 59 were seen on multiple occasions for a total of 337 measurements over a period of four years, between 2014 and 2018. At the time of assessment, CKCS owner consent for the collection of blood samples and the use of acquired data for future research was obtained. The group included dogs that were referred to the University of Sydney Veterinary clinic due to MMVD, were requested to be tested for MMVD by CKCS breeders, and others that were sought out for the study. Diagnosis of MMVD was based on the presence of central characteristics of MMVD including mitral valve thickening, irregularity, prolapse and regurgitation. During echocardiographic assessment, key parameters of left heart size were recorded including left atrium (LA), aortic root (Ao) and left ventricular end diastolic diameter (LVDd). From these parameters, a standardised measurement was created for LA/Ao. LVDd was normalised for weight (LVIDdn =

LVIDd(cm)/weight(kg) $\left.)^{0.294}\right)^{1,36}$. In order to ensure unbiased results, all data was collected under the observation of a single small animal cardiologist. All samples were then grouped based on MMVD severity according to the ACVIM descriptions ${ }^{23}$. Diagnosis of CHF because of MMVD was based on a history of CHF, physical examination (dyspnea and/or tachypnea, abnormal lung sounds, A grade $4 / 6$ systolic murmur and tachycardia), echocardiographic and doppler evidence of cardiac remodelling due to volume overload (severe mitral valve regurgitation and left ventricular filling) or radiographic evidence.

### 2.3.3 Statistical Analysis

Data were analysed with an intention to phenotype CKCS for inclusion in genetic mapping studies. For this reason, prognostic data were first analysed collectively, to identify the predictive strength of echocardiographic measures in a full model. Data were then split into individual models, for both LA/Ao and LVIDdn, to identify quantitative variables that should be included as covariates in trait specific association analyses. Unless otherwise stated, all statistical analyses were conducted in $\mathrm{R}(\mathrm{v} 3.6 .3)^{37}$. The normality of data was evaluated using the Shapiro-Wilk test. For baseline descriptive results, normally distributed data were presented as mean $\pm$ standard deviation, whereas data without a normal distribution were presented as medians (Interquartile range; IQR). Spearman rank correlation coefficients were generated in a pairwise manner for quantitative variables (ACVIM class, LA/Ao, LVIDdn, Age, and weight) and plotted using R package GGally ${ }^{38}$. Multivariable logistic regression models were used to identify the explanatory variables associated with the development of CHF. Univariable logistic regression was then conducted on individual echocardiographic measures and used to determine a predictive threshold for CHF within the Australian CKCS cohort, by calculating the 0.5 value of the regression intercept ( $\beta 0$ ) and slope ( $\beta 1$ ). Next, a multivariate generalised linear model (GLM) was applied on ACVIM classes. To apply the GLM, ACVIM classes were transformed into numerical categories, with ACVIM A corresponding to 1, ACVIM B1 corresponding to 2 , ACVIM B2 corresponding to 3 , ACVIM C corresponding to 4 and ACVIM D corresponding to 5. Finally, a multivariate GLM was conducted on LA/Ao and LVIDdn (employing age, sex and weight as risk factors), to identify explanatory variables that should be used as covariates in mapping studies. A backwards elimination was applied to all models, dropping variables with low significance ( $\mathrm{P}>0.05$ ).

### 2.4 Results

### 2.4.1 Base-line characteristics and correlation analyses

All dogs enrolled in the study are purebred CKCS from breeders or private owners across Australia. A total of 337 measurements (from 240 dogs) were recorded over the study period; baseline characteristics are reported for each ACVIM class (Table 2.1). Briefly, the median (IQR) age of the study cohort was $9.25(7.75,10.83)$ with a bodyweight of $8.70(7.80,9.60)$. The largest proportion of dogs were assigned to class B1 (47\%). A small proportion of the study
cohort (8\%) had no observable signs of MMVD. Four of the 28 dogs that showed no signs of MMVD were assessed at least twice during the study period; none were assigned to class A consistently. Only 3 dogs (2\%) assessed over the age of 10 presented as free from MMVD and were assigned to class A. Fifty-three dogs (6\%) were grouped as ACVIM class C or D, indicating evidence of CHF (Table 2.2). Females were overrepresented in the study group (60\%) and were grouped slightly less in the CHF cohort (42\%). Regarding echocardiographic observations, both LA/Ao and LVIDdn were found to increase with each ACVIM grade. The median and interquartile ranges for LA/Ao and LVIDdn did not overlap between CHF and non-CHF observations. A strong correlation was observed between each pairwise comparison of ACVIM class, Age, LA/Ao and LVIDdn ( $\mathrm{r}^{2}>0.138, \mathrm{P}<0.001$ ) (Figure 2.2). A weaker correlation is reported for weight and ACVIM classification ( $r^{2}=0.13, P<0.05$ ), as well as weight and $L A / A O\left(r^{2}>0.14\right.$, $\mathrm{P}<0.05$ ).

Table 2.1 Descriptive statistics of dogs included in the study. Samples have been grouped by ACVIM class. Categorical data is presented as a count and proportion (\%). The remaining variables did not fit a normal distribution and are reported as median (IQR).

| ACVIM $^{\mathrm{a}}$ |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | A | B 1 | B 2 | C | D | Total |
|  | $(\mathrm{N}=28)$ | $(\mathrm{N}=161)$ | $(\mathrm{N}=95)$ | $(\mathrm{N}=49)$ | $(\mathrm{N}=4)$ | $(\mathrm{N}=337)$ |
| Male | $9(32)$ | $53(33)$ | $41(43)$ | $28(57)$ | $3(75)$ | $134(40)$ |
| Female | $19(68)$ | $108(67)$ | $54(57)$ | $21(43)$ | $1(25)$ | $203(60)$ |
| Age (years) | $5.71(3.33$, | $9.08(7.00$, | $9.58(8.71$, | $9.75(8.75$, | $9.75(8.98$, | $9.25(7.75$, |
|  | $8.00)$ | $10.67)$ | $11.62)$ | $10.67)$ | $10.23)$ | $10.83)$ |
| Bodyweight | $8.95(7.65$, | $8.40(7.50$, | $9.00(8.00$, | $8.70(7.80$, | $3 ; 10.30$ | $8.70(7.80$, |
| (kg) | $9.20)$ | $9.30)$ | $10.00)$ | $10.60)$ | $(9.80,10.55)$ | $9.60)$ |
| LA/Ao ${ }^{\text {b }}$ | $1.29(1.23$, | $1.34(1.23$, | $1.79(1.61$, | $2.43(2.30$, | $3.11(3.03$, | $1.48(1.29$, |
|  | $1.38)$ | $1.42)$ | $1.95)$ | $2.95)$ | $3.27)$ | $1.87)$ |
| LVIDdn $^{\text {c }}$ | $1.55(1.46$, | $1.63(1.49$, | $1.95(1.77$, | $2.43(2.25$, | $2.91(2.78$, | $1.75(1.58$, |
|  | $1.62)$ | $1.74)$ | $2.18)$ | $2.67)$ | $2.93)$ | $2.09)$ |

${ }^{a}$ American College of Veterinary Internal Medicine
${ }^{b}$ Left atrium to aortic root ratio
${ }^{c}$ Weight normalised left ventricular end diastolic diameter

Table 2.2 Descriptive statistics for dogs included in the study presented as non-CHF and CHF samples. Categorical data is presented as a count and proportion (\%). The remaining variables did not fit a normal distribution and are reported as median (IQR).

|  | Non-CHF $^{\text {a }}(\mathrm{N}=\mathbf{2 8 4})$ | $\mathrm{CHF}^{\mathrm{a}}(\mathrm{N}=53)$ |
| :---: | :---: | :---: |
| Male | $103(36)$ | $31(58)$ |
| Female | $181(64)$ | $22(42)$ |
| Age (years) | $9.08(7.50,10.83)$ | $9.75(8.75,10.58)$ |
| Bodyweight (kg) | $8.51(7.77,9.50)$ | $52 ; 8.80(7.88,10.65)$ |
| LA/Ao $^{\text {a }}$ | $1.41(1.27,1.63)$ | $2.59(2.30,3.00)$ |
| LVIDdn $^{\text {b }}$ | $1.69(1.55,1.89)$ | $2.47(2.28,2.74)$ |

${ }^{a}$ Congestive heart failure
${ }^{b}$ Left atrium to aortic root ratio
${ }^{c}$ Weight normalised left ventricular end diastolic diameter


Figure 1.2. Pairwise correlation matrix illustrating the relationship between continuous variable data. The distribution of each variable is plotted as a histogram along the diagonal. Below the diagonal are bivariate scatter plots with a fitted line. Above the diagonal is the result of the Spearman's correlation ( $r$ ) test with the significance level as stars. ${ }^{* * *} \mathrm{P}<.001,{ }^{* *} \mathrm{P}<.01,{ }^{*} \mathrm{P}<.05$, no stars $\mathrm{P}>.05$. figure created with GGally

### 2.4.2 Predictive accuracy of echocardiographic variables for the occurrence of CHF

## in CKCS

Following backward stepwise elimination of predictive variables, highly correlated echocardiographic measures of left atrial enlargement (LA/Ao) and left ventricular enlargement (LVIDdn) remained the only significant predictors of CHF secondary to MMVD (Table 2.3) ( $r>0.71, \mathrm{P}<0.001$ ). A univariate analysis of both variables was then conducted. Both LA/Ao (OR=0.76; Cl 1.75-2.078, $\mathrm{P}<0.001$ ) and LVIDdn ( $\mathrm{OR}=2.13$; $\mathrm{Cl} 1.78-2.67, \mathrm{P}<0.001$ ) remained significant predictors of CHF secondary to MMVD. By means of the $\beta$ coefficients from the fitted models in the univariate analyses, we determined a predictive threshold for CHF for each echocardiographic measure. We suggest cut-offs of LA/Ao>2.36 and LVIDdn>2.39 for prediction of CHF in our sample population (Figure 2.3).


Figure 2.3 Fitted Logistic regression model for CHF using echocardiographic measures as a predictive variable. (a) Left atrium to aortic root ratio (LA/Ao) (b) Weight normalised left ventricular end diastolic diameter (LVIDdn). Data points at the top and bottom of the plot represent the true echocardiographic measures for CKCS used in the Logistic model. Fitted values are marked along the regression curve with an $X$. The dashed red line running vertically along the X axis represents the predictive threshold for CKCS with CHF; 2.36 and 2.39 for LA/Ao and LVIDdn respectively.

Table 2.3 Logistic regression analysis following backward elimination of non-significant variables identifies echocardiographic measures as the only significant predictors of congestive heart failure. This table includes results from three analyses, one multivariate (MV) analysis to assess all predictors and two univariate (UV) analyses, one on each echocardiographic variable, left atrium to aortic root ration (LA/Ao) and weight normalised left ventricular end diastolic diameter (LVIDdn). Echocardiographic variables associated with the left-sided heart enlargement are the only significant predictors of CHF secondary to MMVD. OR, odds ratio; CI, confidence intervals; MV, Multivariate model; UV; Univariate model; significance levels *** $\mathrm{P}<.001,{ }^{* *} \mathrm{P}<.01,{ }^{*} \mathrm{P}<.05$, no stars $\mathrm{P}>.05$

| Model | Variable | $\beta$ coefficient | SE | OR | $\mathrm{Cl}(2.5 \%)$ | $\mathrm{Cl}(97.5 \%)$ | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| MV | (Intercept) | -17.0 | 2.50 |  |  |  | $1.09 \times 10^{-11 * * *}$ |
| MV | LA/Ao | 3.85 | 0.89 | 1.47 | 1.25 | 1.78 | $1.59 \times 10^{-05 * * *}$ |
| MV | LVIDdn $^{\mathrm{b}}$ | 3.61 | 1.21 | 1.44 | 1.15 | 1.87 | $0.00288^{* *}$ |
| UV | (Intercept) | -12.73 | 1.61 |  |  |  | $3.12 \times 10^{-15 * * *}$ |
| UV | LA/Ao | 5.62 | 0.76 | 1.75 | 1.54 | 2.08 | $2.03 \times 10^{-13 * * *}$ |
| UV | (Intercept) | -17.61 | 2.30 | -7.65 |  |  | $1.95 \times 10^{-14 * * *}$ |
| UV | LVIDdn $^{\text {b }}$ | 7.56 | 1.03 | 2.13 | 1.78 | 2.67 | $2.09 \times 10^{-13 * * *}$ |

${ }^{a}$ American College of Veterinary Internal Medicine
${ }^{\text {b }}$ Left atrium to aortic root ratio
${ }^{\text {c }}$ Weight normalised left ventricular end diastolic diameter

### 2.4.3 Predictive accuracy of variables for grading MMVD progression

Linear regression was conducted on three dependant variables ACVIM, LA/Ao and LVIDdn (Table 2.4). For all three regression analyses, the sex of the CKCS was not a strong predictor of outcomes. When utilising the ACVIM staging system as the dependant variable, all other variables were significant predictors of incrementing ACVIM classes, however Age (OR=1.05, $\mathrm{Cl}=1.04-1.07, \mathrm{p}<0.001$ ), LAAO ( $\mathrm{OR}=2.37, \mathrm{Cl}=2.03-2.76, \mathrm{p}<0.001$ ), and LVIDdn(OR=1.80, $\mathrm{Cl}=1.47-2.21, \mathrm{P}<0.001$ ) had a much stronger level of significance when compared to bodyweight ( $\mathrm{OR}=1.03, \mathrm{Cl}=1.01-1.06, \mathrm{p}<0.05$ ). A reduced significance for bodyweight in advancing disease was supported by exclusion of the measure as a predictive variable when modelling MMVD using LA/Ao and LVIDdn as the response variable. Consistent with the expectation, age was a strong predictor of advancing MMVD across all analyses.

Table 2.4 Final results of linear regression analysis following backwards elimination of nonsignificant variables. Linear regression was used to identify variable that are strongly associated with advancing myxomatous mitral valve disease as modelled by graded disease (ACVIM) and left-sided heart enlargement (LA/Ao \& LVIDdn). Age was strongly associated to disease severity in all models. OR, odds ratio; Cl, confidence intervals; significance levels *** $\mathrm{P}<.001,{ }^{* *} \mathrm{P}<.01,{ }^{*} \mathrm{P}<.05$, no stars $\mathrm{P}>.05$

| Model | Variable | $\beta$ coefficient | SE | OR | $\mathrm{Cl}(2.5 \%)$ | $\mathrm{Cl}(97.5 \%)$ | P |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ACVIM $^{\text {a }}$ | (Intercept) | -0.79 | 0.185903 |  |  |  | $2.89 \times 10-05 * * *$ |
| ACVIM $^{\text {a }}$ | Age | 0.05 | 0.009334 | 1.05 | 1.04 | 1.07 | $3.36 \times 10^{-08 * * *}$ |
| ACVIM $^{\text {a }}$ | LA/Ao $^{\text {b }}$ | 0.86 | 0.078402 | 2.37 | 2.03 | 2.76 | $2.00 \times 10^{-16 * * *}$ |
| ACVIM $^{\text {a }}$ | LVIDdn $^{\text {c }}$ | 0.59 | 0.104154 | 1.80 | 1.47 | 2.21 | $3.51 \times 10^{-08 * * *}$ |
| ACVIM $^{\text {a }}$ | Bodyweight | 0.033 | 0.014315 | 1.03 | 1.01 | 1.06 | $0.0232^{*}$ |
| LA/Ao $^{\text {b }}$ | (Intercept) | 1.43 | 0.10236 |  |  |  | $<2 \times 10^{-16 * * *}$ |
| LA/Ao $^{\text {b }}$ | Age | 0.03 | 0.01078 | 1.03 | 1.01 | 1.05 | $0.0112^{* *}$ |
| LVIDdn $^{c}$ | (Intercept) | 1.58 | 0.076579 |  |  |  | $2.00 \times 10^{-16}$ |
| LVIDdn $^{\text {c }}$ | Age | 0.03 | 0.008062 | 1.03 | 1.02 | 1.05 | $0.000142^{*}$ |

${ }^{\text {a }}$ American College of Veterinary Internal Medicine
${ }^{\text {b }}$ Left atrium to aortic root ratio
${ }^{c}$ Weight normalised left ventricular end diastolic diameter

### 2.5 Discussion

We evaluated the prognostic significance and predictive capacity of common echocardiographic measures of heart enlargement in CKCS with MMVD. This research identified key characteristics for phenotyping MMVD in CKCS for the purpose of genetic mapping studies. Our modelling efforts identified predictive variables, or risk factors, of advancing MMVD that should be incorporated into phenotypic modelling efforts. We found that echocardiographic measures LA/Ao and LVIDdn are highly correlated in the study population and that both measures are significant predictors of CHF. We propose these measures should be used in a continuous variable genome-wide association analysis, correcting for age. The studied population presented with typical characteristics of MMVD reported within literature, in that most dogs were of medium age, had standard bodyweight, and showed evidence of mitral regurgitation ${ }^{5,7,8}$. A heart murmur was evident in $98 \%$ of dogs over ten years of age. This is a distinctive feature of the CKCS, where virtually all geriatric dogs show signs of MMVD ${ }^{7,9,10,39}$. While evidence of cardiac remodelling was substantial, far fewer CKCS (16\%) were symptomatic for CHF. The ability to identify key clinical features that can be used to differentiate asymptomatic dogs with MMVD from those that develop CHF is of key interest for improving dog wellbeing.

Continuous variable GWAS can be applied to phenotypically diverse traits for the identification of quantitative trait loci. The development of MMVD is not strictly quantitative in that some dogs can develop the disease while others do not. However, the prevalence of the disease,
particularly in small breeds, is widespread and heterogenous in the rate of progression and endpoint of disease ${ }^{17-19}$. The ACVIM grading system developed to diagnose and treat affected dogs emphasises the use of echocardiographic measures for the accurate classification of disease severity ${ }^{1}$. The results presented here are in favour of this outlook. As echocardiographic measures are a major tool for clinically evaluating MMVD, measures like LA/Ao and LVIDdn are frequently reported in a clinical context. This is ideal for the future of MMVD mapping research as the measures are readily applied to a continuous variable GWAS.

Strong artificial selection for breed-defining phenotypes has increased the prevalence of major-effect loci in the dog, evident by the few quantitative trait loci that govern diverse traits including chondrodysplasia ${ }^{40,41}$, tail curvature ${ }^{42}$, ears shape ${ }^{42-44}$, coat texture ${ }^{45,46}$ and body size ${ }^{42,44,46-49}$. Despite the complexity of MMVD, a role of large-effect loci is indicated in the CKCS by the successful reduction of disease prevalence when selecting against severe disease in a regional population ${ }^{50}$. This makes GWAS a promising approach for identifying a genetic basis for severe forms of the disease. The use of quantitative measures, like LA/Ao and LVIDdn, are critical in accurately phenotyping complex diseases. This is because quantitative measures allow for fine scaling and ranking of individuals that is not possible when phenotyping animals in a graded manner. An example of this in canine health research is the benefit of using PennHIP distraction index, a quantitative measure, over the Orthopaedic Foundation hip joint scoring system due to its greater accuracy in detection and prediction of canine hip dysplasia ${ }^{51-}$ ${ }^{53}$. It has been suggested that application of echocardiographic variables of MMVD can benefit modelling by minimising the difficulty in identifying a perfect within-breed control cohort ${ }^{34,54}$. The result of this research supports the use of LA/Ao and LVIDdn as quantitative variables in MMVD mapping analyses whilst also proposing a threshold for classifying samples as cases in a CHF-GWAS.

Dogs with eccentric left-sided cardiac remodelling are at an increased risk of developing CHF and have a reduction in survival period ${ }^{10,18,24-27}$. To monitor disease progression, the ACVIM guidelines recommend echocardiographic detection of left-sided cardiac remodelling, via increasing LA and LV measures, for the identification and classification of subclinical dogs with MMVD ${ }^{28-31}$. Here, we assessed two echocardiographic measures, LA/Ao and LVIDdn, that were highly correlated with one another and ACVIM grade. Both measures are strong predictors of graded disease and the development of CHF as diagnosed by certified cardiologist based on evidence of left ventricular filling and volume overload. Univariate analysis of echocardiographic measures suggests that for every 0.1 unit increase in LA/Ao or LVIDdn, CKCS have 1.75 or 2.73 increased odds of developing CHF, respectively. To forecast the development of CHF in mapping efforts, we suggest the use of predictive thresholds 2.36 and 2.39 for LA/Ao and LVIDdn respectively. We predict that using these thresholds to define cases and controls in genome-wide association study will aid in the identification CHF-risk loci.

A distinct relationship between age and progression of MMVD is well documented 11,12,55-59. However, while CHF is expected to occur more frequently in dogs above eight years of age ${ }^{18}$,
age itself is not a significant predictor of mortality or development of $\mathrm{CHF}^{25,60,61}$. This research identified age as a significant predictor for the progression of MMVD through ACVIM grades and for increasing measures of LA/Ao and LVIDdn. However, irrespective of the increasing severity of MMVD in mature dogs, our data established that age is a poor predictor for the development of CHF in the studied population.

Previous research proposes that the prevalence, age of onset, rate of progression and chances of early mortality is higher in male dogs ${ }^{7,8,55,58}$. CKCS-specific research has indicated that for males there is evidence of earlier onset, more rapid progression through ACVIM grades and more frequent heart chamber enlargement ${ }^{11,62}$. A recent study in CKCS reported higher values of LA/Ao and LVIDdn in male dogs, which were common measurements considered in this research ${ }^{62}$. However, even though females were overrepresented in our study cohort (60\%) and made up a smaller part of CHF samples (42\%), our results were contrary to those reported and suggested a low predictive value of sex for all models.

On initiation of the research project, dogs were assessed specifically for echocardiographic measures recommended for treatment of severe MMVD ${ }^{26}$, and for use in a genetic mapping study. As such, LA/Ao and LVIDdn, two strong predictors of left-sided heart enlargement, were picked to be reported. However, LA/Ao and LVIDdn are just two of many echocardiographic measures. Our results are highly representative of the academic cohort and our intention is not to suggest the use of these measurements over any other but rather highlight strength and predictive power of these common variables within the study population. While we were able to limit biases created using multiple clinicians, the collection of samples was not consecutive and thus some selection biases may have occurred. Our study included a disproportionate number of females compared to males and may reflect a greater participation from breeders compared to private owners. As the study was not of a retrospective design, medications known to aid in the progression of MMVD were not withheld from dogs or documented throughout the analysis and thus we could not predict an effect of this variable on outcomes. Finally, the main limitation of this research is the uneven number of dogs assigned to each ACVIM class. Through the study period and generally reflective of disease, dogs with no signs of MMVD (ACVIM A) and dogs in more advanced stages of MMVD (ACVIM C and D) were less frequently identified. In this regard, though the models showed a strong fit, the presence of statistical type II errors cannot be ruled out. Increasing subjects for each grade would have benefited outcomes.

This research demonstrates utility of variables, LA/Ao and LVIDdn, to predict the progression of MMVD through the various stages of disease progression as well as those likely to develop CHF. The predictive thresholds determined can be applied to better assess case-control status for genome-wide association analysis. Other key prognostic variables, particularly age, should be used as covariates for association analysis of quantitative traits LA/Ao and LVIDdn.

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# Chapter 3: Candidate gene analysis of myxomatous mitral valve diseases in Cavalier King Charles Spaniels 

### 3.1 Abstract

Myxomatous mitral valve disease (MMVD) is the most frequent cause of cardiac morbidity and mortality in the dog. Though little is known about the underlying aetiology of the disease, breed-specific characteristics and prevalence suggest that there is a significant genetic basis. Breeds with a high prevalence for the disease, like the Cavalier King Charles Spaniel (CKCS), offer a tremendous opportunity to uncover genetic components contributing to the disease aetiology. Multiple studies from various breeds have been conducted that highlight potential loci associated with MMVD traits, but to date researchers have neither described or validated variants with a putative functional role. Outside of attempts to map genetic components of disease, multiple studies have been conducted that assess the expression of genes and proteins in healthy dogs and those with advancing MMVD. This field of research has highlighted genes and signalling pathways expected to contribute to disease pathogenesis. Gene families and signalling pathways observed consistently across expression studies can help inform hypothesis-based research. Using evidence of several pathways implicated in MMVD disease progression, this research developed a list of candidate genes to test for association with MMVD phenotypes. In this chapter genes were selected from the transforming growth factor Beta (TGF- $\beta$ ), serotonergic signalling, extracellular matrix and calcium signalling pathways. Using genotype data from 178 CKCS, genomic markers within 500 kilobases of candidate genes were extracted and tested for association with the development of congestive heart failure and echocardiographic measures of left-sided cardiac remodelling, LA/Ao and LVIDdn. Haplotype analysis was conducted in the vicinity of single nucleotide variants that passed the genome-wide significant threshold. Using five whole genome sequenced CKCS, variants were assessed for putative functional effects. A genome-wide significant signal was observed within the vicinity of candidate gene GNG7. Further analysis failed to indicate genetic causation in GNG7 or any of the other genes investigated in the dogs studied.

### 3.2 Introduction

Myxomatous mitral valve disease (MMVD; OMIA 000654-9615) is the most frequently reported cardiac disease in dogs (Canis lupus familiaris) and the most common pathophysiological cause of congestive heart failure (CHF) ${ }^{1-3}$. The condition begins with the myxomatous degeneration of valvular leaflets and increasing deformation of the valves allows backflow of blood (mitral valve regurgitation) ${ }^{4,5}$. As the condition progresses, signs of secondary fibrosis, eccentric hypertrophy and left sided volume overload can occur ${ }^{6,7}$. In severe cases, disease results in mortality due to left-sided $\mathrm{CHF}^{6,7}$. MMVD has a pedigree related
disease susceptibility and increasing prevalence with age ${ }^{8-10}$. No breed is more outstandingly affected than the Cavalier King Charles Spaniel (CKCS) ${ }^{11-16}$.

MMVD is a heterogeneous condition. Given the prevalence of MMVD in the canine population, there is a significant lack of information regarding the genetic basis of disease pathogenesis and progression ${ }^{17,18}$. Although the genetic aetiology of MMVD is unknown, there is a consensus in literature to support a process whereby valvular cells undergo phenotypic transformation, resulting in remodelling of the extracellular matrix (ECM) ${ }^{5,19-23}$. This finding is generally supported by differential expression of ECM genes and proteins in disease valves compared to unaffected valves (Chapter 1. Table 1). Transforming growth factor Beta (TGF- $\beta$ ) and serotonergic signalling pathways represent convincingly hypothesised effectors of ECM remodelling ${ }^{4,17,24-28}$. Genes in these signalling pathways represent candidates for the pathogenesis of MMVD and advancing disease.

Myocardial dysfunction is not a direct consequence of MMVD but is observable in dogs with advancing disease, especially in late stages ${ }^{29-32}$. Left ventricular systolic dimensions have been associated with long-term mortality rates in the CKCS and it has been hypothesised that the breed suffers from systolic dysfunction at an early age ${ }^{33}$. Recently, calcium signalling was identified as a top canonical pathway in the $\mathrm{CKCS}^{34}$. Genes within this pathway were significantly down-regulated when compared to both healthy and affected mitral valves from other dog breeds ${ }^{34}$. It is possible that genes in the calcium signalling pathway exacerbate MMVD, promote an advancement towards CHF or stimulate early onset in the CKCS.

The current study was conducted with the purpose of discovering genetic loci that increase the risk of CHF in CKCS with MMVD using a candidate gene approach. We focused on candidate genes from pathways implicated in the development and progression of MMVD, namely the TGF-beta signalling, seratogenic signalling, ECM-receptor interaction and calcium signalling pathways.

### 3.3 Methods and materials

### 3.3.1 Ethics, clinical diagnosis and sample collection

As previously described (Chapter 2.2.1), all recommendations from the Australian Code for the Care and Use of Animals for Scientific Purposes were adhered to. This study included 178 Australian owned CKCS. Informed consent was obtained from the owners of the CKCS for collection of blood samples and the use of acquired data throughout this study. A diagnosis on the presence of MMVD was made by a small animal cardiologist as presented in the methodology of Chapter 2 (Chapter 2.2.2).

### 3.3.2 Genotyping and next generation sequencing of samples

Genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Invitrogen, Hilden, Germany), or submitted to the genotyping service provider as whole blood on Whatman Flinders Technology Associates (FTA) cards provided by the genotyping service provider. Genotyping array data were obtained from the Illumina CanineHD BeadChip (Illumina, San Diego, CA) by Neogen (Lincoln, NE USA).

Approximately $5 \mu \mathrm{~g}$ of DNA from three CKCS included in the analysis was submitted for library preparation and whole genome sequencing at the Australian Genome Research Facility (AGRF; University of Queensland, Brisbane, Australia). Illumina paired-end libraries were prepared and sequenced with 150 paired-end reads (55-56x coverage). A further two CKCS, available through Sequence Read Archive (SRA) were also obtained for use in this research (SRX4035783 and SRX4035784; 28x and 30x coverage respectively).

### 3.3.3 Population structure

Quality control was carried out on all samples using PLINK 1.935. SNPs were excluded with a minor allele frequency (MAF, --maf) of less than 0.1 and a genotyping call rate (--geno) less than $90 \%$. We identified all possible duplicate samples based on pairwise genetic distances (-genome) and excluded one sample from each pair with an identity by descent (IBD) estimate greater than 0.65 . Population stratification was evaluated using a multidimensional scaling (MDS) plot with two dimensions (--mds).

### 3.3.4 Selection of candidate genes

Kyoto Encyclopedia of Genes and Genomes (KEGG), is a public resource and collection of databases representing biological systems ${ }^{36}$. Utilising the KEGG pathways database, a list of candidate genes from signalling pathways that have been implicated in MMVD pathogenesis and progression was developed. Given the extensive linkage disequilibrium observed in dogs, genomic markers 500 kilobases (kb) upstream and downstream of candidate genes on the genotyping array were extracted and used in a candidate gene association analysis ${ }^{37}$.

### 3.3.5 Candidate gene association and haplotype analysis

Testing for association between severe MMVD and markers on the canine genotyping array was performed using Efficient Mixed-Model Association eXpedited (EMMAX) ${ }^{38}$ software. The EMMAX model implements a standard linear mixed model approach with a single phenotype, correcting for stratification using a kinship matrix. The top principal component, based on variance-standardised relationship matrix in Plink (--pca), was included as a covariate to control
for any remaining cryptic relatedness. Association analyses to identify loci and genes associated with severe MMVD was performed on three MMVD phenotypes; LA/Ao, LVIDdn and CHF. For echocardiographic measures, LA/Ao and LVIDdn, a quantitative trait analysis was conducted on each measure. Given the strong association between age and left atrial enlargement (Chapter 2.4.3), age was included as a covariate. A case-control model was used to test for association of CHF and markers on the genotyping array, using left atrial enlargement as a covariate. LA/Ao and LVIDdn are highly correlated ( $r>0.8, \mathrm{P}<0.001$; Chapter 2.4.1). As such LA/Ao, the strongest predictor of CHF (Chapter 2.4.2), was included as a covariate. Dogs were included as cases in the case-control association if they had been diagnosed with CHF by small animal cardiologist or if their left ventricular echocardiographic measures were indicative of heart failure according to logistic analysis; LA/Ao> 2.36 Or LVIDdn >2.4 (Chapter 2.4.2), hereon referred to as CHF samples. To control for the testing of multiple hypotheses, significant and suggestive thresholds were Bonferroni-corrected, $3.22 \times 10^{-}$ ${ }^{6}$ (Bonferroni cut-off of $\alpha=0.05, \mathrm{n}=15,545$ ) and $4.25 \times 10^{-5}$ (Bonferroni cut-off of $\alpha=1.0$, $\mathrm{n}=15,545$ ), respectively. For loci passing a significant threshold haplotype analysis was conducted using HAPLOVIEW (v4.2) ${ }^{39}$. CHF-associated haplotype blocks were examined in all CKCS individuals and were defined using the four-gamete rule ${ }^{40}$.

### 3.3.6 Risk variant discovery and annotation

WGS CKCS were aligned to the CanFam3.1 reference genome using Burrows-Wheeler Alignment (BWA) mem version 0.7.15 ${ }^{41}$, with default parameters for paired-end sequencing. Indel realignments and base quality score recalibration was performed using Genome Analysis Toolkit (GATK) version 3.8.1 ${ }^{42}$. Variant calls were made according to best practices using GATK's HaplotypeCaller. A variant call format (VCF) file was generated using GATK's variant quality score recalibration (VQSR) tool, utilising sites from Ensembl's variant database and the Illumina CanineHD BeadChip as training resources. To ensure high quality variants were included for downstream analysis, the VCF was passed to Variant Filtration tool and filtered by quality (QUAL>40.0), Quality by depth (QD>2.0), Root mean square of the mapping quality (MQ>40.0) and Fishers exact strand bias (FS<50). Phenotypic presentation of MMVD is complex. As such, variants within associated haplotype blocks were filtered based on the presence or absence of risk haplotypes irrespective of clinical presentation. Remaining variants were analysed with Ensembl's Variant Effect predictor (VEP) tool ${ }^{43}$ and uploaded as custom tracks to University of California Santa Cruz (UCSC) genome browser where they were annotated with the Variant annotation integrator (VAI) ${ }^{44}$.

### 3.4 Results

### 3.4.1 Candidate gene association

178 CKCS that were genotyped on the Illumina canineHD beachchip genotyping array passed quality control. The study included 78 males and 96 females with an average age of 10.24 years. Characteristics of the cohorts are summarised according to ACVIM grading system (Table 3.1). All samples were included for each association analysis. The CHF cohort comprised of 51 cases and 127 controls. Association of genotype markers with echocardiographic traits LA/Ao and LVIDdn was conducted as a continuous variable association analysis. Multidimensional scaling indicated minimal population stratification with all samples clustering closely and no notable outliers (Figure 3.1a). Cases and controls were evenly distributed across the cohort. $P$-values of the quantile-quantile (QQ) plot for each association test showed no obvious deviation except in the right tail of the distribution (mean $\lambda=1.04$; Fig 3.1b).

Table 3.1 Characteristics for the dogs included in the candidate gene association study. These are reported as a proportion (\%) for categorical variables and mean (standard deviation; SD), median (lower quartile; upper quartile; UQ ) for continuous variables. n represents the number of dogs included in each group.

| $\mathrm{ACVIM}^{\text {a }}$ |  | $\begin{gathered} \hline A \\ (N=3) \end{gathered}$ | $\begin{gathered} \mathrm{B} 1 \\ (\mathrm{~N}=68) \end{gathered}$ | $\begin{gathered} \mathrm{B} 2 \\ (\mathrm{~N}=65) \end{gathered}$ | $\begin{gathered} C \\ (N=38) \end{gathered}$ | $\begin{gathered} \hline D \\ (N=4) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Sex | M (\%) | 1 (33) | 26 (38) | 29 (45) | 21 (55) | 3 (75) |
|  | F (\%) | 2 (67) | 42 (62) | 36 (55) | 17 (45) | 1 (25) |
| Age | mean (sd) | 8.58 (+- 0.67) | $\begin{aligned} & 10.39 \text { (+- } \\ & 2.18) \end{aligned}$ | $\begin{gathered} 10.56 \text { (+- } \\ 1.80) \end{gathered}$ | 9.69 (+- 1.82) | 9.06 (+- 1.03) |
|  | median (IQR) | $\begin{gathered} 8.58 \\ (8.25,8.91) \end{gathered}$ | $\begin{gathered} 10.04 \\ (8.54,11.77) \end{gathered}$ | $\begin{gathered} 10.33 \\ (9.17,11.58) \end{gathered}$ | $\begin{gathered} 9.5 \\ (8.60,10.77) \end{gathered}$ | $\begin{gathered} 8.96 \\ (8.67,9.36) \end{gathered}$ |
| Body weight (kg) | mean (sd) | 9.70 (+- 2.07) | 8.68 (+- 1.89) | 8.88 (+- 1.69) | 9.42 (+-2.15) | 9.25 (+-1.62) |
|  | median (IQR) | $\begin{gathered} 10 \\ (8.75,10.80) \end{gathered}$ | $\begin{gathered} 8.5 \\ (7.77,9.22) \end{gathered}$ | $\begin{gathered} 8.5 \\ (7.80,9.30) \end{gathered}$ | $\begin{gathered} 8.94 \\ (8.00,10.47) \end{gathered}$ | $\begin{gathered} 9.6 \\ (8.73,10.12) \end{gathered}$ |
| $L A / A o^{\text {b }}$ | mean (sd) | 1.26 (+-0.12) | 1.31 (+-0.15) | 1.76 (+- 0.32) | 2.52 (+-0.56) | 3.29 (+-0.32) |
|  | median (IQR) | $\begin{gathered} 1.3 \\ (1.22,1.33) \end{gathered}$ | $\begin{gathered} 1.31 \\ (1.21,1.41) \end{gathered}$ | $\begin{gathered} 1.74 \\ (1.53,1.94) \end{gathered}$ | $\begin{gathered} 2.41 \\ (2.14,2.93) \end{gathered}$ | $\begin{gathered} 3.3 \\ (3.03,3.56) \end{gathered}$ |
| LVIDdn ${ }^{\text {c }}$ | mean (sd) | 1.43 (+- 0.11) | 1.57 (+- 0.20) | 2.03 (+-0.24) | 2.40 (+-0.43) | 2.75 (+- 0.24) |
|  | median (IQR) | $\begin{gathered} 1.38 \\ (1.37,1.47) \end{gathered}$ | $\begin{gathered} 1.6 \\ (1.47,1.72) \end{gathered}$ | $\begin{gathered} 2.01 \\ (1.85,2.23) \end{gathered}$ | $\begin{gathered} 2.36 \\ (2.15,2.59) \end{gathered}$ | $\begin{gathered} 2.83 \\ (2.67,2.92) \end{gathered}$ |

[^1]

Figure 3.1 (a) MDS-plot of 178 CKCS included in the candidate gene association analysis. Cases represent samples diagnosed with CHF by small animal cardiologist or if key prognostic variable LA/Ao>2.36 or LVIDdn>2.4 (b) Quantile-quantile plot showing limited inflation of the test statistics

A candidate gene association was conducted using multiple phenotypes including LA/Ao, CHF and LVIDdn. Genes ( $\mathrm{n}=485$ ) from four KEGG pathways were included in the analysis; TGF-beta signalling (KEGG ID: cfa04350), serotonergic signalling (KEGG ID: cfa04020), calcium signalling (KEGG ID: cfa04020) and ECM-receptor interaction pathways (KEGG ID: cfa04512). After frequency and genotype pruning, 17,718 SNVs within 500 kb of candidate genes remained for analysis. Of the analyses conducted, a single marker at CFA20:56,661,518 (chr20_56661518; $P_{\text {raw }}=2.68 \times 10^{-06}$ ) passed the Bonferroni corrected significance threshold and was significantly associated with LA/Ao (Table 3.2). At the given loci, another marker in strong LD with the top associated SNV had a highly suggestive association (CFA20:56,483,566; BICF2P866985; Praw $=$ $4.24 \times 10^{-06} ; r^{2}=0.78$ ) and was located within the intonic region of serotonergic signalling gene, G Protein Subunit Gamma 7 (GNG7). An additional single nucleotide variant (SNV) had a low suggestive threshold in the CHF association and was observed $\sim 1.5$ megabases (Mb) downstream from the top associated locus at CFA20:58023254 (BICF2P360101; Praw $=$ $3.62 \times 10^{-05}$ ). No variants passed a suggestive threshold for LVIDdn.

Table 3.2 A single genomic marker included in the candidate gene association analysis passed the significant threshold. Significant and suggestive thresholds were determined through Bonferroni-correction. Genomic markers passing a suggestive threshold are reported. A1 and A2 represent the major and minor allele respectively. Minor allele frequency (MAF) and number of samples with uncalled variants (missing) are reported. A single variant passing the Bonferroni corrected significance level is highlighted in bold.

| Phenotype | SNPID | CHR | BP | A1 | A2 | MAF | MISSING | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LA/Ao $^{\text {a }}$ | chr20_56661518 | 20 | 56661518 | C | T | 0.1048 | 11 | $2.68 \times 10^{-06}$ |
| LA/Ao | BICF2P866985 | 20 | 56483566 | G | A | 0.1264 | 0 | $4.24 \times 10^{-06}$ |
| CHF $^{\text {b }}$ | BICF2P360101 | 20 | 58023254 | C | T | 0.494 | 10 | $3.62 \times 10^{-05}$ |

${ }^{a}$ Left atrium to aortic root ratio
${ }^{\mathrm{b}}$ Congestive heart failure

### 3.4.2 Haplotype analysis

Haplotype analysis was conducted at the top loci on CFA2O for the identification CHFassociated haplotypes. Two haplotype blocks are reported (Table 3.3), each which contained a SNVs passing the suggestive threshold. The strongest associated haplotype spanned the interval CFA20:56464693-56628267 ( $\mathrm{P}_{\mathrm{raw}}=8.93 \times 10^{-05}$ ) and overlaps the candidate gene GNG7. The risk haplotype was present in $23.5 \%$ of CHF cases and $8.3 \%$ of controls. Across the haplotype region 456 variants were annotated using VEP and VAI. None of the variants annotated in the candidate gene, GNG7, are predicted to have a functional effect.

Table 3.3 Haplotype analysis of the top associated signal identifies a risk haplotype overlapping GNG7. Results of haplotype association test implemented in haploview.

| Haplotype block | Size <br> $(\mathrm{kb})$ | Observed <br> blocks | Associated <br> haplotype | Freq | Case:control <br> Frequency | Haplotype <br> P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $56464693-$ | 164 | 4 | CTAGCC | 0.126 | $0.235,0.083$ | $8.93 \times 10^{-05}$ |
| 56628267 |  |  |  | 0.109 | $0.192,0.076$ | 0.0016 |
| $56644215-$ | 17 | 3 | GT |  |  |  |
| 56661518 |  |  |  |  |  |  |

### 3.5 Discussion

Association analysis of candidate genes from four signalling pathways implicated in the pathogenesis of MMVD was conducted. Exploitation of echocardiographic measure LA/Ao as a quantitative phenotype identified an associated locus on CFA20. At the associated locus, a risk haplotype, overlapping serotonergic signalling gene GNG7, segregated in $23.5 \%$ of CHF samples. Across sources, GNG7 is reported as a long non-coding RNA as well as a protein coding gene (NCBI geneid: 612139). Different sources of gene annotations have high overlap, however characteristics of gene annotations can differ due to variation in annotation strategies and data sources ${ }^{45}$. The Improved Canine Annotation available at UCSC, constructed by the

Broad Institute and Uppsala University, contains transcripts that are considered protein coding in the $\operatorname{dog}^{46}$. This source reports high expression of the GNG7 transcript in heart tissue, proposing a potential role in cardiac function. However, no putative functional variants were identified in the study cohort.

Array markers surrounding genes from the serotonergic signalling pathway were included in this study due to the increasing evidence that serotonin signalling has a prominent role in the initiation of MMVD ${ }^{47-50}$. Serotonin signalling is of key interest in MMVD due to its association with valvopathies and because of its interaction with TGF- $\beta$ signalling, another prominent signalling pathway in disease pathogensis ${ }^{26-28}$. Serotonergic signalling gene, GNG7, is a subunit of a heterotrimeric $G$ protein complex, which induces G-protein-coupled receptor (GPCR) activation. GPCRs are major signalling mediators that have prominent roles in most physiological processes, including dynamic roles in healthy and diseased hearts ${ }^{51,52}$. Presently, no research exists that specifies a role of GNG7 in serotonin signalling ${ }^{53}$ and the gene has not been directly implicated with MMVD or similar phenotypes. However, thousands of possible heterotrimeric combinations exist that contribute to efficient transmembrane signalling ${ }^{53}$. It is possible that GNG7 plays a role in physiological processes affecting disease pathogenesis that are currently not known. To date, no variation in the GNG7 gene or protein has been observed in canine MMVD studies, which may highlight the marker as a false-positive association. No putative functional variants for GNG7 were found.

Selection of candidate genes for association studies is commonly based on knowledge of genes with a known or predicted effect on the studied trait ${ }^{54}$. The approach is useful for quickly determining an association of genes or genetic variants with a phenotype of interest. However, the proportion of known causative genes governing complex traits is often small ${ }^{55}$. A previous candidate gene approach was conducted in CKCS and Dachshund breeds using whole genome sequenced variant data ${ }^{56}$. The study hypothesised that genes associated with human orthologous phenotypes would be common to canine MMVD ${ }^{56}$. The paper considered 17 candidate genes and found no convincing evidence of a genetic component for MMVD pathogenesis. The candidate gene approach conducted in this chapter employed a novel method that facilitated the identification of a larger catalogue of candidate genes. To assist in gene selection, the KEGG pathways database was used to identify genes from signalling pathways implicated in canine MMVD. This meant genes included in this approach did not rely on knowledge of genes previously associated with similar phenotypes. While this had the benefit of assessing a larger number of genes, signals did not segregate with strong candidates for the disease. A concern with this approach is that annotation of online databases, like KEGG, are ongoing processes and do not necessarily provide the full scope of genes in signalling pathways and are often biased towards well known diseases and genes ${ }^{57}$.

Despite the CKCS representing a high-risk breed for MMVD, a candidate gene approach is most successful at identifying large effect mutations in monogenic traits ${ }^{55}$. This research modestly supported the hypothesis that a genomic basis for severe forms of MMVD exists within major MMVD signalling pathways. Using LA/Ao as a quantitative phenotype, we identified a riskhaplotype at CFA2O associated with left atrial enlargement but were unsuccessful in detecting risk variants in the candidate gene GNG7. Limited evidence was identified in the current cohort that supported a functional effect of GNG7 in the progression of MMVD to CHF. An association of the risk-haplotype with CHF in an external cohort of phenotype individuals is necessary to validate the observed locus. Given the cumulative effects of multiple loci and the phenotypic heterogeneity observed in complex diseases, like MMVD, a genome wide association study is justified.

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# Chapter 4 Mapping the genetic basis of severe myxomatous mitral valve disease and congestive heart failure in Cavalier King Charles Spaniels 

### 4.1 Abstract

Myxomatous mitral valve disease (MMVD) is the most frequent cause of heart disease in dogs. It is a slow, progressive disorder initiated by myxomatous degeneration of the mitral valve leaflets frequently leading to mitral regurgitation and left sided cardiac remodelling. The disease is recognised as the most prominent cause of cardiac related deaths in dogs that generally occurs because of congestive heart failure (CHF). Although a heightened within breed prevalence of MMVD indicates a strong heritable component, a genetic basis for the disease has never been identified. Cavalier King Charles Spaniels (CKCS) are the most overrepresented breed affected by MMVD. In affected dogs, it is not uncommon to experience a healthy lifespan in the asymptomatic period of disease. However, for a small proportion of dogs, acute disease results in CHF. In this research, a genome-wide association study was conducted in attempts to identify risk-loci associated with severe forms of MMVD and the development of CHF. An Australian population of CKCS were phenotyped for MMVD using echocardiography. Parallel multi-trait GWAS were conducted on CKCS genotyped with the Illumina CanineHD array. Our results indicated five MMVD-associated loci at chromosomes 1, 13, 14, 20, and 24. Positional candidate genes were identified within the associated loci including OBSCN, LMNB2, SULF2 and ADAMTS3. For each locus, risk haplotypes were reported as well as concordant variants from five whole genome sequence samples. Candidate putative functional variants were identified in OBSCN and LMNB2. Both genes have been linked to cardiac phenotypes. The results of this research highlight the potential for identification of complex disease variants using a multi-trait analysis. Variation in candidate genes support the notion that CKCS experience disrupted calcium signalling and cardiac muscle contraction.

### 4.2 Introduction

Myxomatous mitral valve disease (MMVD; OMIA 000654-9615) is a cardiac disorder with variable progression and the most frequent cause of cardiovascular disease in dogs (Canis lupus familiaris ${ }^{1,2}$. It occurs as the result of Intravalvular degenerative processes, causing loss of valvular mechanical ability and subsequent regurgitation of blood, referred to as mitral regurgitation (MR)³. Following primary mitral valve insufficiency, adaptive compensatory changes take place. As the severity of MMVD advances, clinical signs include progressive MR, eccentric cardiac hypertrophy, left atrial enlargement and systolic dysfunction ${ }^{4-6}$. In the worst instances, extensive remodelling of the heart can lead to congestive heart failure (CHF) and cardiac mortality ${ }^{7,8}$.

MMVD worsens with age and is observed in geriatric dogs regardless of breed ${ }^{3,9}$. While MMVD is observed in all breeds, it has an exceptionally high prevalence rate in small to medium-sized dogs ${ }^{10-12}$. This breed association is evidence for a genetic basis of the disease. The Cavalier King Charles Spaniels (CKCS) has a higher prevalence of MMVD and earlier onset compared with other breeds ${ }^{13-18}$. Both the presence and severity of MMVD in the CKCS is highly heritable ${ }^{16}$. Possibly due to the relatively fixed nature of MMVD in the breed, heart disease is a prominent cause of mortality in the CKCS, even in young dogs ${ }^{2,19}$. As such, the CKCS breed has been the subject of numerous attempts to identify the genetic basis of MMVD with limited success ${ }^{20-22}$.

As a result of domestication and breed line formations in the dog, linkage disequilibrium (LD) within pedigreed populations is extensive ${ }^{23-25}$. Within-breeds genetic heterogeneity is significantly reduced making them effectively genetically isolated populations that require fewer markers and samples to efficiently map traits than humans ${ }^{26}$. As such, dogs are an effective model species for genome-wide association studies (GWAS), particularly when the phenotype of interest is variable within a breed ${ }^{26}$. Although the mode of inheritance for MMVD is yet to be determined, it is regarded as a multifactorial, polygenic threshold trait ${ }^{27,28}$. The high prevalence of MMVD in the CKCS and evidence for an age-related penetrance suggest that the disease may be fixed within this breed. Complex, multigenic diseases that demonstrate a high level of fixation within breeds, like MMVD in the CKCS ${ }^{21,22}$, can complicate disease modelling. Failure to capture, replicate or validate genomic signals for MMVD through GWAS is likely due to the heterogeneity of the disease phenotype, sampling effects, and variation in the models used ${ }^{20-22}$. Identifying causative genes for MMVD in the CKCS through GWAS is unlikely. Still, with accurate phenotyping the breed offers the opportunity to uncover modifier loci responsible for variable disease expression.

Typically, MMVD has a slow rate of progression and affected individuals can be expected to live a relatively asymptomatic and natural lifespan ${ }^{7,29,30}$. But for dogs that do progress beyond the preclinical stages of MMVD and develop signs of CHF, the survival period is short regardless of medical intervention $7,8,31,32$. While MMVD is clinically well described, the underlying processes driving the rapid progression of mild symptoms to CHF have not been revealed. Genetic characterisation of factors influencing the progression of MMVD is important in understanding disease pathogenesis as well as improving canine welfare and longevity, especially in breeds like the CKCS where heart disease is a major contributing factor of mortality ${ }^{2,19}$. To date, no genetic tests have been developed to detect increased risk of early mortality or disease severity in at-risk dogs. Identification of loci that affect the progression and severity of MMVD in CKCS may help increase the effectiveness of breeding protocols and assist in improving the welfare of this breed. In this chapter I conducted a GWAS on a population of Australian CKCS, with a primary focus on incrementing disease severity and the development of CHF. MMVD disease severity was modelled using linear and logistic regression analyses (presented in Chapter 2). The aim of this paper is to identify genetic loci and variants associated with severe MMVD using echocardiographic data to inform phenotype.

### 4.3 Methods and materials

### 4.3.1 Data collection

The methodology described for this research was conducted on a population of Australian CKCS previously described and conforms with ethical practices (Chapter 2.2.1). Diagnosis (Chapter 2.2.2), genotyping, whole genome sequencing (WGS)(Chapter 3.2.2), WGS alignment and variant calling (Chapter 3.2.6) are consistent between analyses. Any variation in processes is detailed.

### 4.3.2 Genome wide association analysis, haplotype discovery and variant annotation

GWAS were conducted on samples genotyped using the CanineHD BeadChip (Illumina, San Diego, CA) SNP array. A phenotype-genotype association analysis was conducted using the Efficient Mixed-Model Association eXpedited (EMMAX) ${ }^{33}$ software, on three phenotypes previously described (Chapter 3.2.5). Briefly, samples were collected across Australia and classified based on disease severity, according to the American College of Veterinary Internal Medicine (ACVIM) classification scheme ${ }^{6}$. At the time of sample collection, diagnoses were made based on the level of cardiac remodelling using two measures recommended for clinical diagnosis, left atrium to aortic root ratio (LA/Ao) and left ventricular end diastolic dimension, normalised for body weight (LVIDdn) ${ }^{6}$. A case-control GWAS was conducted on CKCS where cases represented dogs with evidence of CHF, or with left atrial enlargement surpassing a predictive threshold for the development of congestive heart failure (LA/Ao>2. And LVIDdn>2.39), hereby referred to as CHF dogs. Two further GWAS were conducted as a continuous variable analysis utilising individual quantitative measures of cardiac remodelling, LA/Ao and LVIDdn.

Quality control of genomic markers was performed using PLINK 1.9 ${ }^{34}$. Single nucleotide variants (SNVs) were pruned at a minor allele frequency (MAF) of 0.1 (--maf) and with an individual call rate above $90 \%$ (--geno). The genome-wide significance threshold was determined using the empirical $95 \%$ confidence interval (CI), determined by running the GWAS 1,000 times with randomly permuted phenotypes generated in PLINK (--make-perm-pheno). The genome-wide significance threshold was set as associations exceeding the $97.5 \%$ upper empirical Cl and varies by model ( $\mathrm{P}<5.96 \times 10^{-5}$ for CHF, $\mathrm{P}<5.29 \times 10^{-5}$ for $L A / A o$ and $\mathrm{P}<5.84 \times$ $10^{-5}$ for LVIDdn). The extent of the associated regions was refined in two-steps with LD clumping in PLINK (--clump). Initially, a region of weak LD (r2>0.2) was defined up to 5 Mb of each top SNV and narrowed down to regions of high LD ( $22>0.8$ ) within 2 Mb of the highest associated SNV at each locus.

SNVs within the associated region were submitted for haplotype analysis in HAPLOVIEW (v4.2) ${ }^{35}$. Haplotype blocks were examined in all samples and were defined using the four-
gamete rule ${ }^{36}$. A set of high-quality variants from five WGS CKCS, previously described (Chapter 3.3.6), was used for variant discovery. Haplotypes harbouring SNVs that passed the genomewide significance threshold are reported. WGS variants were filtered for variants within the defined haplotypes. Given the complex phenotype of MMVD, variants within the haplotype blocks were filtered based on the presence or absence of risk haplotypes. SNV with the highest $p$ value within haplotypes were selected as tag SNVs. In the absence of WGS samples homozygous for the risk haplotype, putative functional variants are reported that segregate with the tag SNV. The remaining variants were analysed with Ensembl's Variant Effect predictor (VEP) tool ${ }^{13}$ and uploaded as custom tracks to University of California Santa Cruz (UCSC) genome browser where they were annotated with the Variant annotation integrator (VAI) ${ }^{38}$ tool. Variants detected with putative functional effects were evaluated. The potential impact of amino acid substitutions on protein function were predicted with the Sort Intolerant From Tolerant (SIFT) algorithm ${ }^{39}$. The Broad Institute and Uppsala University comprehensive catalogue of genes and transcripts was used to determine protein coding transcripts and tissue expression ${ }^{40}$.

### 4.4 Results

A multi-trait GWAS was conducted in an Australian population of CKCS. The study tested for associations between germline variants and the development of severe forms of MMVD. All samples were classified based on disease severity according to the ACVIM grading system using echocardiography (Chapter 3 Table 3.1). Included in the GWAS was 178 CKCS genotyped on the Illumina canine HD array. The final dataset included 90,501 array markers. Some evidence of population stratification can be seen within the study cohort (Chapter 3 Figure 3.1a). False signals caused by cryptic relatedness and population structure were controlled by using a mixed model approach with the top principal component as a covariate ( $\lambda<1.1$ Figure 4.1a-c). Across the three phenotypes studied, there was a total of six loci passing a genome-wide significant threshold (Figure 4.1d-f). A total of 71 variants passed the genome-wide significant threshold for genotype-phenotype association (Table S1). Of the top associated variants, 22 are located within predicted genes, $18(81.81 \%)$ are intronic, two (9.09\%) are missense, one (4.54\%) is synonymous, and one (4.54\%) in an untranslated region (UTR). Missense variants were identified in Obscurin (OBSCN) and Laminin subunit beta-2 (LMNB2) genes. The UTR SNV falls within an olfactory receptor family 2 subfamily T member 4C (OR2T4C).


Figure 4.1 Genome wide association analysis identifies 5 loci associated with myxomatous mitral vale disease (MMVD). The QQplots shows no evidence of stratification relative to the expected distribution except in the right tail of distribution for MMVD traits (a) congestive heart failure (CHF) (b) left atrium to aortic root ratio (LA/Ao) and (c) left ventricular end diastolic dimension, normalised for body weight (LVIDdn). Manhattan plots show the -log10p distribution of array markers associated with (d) CHF (e) LA/Ao and (f) LVIDdn. The dashed grey line indicates the 95\% empirically determined significance threshold for each trait. Markers passing the genome wide significance threshold are highlighted in red.

### 4.4.1 Congestive heart failure GWAS

A total of 178 samples were included in the case-control analysis to test for association between genomic markers and the development of CHF. Of these, 51 were cases and 127 were controls (Table 4.1). In the GWAS cohort, males had slightly higher echocardiographic
measures for both LA/Ao and LVIDdn ( $\mathrm{P}<0.05$; Figure 4.2a\&b). This was reflected in the phenotyping of CHF where males made up the greatest proportion of samples in the CHF cohort and a lower proportion of the controls. In genotyped CKCS there was a significant difference in the ages of the CHF and non-CHF groups (Wilcoxon $\mathrm{p}<0.05$; Figure 4.2c), where cases are younger than control samples. The proportion of younger cases in the CHF model provides a powerful comparison of young cases and older controls, fitting for the early onset of disease in CKCS.

Table 4.1 Characteristics of the dogs included in the congestive heart failure (CHF) genome wide association study. Reported as a proportion (\%) for categorical variables and mean (standard deviation; SD), median (lower quartile; LQ, upper quartile; UQ) for continuous variables. n represents the number of dogs included in each group, where cases are dogs with clinical signs of CHF or passing the predictive threshold determined by logistic regression.

|  |  | Case $(\mathrm{n}=51)$ | Control $(\mathrm{n}=127)$ |
| :---: | :--- | :--- | :--- |
| Sex | Male (\%) | $29(57)$ | $51(40)$ |
|  | Female (\%) | $22(43)$ | $76(60)$ |
|  | mean (sd) | $9.72+-1.80$ | $10.46+-2.00$ |
| Body weight (kg) | median (IQR) | $9.25(8.67,10.54)$ | $10.25(8.96,11.66)$ |
|  | mean (sd) | $9.19+-1.91$ | $8.85+-1.87$ |
|  | median (IQR) | $8.97(7.95,10.35)$ | $8.50(7.80,9.34)$ |
| LA/Ao ${ }^{\text {a }}$ | mean (sd) | $2.54+-0.56$ | $1.47+-0.27$ |
|  | median (IQR) | $2.41(2.14,2.95)$ | $1.43(1.27,1.64)$ |
| LVIDdn $^{\text {b }}$ | mean (sd) | $2.43+-0.39$ | $1.74+-0.28$ |
|  | median (IQR) | $2.41(2.21,2.60)$ | $1.73(1.57,1.88)$ |

[^2]

Figure 4.2 Significant variation is observed between samples included for genome wide association analyses. Boxplots illustrate the distribution of variable traits between Cavalier King Charles Spaniel (CKCS) groups. The assessed variable is plotted along the y axis and the groups compared are plotted on the xaxis. (a) Distribution of echocardiographic measure left atrium to aortic root ratio (LA/Ao), between male (M) and female (F) CKCS. (b) Distribution of echocardiographic measure left ventricular end diastolic dimension, normalised for body weight (LVIDdn) between M and F CKCS. (c) Distribution of age in years between samples included as cases and controls in the CHF association analysis. *Indicates statistical significance ( $\mathrm{p}<0.05$ ) using a Wilcoxon signed-rank test.

The locus most associated with the development of CHF in the CKCS was observed at chromosome 14 (CFA14). This signal observed at CFA14 the strongest across all three analyses with 59 SNVs passing the genome-wide significance threshold. The most significantly associated marker from the analysis was located at CFA14:669,043 (BICF2P757489; Pgenome $\left.=1.17 \times 10^{-08}\right)$. Extensive LD was observed at the locus. Variants in high LD ( $\mathrm{r}^{2}>0.8$ ) with the top marker spanned up to 4.97 megabases (Mb)(CFA14:581,822-5,559,055). A second clump of SNVs starting within the primary associated region but spanning a further 1.29 Mb also passed the genome-wide significance threshold but fell slightly below the LD criteria for defining the associated region ( $r^{2}>0.75$ with the top associated SNV). The associated region, inclusive of the extended markers, was put forward for haplotype analysis (CFA14:581,822$6,844,213)$. Across the studied region 17 haplotype blocks were observed that contained variants passing the genome-wide significance threshold (Table 4.2). Haplotypes blocks cumulatively span 3.2 Mb (51\%) of the associated region. Seven haplotypes overlapped a genomic region dense with olfactory receptor genes and coding sequence variants observed across the region were predominantly found in olfactory receptors genes (Table S2). Twentyfive further coding sequence variants from 11 genes were identified within the remaining haplotype blocks (Table 4.3).

Table 4.2 Risk-haplotypes for loci associated with congestive heart failure in the Cavalier King Charles Spaniel. Haplotype range indicates the base pair positions for the first and last of single nucleotide variants (SNV) included in the haplotype block. P values represent significance value of the tag SNV in the genome wide association analysis.

| Range | Haplotype | Freq. | Case, Control Frequencies | Tag SNV | $P$ Value | Genes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} 14: 581822- \\ 703675 \end{gathered}$ | GCCTCGTACTCAC | 0.278 | 0.471, 0.201 | BICF2P757489 | $1.1 \times 10^{-08}$ | OBSCN, TRIM11, H2AW, H2BU1, H2BU2 |
| $\begin{gathered} \text { 14:721992- } \\ 825927 \end{gathered}$ | GGAGGA | 0.309 | 0.500, 0.232 | BICF2P289847 | $1.43 \times 10^{-07}$ | $\begin{gathered} \text { OBSCN, GUK1, GJC2, } \\ \text { IBA57 } \end{gathered}$ |
| $\begin{gathered} 14: 897657- \\ 945637 \end{gathered}$ | ATC | 0.311 | 0.498, 0.236 | BICF2G630517194 | $1.34 \times 10^{-06}$ | OR genes |
| $\begin{gathered} 14: 1418008 \\ -2028857 \end{gathered}$ | ATTCGGACAGCGTGAGTC | 0.262 | 0.424, 0.197 | chr14_1626211 | $1.95 \times 10^{-06}$ | OR genes, TRIM58 |
| $\begin{gathered} 14: 2126986 \\ -2291305 \end{gathered}$ | CCCTATTAAG | 0.317 | 0.488, 0.248 | BICF2P90189 | $5.82 \times 10^{-06}$ | OR genes |
| $\begin{gathered} 14: 2309870 \\ -2418990 \end{gathered}$ | CCCAGAT | 0.314 | 0.500, 0.238 | BICF2S23516044 | $2.98 \times 10^{-07}$ | OR genes |
| $\begin{gathered} 14: 2467305 \\ -2526516 \end{gathered}$ | TCTG | 0.314 | 0.500, 0.240 | BICF2S22917146 | $3.08 \times 10^{-07}$ | OR genes |
| $\begin{gathered} 14: 2562311 \\ -2819625 \end{gathered}$ | CGGACCGTTGTC | 0.308 | 0.489, 0.236 | chr14_2819625 | $6.57 \times 10^{-07}$ | OR genes |
| $\begin{gathered} 14: 2934833 \\ -2981896 \end{gathered}$ | TCT | 0.312 | 0.500, 0.236 | chr14_2934833 | $2.98 \times 10^{-07}$ |  |
| $\begin{gathered} 14: 2990449 \\ -3264856 \end{gathered}$ | CATGGGCGG | 0.309 | 0.490, 0.236 | BICF2G630517833 | $3.98 \times 10^{-07}$ | AKR1B1, SLC35B4, LRGUK |
| $\begin{gathered} 14: 4569454 \\ -4695786 \end{gathered}$ | GCGTTGTTGGAT | 0.284 | 0.458, 0.214 | BICF2G630519056 | $4.30 \times 10^{-06}$ | CHCHD3, PLXNA4 |
| $\begin{gathered} 14: 4712847 \\ -4810511 \end{gathered}$ | AGTAGG | 0.309 | 0.480, 0.240 | BICF2P1409592 | $4.30 \times 10^{-06}$ | PLXNA4 |
| $\begin{gathered} 14: 4841400 \\ -5017714 \end{gathered}$ | CTCTAGGAGGATTGACAT | 0.312 | 0.480, 0.244 | BICF2P84129 | $3.47 \times 10^{-06}$ | PLXNA4 |
| $\begin{gathered} 14: 5162250 \\ -5248244 \end{gathered}$ | CGCCCA | 0.281 | 0.461, 0.209 | BICF2P1095320 | $3.30 \times 10^{-07}$ |  |
| $\begin{gathered} 14: 5264341 \\ -5593889 \end{gathered}$ | CCGATCACGTCGAG | 0.281 | 0.461, 0.209 | BICF2S2364353 | $2.82 \times 10^{-07}$ | PODXL |
| $\begin{gathered} 14: 5859462 \\ -5932681 \end{gathered}$ | TGGATGCAG | 0.281 | 0.461, 0.209 | BICF2P850224 | $3.30 \times 10^{-05}$ |  |
|  |  |  |  |  |  | $\begin{gathered} \text { COPG2, } \\ \text { MEST, CEP41, } \\ \text { ENSCAFG00000008 } \end{gathered}$ |
| $\begin{gathered} 14: 6379001 \\ -6915563 \end{gathered}$ | GACCGGTTCGGGGGGGAA GGTGCC | 0.27 | 0.441, 0.201 | BICF2G630519491 | $8.78 \times 10^{-07}$ | 551, <br> ENSCAFG00000009 988, CPA1, CPA5, CP A4, SSMEM1, TMEM 209, KLHDC10, ZC3 HC1, UBE2H |
| $\begin{aligned} & \text { 20:5801474 } \\ & \text { 2-58103316 } \end{aligned}$ | ACTCGTATAGCAGG | 0.491 | 0.559, 0.453 | BICF2P360101 | $1.31 \times 10^{-05}$ | $\begin{aligned} & \text { ENSCAFG00000043 } \\ & 280, \text { C2CD4C, } \\ & \text { THEG, MIER2, PLPP2 } \end{aligned}$ |

Table 4.3 Coding variants matching CHF-risk haplotypes on chromosome 14. Variant annotation was conducted using Variant Effect Predictor (VEP) and Variant Annotation Integrator (VAI). Programs predict functional consequences based on transcript annotations from multiple sources. Transcript annotations used to predict functional consequences include Ensembl (E), National Center for Biotechnology information (N), and University of California Santa Cruz (U) and are reported under transcript source.

| Variant ID | CHR | POS | REF | ALT | Gene | Putative Function | Amino Acid | SIFT | Transcript Source |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| rs851214998 | 14 | 599485 | C | T | H2BU2 | synonymous | D |  | E,N |
|  | 14 | 604062 | C | T | H2BU1 | synonymous | V |  | E,N |
| rs851936980 | 14 | 604149 | C | T | H2BU1 | synonymous | K |  | E,N |
|  | 14 | 634098 | C | T | TRIM17 | 5'UTR |  |  | E,N |
| rs851022577 | 14 | 635213 | G | A | TRIM17 | missense | G/D | 0 | E,N |
| rs851984265 | 14 | 646656 | T | C | TRIM17 | synonymous | D |  | E,N |
| rs852522978 | 14 | 646962 | G | T | TRIM17 | 3'UTR |  |  | E,N |
| rs851521752 | 14 | 664481 | G | - | OBSCN | 3'UTR |  |  | N |
| rs852107580 | 14 | 669043 | C | G | OBSCN | missense | G/R | 0.11 | N |
| rs851492077 | 14 | 669878 | C | A | OBSCN | synonymous | P |  | N |
| rs850708053 | 14 | 696128 | G | T | OBSCN | missense | T/K | 0.15 | N |
| rs850956456 | 14 | 715836 | G | A | OBSCN | splice region |  |  | N |
|  | 14 | 721020 | C | T | OBSCN | missense | G/S | 0.31 | N |
| rs22343182 | 14 | 800005 | C | A | IBA57 | 3'UTR |  |  | N |
|  | 14 | 826849 | C | G | GUK1 | 5'UTR |  |  | E,N |
|  | 14 | 826849 | C | G | MRPL55 | splice region |  |  | E,N |
| rs852129395 | 14 | 2991898 | C | G | AKR1B1 | 5'UTR |  |  | E,N,U |
| rs851065876 | 14 | 2991980 | G | A | AKR1B1 | missense | V/I | 1 | E,N,U |
|  | 14 | 2992420 | A | G | AKR1B1 | 5'UTR |  |  | N |
|  | 14 | 2992445 | G | T | AKR1B1 | splice region |  |  | N |
|  | 14 | 4683923 | C | G | PLXNA4 | 5'UTR |  |  | N |
| rs851422603 | 14 | 5004071 | G | C | PLXNA4 | synonymous | S |  | E,N |
| rs852901111 | 14 | 5004074 | C | T | PLXNA4 | synonymous | G |  | E,N |
| rs851466365 | 14 | 5614640 | G | A | PODXL | synonymous | T |  | E,N, U |
|  | 14 | 6562819 | G | A | CPA1 | missense | P/L | 1 | E,N |

[^3]The most significantly associated array marker from the CHF analysis, BICF2P757489 (CFA14g.669043C>G; $\mathrm{P}_{\text {genome }}=1.17 \times 10^{-08}$ ), is a putative functional variant for the development of CHF. The genomic marker captures a missense variant in the coding region of the gene OBSCN. The Broad Institute and Uppsala University comprehensive catalogue of genes and transcripts reports the highest observed expression of OBSCN in muscle and heart tissue of the dog ${ }^{40}$. The CKCS population frequency of the SNV was 0.29 . CKCS genotypes, as determined by genomic markers, are designated as wild-type and mutant, where the wild-type allele is considered the reference allele and the mutant is the alternate. Within the studied cohort, 12 dogs ( $14.33 \%$ ) were homozygous for the OBSCN polymorphism, 85 (49\%) were homozygous wild-type, and 75 (43\%) were heterozygous wild-type. The genotype was uncalled in six samples. An additional genomic marker within an intronic region of OBSCN, located ~16 kilobases (kb) downstream from the top variant at CFA14:685,005 (BICF2P813381; $P_{\text {genome }}=9.14 \times 10^{-08}$ ) also passed the genome-wide significance threshold. This second marker is in complete $L D\left(r^{2}=1\right)$ with the putative variant and had no missing genotypes across all samples. The intronic marker was used as a proxy for the missense variant in further analysis. Cardiac remodelling observed in CKCS homozygous for the observed mutation was significantly greater than both the homozygous wild-type and heterozygous wild-type groups (Wilcoxon $\mathrm{P}<0.005$; Figure 4.3a-d). No significant difference was found between the wildtype groups (Wilcoxon $\mathrm{P}>0.05$ ). A further five variants were identified in the coding region of OBSCN in WGS samples that matched the genotype of the top associated marker. One (CFA14g.669878C>A) is predicted to be a synonymous variant, two (CFA14g.696128G>T and CFA14g.721020C>T) are missense variants, and another (CFA14g.715836G>A) is a splice region variant.

A single marker associated with CHF passed the genome-wide significance threshold at CFA20:58023254 (BICF2P912253; Pgenome=1.13×10-5). The haplotype containing the genomic marker spans CFA20:58014742-58103316. The risk haplotype was observed in $55.9 \%$ of cases and $45.3 \%$ of controls. In WGS samples, 29 coding sequence variants segregated with the tag SNV for a total of 31 predicted consequences (Table S3). The majority of consequences were expected to be neutral (41.3\%). The remaining SNVs are six (20\%) missense variants, two (6.89\%) splice region variants and nine (31.03\%) UTR variants in the genes SHC Adaptor Protein 2 (SHC2), C2 Calcium Dependent Domain Containing 4C (C2CD4C), Testicular haploid expressed gene (THEG), Mesoderm induction early response 2 (MIER2), and Phospholipid Phosphatase 2 (PLPP2). Genes observed at the CFA20 signal are not expected to play a major role in pathophysiology of the heart. A final marker passed the genome-wide significance threshold at CFA1:119250918 (BICF2P360101; Pgenome $=3.64 \times 10^{-5}$ ). This region was excluded from further analyses as the SNV was not in high LD with any of the surrounding markers.


Figure 4.3 A putative functional variant in the obscurin (OBSCN) gene is associated with congestive heart failure (CHF) in Cavalier King Charles Spaniels (CKCS) with myxomatous mitral valve disease. Using Illumina marker BICF2P813381 as a proxy for the highly correlated ( $r^{2}=1$ ) CHF-associated marker BICF2P757489 and missense variant associated with CHF, boxplots illustrate CKCS homozygous for the alternate allele have significantly higher measures of echocardiographic variables. (a) left atrium to aortic root ratio (LA/Ao) (b) Age corrected LA/Ao (c) left ventricular end diastolic dimension, normalised for body weight(LVIDdn) (d) Age corrected LVIDdn. Genotypes as determined by genomic markers are designated as wild-type (Wt) and mutant (Mut), where the wild-type allele is considered the reference allele and the mutant is the alternate. *Statistical significance using Wilcoxon signed-rank test *** p<0.001 and ** $p<0.01$

### 4.4.2 Quantitative GWAS using echocardiographic measures of cardiac remodelling

To identify loci associated with the increasing severity of MMVD, two continuous trait GWAS were conducted on CKCS using quantitative measures, LA/Ao and LVIDdn, including age as a covariate. Three associated loci were detected across both GWAS (Figure 4.1b\&c). The top locus observed between the two studies was associated with the echocardiographic measure LA/Ao, where CFA24:34932842 (BICF2P912253; $\mathrm{P}_{\text {genome }}=1.22 \times 10^{-5}$ ) was the most significantly associated marker. Across the associated loci, five haplotype blocks were identified (Table 4.4). The haplotype blocks were $\sim 130.29 \mathrm{~kb}$ long on average, which is not significantly different from the case-control analysis (Wilcoxon $\mathrm{p}=0.73$ ). However, the genomic range of LD surrounding the top associated variant was less extensive and haplotype blocks were fewer within the quantitative trait loci. Seven coding-region variants were identified within the representative haplotype blocks (Table 4.5).

Table 4.4 Haplotype blocks within regions associated with echocardiographic (Echo) measures of MMVD. Haplotype range indicates the base pair positions for the first and last of single nucleotide variants (SNV) included in the haplotype block. P values represent significance value of the tag SNV from two separate continuous variable genome-wide association analyses; indicated by the associated echo measure.

| Associated Echo ${ }^{\text {a }}$ <br> Measure | Range | Haplotype | Freq. | Tag SNV | $P$ Value | Genes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LVIDdn | $\begin{gathered} \text { 13:61496628- } \\ 61539196 \end{gathered}$ | TCCTT | 0.244 | BICF2P555379 | $\underbrace{2.51 \times 10^{-}}_{05}$ | ADAMTS3 |
| LA/Ao | $\begin{gathered} \text { 20:56464693- } \\ 56628267 \end{gathered}$ | CTAGCC | 0.13 | BICF2P360101 | $\begin{aligned} & 1.31 \times 10^{-} \\ & 05 \end{aligned}$ | GNG7, DIRAS1 |
| LA/Ao | $\begin{gathered} \text { 20:56644215- } \\ 56661518 \end{gathered}$ | GT | 0.11 | BICF2P866985 | $\underset{05}{2.17 \times 10^{-}}$ | LMNB2 |
| LA/Ao | $\begin{gathered} 24: 34737337- \\ 34947424 \end{gathered}$ | GCTTTTGTTCCG | 0.22 | BICF2P912253 | $\underset{05}{1.22 \times 10^{-}}$ | SULF2 |
| LA/Ao | $\begin{gathered} 24: 34999012- \\ 35216909 \end{gathered}$ | ATCTTTGACAGT | 0.236 | TIGRP2P318119 | $\underset{05}{5.22 \times 10}$ | SULF2 |

[^4]Table 4.5 Coding variants matching MMVD-risk haplotypes on chromosome 13, 20 and 24. Variant annotation was conducted using Variant Effect Predictor (VEP) and Variant Annotation Integrator (VAI). Programs predict functional consequences based on transcript annotations from multiple sources. Transcript annotations used to predict functional consequences include Ensembl (E), National Center for Biotechnology information (N), and University of California Santa Cruz (U) and are reported under transcript source.

| Variant ID | CHR | POS | REF | ALT | Gene | Putative <br> Function | Amino <br> Acid | SIFT | Variant <br> Source |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| rs851561774 | 13 | 61539196 | C | T | ADAMTS3 | 5'UTR |  | N |  |
| rs852413501 | 13 | 61539199 | A | G | ADAMTS3 | $5^{\prime}$ 'UTR |  | N |  |
| rs852587856 | 20 | 56661518 | C | T | LMNB2 | missense | S/L | 0.01 | E,N |
| rs851285257 | 24 | 34736272 | A | G | SULF2 | synonymous | R | E,N,U |  |
| rs9009460 | 24 | 34752654 | C | T | SULF2 | synonymous | T | E,N,U |  |
| rs23166895 | 24 | 34826764 | G | T | SULF2 | 5'UTR |  | N |  |
| rs852282457 | 24 | 34850300 | G | C | SULF2 | 5'UTR |  | N |  |

[^5]The most significantly associated genetic marker, located at CFA20:56661518 (chr20_56661518; $\mathrm{P}_{\text {genome }}=1.71 \times 10^{-5}$ ), is a putatively functional variant. The captured variant is a missense substitution (CFA20.56661518C>T) of the gene LMNB2. SIFT classifies the substitution as deleterious to protein function. The frequency of the observed variant is 0.104 in the studied population. Only two CKCS are homozygous for the risk allele, both with evidence of CHF. The genomic marker was uncalled in 11 samples. Of the remaining dogs, heterozygous wild-type and homozygous-mutant samples demonstrate more advanced cardiac remodelling than homozygous wild-type samples ( $\mathrm{P}<0.05$; Figure 4.4a-d). Advancing MMVD was particularly apparent for the associated trait, LA/Ao.


Figure 4.4 A missense variant in the lamin subunit beta-2 (LMNB2) gene is associated with left sided cardiac remodelling in Cavalier King Charles Spaniels (CKCS) with myxomatous mitral valve disease. CKCS carrying the alternate allele for the left atrium to aortic root ratio (LA/Ao) associated marker chr20_56661518 and LMNB2 missense variant have significantly higher measures of echocardiographic variables. (a) LA/Ao (b) Age corrected LA/Ao (c) left ventricular end diastolic dimension, normalised for body weight (LVIDdn) (d) Age corrected LVIDdn. Genotypes as determined by genomic markers are designated as wild-type (Wt) and mutant (Mut), where the wild-type allele is considered the reference allele and the mutant is the alternate. The genomic marker was uncalled in 11 samples (NA). *Statistical significance using Wilcoxon signed-rank test ( $p<0.05$ ) and non-significant differences ( $n s ; p>0.05$ )

### 4.5 Discussion

Through parallel multi-trait analyses, we identified the genomic regions associated with the progression of MMVD and development of CHF in the CKCS. Population structure, extensive regions of fixation, and cryptic relatedness in the dog can complicate GWAS and lead to false positive results. To correct for spurious results caused by stratification, we conducted the GWAS in EMMAX, employing a kinship matrix and using the top principal component as a covariate ${ }^{41,42}$. Given the extensive LD observed in dog breeds, genomic markers are not individual tests and so Bonferroni correction for significance is considered too stringent ${ }^{23,43}$. For this reason, we used empirically defined Cls to set a conservative genome-wide significance threshold ${ }^{43,44}$. Modelling MMVD with an emphasis on echocardiographic traits, we were able to identify five loci, positional candidate genes and candidate functional variants associated in the pathogenesis of MMVD in the CKCS breed.

Phenotypes applied in the GWAS were modelled and refined within the study cohort and are expected to reduce phenotyping error and aid in accurate detection of risk-loci (Chapter 2). Logistic regression analysis applied to this population found age was not a significant predictor of CHF but is a significant predictor of cardiac remodelling (Chapter 2.3.3). In the genotyped subset of CKCS used in this research we found that there was a significant difference in age between CHF and non-CHF samples, though cases are younger than control samples. It is possible that through our comparison of younger cases and older controls we identified loci associated with an early onset form of MMVD. Retrospective analysis or prevalence estimates of associated variants in an age-controlled cohort of at-risk dogs are recommended to validate variants.

As a result of canine domestication and breed selection, the underlying genetic architecture of the dog is beneficial in genetic mapping studies ${ }^{23,26}$. An enduring pattern of complex traits in dogs is that relatively few loci of large effect appear to govern most phenotypic differences among breeds ${ }^{45,46}$. As such, genes influencing complex traits with a quantitative phenotype, such as height, coat colour, and skull shape have been mapped with high accuracy ${ }^{45-52}$. This was the basis for the use of echocardiographic measures to map MMVD severity in the CKCS. LA/Ao and LVIDdn are frequently reported measures of cardiac remodelling and are strong predictors of advancing disease ${ }^{6,7,53}$. Although the use of echocardiographic measures did not result in the same statistical power as the case/control analysis, LD observed at each locus was less extensive, and a strong candidate mutation for disease progression was captured by the genomic markers. As a greater number of samples are included in MMVD mapping efforts, it could be expected that highly correlated traits like LA/Ao and LVIDdn will identify overlapping signals associated with MMVD disease progression with a greater significance. Given the prevalence of the MMVD in small dogs, it is possible that a common ancestral mutation is implicated in disease severity and is widely dispersed in the canine population. However, the current data and the continuing difficulty to replicate genomic signals within breed ${ }^{20-22}$
suggests that regardless of the heightened heritability of early onset disease and severity in the CKCS ${ }^{16}$, it is unlikely that only a few genetic variants are implicated in chronic disease.

Mapping studies utilising multiple breeds with the same trait benefit from improved statistical power and accuracy ${ }^{26}$. Quantitative variables such as those implemented in the current study are commonly reported during routine assessment of canine MMVD and would be easily applied across a multibreed cohort. Future studies should attempt to validate and explore variants and loci highlighted in this research by common measures of disease severity. To date, MMVD mapping and variant detection has been conducted in four dog breeds including CKCS, Whippets, Maltese terriers and dachshunds ${ }^{22,54-56}$. Promoting the use of common, objective measures like those utilised in this study would benefit disease research by permitting crossbreed meta-analyses. None of the loci published to date have overlapped between breeds. It is possible that this directly reflects MMVD as a complex trait caused by random assortment of low frequency risk factors. This supports the hypothesis of MMVD as a polygenic trait ${ }^{27,28}$.

The strongest signal and most convincing CHF-association was observed at the CFA14 locus where the top genomic marker (BICF2P757489) identified in the associated region was a missense substitution, CFA14g. $669043 \mathrm{C}>\mathrm{G}$, in the OBSCN gene. OBSCN is a giant sarcomeric protein that plays a prominent role in cardiac structure and function ${ }^{57-59}$. OBSCN is a prime gene of interest in the pathogenesis of heart disease as multiple causal associations for human inherited cardiomyopathies have been reported ${ }^{60,61}$. To date multiple missense, frameshift, and splicing mutations have been linked to occurrences of hypertrophic cardiomyopathy and dilated cardiomyopathy, and have also been associated with left ventricular compaction, lone atrial fibrillation and chronic systolic heart failure ${ }^{60-63}$. OBSCN plays a prominent regulatory role in the heart, particularly in calcium signalling ${ }^{64-66}$. Mouse models have demonstrated that dysfunction of OBSCN can result in cardiac remodelling with a possible age and sex effect ${ }^{\text {64-66 }}$. Genes involved in calcium signalling, cardiac muscle contraction, control and differentiation are significantly down-regulated in the CKCS when compared to both healthy and affected mitral valves from other dog breeds ${ }^{67}$. It is possible that the OBSCN variants observed in the CKCS contribute to abnormal cell signalling in dogs with cases of severe disease, but additional research is required to validate this hypothesis. However, it can be hypothesised that variation in the OBSCN gene may exacerbate cardiac remodelling in CKCS with MMVD and promote early onset disease or an increased risk of CHF. Three further functional variants OBSCN were predicted within the CHF-associated haplotype block. The gene and reported variants warrant further analysis in dogs with MMVD. Two loci at CFA14 have been previously identified in CKCS through GWAS and homozygosity analysis ${ }^{22}$. There is no evidence of overlap with the locus reported in this study.

At CFA20, a putative functional variant in the LMNB2 gene, CFA20.56661518C>T, that encodes a major lamin isoform passed the genome-wide significance threshold. The locus was significantly associated with echocardiographic variable LA/Ao. The observed variant is a
missense variant that is predicted to have a deleterious effect on the LMNB2 protein function. Lamins are widely expressed intermediate filament proteins that provide structural integrity to the nucleus and cytoskeleton, impact gene regulation and genome stability, and are involved in cell signalling ${ }^{68}$. Many disorders have been linked to genetic variants in lamin genes, chiefly lamin A/C (LMNA), colloquially referred to as laminopathies ${ }^{68,69}$. A major disease category of laminopathies include striated and cardiac muscle diseases, where LMNA variation is associated with cardiac phenotypes ${ }^{69-73}$. It is not uncommon for laminopathies to be accompanied by phenotypes affecting cardiac valves, including the mitral valve ${ }^{70,74-78}$. Variation in the LMNB2 gene has not been linked to cardiac disease, but it was recently proposed to play an essential role in mammalian cardiomyocyte karyokinesis and might contribute to the regenerative potential of cardiomyocytes ${ }^{79}$.

It has been hypothesised that selection for favourable coat colours and against severe MMVD may have had an important role in the development of Chiari-like malformation and syringomyelia in the CKCS breed ${ }^{80}$. The heightened prevalence of Chiari-like malformation and syringomyelia in the CKCS ${ }^{80-82}$ as well as anecdotal suggestion of a series of changes in the facial conformation of the CKCS has recently resulted in a heightened interest in genetic components contributing the variation in the CKCS skull shape ${ }^{83,84}$. Heterogeneity in the LMNB2 gene has recently been associated with microcephaly, a condition affecting head size, in humans ${ }^{85}$. The ${ }^{83,84}$. Variants in LMNB2 should be further investigated with attention to both MMVD and craniofacial phenotypes.

The pathophysiology of MMVD is complex and far from fully understood. Advanced stages of MMVD are categorised by the excessive deposition of proteoglycans and the disorganisation of the extracellular matrix (ECM). Two loci associated with increasing echocardiographic measures LA/Ao and LVIDdn were identified at CFA24 and CFA13 respectively and captured candidate genes for ECM remodelling. The associate locus at CFA24 contained the candidate gene sulfatase 2 (SULF2) which encodes a Heparan sulfate 6-O-endosulfatase enzyme. Heparan sulfate proteoglycans (HSPGs) are major elements of the ECM and a biological substrate of SULF2 ${ }^{86}$. The sulfation status of HSPGs is modulated by sulfatases and can have critical impacts on signalling pathways ${ }^{87-91}$. The effects of sulfatases on the binding of growth factors, particularly TGF- $\beta$, has meant SULF2 is a driver of transformed cellular phenotypes, evident by its role in multiple cancers ${ }^{88,92-95}$. A hallmark feature of MMVD is the development of nodules along the mitral valve leaflet, expansion of the spongiosa, and disorganisation of the fibrosa ${ }^{96}$. A critical step in the observed changes is the transformation of valvular interstitial cells into an active state, where upregulation of key signalling pathways, TGF- $\beta$ and serotonin, promote substantial ECM remodelling ${ }^{5,97-101}$. Variation in gene expression during this process suggest valvular cells undergo an endothelial to mesenchymal transition ${ }^{102-105}$. Notably, activity of SULF2 can regulate TGF- $\beta$ activity and inhibit transformation of cells into a mesenchymal state as well as increase the proliferative activity of cells ${ }^{87,90}$. SULF2 is also expected to play a regenerative role in hearts and attenuate left ventricular remodelling, post-infarction by
mediating angiogenesis and profibrotic activity of TGF- $\beta 1^{106}$. Currently, variable expression of the SULF2 gene and protein has not been observed in MMVD studies but evidence of the genes role in cell signalling and behavioural transformation makes it a candidate gene for advancing MMVD.

A single marker identified in the ADAMTS3 gene was significantly associated with echocardiographic variable LVIDdn. ADMTS3 belongs to the 'A disintegrin and metalloproteinase with thrombospondin motifs' (ADAMTS) family of proteins. ADAMTS and other closely related metalloproteinase families play a prominent role in the turnover and remodelling of the ECM ${ }^{107-109}$ and are variably expressed in proteomic and transcriptomic MMVD studies ${ }^{105,110}$. It is expected that differential expression of metalloprotease genes is a consequence of advancing MMVD, rather than a cause. Still, variation in ADAMTS3 is linked to cardiovascular health with variable expression observed in acute myocardial infarction, blood vessel homeostasis and lymphangiogenesis ${ }^{111,112}$. In humans, variation in the ADAMTS3 gene can cause Hennekam lymphangiectasia-lymphedema syndrome (HKLLS), characterised by lymphatic dysplasia and characteristic facial dysmorphism ${ }^{113,114}$. ADAMTS3 is highly expressed in the craniofacial region and is link to craniofacial phenotypes ${ }^{115,116}$. In dogs, variation in ADAMTS3 is associated with obstructive airway syndrome in the Norwich Terrier but the study found no morphological differences in the skull shape of affected and unaffected dogs ${ }^{117}$. Neither variation in the ADAMTS3 coding sequence nor gene expression have been implicated in the pathogenesis of MMVD in dogs or species with analogous phenotypes. As with the CFA14 locus, the signal observed at this locus does not overlap with the previously reported CKCS MMVD associated CFA13 locus ${ }^{22}$.

Whole genome sequencing and variant annotation is a powerful tool for understanding the inheritance of complex diseases. But the vast amount of information produced by such data introduces new challenges that require novel computational approaches for functional annotation and identification of causal variants in diseases of interest. The difficulty in identifying variants that underlie disease causation is even more difficult in complex disease like MMVD and CHF that involve a complex interplay of genetics with varying modes of selection, as well as metabolic and acquired factors ${ }^{118,119}$. Here, several candidate genes were outlined that might influence the development of CHF in CKCS with MMVD. SIFT was utilised as the main source of variant effect prediction due to its frequent use in canine publications and seamless integration into Ensembl's VEP tool ${ }^{37}$. An external cohort of comprehensively phenotyped individuals is needed to validate the genetic loci and risk factors presented. Furthermore, quantification of variations across species with distinct demographic histories is recommended and could assist in identifying non-coding variants of interest. Further analysis and prioritisation of genetic variants should be considered as novel computational approaches are developed and data are acquired.

This study identified five genomic regions associated with severe forms of MMVD and the development of CHF. Several candidate genes and mutations were identified that highlight
cardiomyocyte organisation, signal transduction, cell phenotype transformation and ECM remodelling as genetic components of advancing MMVD. Two putative functional variants were identified, CFA14g.669043C>G and CFA20.56661518C>T, that are predicted missense variants in the OBSCN and LMNB2 genes respectively. Both candidate variants are captured by genomic markers included on the Illumina canine HD beadchip. Risk loci and variants presented in this research should be validated using a larger cohorts of samples phenotyped according to left ventricular remodelling using echocardiographic variables. The incomplete segregation of MMVD risk loci across the studied samples support the disease as a polygenic threshold disorder. Dual functions of candidate genes in MMVD disease severity and craniofacial phenotypes may imply that selection on morphological phenotypes supported an increased occurrence of risk haplotypes in the CKCS. Contrariwise selection away from severe MMVD or early onset disease might have influenced CKCS skull morphology. Morphometric analysis of CKCS skull shapes are necessary to support this hypothesis.

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# Chapter 5. Runs of homozygosity analysis in the Cavalier King Charles Spaniel identifies candidate genes for the pathogenesis myxomatous mitral valve disease 


#### Abstract

5.1 Abstract

The unique history of canine domestication and breed-line development has resulted in dogs breeds exhibiting a diverse repertoire of phenotypic variation. Genomic signatures of selection that reflect this history, are most notably observed in runs of homozygosity (ROH). Identifying regions of selection within dog breeds is a valuable process for gaining insight into the genetic variation that governs breed-specific traits and common diseases that have potentially hitchhiked alongside the selection processes. Myxomatous mitral valve disease (MMVD) is the most frequently diagnosed cardiovascular disease in dogs. The Cavalier King Charles Spaniel (CKCS) is a breed universally affected by MMVD, and genes involved in disease pathogenesis are expected to be near fixation in the population. Given the fixed nature of MMVD in the CKCS, this research sought to identify signatures of selection in this breed through ROH.


ROH were detected in Australian CKCS, genotyped on the Illumina canineHD array and validated in a second cohort of CKCS. ROH with a high incidence in both cohorts were investigated for candidate genes and variants for the pathogenesis of MMVD. MMVD affects all dog breeds, but prevalence estimates are significantly higher in small breeds, like the CKCS. Variants that may have hitchhiked with nearby positively selected size variants were assessed. A single ROH overlapping the FGF4-retrogene, a known size variant that has been implicated in valvulogenesis, was observed at a high frequency in both cohorts. A further candidate gene for the pathogenesis of MMVD, COL11A1, was observed in the longest validated ROH. Three COL11A1 splice region variants were observed in CKCS whole genome sequence data. Mutations in COL11A1 are implicated in connective tissue syndromes in humans that may present with valvular phenotypes and other features observed in the CKCS.

### 5.2 Introduction

Myxomatous mitral valve disease (MMVD; OMIA 000654-9615) is a chronic, progressive disorder that affects elderly dogs (Canis lupus familiaris) of all sizes, but the highest incidence and greatest susceptibility to the disease is confined to smaller breeds ${ }^{1-3}$. The elevated prevalence of MMVD in small stature breeds has led to the hypothesis that pathogenic variants may have hitchhiked with genes influencing small size ${ }^{4}$. MMVD is the most common cardiac disease in dogs ${ }^{5-7}$. Frequently, the disease is diagnosed with auscultation and is characterised by a systolic murmur caused by the back-flow of blood, known as mitral regurgitation (MR). In most cases, the presence of MR is of minimal concern, but MMVD has the potential to progress
into eccentric left-sided cardiac remodelling that can result in congestive heart failure and premature death ${ }^{8-11}$.

Selective breeding for behavioural and breed defining morphological traits has resulted in genetic bottlenecks that divide purebred dogs into distinct populations with reduced phenotypic and genetic heterogeneity, and extensive linkage disequilibrium (LD) ${ }^{12-14}$. Inadvertently, this process can result in disease-associated risk alleles hitchhiking alongside desirable traits ${ }^{15-17}$. While persistent selective pressure can result in an accumulation of disease risk-alleles, background ancestral variation in the dog remains high, reducing LD across breeds and making it an ideal genetic system for studying complex traits ${ }^{18}$. Taking advantage of the unique genetic background of the dog, significant advances have been made in identifying heritable loci that govern disease risk and other observable phenotypes ${ }^{18-22}$. Genome wide association studies (GWAS) represent a key strategy in mapping heritable traits. However, GWAS largely requires access to equal sizes of affected and unaffected dogs within a breed, and in circumstances where causative genetic polymorphisms have become fixed within a breed by drift or artificial selection, a case/control GWAS is challenging. This is a prominent roadblock when studying MMVD in breeds like the Cavalier King Charles Spaniel (CKCS) where the underlying degeneration of mitral valves is consistent within the breed ${ }^{23,24}$.

Because prevalence of MMVD is so high in the CKCS, identifying fixed genomic regions could be advantageous in the understanding the genetic aetiology. Observing regions under artificial selection has the potential to help identify genomic loci contributing to disease susceptibility and complex traits ${ }^{17}$. Selective sweeps are defined as a reduction in genetic variation surrounding a beneficial or artificially selected mutation and are prominent in populations that have undergone recent intensive selection. Runs of homozygosity ( ROH ) are a characteristic of populations with a low effective population size and are a key signature of artificial selection ${ }^{25,26}$. ROH describe tracts of genomic homozygosity within breeds that are identical by descent, manifesting because of common ancestry among breed representatives. The size of the tract provides some indication of how recently the selection was applied with longer tracts indicating more recent or stronger selective pressure ${ }^{26,27}$. Canine research supports the notion that deleterious variants and disease-associated genes are enriched in regions of intense artificial selection ${ }^{15,28}$. For traits and diseases considered fixed within a population, like MMVD in the CKCS, it is reasonable to suggest that causative loci exist within ROH.

The CKCS is a toy breed that genetically clusters within the Spaniel group ${ }^{29}$. Historically, the breed is believed to have undergone several conformational changes, mostly affecting head shape ${ }^{30}$. It is possible that during the breed development process, CKCS have acquired an increased prevalence of heritable disorders like MMVD and syringomyelia ${ }^{31}$, because of disease causing variants that have hitchhiked with those under strong selection for hallmark
phenotypic traits. It is also possible that genes involved in heritable diseases share physiological function with genes under selection. The CKCS is recognised as being affected by MMVD more frequently and at a much younger age than other breeds ${ }^{32-35}$. Both the presence and severity of MMVD in the CKCS breed is highly heritable ${ }^{36}$. For this reason, the CKCS is the most represented breed in canine MMVD research ${ }^{37-42}$. The modern-day CKCS remains a highly popular breed in both the United Kingdom and Australia ${ }^{43,44}$. While breeders prioritise avoiding inbreeding, genetic evidence still indicates that the breed has low heterozygosity and a low rate of LD decay ${ }^{45-47}$. Several breed-related health issues are noteworthy in the CKCS ${ }^{31}$ including; myxomatous mitral valve disease ${ }^{37-42}$, Chiari-like malformation, syringomyelia ${ }^{48,49}$, retinal dysplasia, cataracts ${ }^{50}$ and chronic pancreatitis ${ }^{51}$. All of these health related issues are overlapping features of heritable disorders of connective tissue ${ }^{52}$. Detrimental variants in genes regulating connective tissue remodelling and repair may impact the prevalence of such disorders.

This research aims to identify candidate genes and variants involved in the pathogenesis of MMVD by identifying signatures of artificial selection in the CKCS. Given the fixed nature of the disease within the breed, we propose assessing ROH in the CKCS for candidate genes and variants affecting MMVD risk. Due to the exaggerated prevalence of the disease in small dogs, this research attempts to identify variants common in small dogs that may have hitchhiked with nearby positively selected size variants.

### 5.3 Methods and materials

### 5.3.1 Ethics, clinical diagnosis, and data collection

The methodology described in Chapter 5 was conducted on a population of Australian CKCS previously reported and conforms with ethical practices (Chapter 2.2.1). Clinical evaluation of MMVD (Chapter 2.2.2), collection of blood and genotyping (Chapter 3.2.2), next generation sequencing (Chapter 3.2.2), whole genome sequence (WGS) alignment and variant calling (Chapter 3.2.6) have been reported.

Genotype data from a secondary group of CKCS ( $\mathrm{n}=96$ ) from the United Kingdom available through the public Gene Expression Omnibus database (accession GSE102906) were included as a validation cohort. The data was previously applied to a genetic investigation of syringomyelia ${ }^{53,54}$.

### 5.3.2 CKCS runs of homozygosity analysis

Identification of autozygous genomic regions was conducted in two stages. The first stage used locally acquired CKCS genotype data. In the second stage, the same analysis was applied to public-domain CKCS data from a geographically removed population. ROH analysis was conducted in both cohorts using Plink ${ }^{55}$ (--homozyg) in accordance with a protocol previously described and validated ${ }^{56}$. Settings for the minimal density of single nucleotide variants (SNV) (--homozyg-density) and maximal gap size (--homozyg-gap) were empirically determined ${ }^{56}$. To improve accuracy of inbreeding coefficients, input settings were applied at thresholds allowing for the highest coverage of the genome. Scanning window length (--homozyg-window-snp) and scanning window threshold (--homozyg-window-threshold) were calculated using the suggested formula ${ }^{56}$. In accordance with the published protocol by Meyermans et al. (2019), SNPs were not subjected to minor allele frequency (MAF) or LD pruning ${ }^{56}$. The minimal number of SNPs in a ROH was determined for each analysis using a previously described and adapted formula ${ }^{57}$. Further settings allowed for one heterozygous SNP (--homozyg-window-het) and one missing SNP (--homozyg-window-missing) per scanning window for a maximum of one heterozygous SNP in the final segment (--homozyg-het). The incidence of genomic markers in a ROH is calculated as the proportion of times it occurs in a ROH across the analysed population. Genomic regions containing the highest incidence (top 1\%) of SNVs observed in a ROH were considered ROH hotspots ${ }^{57}$. The extent of hotspots was measured by combining SNVs with neighbouring genomic markers that passed the incidence threshold. Autozygous regions underwent further examination if they were consistent in both the initial CKCS and validation cohorts. Inbreeding coefficients (FROH) were calculated for both length of the autosomal genome ( $\mathrm{FROH}_{\text {aut }}$ ) and the length of the genome covered by the array $\left(\mathrm{FROH}_{\text {cov }}\right)^{56}$. Using a Pearson's correlation test, we assessed the relationship between inbreeding coefficients, $\mathrm{FROH}_{\text {aut }}$ and $\mathrm{FROH}_{\text {cov. }}$ A Wilcoxon signed-ranks test was used to determine if ROH results were significantly different between the studied cohorts. Validated ROH tracts were examined for syntenic regions in humans using the Ensembl Bioinformatics database ${ }^{58}$.

### 5.3.3 CKCS ROH genes consistent with breed standard hallmark traits

Breed hallmark traits that were identified by observation of CKCS breed standards outlined by The Kennel Club (KC; https://www.thekennelclub.org.uk/breed-standards/toy/cavalier-king-charles-spaniel/) and Australian National Kennel Council (ANKC; http://ankc.org.au/Breed/Detail/18). ROH presence and tract size were recorded for genes and chromosomal regions with known association to CKCS breed hallmark traits were identified in the literature (Table 5.1).

Table 5.1 Gene associated with Cavalier King Charles Spaniel hallmark traits for breed.

| Phenotype | Gene | Genomic coordinates (Canfam3.1) | Reference |
| :---: | :---: | :---: | :---: |
| Coat colour - Black and Tan | ASIP | 24:23354888-23393896 | 59 |
| Coat colour - Ruby | MC1R | 5:63694296-63695249 | 60 |
| Coat colour Blenheim/Tricolour | MITF | 20:21883312-22101930 | 18 |
| Skull shape - Skull flat between ears. Nose ( 3.8 cm ) | BMP3 | 32:5207833-5231966 | 61 |
|  | SMOC2 | 1:56009740-56168234 | 62 |
| Coat - long and silky | FGF5 | 32:4533042-4556071 | 63,64 |
| Size - small well- | GHR | 4:67022252-67290473 | 65 |
|  | HMGA2 | 10:8352270-8491307 | 65 |
|  | IGF1 | 15:41202518-41275794 | 66 |
|  | IGF2BP2 | 34:18369684-18522157 | 67 |
|  | IGF1R | 3:41794623-42090387 | 68 |
|  | LCORL | 3:91132373-91271686 | 69 |
|  | SMAD2 | 7:43700445-43769983 | 65 |
|  | STC2 | 4:39152503-39162343 | 65 |
|  | ZNF608 | 11:14301410-14405797 | 69 |
|  | FGF4 | 18:48413694-48415206 | 21 |
|  | FGF4* | 12:33710168-33710178 | 70 |
|  | FGF4* | 18:23431136-23431136 | 21 |
| Ears - Pendulous | MSRB3 | 10:7971606-8151219 | 69 |

*Retrogenes associated with phenotype

### 5.3.4 CKCS ROH genes consistent with MMVD gene ontology

Custom lists of Gene Ontology (GO) annotations associated with MMVD and genes differentially expressed in MMVD transcriptomic studies were produced ${ }^{71-74}$ (Table S1 \& S2).

Genes located within CKCS ROH hotspots were assessed for overlap with annotated terms using Gonet annotation analysis with a custom list ${ }^{75}$.

### 5.3.5 Discovery of private or rare CKCS variants

Data from next generation sequencing (Chapter 3.2.2), WGS alignment and variant calling (Chapter 3.2.6) were applied to the research described in this chapter. This includes access to WGS alignments and variant call data obtained from five CKCS samples.

CKCS and other small breed dogs are predicted to have a higher frequency of variants driving the development of MMVD compared to larger breeds. Using data from a variant catalogue developed from 722 Canid sequences available on NCBI ${ }^{69}$ (accession number: PRJNA448733), hereby referred to as 'the canine catalogue', we compared the frequency of putative functional variants in the CKCS with other breeds. Prior to assessing the frequency of variants in canine catalogue, dogs were excluded from the analysis if they did not belong to the subspecies Canis Lupus Familiaris or if their breed was mixed or unknown. No more than four samples per breed were included to moderate overrepresentation and prevent breed-driven results. Samples were preferentially selected for highest coverage and a gender balance. Detection of rare CKCS alleles in the remaining dataset was conducted in four steps. First, to ensure variants had a high level of fixation in the CKCS, Bi-allelic variants with a frequency greater than 0.8 were extracted from the high-quality CKCS WGS variant dataset, previously described (Chapter 3.2.6), using VCFtools ${ }^{76}$. Variants were then analysed with Ensembl's Variant Effect predictor (VEP) tool ${ }^{77}$. Next, VEP output was filtered to retain variants with probable functional consequences using the following impact terms: missense_variant, start_lost, stop_lost, stop_retained_variant, stop_gained, splice_region_variant, splice_acceptor_variant, splice_donor_variant, 3_prime_UTR_variant, 5_prime_UTR_variant. Finally, using the genomic coordinates of filtered variants in the CKCS, variants were extracted from the canine catalogue ${ }^{69}$. The frequency of the remaining variants was compared between small dogs predisposed to MMVD, less than nine kilograms ${ }^{4}$, and larger breeds. Rare variants, with a MAF less than 0.05 , in the larger breed cohort were reported.

### 5.3.6 Haplotype analysis of candidate genes

Haplotype analysis of candidate genes was conducted using Haploview software ${ }^{78}$. Array markers 500 kb upstream and downstream of the gene were extracted from 274 CKCS from both the initial and validation cohort. Haplotypes were generated based on $95 \%$ confidence bounds on LD parameter D prime ${ }^{79}$. Variants segregating within the observed haplotypes were extracted from the CKCS WGS VCF using VCF tools ${ }^{76}$ and analysed with VEP. Variants with a putative functional consequence were reported. The genomic markers captured within the CKCS haplotype were extracted from all samples included in the canine catalogue using VCF tools. Individuals in the canine catalogue homozygous across the haplotype were filtered for further analysis. Dogs with matching haplotypes to those observed in the CKCS were used to validate putative functional variants.

### 5.4 Results

### 5.4.1 CKCS runs of homozygosity

Final input parameters for the primary and validation ROH analyses were determined to maximise genome coverage (Table S3). Observed ROH were broadly consistent across both cohorts (Table 5.2). Genome coverage, represents the proportion of the genome analysed based on the input parameters and reflects the validity of ROH analysis ${ }^{56}$. Genome coverage for both cohorts averaged $99.8 \%$. Acoss both cohorts, we identified 43,033 individual ROH for all autosomes. The distribution of the ROH tract sizes were consistent across the initial and validation cohorts (Figure 5.1a). The average ROH tract size was $\sim 5,087 \mathrm{~kb}$. The vast majority of ROH ( $88.37 \%$ ) of individual runs were less than 10 Mb long. Two inbreeding coefficients were reported for each group, $\mathrm{FROH}_{\text {aut }}$ and $\mathrm{FROH} \mathrm{cov}_{\text {cov }} \mathrm{FROH}_{\text {aut }}$ represents the most reported statistic for inbreeding and uses length of the autosomal genome, while $\mathrm{FROH}_{\text {cov }}$ considers genome coverage based on individual datasets. Within cohorts, inbreeding coefficients were highly correlated ( $r=1.0 ; \mathrm{P}<0.001$ ), we chose to present the value for the autosomal genome. Across populations, inbreeding coefficient FROH was marginally higher in the Australian cohort though not significantly different ( $z=1.6211, p=0.105$; Figure 5.1b). The threshold for defining ROH hotspots was $90.44 \%$ and $83.87 \%$ for the initial and validation cohort respectively. ROH hotspots were accepted as validated if common markers passed incidence threshold in both cohorts. A total of eight ROH passed the considered threshold in the initial cohort, of which six were validated in the secondary analysis (Figure 5.2; Table 5.3). Two hotspots with the highest incidence ( $>95 \%$ ) on chromosomes 7 (CFA7) and chromosome 12 (CFA12) were observed in both cohorts.

Table 5.2 Summary of results for runs of homozygosity in Cavalier King Charles Spaniel conducted in plink

|  | Initial | Validation |
| :--- | :---: | :---: |
| Samples | 178 | 93 |
| Genomic markers | 194,794 | 162,139 |
| Runs of homozygosity | 29,533 | 13,500 |
| Average runs per sample | 170.17 | 151.90 |
| Average size of runs (kb) | 4966.53 | 5319.78 |
| Genomic coverage (\%) | 99.85 | 99.84 |
| Inbreeding coefficient | 0.38 | 0.36 |
| (FROH) |  |  |



Figure 5.2 Results of runs of homozygosity analysis are consistent across two populations of Cavalier King Charles Spaniels. (a) distributions of the ROH in the initial and validation cohort of Cavalier King Charles Spaniels. Highest ROH observations were found in the size range $1-5 \mathrm{Mb}$. (b) Inbreeding coefficient measurements were equivalent between both populations. FROHaut and FROHcov are highlighly correlated ( $r=1$ )


Figure 5.1 Autosomal distribution of single nucleotide variants (SNV) in runs of homozygosity ( ROH ). The incidence of markers in an ROH was calculated as the percentage of animals with the variant observed within a ROH. (a) initial cohort (b) validation cohort. Highlighted in red are variants passing the top $1 \%$ incidence threshold for both populations.

Table 5.3 Summary of ROH hotspots validated in two cohorts of Cavalier King Charles
Spaniels. The position of the ROH hotspot is defined by the common variants passing the top $1 \%$ incidence in each cohort.

| ROH hotspot position | Size(kb) | number of ROHs Initial | $\begin{gathered} \text { Average size } \\ \text { ROH (Kb) } \\ {[\mathrm{min}-\mathrm{max}]} \\ \text { Initial } \end{gathered}$ | number of ROHs Validatio n | $\begin{aligned} & \text { Average size } \\ & \text { ROH (Kb) } \\ & {[\text { min - max] }} \\ & \text { Validation } \end{aligned}$ | Genes |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { 2:2337558- } \\ 2760263 \end{gathered}$ | 423 | 166 | $\begin{gathered} 4052(1700- \\ 4170) \end{gathered}$ | 80 | $\begin{gathered} 3815 \text { (1360- } \\ 4200) \end{gathered}$ | SNRPC, PARD3 |
| $\begin{gathered} \text { 6:44133189- } \\ 50897341 \end{gathered}$ | 6764 | 277 | $\begin{aligned} & 12172 \\ & (1173- \\ & 72794) \end{aligned}$ | 124 | $\begin{gathered} 13823(1325- \\ 50854) \end{gathered}$ | PLPPR5, PLPPR4, PALMD, FRRS1, AGL, SLC35A3, AC118553.2, SASS6, TRMT13, LRRC39, DBT, RTCA, CDC14A, GPR88, VCAM1, EXTL2, SLC30A7, DPH5, S1PR1, OLFM3, COL11A1, RNPC3, PRMT6, NTNG1, MFSD14A |
| $\begin{gathered} 7: 66802311- \\ 67951538 \end{gathered}$ | 1149 | 172 | $\begin{gathered} 5066 \text { (1141- } \\ 39705) \end{gathered}$ | 90 | $\begin{gathered} 5623 \text { (1319- } \\ 55747) \end{gathered}$ | ADCYAP1, YES1, ENOSF1, TYMS, CLUL1, CETN1, COLEC12, THOC1, USP14, ROCK1, ATP5PD, PLP2 |
| $\begin{gathered} \text { 11:37308771- } \\ 38815495 \end{gathered}$ | 1507 | 171 | $\begin{aligned} & 14975 \\ & (2232- \\ & 60661) \end{aligned}$ | 84 | $\begin{gathered} 13679(1644- \\ 46775) \end{gathered}$ | SAXO1, ADAMTSL1, SH3GL2, CNTLN |
| $\begin{gathered} \text { 12:33673559- } \\ 35296744 \end{gathered}$ | 1623 | 175 | $\begin{gathered} 8133 \text { (1612- } \\ 44600) \end{gathered}$ | 92 | $\begin{aligned} & 9027 \text { (1679- } \\ & 72480) \end{aligned}$ | FGF4-retrogene, KCNQ5, RIMS1, RIMS1, OGFRL1 |
| $\begin{gathered} 35: 12068890- \\ 12891676 \end{gathered}$ | 823 | 171 | $\begin{gathered} 5186 \text { (1224- } \\ 24268) \end{gathered}$ | 83 | $\begin{gathered} 5283 \text { (1238- } \\ 22264) \\ \hline \end{gathered}$ | GFOD1, TBC1D7, PHACTR1 |

### 5.4.2 ROH in vicinity of breed hallmark trait loci

The incidence of ROH overlapping genes associated with traits observable in the CKCS were calculated (Table 5.4). A ROH hotspot spanning 1623 kb overlapped the fibroblast growth factor 4 (FGF4) retrogene and was observed at a high incidence in both cohorts (>96\%). The average minor allele frequency of genomic markers covering the CFA12 ROH hotspot was 0.003 (Table S4). Evidence of an insertion of the FGF4 retrogene at CFA12:33,710,178 was observed in all WGS CKCS (Figure S1).

The genomic region with the next highest incidence of ROH in both cohorts overlapped the Growth Hormone Receptor (GHR) gene but incidence of ROH were significantly lower in both cohorts. Two variants previously described in the GHR gene, GHR(1) (CFA4 g.67040898C>T) and GHR(2) (CFA4 g.67040939G>A), were homozygous across all five WGS CKCS ${ }^{65}$. No further ROH harbouring loci for common morphological traits were observed at a high frequency.

Table 5.4 Summary of runs of homozygosity overlapping genes associated with hallmark traits in the Cavalier King Charles Spaniel. ROH incidence passing the top 1\% threshold in both cohorts in bold.

| Gene | ROH <br> Incidence <br> $(\%)$ | Initial <br> median size (kb) | ROH <br> Incidence (\%) | Validation <br> median size (kb) |
| :---: | :---: | :---: | :---: | :---: |
| ASIP | 35.96 | 8278 | 26.88 | 12396 |
| MC1R | 29.78 | 8651 | 23.66 | 9288 |
| MITF | 40.45 | 6038 | 58.06 | 2520 |
| BMP3 | 43.26 | 4685 | 50.54 | 4498 |
| SMOC2 | 29.78 | 14375 | 36.56 | 23557 |
| FGF5 | 42.70 | 4546 | 43.01 | 4967 |
| GHR | 79.21 | 3202 | 83.87 | 4957 |
| HMGA2 | 43.26 | 2045 | 38.71 | 3022 |
| IGF1 | 21.91 | 5893 | 31.18 | 3934 |
| IGF2BP2 | 30.90 | 6502 | 40.86 | 5024 |
| IGF1R | 60.11 | 7155 | 61.29 | 9970 |
| LCORL | 24.16 | 3097 | 31.18 | 3324 |
| SMAD2 | 47.19 | 7734 | 46.24 | 5013 |
| STC2 | 48.88 | 9458 | 50.54 | 10146 |
| ZNF608 | 41.01 | 5721 | 23.66 | 7213 |
| FGF4 | 28.09 | 5086 | 18.28 | 3707 |
| FGF4_(CFA12) | 97.75 | 3954 | 96.77 | 6041 |
| FGF4_(CFA18) | 39.89 | 6777 | 30.11 | 7783 |
| MSRB3 | 66.29 | 1955 | 40.86 | 2783 |

### 5.4.3 CKCS ROH genes consistent with MMVD GO

The curated list of GO terms derived from MMVD gene expression studies contains 135 GO annotations from all three domains: biological process, molecular function and cellular component. The majority of GO terms ( $\mathrm{n}=103$ ), fall under biological processes followed by cellular components ( $n=18$ ) and molecular function ( $n=14$ ). From the curated list, 69 (51\%) of GO terms shared overlap with at least one gene observed in CKCS ROH. GO term and gene overlap ranged from 0 to 9 with an average of 1 gene per annotation (Table S5). The GO term with the greatest overlap ( 9 genes) was extracellular space. Of the 50 genes included in the

GOnet analysis, 30 (60\%) had overlap with at least one GO term. On average, genes overlapped 4.73 Goterms. Genes, Vascular Cell Adhesion Molecule 1 (VCAM1), Sphingosine-1-Phosphate Receptor 1 (S1PR1), Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1), Adenylate Cyclase Activating Polypeptide 1 (ADCYAP1), and Fibroblast Growth Factor 4 (FGF4) intersected the most MMVD Goterms with $24,21,16,11$ and 10 respectively. Five genes in ROH hotspots, including the two most represented genes from the GoNet analysis, VCAM1 and S1PR1, have previously been identified as differentially expressed genes in MMVD studies ${ }^{71-74}$ (Table S6). Excluding S1PR1, all genes showed consistent directional changes in expression regardless of study or model.

### 5.4.4 Variant calling and discovery of private or rare CKCS variants

A summary of genomic variants annotated across consensus ROH, before and after biallelic MAF-filtering, is provided in supplementary materials (Table S7). After MAF filtering, 21,082 biallelic variants, assigned to 36,220 functional classes, remained across five CKCS. The number of functional classes is higher than the total number of variants because overlapping genes and transcripts result in multiple annotations. Within the consensus regions, 11,317 (53.7\%) variants were novel variants and 9,765 ( $46.3 \%$ ) had been previously annotated. Using the impact classification scheme defined by VEP, 36,220 variants (99.47\%) are predicted to have no impact on protein function and are predominantly located in intronic or intergenic regions. Variants annotated to protein coding regions included 87 (54.03\%) synonymous variants, 55 $(34.26 \%)$ missense variants, 10 ( $6.211 \%$ ) frameshift variants, 5 (3.1\%) in-frame insertions, and $4(2.48 \%)$ in-frame deletions. Two splice region variants were predicted and accounted for less than $1 \%$ of all variants.

Variants with putative functional consequences in the CKCS were observed in comparison with dogs from the canine catalogue. After filtering to exclude non-domesticated canids, mixedbreed dogs and samples from over-represented breeds, 314 dogs from 143 unique breeds remained for comparison with the CKCS (Table S8). In the remaining dataset, 261 dogs (121 breeds) represented dogs with a breed average weight over 9 kg while the remaining 53 dogs (22 breeds) fell into the weight range consistent with a high-risk for MMVD ${ }^{4}$. After filtering variants with a MAF <0.05 in the dogs greater than 9 kg , only two variants with putative functional consequences remained (Table 5.5). Both variants are five prime untranslated region (UTR) variants observed at a low frequency in both large and small breeds.

Table 5.5 Rare variants in protein coding genes in Cavalier King Charles Spaniels runs of homozygosity. Variants are five prime untranslated region variants

| Chromosome | Position <br> (Canfam3.1) | Variant ID | REF | ALT | CKCS <br> AF | MAF <br> dog <br> $>9 \mathrm{~kg}$ | MAF <br> dog <br> $<9 \mathrm{~kg}$ | Gene | Ensembl GeneID |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 6 | 49232668 | rs850617503 | C | T | 1 | 0.045 | 0.109 | EXTL2 | ENSCAFG000000020002 |
| 6 | 44397940 | . | C | A | 0.9 | 0.046 | 0.029 | NTNG1 | ENSCAFG000000019958 |

### 5.4.5 Candidate gene COL11A1

Given the nature of extracellular matrix remodelling in MMVD, collagen genes represent strong candidates for disease pathogenesis ${ }^{80-84}$. The swept region on CFA6 contained collagen gene Collagen Type XI Alpha 1 Chain (COL11A1). Haplotype analysis was performed using 90 genomic markers surrounding COL11A1, between and inclusive of CFA6:47000519 and CFA6:47986362. A single haplotype block spanning CFA6:47477037-47583958 ( $\sim 35.6 \mathrm{~kb}$ ), was identified. Two CKCS haplotypes are reported (Table 5.6). Of the observed haplotypes, one was significantly overrepresented in the CKCS population and no samples were homozygous for the alternate. A single marker within the observed haplotype (CFA6:47559821) did not differentiate within the CKCS and was not represented in the canine catalogue, suggesting it is monomorphic in canids and was excluded. The haplotype block was observed in samples in the canine catalogue. Two hundred and twenty-one dogs homozygous across the haplotype block were filtered to avoid ambiguity of haplotype calling. A further 15 haplotypes were detected in the extended dataset (Table S9), of which five are unique to non-domesticated canid species.

Table 5.6 Haplotypes overlapping gene COL11A1 in 274 Cavalier King Charles Spaniels

| Chromosome | Position | Haplotype block | Frequency |
| :---: | :---: | :---: | :---: |
| 6 | $47477037-47583958$ | CCTTTTAGCT | 0.937 |
|  |  | TTGGAACATC | 0.048 |

In the five WGS CKCS, three samples were homozygous for the frequently observed haplotype and two were heterozygous. After extracting variants within the genomic markers bordering the haplotype, 371 variants segregated with the haplotypes. Most variants identified were intronic variants (98\%). Three synonymous SNVs and three splice site variants segregated in the coding region of COL11A1 (Table 5.7). Two of the three splice site variants occur outside of the haplotype block, between the last SNV in the block and the neighbouring array marker. Within the canine catalogue, 38 and 16 dogs of various breeds were homozygous for the common and rare CKCS haplotypes respectively. Splice site variants from 58 dogs ( $95 \%$ ) had genotypes consistent with expectation (Table S10). In samples homozygous for the common CKCS haplotype, the splice site variants segregated in perfect concordance, excluding one sample with a missing call. For samples with the rare CKCS haplotype, CFA6:g.47507204C>T
was in high concordance, but two samples were heterozygous, one which had a further heterozygous call at splace variant CFA6:g.47591604A>G.

Table 5.7 Putative functional variants in COL11A1 concordant with the haplotypes observed in the Cavalier King Charles Spaniel. Alleles in bold segregate with the overrepresented haplotype in the Cavalier King Charles Spaniel.

| Position | Variant ID | REF | ALT | Putative function | Frequency <br> canine <br> catalogue |
| :--- | :--- | :--- | :--- | :--- | :--- |
| 47507204 | rs24324848 | C | T | Splice region <br> variant <br> Splice region <br> variant <br> Splice region <br> variant | 0.3331 |
| $47590602^{*}$ | rs24299845 | G | A | 0.412 |  |
| $47591604^{*}$ | rs852596807 | A | G | 0.4115 |  |

*Variants identified between the last genomic marker of the haplotype and the neighbouring genomic marker

The splice variants were observed in the remaining haplotypes observed in the canine catalogue samples. To the exclusion of one dog in the homozygous cohort, the observed genotype for CFA6:g.47507204C>T was consistent for all samples with a shared haplotype. Inheritance of the remaining splice variants was inconsistent. Across all samples in the canid catalogue, array marker at CFA6:47507204 is in high LD with splice variant CFA6:g.47507204C>T ( $r^{2}=0.959$ ). Notably, for 36 homozygous samples from 10 uncommon haplotype groups, the genomic marker and splice variant have an inverse relationship from the rest of the cohort. The samples are predominantly non-domesticated canids, but also includes village dogs (Borneo and China), Chongqing Dog, Chow Chow, Tibetan Mastiff, Alaskan Husky, Sberian Husky, Great Danes and an Italian Greyhound.

### 5.5 Discussion

This research identified genomic regions with a high level of fixation in the CKCS and proposed candidate genes and loci contributing to MMVD pathogenesis. The current study highlighted a ROH in the CKCS breed overlapping the FGF4 retrogene, previously implicated in body size in small and medium sized dog breeds ${ }^{70,85}$. The research also identified a strong candidate gene for MMVD, COL11A1, in a swept region on CFA6. An overrepresented COL11A1 haplotype in the CKCS harbouring a splice region variant was described.

Selective sweeps are a reduction in genetic diversity surrounding a beneficial or desirable mutation and are indicative of positive selection. Hitchhiking alleles are those in the vicinity of favourable variants that inadvertently undergo a shift in allelic frequency alongside selected variants ${ }^{86}$. Kennel clubs set out breed standards for the registration of purebred dogs. Genes
that govern phenotypic traits that meet breed standards likely to be under positive selection. Selection for a small size in dogs is predicted to have resulted in the hitchhiking of variants associated with MMVD ${ }^{4}$. As such, the incidence of ROHs in genomic regions harbouring genes associated with breed defining traits were assessed in the CKCS to infer a basis for the observed ROH. The CKCS showed limited evidence of selective sweeps in genomic regions of genes associated with traits under selection in the breed. This is possibly influenced by quantitative loci governing architype traits, like size ${ }^{65}$, or because heterogeneity within some breed standard traits, like coat colour exist ${ }^{87}$. Similarly, LD surrounding loci historically under selection might have since been broken down by recombination so that they were too small to be to be detect using parameters specified in this study ${ }^{88,89}$. Still, the ROH overlapping the FGF4 retrogene had a high frequency in the CKCS cohorts (>99.99\%) and the retrogene is expected to be fixed in the CKCS. This is consistent with previous research where the FGF4 retrogene on CFA12 was consistently observed in all CKCS samples ${ }^{90,91}$. The genomic region containing the FGF4 retrogene has also been associated with large pendulous ears ${ }^{69}$, another key trait of the CKCS. Though no variants have been implicated, it is possible that the FGF4 retrogene also contributes to this trait ${ }^{92}$.

Two functional retrogenes derived from FGF4 are recognised in the dog genome ${ }^{45,67}$. Both retrogenes contribute to the overexpression of FGF4, resulting in altered limb length ${ }^{21,70,90}$. At CFA12, the FGF4 retrogene is also associated with premature intervertebral disc degeneration ${ }^{21,70,93}$. Notably, the highest incidences of MMVD occurs in chondrodystrophic dog breeds ${ }^{2,3,5}$. Both the CKCS and Dachshund, a considerably chondrodystrophic dog, are similarly predisposed to an early onset form of MMVD ${ }^{94,95}$. FGF signalling plays various roles in heart development and disease ${ }^{96,97}$. In studies focusing on chicken valvulogenesis, FGF4 signalling is proposed to play a role in cardiac valve development ${ }^{98,99}$. Overexpression of FGF4 can result in precocious expansion of valvular leaflets during formation ${ }^{99}$ and is also expected to influence gene expression profiles of the extracellular matrix ${ }^{98}$. Remarkably, even in young healthy CKCS, mitral valve morphology differs from that of other healthy dogs but is characteristically similar to dogs affected by MMVD ${ }^{42,100}$. Given the overwhelming prevalence of MMVD in the CKCS and results indicative of fixation of the FGF4 retrogene in this breed, future research should evaluate the effects of the FGF4 retrogene on mitral valve morphology. Given a role of FGF4 in valvulogenesis and results indicating the FGF4 retrogene is fixed in this breed, this research might suggest that valvular dysfunction predisposing the CKCS to MMVD is apparent from birth.

The size of ROH can suggest the timing of selection with longer tracts indicating a recent selective pressure ${ }^{26,27}$. Canine research suggests deleterious variants accumulate in long runs of homozygosity ${ }^{101}$. The longest ROH observed in the CKCS was on CFA6 ( 6764 kb ), implying recent selection in the CKCS. The extensive length of this run makes it a strong candidate locus for deleterious variants contributing to disease pathogenesis. The ROH on CFA6 had no clear candidate genes under positive selection in the CKCS but encompassed two genes, VCAM1 and

S1PR1, that had the most overlap with GOterms previously implicated with MMVD and that are variably expressed in MMVD pathogenesis ${ }^{71-74}$. VCAM1 is a vascular adhesion molecule that has increased expression with advancing stages of MMVD and in CKCS compared to healthy dogs ${ }^{73,74}$. Recent investigation of VCAM1 suggests expression is marked in both inflamed and degenerative valves ${ }^{102-105}$ and it has been predicted to play a role in the pathogenesis of mitral regurgitation ${ }^{106}$. S1PR1 is a G protein-coupled receptor that protects the heart during the pathogenesis of disease ${ }^{107-109}$. Recent research also proposes a role of S1PR1 signalling in attenuating valvular damage in rheumatic heart disease studies ${ }^{107,110,111}$. It is probable that an increased expression of VCAM1 and S1PR1 is a consequence of MMVD rather than causative and requires further investigation.

Collagens make up a considerable component of the mitral valve extracellular matrix (ECM) ${ }^{112}$. As MMVD progresses, it is characterised by the disorganisation of the ECM, including a buildup of excess proteoglycans and altered collagen ${ }^{113-116}$. In human MMVD, the disease can occur as an individual ailment (namely Barlow's disease) or be part of a larger connective tissue syndrome ${ }^{117-119}$. The high prevalence of MMVD, as well as Chiari-like malformation in the CKCS, may lend evidence to a global connective tissue disorder in the breed. COL11A1 was identified in a swept region on CFA6. No rare variants for COL11A1 were observed in the CKCS here or in a previous candidate gene approach ${ }^{84}$. Both papers used relatively strict cut-offs for variant filtration and operated under the assumption that genes implicated in MMVD in the breed would occur at a low frequency in external breeds. Still, within the study population, an overrepresented haplotype was identified with a concordant splice region variant, CFA6:g.47507204C>T. In humans, mutations in COL11A1 can result in the rare connective tissue disorders, Stickler syndrome and Marshall syndrome. Both disorders are identifiable through ocular, craniofacial and joint anomalies, but can include a series of other clinical phenotypes including; hearing loss, spinal abnormalities, short stature, and mitral valve prolapse ${ }^{120-122}$. However, the prevalence of mitral valve prolapse in individuals with these syndromes is disputed ${ }^{123,124}$. Mutations in ECM genes are generally expected to follow Mendelian inheritance but evidence exists that collective effects of multiple less-damaging mutations can influence disease risk, fitting with polygenic inheritance ${ }^{125}$. In the CKCS, a rare missense variant in COL5A1 also exists ${ }^{84}$. Transcriptomic analysis of the of the COL11A1 gene could be used to test the effects of predicted splice site variants.

Two UTR variants in genes Exostosin Like Glycosyltransferase 2 (EXTL2) and Netrin-G1 (NTNG1) were identified with a high incidence in the CKCS and low frequency in other dogs. Although UTR variants are not expected to change the predicted sequence of a protein, it is possible for variants of this type to impact gene expression and disease pathogenesis ${ }^{126}$. Still, neither of the rare gene variants observed in this study represent strong candidates for MMVD and currently gene expression studies do not support differential gene expression in dogs with MMVD ${ }^{71-74}$. Constraints used for the definition of rare alleles in this study were stringent. The analysis was conducted under the assumption that the CKCS is likely harbouring a high impact
mutation contributing to the breeds overall susceptibility. For this reason, variants were filtered using a minor allele frequency of 0.05 . Given the overall heterogeneity of the disease and its complex inheritance, it is possible that dogs in the control population share functional alleles at a higher frequency than expected and were filtered out.

Although the ROH hotspots presented here are assumed to be driven by positive selection, most observed signals show no clear link to underlying biological mechanisms. It is possible that complex traits, like behaviour, with an unobvious phenotypic expression that are desirable and breed-typical contribute to these ROH hotspots ${ }^{127-129}$. ROH hotspots may also be driven by population bottlenecks, repressed recombination, or artefacts caused by copy number variants or SNV gaps ${ }^{130,131}$. The optimisation process used in this study was designed to minimise gap effects ${ }^{56}$. Within a canine population, autozygosity levels may also be driven by popular sire effects ${ }^{132}$, although the CKCS breeding population is at low risk due to its large breeding registry ${ }^{44}$. The initial and validation CKCS cohorts shared similar results for size and length of ROH as well as overall inbreeding coefficients. The inbreeding coefficient for autosomal length ( $\mathrm{FROH}_{\text {aut }}$ ) and length of the covered genome ( $\mathrm{FROH}_{\text {cov }}$ ) were highly correlated in this study. A positive reflection of marker density on the Illumina CanineHD BeadChip genotyping array and a good indication of its suitability to study ROH. Overall, the inbreeding coefficient, ~0.37, for the CKCS is on the high end of values reported in other breeds ${ }^{45,101}$, consistent with comparatively high levels of within-breed LD ${ }^{16}$.

Clinical signs of MMVD are universally observed in the CKCS and causative genetic polymorphisms are expected to be fixed within the breed. The present study identified and validated six genomic regions swept in the CKCS population. Within the swept regions, plausible genes involved in MMVD were documented. A promising variant, the FGF4 retrogene, was identified in a swept region on CFA 12 and is predicted to be fixed in the CKCS population. Among other traits, the FGF4 retrogene is documented as having a significant effect on the size of dogs. Given the prevalence of MMVD in small breeds and a role of FGF4 in valvulogenesis, the current study may provide preliminary evidence for a role of the FGF4 retrogene in the development of MMVD.

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## Chapter 6. Concluding remarks

The overall aim of this thesis was to improve the knowledge gap in the genetic basis of canine myxomatous mitral valve disease (MMVD) using an Australian Cavalier King Charles Spaniel (CKCS) population as a case study and genetic model for disease research. MMVD represents the most frequent cause of cardiovascular morbidity and mortality in the canine population ${ }^{1-}$ ${ }^{3}$, making it a prominent welfare issue and concern within the canine community. MMVD is an acquired disease that results in valvular dysfunction and in the most severe cases, can result in the development of congestive heart failure (CHF) ${ }^{4,5}$. Despite a comprehensive understanding of the clinical aspects of $M M V D^{5}$, information on the genetic mechanisms that drive disease onset and progression are significantly lacking. Heightened within breed prevalence of MMVD, particularly in small breeds, is evidence of a genetic basis for disease ${ }^{3,6-9}$ and suggests that breeding programs could help reduce the presence and severity of disease. However, current genomic research supports a polygenic mode of inheritance and exemplifies the difficulty in identifying disease risk variants in complex traits ${ }^{10-15}$. No genetic tests have been developed to detect increased risk for the development of MMVD or dogs at-risk for early onset and severe forms of the disease. Throughout this research, genomic workflows were used to investigate MMVD in the CKCS, but I trust the approaches used would be easily applied to other breeds as well as a multi-breed cohort. For this project, I first assessed the strength of MMVD phenotypes for use in comparative genomic studies. Then, through bioinformatic approaches, I explored multiple questions that can be answered with a single genomic dataset and access to publicly available data.

Comparative genetic studies offer a major opportunity to highlight the genetic basis of disease, but common genetic approaches rely on accurate phenotyping of disease and access to approximately equal sizes of cases and controls ${ }^{16}$. Two significant roadblocks exist for mapping loci implicated in the pathogenesis of MMVD. One, the exact prevalence of MMVD in certain breeds is not comprehensively evaluated and it is predicted that geriatric dogs from all breeds show clinical signs of MMVD on post-mortem ${ }^{17}$. Two, there is significant heterogeneity in the rate of disease progression ${ }^{18-20}$. For these reasons collection of a perfect control cohort is near impossible and a traditional case-control approach for studying the genetic basis of disease is difficult. Death sequalae to MMVD is typically mediated by CHF ${ }^{19}$. Frequently, dogs with MMVD live long lives such that they never develop CHF and die from unrelated causes ${ }^{18-20}$. For MMVD, which negatively impacts dog longevity, it is logical to modify the focus of genetic research from identifying genetic causation to identifying genetic factors that influence the rate of disease progression and the development of CHF. Identification of risk-loci and variants associated with cardiomegaly and CHF can enable the selection of breeding stock to increase the proportion of animals that can live long asymptomatic lives.

### 6.1 Conclusions derived from Chapter 2

In Chapter 2, I sought to refine the phenotypes used for association mapping throughout this thesis. Over the course of four years, CKCS anthropometric and heart condition data was collected for the same population considered for genetic analysis. At this time, age, sex, weight, and echocardiographic measures LA/Ao and LVIDdn were recorded for each animal alongside a severity score according to the ACVIM system ${ }^{21}$. Using logistic and linear regression analysis, I evaluated the use of echocardiographic variables, LA/Ao and LVIDdn, as predictors of CHF and MMVD severity. As expected, Chapter 2 demonstrated the utility of prognostic variables, LA/Ao and LVIDdn, in phenotyping disease severity and predicting CHF. This research supports the use of echocardiographic measures as a continuous variable trait for association analysis. Additionally, through this research, I was also able to suggest a predictive threshold for the development of CHF using LA/AO and LVIDdn, which could be applied to a case-control mapping study. Unsurprisingly, this research also found that age was a significant predictor of disease severity. For this reason, we chose to include older dogs in our association analyses and apply age as a covariate for quantitative traits LA/Ao and LVIDdn.

### 6.2 Conclusions derived from Chapter 3

Despite extensive literature available regarding pathological, cellular and molecular features of MMVD, the exact mechanism and a genetic basis for the disease is unknown. Previous studies have highlighted important aetiological processes and pathways likely involved in disease pathogenesis ${ }^{22-26}$. The aim of Chapter 3 was to investigate an association between severe MMVD phenotypes (validated in Chapter 2) and candidate genes elected using knowledge of signalling pathways identified by MMVD gene and protein expression studies. I used the Illumina Canine HD array and whole genome sequenced data to identify genomic regions of disease association across a subset of samples included in Chapter 2. To prioritise genes within candidate pathways, this research utilised the publicly available Kyoto Encyclopedia of Genes and Genomes Pathway database. Candidate genes included in this approach did not rely on knowledge of genes previously associated with the same phenotype. Candidate genes from four pathways were included in this Chapter: transforming growth factor Beta (TGF- $\beta$ ), serotonergic signalling, Extracellular matrix-receptor interaction and calcium signalling pathways.

In Chapter 3, a single marker within the vicinity of serotonergic signalling gene GNG7, passed the genome-wide significant threshold for association with LA/Ao. GNG7 has not previously been associated with cardiovascular disease and no evidence for variable expression of this gene in canine MMVD exists. We were unable to identify any genetic causation in GNG7. Common array markers were significantly associated with LA/Ao again in Chapter 4, where a novel variant in a candidate gene outside of the considered pathways, LMNB2, contained a putative functional variant.

### 6.3 Conclusions derived from Chapter 4

Continuing development of genetic resources, like the canine HD array, which now holds greater than 200K genotype markers, has dramatically expanded the capacity for researchers to move beyond candidate gene approaches towards hypothesis free investigations. For this reason, using the same genetic data from my previous investigation (Chapter 3) in Chapter 4, I attempted to identify loci associated with severe MMVD on a genome-wide basis. GWAS have resulted in a deluge of discoveries by means of genetic risk factors for disease as well as morphological traits ${ }^{27-31}$. In the modern dog, population history that established pedigreed standards and well-defined breeds have resulted in populations suitable for association analyses ${ }^{32-34}$. Arguably, GWAS are strongly suited to studying the genetic basis of simply inherited traits. Still, mapping complex traits in dogs is promising with considerable evidence of large effect loci governing complex traits ${ }^{30,31,35-38}$. For dogs affected by MMVD in general, but especially in the CKCS, it could be argued that a causative disease variant is fixed within the genome and that modifier loci are responsible for the heterogeneity of disease outcomes. Using the phenotypes validated in Chapter 2, Chapter 4 focused on identifying loci with the potential to exacerbate features of MMVD and increase risk of premature mortality.

In Chapter 4, I reported five loci on chromosomes 1, 13, 14, 20, and 24 associated with MMVD disease severity. Strong, positional candidate genes for severe forms of MMVD were observed within the associated loci including OBSCN, LMNB2, SULF2 and ADAMTS3. Two of the candidate genes, OBSCN and LMNB2, harboured putative functional variants. The most convincing variant observed in the context of this research was in the OBSCN gene, which was significantly associated with progression of MMVD to CHF. In humans OBSCN has been associated with multiple cardiomyopathies and is expected to play a prominent regulatory role in heart function, particularly in calcium signalling ${ }^{39,40}$. A second gene with a putative functional variant, LMNB2, was associated with cardiac remodelling using quantitative measure LA/Ao. While limited evidence exists for a role of LMNB2 in cardiac remodelling, it was recently suggested to play a role in cardiomyocyte regeneration ${ }^{41}$. The identification of genes involved in cardiac signalling, regeneration and the pathogenesis of cardiomyopathies supports research claiming CKCS experience disrupted calcium signalling and cardiac muscle contraction ${ }^{42}$. Deranged signalling and regenerative capacity of cardiac tissue in CKCS with MMVD might precede the development of CHF and influence the rate of disease progression. Putative variants identified in this chapter should be validated in a larger MMVD cohort. For MMVD which is frequently observed in multiple breeds, validation analyses would benefit from CKCS and across-breed validations ${ }^{16,43}$.

Multiple GWAS for early onset and severe forms of MMVD have been published ${ }^{10-12,14,15}$. However, the study presented in Chapter 4 is the only research to date that has reported putative functional variants at associated loci. Previous attempts at mapping MMVD have used niche phenotyping systems, often with arbitrary cut-offs for case-control analyses. Arguably, the use of echocardiographic measures that are frequently reported in the clinical detection
of MMVD, limits the subjectivity in disease modelling and makes phenotyping more accurate and repeatable. It's likely that the success of the GWAS analyses conducted in Chapter 4 was the use of echocardiographic parameters to phenotype MMVD. Phenotyping samples using echocardiographic measures of cardiac remodelling has the added benefit of being able to combine MMVD datasets if future researchers report in a consistent manner. Group collaboration and the combination of many test subjects has resulted in successful mapping of complex traits in human research and sets a solid framework for investigating complex disease in dog ${ }^{44-49}$.

### 6.4 Conclusions derived from Chapter 5

Identification of autozygous genomic regions can provide insights into genetic variation that underlies breed-specific traits or diseases and is a particularly useful approach when traits are fixed ${ }^{37,38,50-54}$. As a breed, the CKCS is ubiquitously affected by MMVD and causative variants for the disease are expected to be fixed ${ }^{55-60}$. This is a prominent barrier in detecting a genetic basis for MMVD, as common genetic approaches (like GWAS) require access to phenotypically diverse samples to identify loci associated with a specific trait. As such the association analyses from previously discussed chapters, Chapter 3 and Chapter 4, focused on severe forms of MMVD including CHF and increasing measures of cardiac remodelling. Alternatively, the research conducted in Chapter 5 sought to determine genomic regions that have a high level of fixation in the CKCS, through a runs of homozygosity analysis. Within swept regions of the CKCS genome we attempted to locate candidate genes implicated in the development of MMVD. To account for regions that have become fixed in our Australian population, by processes like popular sire effects or limited access to extensive pedigrees based on physical distance, we chose to include a second population of CKCS with genotype data available in the public repertoire as a validation cohort. Six autozygous regions were captured consistently across both cohorts.

MMVD can be observed in geriatric dogs of all breeds, although specific breeds have a heightened prevalence ${ }^{5,17,59}$. MMVD is the frequently observed in small dogs, leading researchers to hypothesise that the genetic basis of MMVD has hitchhiked alongside genes that govern size ${ }^{61}$. In Chapter 5, the research focused identifying variants within CKCS ROH that were common to small breeds. This was conducted in two steps. First, I observed ROH overlapping genomic regions with variants previously implicated in canine size morphology. Second, we filtered rare variants with putative functional effects across our six autozygous regions and estimated their frequency in small dogs compared to larger breeds using data from the publicly available 722 dog consortium variant call file. No rare variants observed in this study had a high frequency in small breeds compared to large breeds. But the genomic region harbouring the FGF4-retrogene on CFA12 was fixed across all CKCS samples. FGF4 functional retrogenes are common in small dogs and the gene has been linked to valvulogenesis ${ }^{62-64}$.

Results of this method might imply that the FGF4-retrogenes contribute to MMVD pathogenesis.

Canine specific research has indicated that long runs of homozygosity disproportionately harbour deleterious variants compared to short tracts ${ }^{54}$. In Chapter 5, I also found an extensive ROH on CFA6, approximately four times greater than the second longest ROH. Observed within this ROH was a strong candidate gene for the development of MMVD, COL11A1. This gene has been previously implicated in connective tissue disorders in humans, such as Ehlers-Danlos Syndrome and Stickler Syndrome ${ }^{65-67}$, that can present with similar phenotypes to diseases observed in the CKCS, like MMVD and Chiari-like malformation. Three splice region variants were identified in COL11A1. None of the reported variants were observed at a low frequency when compared with 722 dog consortium. Still, it is possible that COL11A1 plays a role in CKCS connective tissue disease phenotypes.

### 6.5 Final remarks

Bioinformatic workflows are ever growing and increasingly becoming more important in everyday research. As the scientific world advances, improved sequencing technologies and computational power have allowed greater access to high quality 'omic datasets, especially for non-model species. This has expanded the scope of genetic research available to animal geneticists and increased research opportunities. The work presented in this thesis took advantage of the domestic history of modern dogs that resulted in unique population structures and patterns of genome organisation that make the canine genome particularly amenable to genetic research. In studying the genetic basis of MMVD, I was able to demonstrate the value of this species in investigating complex disease.

The work produced within this thesis represents the first time precise and validated phenotypic definitions of MMVD have been described and applied to the genomic analysis of this disease. Using validated methodologies, it was possible to produce results and conclusions that significantly add to the understanding of the genetics and genomics of MMVD in the CKCS. The main features of this thesis include; the validation of two echocardiographic measures as predictors of MMVD progression and CHF in CKCS; testing for association in novel candidate genes in biologically relevant gene pathways; a GWAS resulting in the identification of five chromosomal loci associated with the disease state, where putative functional variants were reported in two phenotypically relevant genes; and the analysis for signatures of selection via runs of homozygosity resulting in the identification of candidate genes for MMVD pathogenesis. This thesis outlines a process that can be applied to the trait in future research and ideally to other breeds. A prominent outcome of this research includes the identification of putative functional variants with a predicted pathogenic effect in two genes, OBSCN and LMNB2, with phenotypic relevance to the disease. Finally, it has been long hypothesised that the development of MMVD was selected for alongside small size in dogs. Using genomic signatures of selection, we were able to identify a long run of homozygosity surrounding the

FGF4-retrogene on CFA12, a gene associated with small stature in dogs that has also been implicated with a role in valvulogenesis. It is possible that this retrogene, with clear evidence for selection in the CKCS, may be involved in the pathogenesis of MMVD in this breed and other breeds with a high incidence of MMVD and selection for this retrogene, such as the Dachshund

In addition to the discoveries relating to MMVD in the CKCS, during my candidature I also applied strategies developed in this thesis to map a disease variant in a second dog breed that published in scientific reports (Appendix III). In the manuscript titled 'A large deletion on CFA28 omitting ACSL5 gene is associated with intestinal lipid malabsorption in the Australian Kelpie dog breed' a GWAS was applied to investigate intestinal lipid malabsorption in a population of Australian Kelpies (AK). At the top associated locus a 103.3 kb deletion (NC_006610.3CFA28:g.23380074_23483377del), containing genes Acyl-CoA Synthetase Long Chain Family Member 5 (ACSL5) and Zinc Finger DHHC-Type Containing 6 (ZDHHC6) was identified through whole transcriptomic analysis of an affected individual. A PCR-based diagnostic test was developed to validate the variant in an extended cohort of AK and is now a commercially available diagnostic test.

To summarise, the research conducted throughout this thesis has demonstrated the utility of genomic tools developed for the domestic dog in investigating complex traits. By utilising the same genomic dataset for Chapters 3 to 5 , my research has exemplified the capacity to answer numerous research questions using multiple bioinformatic approaches. Finally, my research was frequently accompanied by data available in the public repertoire, demonstrating the importance of shared resources in improving research outcomes.

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## Appendix I: Supplementary data for chapter 4

Table S1. Variants passing the genome-wide significant threshold for association with three MMVD phenotypes including Congestive heart failure (CHF), Left atrium to aortic root ration (LA/Ao), weight normalised Left ventricular end diastolic diameter (LVIDdn)

| Model | CHR | POS | Array Marker | A1 | A2 | Putative Function | Gene | Gene Prediction | Amino <br> Acid <br> Change | MAF | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CHF | 14 | 669043 | BICF2P757489 | G | C | missense | OBSCN |  | G/R | 0.29 | $1.17 \mathrm{E}-08$ |
| CHF | 14 | 685005 | BICF2P813381 | T | C | intron | OBSCN | E,N | - | 0.28 | $9.14 \mathrm{E}-08$ |
| CHF | 14 | 581822 | BICF2P1196270 | G | A |  |  |  |  | 0.31 | $1.43 \mathrm{E}-07$ |
| CHF | 14 | 606955 | BICF2P1391846 |  | A |  |  |  |  | 0.31 | $1.43 \mathrm{E}-07$ |
| CHF | 14 | 628308 | BICF2P386682 | C | T |  |  |  |  | 0.31 | $1.43 \mathrm{E}-07$ |
| CHF | 14 | 786206 | BICF2P289847 |  | G |  |  |  |  | 0.31 | $1.43 \mathrm{E}-07$ |
| CHF | 14 | 5559055 | BICF2S2364353 |  | G |  |  |  |  | 0.28 | $2.82 \mathrm{E}-07$ |
| CHF | 14 | 2309870 | BICF2S2351604 | C | T |  |  |  |  | 0.31 | $2.98 \mathrm{E}-07$ |
| CHF | 14 | 2934833 | chr14_2934833 | T | C |  |  |  |  | 0.31 | $2.98 \mathrm{E}-07$ |
| CHF | 14 | 2948414 | BICF2G6305178 |  | T |  |  |  |  | 0.31 | $2.98 \mathrm{E}-07$ |
| CHF | 14 | 2467305 | BICF2S2291714 | T | C | 3'UTR | OR2T4C | N | - | 0.31 | $3.08 \mathrm{E}-07$ |
| CHF | 14 | 5167461 | BICF2P1095320 | G | A |  |  |  |  | 0.29 | $3.30 \mathrm{E}-07$ |
| CHF | 14 | 2990449 | BICF2G6305178 |  | A |  |  |  |  | 0.31 | $3.98 \mathrm{E}-07$ |
| CHF | 14 | 5444436 | G814f50S305 |  | G |  |  |  |  | 0.29 | $4.20 \mathrm{E}-07$ |
| CHF | 14 | 2500989 | BICF2G6305176 |  | T |  |  |  |  | 0.31 | $4.35 \mathrm{E}-07$ |
| CHF | 14 | 645047 | BICF2P693197 | T | C | intron | TRIM17 | E | - | 0.33 | $4.44 \mathrm{E}-07$ |
| CHF | 14 | 2819625 | chr14_2819625 | C | T |  |  |  |  | 0.32 | $6.57 \mathrm{E}-07$ |
| CHF | 14 | 2717089 | BICF2G6305177 |  | C |  |  |  |  | 0.31 | $6.83 \mathrm{E}-07$ |
| CHF | 14 | 5342684 | BICF2S2291087 |  | G |  |  |  |  | 0.29 | 7.08E-07 |
| CHF | 14 | 6830641 | BICF2G6305194 |  | A |  |  |  |  | 0.31 | $8.78 \mathrm{E}-07$ |
| CHF | 14 | 6844213 | BICF2P271536 | T | C | intron | ZC3HC1 | E,N | - | 0.31 | $8.78 \mathrm{E}-07$ |
| CHF | 14 | 904357 | BICF2G6305171 |  | C |  |  |  |  | 0.31 | $1.34 \mathrm{E}-06$ |
| CHF | 14 | 2354354 | BICF2G6305175 | C | T |  |  |  |  | 0.33 | $1.73 \mathrm{E}-06$ |
| CHF | 14 | 6668504 | BICF2G6305194 | G | A | intron | SSMEM1 | N | - | 0.30 | $1.89 \mathrm{E}-06$ |
| CHF | 14 | 1626211 | chr14_1626211 |  | G |  |  |  |  | 0.31 | $1.95 \mathrm{E}-06$ |
| CHF | 14 | 1709234 | chr14_1709234 |  | G |  |  |  |  | 0.31 | $1.95 \mathrm{E}-06$ |
| CHF | 14 | 1760541 | chr14_1760541 | C | A |  |  |  |  | 0.31 | $1.95 \mathrm{E}-06$ |
| CHF | 14 | 1797074 | chr14_1797074 | G | T |  |  |  |  | 0.31 | $1.95 \mathrm{E}-06$ |
| CHF | 14 | 1857818 | BICF2S2324424 |  | G |  |  |  |  | 0.31 | $1.95 \mathrm{E}-06$ |
| CHF | 14 | 4866764 | BICF2P84129 | C | G | intron | PLXNA4 | E,N | - | 0.31 | $3.47 \mathrm{E}-06$ |
| CHF | 14 | 2562311 | BICF2S2342169 | C | T |  |  |  |  | 0.30 | 4.04E-06 |
| CHF | 14 | 2802415 | BICF2G6305177 | C | T |  |  |  |  | 0.45 | 4.23E-06 |
| CHF | 14 | 4675587 | BICF2G6305190 |  | A |  |  |  |  | 0.31 | 4.30E-06 |
| CHF | 14 | 4685857 | BICF2G6305190 | A | G | intron | PLXNA4 | E,N | - | 0.31 | 4.30E-06 |
| CHF | 14 | 4757486 | BICF2P1409592 | T | C | intron | PLXNA4 | E,N | - | 0.31 | 4.30E-06 |
| CHF | 14 | 4845378 | BICF2P752059 |  | C |  |  |  |  | 0.31 | 4.30E-06 |
| CHF | 14 | 4889665 | BICF2P942451 | A | T | intron | PLXNA4 | E,N | - | 0.31 | 4.30E-06 |
| CHF | 14 | 5017714 | BICF2P459975 | T | C | intron | PLXNA4 | E,N | - | 0.31 | 4.30E-06 |


| CHF | 14 | 2783652 | BICF2G6305177 | T | G |  |  |  |  | 0.45 | 4.77E-06 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CHF | 14 | 2621160 | BICF2P830830 | A | G |  |  |  |  | 0.45 | 5.27E-06 |
| CHF | 14 | 2698552 | BICF2G6305177 | A | T |  |  |  |  | 0.45 | $5.41 \mathrm{E}-06$ |
| CHF | 14 | 2185062 | BICF2P90189 | C | T |  |  |  |  | 0.35 | 5.82E-06 |
| CHF | 14 | 2191219 | BICF2G6305173 | T | G |  |  |  |  | 0.35 | 5.82E-06 |
| CHF | 14 | 2213041 | BICF2G6305173 | A | G |  |  |  |  | 0.35 | $5.82 \mathrm{E}-06$ |
| CHF | 14 | 2224605 | BICF2G6305173 | T | C |  |  |  |  | 0.35 | $5.82 \mathrm{E}-06$ |
| CHF | 14 | 2248006 | BICF2G6305174 | T | C |  |  |  |  | 0.35 | 5.82E-06 |
| CHF | 14 | 2263592 | BICF2G6305174 | A | G |  |  |  |  | 0.35 | 5.82E-06 |
| CHF | 14 | 2291305 | BICF2G6305174 | G | A |  |  |  |  | 0.35 | 5.82E-06 |
| CHF | 14 | 5615896 | BICF2P381585 | G | T | intron | PODXL | E, U | - | 0.34 | 6.14E-06 |
| CHF | 14 | 5029359 | BICF2P514405 | A | G | intron | PLXNA4 | E,N | - | 0.34 | 7.71E-06 |
| CHF | 14 | 4621355 | BICF2P681553 | T | C |  |  |  |  | 0.31 | 8.07E-06 |
| CHF | 14 | 4906217 | BICF2G6305191 | G | A | intron | PLXNA4 | E,N | - | 0.31 | 8.07E-06 |
| CHF | 14 | 2393780 | BICF2S2342856 | G | A |  |  |  |  | 0.45 | 1.07E-05 |
| CHF | 14 | 4643701 | BICF2G6305189 | T | C |  |  |  |  | 0.28 | $1.11 \mathrm{E}-05$ |
| CHF | 14 | 2643986 | BICF2S2365948 | G | A |  |  |  |  | 0.45 | 1.21E-05 |
| LA/Ao | 24 | 34932842 | BICF2P912253 | C | T |  |  |  |  | 0.31 | $1.22 \mathrm{E}-05$ |
| CHF | 20 | 58023254 | BICF2P360101 | T | C |  |  |  |  | 0.49 | $1.31 \mathrm{E}-05$ |
| LA/Ao | 20 | 56661518 | chr20_5666151 | T | C | missense | LMNB2 | E,N | S/L | 0.10 | $1.71 \mathrm{E}-05$ |
| CHF | 14 | 2270530 | BICF2G6305174 | A | G |  |  |  |  | 0.31 | 2.02E-05 |
| LA/Ao | 20 | 56483566 | BICF2P866985 | A | G | intron | GNG7 | E,N | - | 0.13 | $2.17 \mathrm{E}-05$ |
| LA/Ao | 24 | 34869455 | BICF2P1268671 | C | A |  |  |  |  | 0.30 | 2.30E-05 |
| LVIDdn | 13 | 61496628 | BICF2P555379 | T | C | intron | ADAMTS3 | E,N | - | 0.24 | $2.51 \mathrm{E}-05$ |
| LA/Ao | 24 | 34752654 | TIGRP2P317522 | C | T | synonymous | SULF2 | E,N,U | T/T | 0.29 | $2.62 \mathrm{E}-05$ |
| LA/Ao | 24 | 34775608 | TIGRP2P317533 | T | C | intron | SULF2 | E,N,U | - | 0.29 | $2.62 \mathrm{E}-05$ |
| LA/Ao | 24 | 34790784 | BICF2P132951 | T | C | intron | SULF2 | E,N,U | - | 0.29 | $2.62 \mathrm{E}-05$ |
| LA/Ao | 24 | 34816494 | BICF2P494910 | T | A | intron | SULF2 | E,N,U | - | 0.29 | $2.62 \mathrm{E}-05$ |
| CHF | 14 | 2339529 | chr14_2339529 | A | C |  |  |  |  | 0.44 | $2.93 \mathrm{E}-05$ |
| CHF | 14 | 5830017 | BICF2P850224 | A | C |  |  |  |  | 0.47 | 3.30E-05 |
| CHF | 1 | 119250918 | BICF2S2293194 | A | T | intron | TDRD12 | E,N | - | 0.48 | 3.64E-05 |
| CHF | 14 | 2665383 | BICF2G6305177 | C | T |  |  |  |  | 0.50 | 3.85E-05 |
| LA/Ao | 24 | 35206390 | TIGRP2P318119 | G | A |  |  |  |  | 0.35 | 5.22E-05 |

Table S2. Variants in olfactory receptor genes that match CHF-risk haplotypes. Variant annotation was conducted using Variant Effect Predictor (VEP) and Variant Annotation Integrator (VAI). Programs predict functional consequences based on transcript annotations from multiple sources. Transcript annotations used to predict functional consequences include Ensemble (E), National Center for Biotechnology information (N), and University of California Santa Cruz (U) and are reported under variant source.

| Variant ID | CHR | POS | Allele | Gene |   Amino <br> Futative <br> acid   | Variant Source |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| rs851214998 | 14 | 599485 | T | LOC1006836 | XM_0034318 synonymous_ D/D | N |
| chr14_60406: | 14 | 604062 | T | LOC482202 | XM_539321. synonymous V/V | N |
| rs851936980 | 14 | 604149 | T | LOC482202 | XM_539321. ${ }^{\text {synonymous_ K/K }}$ | N |
| rs850983105 | 14 | 1572608 | A | OR2W3 | XM_0034318 synonymous_ N/N | E,N |
| chr14_16951، | 14 | 1695143 | C | LOC607634 | XM_0224271 synonymous_ R/R | N |
| rs850817693 | 14 | 1732158 | T | LOC1008567 | XM_0056287 missense_va H/Q | N |
| rs852293968 | 14 | 1732579 | T | LOC10085 | XM_0056287 missense_va G/D | N |
| rs851880642 | 14 | 1897064 | T | COR2AV2 | XM_844479. missense_va L/F | N |
| rs852157133 | 14 | 1994994 | C | LOC1006853 | XM_0034318 missense_va M/T | N |
| rs850942138 | 14 | 1994995 | A | LOC1006853: | XM_0034318 missense_va M/I | N |
| rs853103865 | 14 | 2127908 | T | OR2T15 | XM_539347.: missense_va C/F | , N |
| rs852131491 | 14 | 2181384 | A | OR2L13 | ENSCAFT000 missense_va R/L | E |
| rs853046839 | 14 | 2222559 | C | OR2T22 | XM_0224271 synonymous I/I | , N |
| rs850534321 | 14 | 2223104 | G | OR2T22 | XM_0224271 missense_va $\mathrm{Q} / \mathrm{R}$ | E,N |
| rs850807966 | 14 | 2351787 | A | OR2M9 | XM_539353.: synonymous_ P/P | E,N |
| rs852221250 | 14 | 2352400 | A | OR2M9 | XM_539353.: missense_va T/l | E, N |
| rs850747725 | 14 | 2352472 | G | OR2M9 | XM_539353.: missense_va V/A | E,N |
| rs852016364 | 14 | 2385542 | A | LOC1065596 | XM_0141188 missense_va V/I | N |
| rs851506475 | 14 | 2466122 | A | OR2T4C | ENSCAFT000 start_lost M/I | E |
| rs22318561 | 14 | 2467305 | T | LOC482236 | XM_539355.! 3_prime_UT\| - | N |
| rs851029001 | 14 | 2595298 | C | OR2T6 | XM_844720.: synonymous C/C | E,N |
| rs851365258 | 14 | 2663514 | A | OR2T2 | XM_539359.: missense_va A/T | E,N |
| rs852892266 | 14 | 2663658 | G | OR2T2 | XM_539359.: missense_va N/D | E,N |
| rs851159810 | 14 | 2663665 | A | OR2T2 | XM_539359.: missense_va R/Q | E,N |
| rs851347718 | 14 | 2663666 | C | OR2T2 | XM_539359.: synonymous_ R/R | E,N |
| rs22309057 | 14 | 2695700 | A | OR2T11 | NM_001256، synonymous L/L | E,N, U |
| rs22309054 | 14 | 2696141 | C | OR2T11 | NM_001256، missense_va L/F | E,N,U |
| rs22302697 | 14 | 2712186 | G | LOC1006865 | XM_0034318 missense_va G/A | E,N |
| rs22302696 | 14 | 2712232 | A | OR2T4B | XM_0034318 synonymous L/L | E,N |
| rs22302695 | 14 | 2712357 | A | OR2T4B | XM_0034318 missense_va S/F | E, N |
| rs22302694 | 14 | 2712451 | C | OR2T4B | XM_0034318 missense_va T/A | E, N |
| rs852392222 | 14 | 2712844 | C | OR2T4B | XM_0034318 missense_va M/V | E,N |
| rs852675194 | 14 | 2712874 | T | OR2T4B | XM_0034318 missense_va V/M | E, N |
| rs852301949 | 14 | 2712988 | T | OR2T4B | XM_0034318 missense_va L/M | E, N |
| rs850771806 | 14 | 2763602 | T | OR2T4D | XM_539362.: missense_va $\mathrm{Q} / \mathrm{K}$ | E, N |
| rs851422085 | 14 | 2764295 | C | OR2T4D | XM_539362.: missense_va M/V | E,N |


| rs850709377 | 14 | 2764308 G | OR2T4D | XM_539362.: synonymous_ V/V | E,N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| rs853173325 | 14 | 2764325 T | OR2T4D | XM_539362.: missense_va V/M | E,N |
| rs850948801 | 14 | 2764440 A | OR2T4D | XM_539362.: synonymous_ F/F | E,N |
| rs852097145 | 14 | 2764497 G | OR2T4D | XM_539362.: missense_va E/D | E,N |
| rs850861730 | 14 | 2764513 C | OR2T4D | XM_539362.: missense_va E/G | E,N |
| rs853005857 | 14 | 2784661 A | OR1412 | XM_0034318 missense_va R/W | E,N |
| chr14_31064i | 14 | 3106483 G | LOC10655 | ( XR_0013176! splice_regior - | N |
| rs852500847 | 14 | 4736948 T | LOC11109 | : XR_0026328 splice_regior - | N |

Table S3. Coding variants matching CHF-risk haplotypes on CFA20. Variant annotation was conducted using Variant Effect Predictor (VEP) and Variant Annotation Integrator (VAI). Programs predict functional consequences based on transcript annotations from multiple sources. Transcript annotations used to predict functional consequences include Ensemble (E), National Center for Biotechnology information (N), and University of California Santa Cruz (U) and are reported under variant source.

|  |  |  |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Variant ID | CHR | POS | REF | ALT | Gene | Putative <br> Function | Amino <br> Acid <br> Change | SIFT | Variant |
| Source |  |  |  |  |  |  |  |  |  |

## Appendix II: Supplementary data for chapter 5

Table S1. Lists of Gene Ontology (GO) terms reported from transcriptomic profiling of canines

| GO:0009897 | external side of plasma membrane | The leaflet of the plasma membrane that faces away from the cytoplasm and any proteins embedded or anchored in it or attached to its surface. | cellular_component |
| :---: | :---: | :---: | :---: |
|  |  | The chemical reactions and pathways involving collagen, any of a group of fibrous proteins of very high tensile strength that form the main component of connective tissue in animals. Collagen is highly enriched in glycine |  |
|  | collagen metabolic process | (some regions are $33 \%$ glycine) and proline, occurring predominantly as 3-hydroxyproline (about 20\%). | biological process |
| GO:0032963 |  | The attachment of a cell, either to another cell or to an underlying substrate such as the extracellular matrix, via cell |  |
| GO:0007155 | cell adhesion | adhesion molecules. | biological_process |
|  |  | The region of the plasma membrane that includes the basal end and sides of the cell. Often used in reference to animal polarized epithelial membranes, where the basal |  |
|  | basolateral plasma membrane | membrane is the part attached to the extracellular matrix, or in plant cells, where the basal membrane is defined with respect to the zygotic axis. | cellular_component |
| GO:0016323 |  | Interacting selectively and non-covalently with a nucleotide, any compound consisting of a nucleoside that is esterified with (ortho)phosphate or an oligophosphate at any hydroxyl |  |
| GO:0000166 | nucleotide binding | group on the ribose or deoxyribose. | molecular_function |
|  | positive regulation of | Any process that activates or increases the activity of a |  |
| GO:0043547 | GTPase activity | GTPase. | biological_process |
|  |  | Catalysis of the hydrolysis of internal, alpha-peptide bonds |  |
|  |  | in a polypeptide chain by a mechanism in which water acts |  |
|  |  | as a nucleophile, one or two metal ions hold the water |  |
|  | metalloendopeptidas | molecule in place, and charged amino acid side chains are |  |
| GO:0004222 | e activity | ligands for the metal ions. | molecular_function |
|  | regulation of cellular senescence | Any process that modulates the frequency, rate or extent of cellular senescence. | biological_process |
| GO:2000772 |  | The component of the plasma membrane consisting of the gene products and protein complexes having at least some |  |
|  | integral component of | part of their peptide sequence embedded in the |  |
| GO:0005887 | plasma membrane | hydrophobic region of the membrane. | cellular_component |
|  |  | The directed movement of endocytosed material through the cell and its exocytosis from the plasma membrane at |  |
| GO:0045056 | transcytosis | the opposite side. | biological_process |
|  |  | Interacting selectively and non-covalently with calcium ions |  |
| GO:0005509 | calcium ion binding | (Ca2+). | molecular_function |
|  | regulation of cell | Any process that modulates the frequency, rate or extent of |  |
| GO:0030334 | migration | cell migration. | biological_process |



| GO:0001666 | response to hypoxia | cellular and organismal level. | biological_process |
| :---: | :---: | :---: | :---: |
|  |  | The immediate defensive reaction (by vertebrate tissue) to infection or injury caused by chemical or physical agents. |  |
|  |  | The process is characterized by local vasodilation, |  |
|  | inflammatory | extravasation of plasma into intercellular spaces and |  |
| GO:0006954 | response | accumulation of white blood cells and macrophages. | biological_process |
|  | cytosolic large |  |  |
| GO:0022625 | ribosomal subunit | The large subunit of a ribosome located in the cytosol. | cellular_component |
|  |  | Any immune system process that functions in the calibrated |  |
|  |  | response of an organism to a potential internal or invasive |  |
| GO:0006955 | immune response | threat. | biological_process |
|  |  | Innate immune responses are defense responses mediated |  |
|  | innate immune | by germline encoded components that directly recognize |  |
| GO:0045087 | response | components of potential pathogens. | biological_process |
|  |  | A process that is carried out at the cellular level which |  |
|  | extracellular matrix | results in the assembly, arrangement of constituent parts, |  |
| GO:0030198 | organization | or disassembly of an extracellular matrix. | biological_process |
|  |  | Any protein complex that undergoes combination with a |  |
|  |  | hormone, neurotransmitter, drug or intracellular messenger |  |
| GO:0043235 | receptor complex | to initiate a change in cell function. | cellular_component |
|  |  | The formation of bone or of a bony substance, or the |  |
|  |  | conversion of fibrous tissue or of cartilage into bone or a |  |
| GO:0001503 | ossification | bony substance. | biological_process |
|  |  | Any process that results in a change in state or activity of a |  |
|  |  | cell or an organism (in terms of movement, secretion, |  |
|  |  | enzyme production, gene expression, etc.) as a result of an |  |
|  |  | insulin stimulus. Insulin is a polypeptide hormone produced |  |
|  |  | by the islets of Langerhans of the pancreas in mammals, |  |
| GO:0032868 | response to insulin regulation of cytoskeleton | and by the homologous organs of other organisms. | biological_process |
|  |  | Any process that modulates the frequency, rate or extent of |  |
|  |  | the formation, arrangement of constituent parts, or |  |
| GO:0051493 | organization positive regulation of inflammatory | disassembly of cytoskeletal structures. | biological_process |
|  |  |  |  |
|  |  | Any process that activates or increases the frequency, rate |  |
| GO:0050729 | response | or extent of the inflammatory response. | biological_process |
|  |  | The process in which the branching structure of the ureteric |  |
|  |  | bud is generated and organized. The ureteric bud is an |  |
|  | branching involved in | epithelial tube that grows out from the metanephric duct. |  |
|  | ureteric bud | The bud elongates and branches to give rise to the ureter |  |
| GO:0001658 | morphogenesis | and kidney collecting tubules. | biological_process |
|  |  | A secretory organelle, typically 50 nm in diameter, of |  |
|  |  | presynaptic nerve terminals; accumulates in high |  |
|  |  | concentrations of neurotransmitters and secretes these into |  |
|  |  | the synaptic cleft by fusion with the 'active zone' of the |  |
| GO:0008021 | synaptic vesicle | presynaptic plasma membrane. | cellular_component |



| GO:0007189 | adenylate cyclaseactivating G proteincoupled receptor signaling pathway | The series of molecular signals generated as a consequence of a $G$ protein-coupled receptor binding to its physiological ligand, where the pathway proceeds through activation of adenylyl cyclase activity and a subsequent increase in the concentration of cyclic AMP (cAMP). | biological_process |
| :---: | :---: | :---: | :---: |
|  |  | The directed movement of a neutrophil cell, the most numerous polymorphonuclear leukocyte found in the blood, in response to an external stimulus, usually an infection or |  |
| GO:0030593 | neutrophil chemotaxis positive regulation of | wounding. <br> Any process that activates or increases the frequency, rate | biological_process |
| GO:0045785 | cell adhesion | or extent of cell adhesion. <br> The series of molecular signals generated as a consequence of a G protein-coupled receptor binding to its | biological_process |
| GO:0007200 | phospholipase Cactivating $G$ proteincoupled receptor signaling pathway | physiological ligand, where the pathway proceeds with activation of phospholipase C (PLC) and a subsequent increase in the concentration of inositol trisphosphate (IP3) and diacylglycerol (DAG). | biological_process |
| GO:0071356 | cellular response to tumor necrosis factor ephrin receptor | Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a tumor necrosis factor stimulus. | biological_process |
| GO:0005003 | activity | Combining with an ephrin to initiate a change in cell activity. The binding of a cell to the extracellular matrix via adhesion | molecular_function |
| GO:0007160 | cell-matrix adhesion blood vessel | molecules. <br> The process in which the anatomical structures of blood vessels are generated and organized. The blood vessel is | biological_process |
| GO:0048514 | morphogenesis | the vasculature carrying blood. <br> A collagen heterotrimer containing type IV alpha chains; [alpha1(IV)]2alpha2(IV) trimers are commonly observed, although more type IV alpha chains exist and may be present in type IV trimers; type IV collagen triple helices | biological_process |
| GO:0005587 | collagen type IV trimer | associate to form 3 dimensional nets within basement membranes. | cellular_component |
| GO:0042593 | glucose homeostasis <br> regulation of heart rate by cardiac | Any process involved in the maintenance of an internal steady state of glucose within an organism or cell. <br> A cardiac conduction process that modulates the frequency | biological_process |
| GO:0086091 | conduction <br> epithelial to mesenchymal | or rate of heart contraction. <br> A transition where an epithelial cell loses apical/basolateral polarity, severs intercellular adhesive junctions, degrades basement membrane components and becomes a | biological_process |
| GO:0001837 | transition negative regulation of | migratory mesenchymal cell. <br> Any process that stops, prevents, or reduces the frequency, | biological_process |
| GO:0016525 | angiogenesis <br> negative regulation of inflammatory | rate or extent of angiogenesis. <br> Any process that stops, prevents, or reduces the frequency, | biological_process |
| GO:0050728 | response | rate or extent of the inflammatory response. | biological_process |

GO:0071456
GO:0033089

GO:0060548
cellular response to hypoxia positive regulation of T cell differentiation in thymus

Any process that results in a change in state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus indicating lowered oxygen tension. Hypoxia, defined as a decline in O 2 levels below normoxic levels of 20.8-20.95\%, results in metabolic adaptation at both the cellular and organismal level.
biological_process

Any process that activates or increases the frequency, rate
or extent of T cell differentiation in the thymus. Any process that decreases the rate or frequency of cell death. Cell death is the specific activation or halting of processes within a cell so that its vital functions markedly negative regulation of cease, rather than simply deteriorating gradually over time, cell death which culminates in cell death. The function of a family of small chemotactic cytokines; their name is derived from their ability to induce directed chemotaxis in nearby responsive cells. All chemokines possess a number of conserved cysteine residues involved in intramolecular disulfide bond formation. Some chemokines are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to a site of infection, while others are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development. Chemokines are found in all vertebrates, some viruses and some bacteria.
Any process that modulates the frequency, rate or extent of behavior, the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) regulation of behavior to internal or external stimuli.

Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a response to wounding stimulus indicating damage to the organism. positive regulation of Any process that activates or increases the frequency, rate cell motility or extent of cell motility. The orderly movement of a myoblast from one site to another, often during the development of a multicellular organism. A myoblast is a cell type that, by fusion with other myoblasts, gives rise to the myotubes that eventually develop into skeletal muscle fibers. biological_process
A change in the morphology or behavior of a cell resulting from exposure to an activating factor such as a cellular or soluble ligand.
The process in which the anatomical structures of the
embryonic viscerocranium are generated and organized during the embryonic phase. The viscerocranium is the part of the skull viscerocranium morphogenesis
comprising the facial bones.
biological_process
biological_process
biological_process
molecular_function
biological_process
biological_process
biological_process
biogical_process
$\qquad$

號
biological_process

| GO:0001568 | blood vessel development | The process whose specific outcome is the progression of a blood vessel over time, from its formation to the mature structure. The blood vessel is the vasculature carrying blood. | biological_process |
| :---: | :---: | :---: | :---: |
|  |  | Any process in which a receptor is transported to, and/or maintained at the synapse, the junction between a nerve |  |
|  | receptor localization to synapse | fiber of one neuron and another neuron or muscle fiber or glial cell. |  |
| GO:0097120 | embryonic cranial <br> skeleton | The process in which the anatomical structures of the cranial skeleton are generated and organized during the |  |
| GO:0048701 | morphogenesis | embryonic phase. | biological_process |
|  | negative regulation of |  |  |
|  | endothelial cell <br> apoptotic proces | Any process that stops, prevents or reduces the frequency, |  |
| GO:2000352 |  | The chemical reactions and pathways resulting in the |  |
|  | nucleoside | breakdown of a nucleoside triphosphate, a compound |  |
|  | triphosphate catabolic | consisting of a nucleobase linked to a deoxyribose or ribose |  |
| GO:0009143 | process | sugar esterified with triphosphate on the sugar. | biological_process |
|  | regulation of cell | Any process that modulates the frequency, rate or extent of |  |
| GO:2000145 | motility | cell motility. | biological_process |
|  | organelle fission | The creation of two or more organelles by division of one organelle. | biological_process |
| GO:0048285 |  | Any process that stops, prevents, or reduces the frequency, |  |
|  | negative regulation of protein kinase B | rate or extent of protein kinase $B$ signaling, a series of reactions mediated by the intracellular serine/threonine |  |
| GO:0051898 | signaling regulation of | kinase protein kinase B. | biological_process |
|  | epithelial cell proliferation | Any process that modulates the frequency, rate or extent of epithelial cell proliferation. | biological_process |
| GO:0050678 |  | The process whose specific outcome is the progression of the circulatory system over time, from its formation to the mature structure. The circulatory system is the organ system that passes nutrients (such as amino acids and electrolytes), gases, hormones, blood cells, etc. to and from |  |
|  | circulatory system <br> development | cells in the body to help fight diseases and help stabilize body temperature and pH to maintain homeostasis. |  |
| GO:0072359 | regulation of blood | Any process that modulates the frequency, rate or extent of | ogical_process |
| GO:0030193 | coagulation | blood coagulation. | biological_process |
|  |  | Combining with a peptide and transmitting the signal across |  |
|  | G protein-coupled peptide receptor | the membrane by activating an associated G-protein; promotes the exchange of GDP for GTP on the alpha |  |
| GO:0008528 | activity | subunit of a heterotrimeric G-protein complex. <br> The process whose specific outcome is the progression of the cardiovascular system over time, from its formation to the mature structure. The cardiovascular system is the | molecular_function |
|  | cardiovascular system | anatomical system that has as its parts the heart and blood |  |
| GO:0072358 | development | vessels. | biological_process |
|  |  | A change in morphology and behavior of a leukocyte resulting from exposure to a specific antigen, mitogen, |  |
| GO:0045321 | leukocyte activation | cytokine, cellular ligand, or soluble factor. | biological_process |


| GO:0034383 | low-density | The process in which a low-density lipoprotein particle is | biological_process |
| :---: | :---: | :---: | :---: |
|  | lipoprotein particle clearance | removed from the blood via receptor-mediated endocytosis and its constituent parts degraded. |  |
|  |  | and its constituent parts degraded. |  |
| GO:0016941 | natriuretic peptide receptor activity | Combining with a natriuretic peptide and transmitting the signal to initiate a change in cell activity. | molecular_function |
|  |  | The chemical reactions and pathways resulting in the |  |
|  | nucleoside | formation of a nucleoside triphosphate, a compound |  |
|  | triphosphate | consisting of a nucleobase linked to a deoxyribose or ribose |  |
| GO:0009142 | biosynthetic process | sugar esterified with triphosphate on the sugar. | biological_process |
|  | positive regulation of |  |  |
|  | transcription from |  |  |
|  | RNA polymerase II |  |  |
|  | promoter involved in | Any positive regulation of transcription from RNA |  |
|  | smooth muscle cell | polymerase II promoter that is involved in smooth muscle |  |
| GO:2000721 | differentiation | cell differentiation. | biological_process |
|  | positive regulation of | Any process that activates or increases the frequency, rate, |  |
| GO:0002687 | leukocyte migration | or extent of leukocyte migration. | biological_process |
|  |  | A transition where a mesenchymal cell establishes apical/basolateral polarity, forms intercellular adhesive |  |
|  | mesenchymal to | junctions, synthesizes basement membrane components |  |
| GO:0060231 | epithelial transition | and becomes an epithelial cell. | biological_process |
|  |  | The movement of an eosinophil in response to an external |  |
| GO:0048245 | eosinophil chemotaxis | stimulus. | biological_process |
|  |  | Any process that stops, prevents or reduces the rate or |  |
|  | negative regulation of | extent of growth, the increase in size or mass of all or part |  |
| GO:0045926 | growth | of an organism. | biological_process |
|  | positive regulation of cytoskeleton | Any process that activates or increases the frequency, rate or extent of the formation, arrangement of constituent |  |
| GO:0051495 | organization | parts, or disassembly of cytoskeletal structures. | biological_process |
|  |  | The movement of a monocyte in response to an external |  |
| GO:0002548 | monocyte chemotaxis | stimulus. | biological_process |
|  | CCR chemokine | Interacting selectively and non-covalently with a CCR |  |
| GO:0048020 | receptor binding | chemokine receptor. | molecular_function |
|  | lymphocyte | The directed movement of a lymphocyte in response to an |  |
| GO:0048247 | chemotaxis | external stimulus. | biological_process |
|  | positive regulation of | Any process that activates or increases the frequency, rate |  |
| GO:0040017 | locomotion | or extent of locomotion of a cell or organism. | biological_process |
|  | positive regulation of |  |  |
|  | cellular component | Any process that activates or increases the frequency, rate |  |
| GO:0051272 | movement | or extent of the movement of a cellular component. | biological_process |
|  | regulation of cellular |  |  |
|  | component | Any process that modulates the frequency, rate or extent of |  |
| GO:0051270 | movement | the movement of a cellular component. | biological_process |
|  |  | Any process that activates or increases the frequency, rate, |  |
| GO:0002684 | positive regulation of ir or extent of an immune system process. Source: GOC:add |  | biological_process |
|  |  | The attachment of a cell or organism to a substrate, another cell, or other organism. Biological adhesion includes |  |
| GO:0022610 | biological adhesion | intracellular attachment between membrane regions. | biological_process |
|  |  | Any process that activates or increases the frequency, rate |  |
| GO:0050867 | positive regulation of c | or extent of activation | biological_process |

A organ system process carried out by any of the organs or tissues of the circulatory system. The circulatory system is an organ system that moves extracellular fluids to and from
circulatory system proc tissue within a multicellular organism
Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus reflecting the presence, absence, or concentration
response to oxygen lev of oxygen
biological_process
biological_process

A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of structures in the space external to the outermost structure of a cell. For cells without external protective or external encapsulating structures this refers to space outside of the plasma membrane, and also covers
reticulum membrane
Regulation of ventricular cardiac muscle action potential Regulation of force of L-methionine
voltage-gated calcium channel complex
cellular_component
heart contraction Any process that modulates the extent of heart contraction, c biological_process
biosynthetic process The chemical reactions and pathways resulting in the from formation of L-methionine, the L-enantiomer of (2S)-2methylthioadenosine amino-4-(methylsulfanyl)butanoic acid. Source: GOC:ecd cardiac chronotropy, regulation of heart contraction rate, regulation of rate of heart contraction The lipid bilayer surrounding the sarcoplasmic reticulum. Source: GOC:rph
cellular_component The aggregation, arrangement and bonding together of proteins to form the actin-based thin filaments of myofibrils
Skeletal muscle thin
filament assembly
Intercalated disc
in skeletal
muscle. Source: GOC:mtg_muscle, GOC:ef, GOC:mah biological_process A complex cell-cell junction at which myofibrils terminate in c cellular_component


|  | Combining with any modified low-density lipoprotein (LDL) |
| :--- | :--- |
| or other polyanionic ligand and delivering the ligand into the |  |
| cell via endocytosis. Ligands include acetylated and oxidized |  |
| LDL, Gram-positive and Gram-negative bacteria, apoptotic |  |
| cells, amyloid-beta fibrils, and advanced glycation end |  |
| products |  |

Table S2. List of Differentially expressed genes in transcriptomic studies of canine Myxomatous Mitral Valve Disease

| Study | Model | Fold | Gene Symbol |
| :---: | :---: | :---: | :---: |
| Markvy et al (2020) | CKCS vs Normal | -14.26 | CASQ2 |
| Markvy et al (2020) | CKCS vs Normal | -7.65 | NEBL |
| Markvy et al (2020) | CKCS vs Normal | -6.78 | CASQ2 |
| Markvy et al (2020) | CKCS vs Normal | -5.01 | LAMA2 |
| Markvy et al (2020) | CKCS vs Normal | -4.5 | FSTL4 |
| Markvy et al (2020) | CKCS vs Normal | -3.74 | FSTL4 |
| Markvy et al (2020) | CKCS vs Normal | -3.41 | LAMA2 |
| Markvy et al (2020) | CKCS vs Normal | -3.24 | KCND2 |
| Markvy et al (2020) | CKCS vs Normal | -2.97 | ADCY2 |
| Markvy et al (2020) | CKCS vs Normal | -2.95 | ADCY2 |
| Markvy et al (2020) | CKCS vs Normal | -2.85 | SLC24A2 |
| Markvy et al (2020) | CKCS vs Normal | -2.73 | PDZD2 |
| Markvy et al (2020) | CKCS vs Normal | -2.68 | SDK1 |
| Markvy et al (2020) | CKCS vs Normal | -2.68 | SLIT2 |
| Markvy et al (2020) | CKCS vs Normal | -2.68 | TMEFF2 |
| Markvy et al (2020) | CKCS vs Normal | -2.64 | NEBL |
| Markvy et al (2020) | CKCS vs Normal | -2.46 | KCND2 |
| Markvy et al (2020) | CKCS vs Normal | -2.4 | PDZD2 |
| Markvy et al (2020) | CKCS vs Normal | -2.24 | KCNQ5 |
| Markvy et al (2020) | CKCS vs Normal | -2.21 | TMEFF2 |
| Markvy et al (2020) | CKCS vs Normal | -2.17 | NID1 |
| Markvy et al (2020) | CKCS vs Normal | -2.15 | SDK1 |
| Markvy et al (2020) | CKCS vs Normal | -2.14 | DOK6 |
| Markvy et al (2020) | CKCS vs Normal | -2.05 | SLIT2 |
| Markvy et al (2020) | CKCS vs Normal | -2.04 | NTN1 |
| Markvy et al (2020) | CKCS vs Normal | -1.99 | NID1 |
| Markvy et al (2020) | CKCS vs Normal | -1.97 | SNTB1 |
| Markvy et al (2020) | CKCS vs Normal | -1.85 | ADCY2 |
| Markvy et al (2020) | CKCS vs Normal | -1.82 | SNTB1 |
| Markvy et al (2020) | CKCS vs Normal | -1.77 | KCNQ5 |
| Markvy et al (2020) | CKCS vs Normal | -1.73 | TANC2 |
| Markvy et al (2020) | CKCS vs Normal | -1.68 | MAML3 |
| Markvy et al (2020) | CKCS vs Normal | -1.64 | NTN1 |
| Markvy et al (2020) | CKCS vs Normal | -1.62 | TANC2 |
| Markvy et al (2020) | CKCS vs Normal | -1.56 | SEL1L3 |
| Markvy et al (2020) | CKCS vs Normal | -1.55 | MAML3 |
| Markvy et al (2020) | CKCS vs Normal | 1.53 | PLCB1 |
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| Markvy et al (2020) | CKCS vs Normal | 4.41 | COL6A5 |
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| Markvy et al (2020) | CKCS vs NON-CKCS | -17 | NRAP |
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| Markvy et al (2020) | CKCS vs NON-CKCS | -3.7 | KCNE1 |
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| Markvy et al (2020) | CKCS vs NON-CKCS | -3.49 | NPR3 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -3.44 | ART3 |
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| Markvy et al (2020) | CKCS vs NON-CKCS | -2.64 | HSPB7 |
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| Markvy et al (2020) | CKCS vs NON-CKCS | -2.58 | XIRP2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.52 | ADGRL3 |


| Markvy et al (2020) | CKCS vs NON-CKCS | -2.52 | KCNJ5 |
| :---: | :---: | :---: | :---: |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.51 | ATP2A2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.44 | DYSF |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.43 | KLHL31 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.43 | CXADR |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.4 | CCDC85A |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.4 | ITGA7 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.38 | CPNE5 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.33 | FITM1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.32 | KCNJ8 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.31 | DECR1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.31 | SLIT2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.25 | ADGRL3 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.23 | SDK1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.17 | RBPMS2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.17 | DES |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.14 | TNXB |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.14 | ESRRG |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.13 | DNAJC6 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.08 | TMEM132C |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.05 | COBL |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.04 | PER2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.03 | ADAMTS8 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -2.01 | FHOD3 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.96 | NID1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.96 | EDNRA |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.96 | SLC37A1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.93 | SLC2A12 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.92 | NGFR |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.91 | HEYL |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.89 | LOC488818 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.86 | PLTP |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.83 | TOX |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.82 | FAM13A |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.82 | PTGDS |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.81 | NID1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.81 | PPP1R12B |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.79 | FITM2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.78 | PPARA |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.76 | RFX2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.76 | GOT1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.75 | BVES |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.75 | CASZ1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.74 | MLLT11 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.72 | RAB33A |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.71 | DRP2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.7 | NCAM1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.67 | MCAM |


| Markvy et al (2020) | CKCS vs NON-CKCS | -1.66 | PROX1 |
| :---: | :---: | :---: | :---: |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.64 | HACD4 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.63 | ATP9A |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.62 | FAM160A1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.62 | CEP126 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.61 | RCAN2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.6 | FAM184B |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.59 | L1CAM |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.58 | ACO2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.55 | IQSEC1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.55 | MDH2 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.55 | CACNA1G |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.54 | TBC1D8 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.53 | SEMA4D |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.53 | SLC8B1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | -1.51 | CCDC92 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.51 | LOC106558240 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.51 | MAT2B |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.52 | ENOPH1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.54 | SWI5 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.56 | CXHXorf21 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.57 | C24H2Oorf24 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.57 | TMEM106C |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.59 | DYNLRB1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.61 | BLVRB |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.61 | ETF1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.65 | MASTL |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.67 | SEC11C |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.67 | NKX3-1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.68 | SYNDIG1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.71 | NOP10 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.72 | LOC102155956 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.83 | HENMT1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.84 | EVI2B |
| Markvy et al (2020) | CKCS vs NON-CKCS | 1.86 | ABCC4 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.23 | C3AR1 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.26 | TMEM261 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.32 | LOC612564 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.46 | LOC100856577 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.48 | IL18 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 2.52 | RASL11A |
| Markvy et al (2020) | CKCS vs NON-CKCS | 3.97 | LOC476900 |
| Markvy et al (2020) | CKCS vs NON-CKCS | 4.77 | CLEC7A |
| Markvy et al (2020) | CKCS valves compared to both normal val Down- regulated ACTA1 |  |  |
| Markvy et al (2020) | CKCS valves compared to both normal val Down- regulated ACTN2 |  |  |
| Markvy et al (2020) | CKCS valves compared to both normal val Down- regulated ADAMTS8 |  |  |
| Markvy et al (2020) | CKCS valves compared to both normal val Down- regulated ADCK3 |  |  |
| Markvy et al (2020) | CKCS valves compared to both normal val Down- regulated ADGRL3 |  |  |

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CKCS valves compared to both normal val Down- regulated ADPRHL1 CKCS valves compared to both normal val Down- regulated ALPK2 CKCS valves compared to both normal val Down- regulated APOBEC2 CKCS valves compared to both normal val Down- regulated ASB12 CKCS valves compared to both normal val Down- regulated ATP1A3 CKCS valves compared to both normal val Down- regulated ATP2A2 CKCS valves compared to both normal val Down- regulated ATP9A CKCS valves compared to both normal val Down- regulated C28H10orf71 CKCS valves compared to both normal val Down- regulated CA14 CKCS valves compared to both normal val Down- regulated CACNA1G CKCS valves compared to both normal val Down- regulated CASQ2 CKCS valves compared to both normal val Down- regulated CCDC92 CKCS valves compared to both normal val Down- regulated CMYA5 CKCS valves compared to both normal val Down- regulated COBL CKCS valves compared to both normal val Down- regulated CORIN CKCS valves compared to both normal val Down- regulated COX6A2 CKCS valves compared to both normal val Down- regulated DECR1 CKCS valves compared to both normal val Down- regulated DRP2 CKCS valves compared to both normal val Down- regulated DSC2 CKCS valves compared to both normal val Down- regulated DSP CKCS valves compared to both normal val Down- regulated DYSF CKCS valves compared to both normal val Down- regulated EDNRA CKCS valves compared to both normal val Down- regulated FAM13A CKCS valves compared to both normal val Down- regulated FAM184B CKCS valves compared to both normal val Down- regulated FHOD3 CKCS valves compared to both normal val Down- regulated FITM1 CKCS valves compared to both normal val Down- regulated FREM1 CKCS valves compared to both normal val Down- regulated GNAO1 CKCS valves compared to both normal val Down- regulated HACD4 CKCS valves compared to both normal val Down- regulated HHATL CKCS valves compared to both normal val Down- regulated HRC CKCS valves compared to both normal val Down- regulated ITGB6 all diseased valves and normal valves -4.86 TNMD all diseased valves and normal valves -4.65 NKAIN2 all diseased valves and normal valves -4.44 CILP all diseased valves and normal valves -3.68 LOC10215413 -3.46 LOC488818 -3.27 NELL2 -3.05 NT5E -2.94 GPR85 -2.82 SCN3B -2.81 TMEFF2 -2.77 FSTL4 -2.62 ADAMTS15 -2.39 KCND2 -2.37 TMEFF2 -2.36 SLC24A2 -2.23 CYP2B6

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all diseased valves and normal valves
KCNQ5
VWDE

ALDH1A1
PNMT
SCIN
PTGFR
AFF2
CRISPLD2
TRPM3
LGI2
KCNQ5
PDZD2
ENOX1
C1QTNF4
DLG2
TRPC5
SCARA5
ENPP2
ITGA2
TANC2
COL11A2
GCNT4
OLFML1
PDE3B
ADAMDEC1
HSP70
ACTG2
ANGPTL1
IL6
cdkn2A
SFRP2
IL18
C4BPA
CCL13
IGKC
RGS2
HTR2B
SNORD14B
CXCL10
CD180
MS4A7
RGS4
HOXD8
CXCL14
MYH11
DNAJB1
IRGM

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| CSTA |
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| OLR1 |
| CPNE4 |
| KCNE4 |
| LOC100855494 |
| MDGA2 |
| PLCXD3 |
| BLVRB |
| CLEC3A |
| PTGS2 |
| FCGR1A |
| MMP12 |
| UCHL1 |
| CTSC |
| DAPP1 |
| MIA |
| FGG |
| C5H17orf61 |
| ANGPT1 |
| DNAH6 |
| EVI2B |
| ENPP6 |
| TLR8 |
| LOC100856456 |
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| ARAP2 |
| CASP8 |
| ZBTB11 |
| LOC100855873 |
| RAB1B |
| CYP4B1 |
| KCNJ15 |
| EPHA3 |
| FCGR3A |
| LST1 |
| MSR1 |
| BANK1 |
| CDH6 |
| LOC478384 |
| RPS27A |
| CLDN1 |
| PDCD1LG2 |
| SELE |
| CXHXorf21 |
| GMFG |
| S100A4 |
| C3AR1 |
| IGJ |

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| ATP8B1 |
| :---: |
| TLR1 |
| SLC7A11 |
| LOC100669834 |
| GPR34 |
| STK17B |
| EMR1 |
| SCN1B |
| HNRNPH2 |
| TACR1 |
| CD48 |
| ARSK |
| LOC100856186 |
| LSMD1 |
| ACP5 |
| CFI |
| PLA2G4A |
| SKAP2 |
| NOV |
| PLXNC1 |
| SLC2A5 |
| AKAP5 |
| BNC2 |
| GCLM |
| CECR1 |
| CD300C |
| MUSTN1 |
| SLC16A12 |
| F13A1 |
| PIR |
| CGREF1 |
| ALOX5AP |
| MRPL51 |
| SERPINB8 |
| OSR1 |
| FAM174A |
| FKBP2 |
| LOC100855806 |
| DYNC111 |
| PCOLCE2 |
| RNASE8 |
| LOC100856330 |
| LPXN |
| KCNMB1 |
| C1QC |
| PDCD10 |
| LSM1 |
| PEPD |

ATP8B1
TLR1 SLC7A11
LOC100669834 GPR34 STK17B SCN1B HNRNPH2 TACR1 ARSK OC100856186

LSMD1
ACP

PLA2G4A

NOV
PLXNC1
SLC2A5

BNC2
GCLM
CECR1

MUSTN1
SLC16A12
13A1

CGREF1
ALOX5AP
MRPL51
SERPINB8

FAM174A
FKBP2
LOC100855806
DYNC1I1
PCOLCE2
RNASE8
LOC100856330

KCNMB1
C1QC

LSM1
PEPD

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MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs
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ARMCX3

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JAK2
TMEM70

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BMPR1B
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MMVD in CKCS compared to normal dogs
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SPI1

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| MMVD in CKCS compared to normal dogs | 1.53 | ACTR2 |

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MMVD in CKCS compared to normal dogs
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Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015) Lu et al (2015)

MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs MMVD in CKCS compared to normal dogs
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INO80D
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CREBBP
SP110
BCL9
LSM10
C26H12orf51 FRAS1
SORBS2
RHBDD2 CD55 KLF3 MKL2
RNPS1
CERS4
DDX39B
SF3A1
SF1
NFATC1
SYVN1
PTPN14
SSH1
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TULP4
SLC43A3
GATAD2B
LAMB1
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EMP3
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PROSER1
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HEG1
SOX9
GAB2
SNTB1
KCNN3
TECR
MYO1E ECM1 MAP4
LRRC37A2
YPEL3
GOT1

| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.66 | CSDA |
| :---: | :---: | :---: | :---: |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.66 | NFE2L1 |
| Lu et al (2015) | MMVD in CKCS compared to normal dogs | -1.65 | RCAN2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.65 | HPRT1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.65 | ACO2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.64 | ENPP2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.63 | ELK3 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.63 | MAPRE2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.62 | ARHGAP21 |
| Lu et al (2015) | MMVD in CKCS compared to normal dogs | -1.61 | MED13L |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.61 | CYB5R3 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.6 | TSC22D2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.6 | TNFAIP1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.6 | ECE1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.6 | PSMF1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.59 | ZNF532 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.59 | SRSF6 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.59 | RAB11B |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.58 | DPYSL2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.58 | PRRC2C |
| Luetal (2015) | MMVD in CKCS compared to normal dogs | -1.56 | ATP10A |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | DEDD |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | FAT4 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | SPPL3 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | PTN |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | INTS3 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.56 | LOC488929 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.55 | VGLL4 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.55 | KIAA0430 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.55 | LOC100856258 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.54 | DDR2 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.54 | TRAFD1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.53 | MMP14 |
| Luetal (2015) | MMVD in CKCS compared to normal dogs | -1.52 | PNISR |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.52 | ENG |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.51 | CAPNS1 |
| Luetal (2015) | MMVD in CKCS compared to normal dogs | -1.51 | HUWE1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.51 | LPAR1 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.51 | ZBTB20 |
| Luet al (2015) | MMVD in CKCS compared to normal dogs | -1.5 | TMED4 |
| Markby et al ., (2020b) | Grade 1 with normal | -10.46 | LOC476900 |
| Markby et al ., (2020b) | Grade 1 with normal | -2.34 | RANBP3L |
| Markby et al ., (2020b) | Grade 1 with normal | -2.33 | MIR218-1 |
| Markby et al ., (2020b) | Grade 1 with normal | -2.09 | FSTL4 |
| Markby et al ., (2020b) | Grade 1 with normal | -2.05 | FSTL4 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.96 | VWDE |
| Markby et al ., (2020b) | Grade 1 with normal | -1.95 | NELL2 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.92 | ADCYAP1 |


| Markby et al ., (2020b) | Grade 1 with normal | -1.86 | NKAIN2 |
| :---: | :---: | :---: | :---: |
| Markby et al., (2020b) | Grade 1 with normal | -1.86 | MIR1838 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.84 | ADCY2 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.8 | ENSCAFG0000002827 $8.1$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.8 | ENSCAFG00000002363 $7.2$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.73 | MIR328 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.73 | $\begin{aligned} & \text { ENSCAFT0000002374 } \\ & \underline{9} \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.68 | ENSCAFG00000000697 $3$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.67 | ADCY2 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.65 | ENSCAFG00000000205 $3$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.65 | RBPJL |
| Markby et al ., (2020b) | Grade 1 with normal | -1.62 | ENSCAFG00000001904 <br> 4 |
| Markby et al ., (2020b) | Grade 1 with normal | -1.61 | ENSCAFG0000001006 $4$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.58 | LOC100684200 |
| Markby et al., (2020b) | Grade 1 with normal | -1.55 | $\begin{aligned} & \text { ENSCAFG00000003242 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | -1.54 | TMEM132C |
| Markby et al., (2020b) | Grade 1 with normal | -1.52 | DOK3; DDX41 |
| Markby et al., (2020b) | Grade 1 with normal | 1.51 | JPH3 |
| Markby et al., (2020b) | Grade 1 with normal | 1.51 | ACHE |
| Markby et al., (2020b) | Grade 1 with normal | 1.51 | HMGB3 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.52 | TIMP4 |
| Markby et al., (2020b) | Grade 1 with normal | 1.52 | GRID2 |
| Markby et al., (2020b) | Grade 1 with normal | 1.52 | $\begin{aligned} & \text { ENSCAFG0000002558 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.53 | CASP14 |
| Markby et al., (2020b) | Grade 1 with normal | 1.53 | $\begin{aligned} & \text { ENSCAFG00000000710 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.54 | CYTL1 |
| Markby et al., (2020b) | Grade 1 with normal | 1.54 | LOC478701 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.54 | $\begin{aligned} & \text { ENSCAFG0000001258 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.55 | GRID2 |
| Markby et al., (2020b) | Grade 1 with normal | 1.56 | F3 |
| Markby et al., (2020b) | Grade 1 with normal | 1.57 | MCAM |
| Markby et al., (2020b) | Grade 1 with normal | 1.58 | $\begin{aligned} & \text { ENSCAFG0000000468 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.58 | ABCC9 |
| Markby et al., (2020b) | Grade 1 with normal | 1.6 | Mar-01 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.6 | ENSCAFG00000001224 $2$ |
| Markby et al., (2020b) | Grade 1 with normal | 1.6 | LOC100856200 |
| Markby et al., (2020b) | Grade 1 with normal | 1.62 | TACR1 |


| Markby et al ., (2020b) | Grade 1 with normal | 1.62 | CADM2 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 1 with normal | 1.62 | ENSCAFG0000002356 $2$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.63 | FHDC1 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.63 | MAL |
| Markby et al ., (2020b) | Grade 1 with normal | 1.65 | PRR15 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.69 | THBS4 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.69 | CXHXorf36 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.7 | SIX1 |
| Markby et al., (2020b) | Grade 1 with normal | 1.71 | SFRP5 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.72 | ENSCAFG0000002088 $6$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.72 | CLEC3A |
| Markby et al., (2020b) | Grade 1 with normal | 1.73 | NRXN1 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.73 | GPC3 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.73 | ENSCAFG0000003732 $2$ |
| Markby et al ., (2020b) | Grade 1 with normal | 1.76 | LRRN1 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.8 | MAGI2 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.81 | VAT1L |
| Markby et al ., (2020b) | Grade 1 with normal | 1.81 | RSAD2 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.82 | SLIT3 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.88 | NRXN1 |
| Markby et al ., (2020b) | Grade 1 with normal | 1.95 | LOC102157036 |
| Markby et al ., (2020b) | Grade 1 with normal | 2.22 | KITLG |
| Markby et al ., (2020b) | Grade 1 with normal | 2.46 | ZNF385B |
| Markby et al ., (2020b) | Grade 1 with normal | 2.49 | ZNF385B |
| Markby et al ., (2020b) | Grade 1 with normal | 2.54 | LOC100687667 |
| Markby et al ., (2020b) | Grade 1 with normal | 2.56 | LYZF2 |
| Markby et al., (2020b) | Grade 2 with normal | -4.62 | LOC476900 |
| Markby et al ., (2020b) | Grade 2 with normal | -3.93 | ENSCAFG0000002649 <br> 8 |
| Markby et al ., (2020b) | Grade 2 with normal | -2.04 | $\begin{aligned} & \text { ENSCAFG0000001911 } \\ & 4 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 2 with normal | -1.79 | NKAIN2 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.78 | GPBAR1 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.74 | NT5E |
| Markby et al ., (2020b) | Grade 2 with normal | -1.72 | CYTH1 |
| Markby et al., (2020b) | Grade 2 with normal | -1.68 | MIR328 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.67 | TMEFF2 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.63 | ENSCAFG0000003979 5 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.62 | ENSCAFG0000002093 <br> 5 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.6 | ENSCAFG00000000316 <br> 8 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.59 | AGAP1 |
| Markby et al., (2020b) | Grade 2 with normal | -1.58 | MIR218-1 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.58 | ENSCAFG0000000693 $6$ |


| Markby et al ., (2020b) | Grade 2 with normal |  | ENSCAFG0000003027 |
| :---: | :---: | :---: | :---: |
|  |  | -1.58 | 6 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.57 | RXRA |
| Markby et al ., (2020b) | Grade 2 with normal | -1.57 | ENSCAFG0000000911 |
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| Markby et al ., (2020b) | Grade 2 with normal | -1.52 | ZCCHC8 |
| Markby et al ., (2020b) | Grade 2 with normal | -1.52 | NCOA2 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.53 | WBSCR27 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.54 | TTPA |
| Markby et al ., (2020b) | Grade 2 with normal | 1.55 | ENSCAFG0000001559 |
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| Markby et al ., (2020b) | Grade 2 with normal | 1.55 | TMEM132B |
| Markby et al ., (2020b) | Grade 2 with normal | 1.57 | TREM2 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.58 | VAT1L |
| Markby et al ., (2020b) | Grade 2 with normal | 1.58 | ELOVL7 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.59 | PCP4L1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.59 | GPC3 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.6 | DYSF |
| Markby et al ., (2020b) | Grade 2 with normal | 1.6 | SLIT3 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.62 | CDKN2A |
| Markby et al ., (2020b) | Grade 2 with normal | 1.62 | FAM159A |
| Markby et al ., (2020b) | Grade 2 with normal | 1.64 | CASP14 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.64 | M ARC1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.64 | MAGI2 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.64 | IFIT1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.67 | CRLF1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.69 | CLEC5A |
| Markby et al ., (2020b) | Grade 2 with normal | 1.73 | PDE6H |
| Markby et al ., (2020b) | Grade 2 with normal | 1.74 | NRXN1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.75 | LRRN1 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.92 | GPC3 |
| Markby et al ., (2020b) | Grade 2 with normal | 1.97 | SLIT3 |
| Markby et al ., (2020b) | Grade 2 with normal | 2.09 | ENSCAFG0000003025 |
|  |  |  | 8 |
| Markby et al ., (2020b) | Grade 2 with normal | 2.14 | GPD1 |
| Markby et al ., (2020b) | Grade 2 with normal | 2.16 | SLC22A1 |
| Markby et al ., (2020b) | Grade 2 with normal | 2.17 | ENSCAFG0000002974 |
|  |  |  | 3 |
| Markby et al ., (2020b) | Grade 2 with normal | 2.27 | CSTA |
| Markby et al ., (2020b) | Grade 2 with normal | 2.45 | LOC100687667 |
| Markby et al ., (2020b) | Grade 2 with normal | 3.19 | ENSCAFG0000003090 |
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| Markby et al ., (2020b) | Grade 2 with normal | 3.2 | ENSCAFG0000002411 |
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| Markby et al ., (2020b) | Grade 2 with normal | 3.29 | LOC608320 |
| Markby et al ., (2020b) | Grade 2 with normal | 3.31 | LOC612122 |
| Markby et al ., (2020b) | Grade 2 with normal | 3.58 | ENSCAFG0000003025 |
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| Markby et al ., (2020b) | Grade 2 with normal | 3.66 | ENSCAFG0000003090 |
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| Markby et al ., (2020b) | Grade 2 with normal | 4.28 | ENSCAFG0000003175 |
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| Markby et al ., (2020b) | Grade 2 with normal | 4.67 | ENSCAFG0000002472 |
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| Markby et al ., (2020b) | Grade 2 with normal | 5.5 | ENSCAFG0000003175 |
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| Markby et al ., (2020b) | Grade 3 with normal | -16.93 | CASQ2 |
| Markby et al ., (2020b) | Grade 3 with normal | -14.74 | ACTN2 |
| Markby et al ., (2020b) | Grade 3 with normal | -11.73 | MYL4 |
| Markby et al ., (2020b) | Grade 3 with normal | -11.49 | MB |
| Markby et al ., (2020b) | Grade 3 with normal | -11.49 | ACTA1 |
| Markby et al ., (2020b) | Grade 3 with normal | -11.24 | ENSCAFG0000001079 |
|  |  |  | 8 |
| Markby et al ., (2020b) | Grade 3 with normal | -10.22 | ENSCAFG0000001402 |
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| Markby et al ., (2020b) | Grade 3 with normal | -9.88 | PGAM2 |
| Markby et al ., (2020b) | Grade 3 with normal | -8.56 | ACTC1 |
| Markby et al ., (2020b) | Grade 3 with normal | -7.81 | CKM |
| Markby et al ., (2020b) | Grade 3 with normal | -7.8 | DSC2 |
| Markby et al ., (2020b) | Grade 3 with normal | -7.66 | ENSCAFG0000000825 |
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| Markby et al ., (2020b) | Grade 3 with normal | -7.28 | TNMD |
| Markby et al ., (2020b) | Grade 3 with normal | -6.45 | NKAIN2 |
| Markby et al ., (2020b) | Grade 3 with normal | -6.45 | ENSCAFG0000000825 |
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| Markby et al ., (2020b) | Grade 3 with normal | -5.86 | CILP |
| Markby et al ., (2020b) | Grade 3 with normal | -5.51 | LAMA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -5.23 | WIF1 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.84 | ENSCAFG0000002806 |
|  |  |  | 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.6 | PPARGC1A |
| Markby et al ., (2020b) | Grade 3 with normal | -4.55 | ENSCAFG0000001417 |
|  |  |  | 8 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.53 | AQP4 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.35 | MMP3 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.02 | LOC488818 |
| Markby et al ., (2020b) | Grade 3 with normal | -4.02 | ENSCAFG0000000825 |
|  |  |  | 3 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.86 | GJB6 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.72 | LAMA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.67 | MIR99A-1 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.47 | ADAMTS15 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.43 | SLITRK6 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.4 | MIRLET7C |
| Markby et al ., (2020b) | Grade 3 with normal | -3.39 | ENSCAFG0000002273 |
|  |  |  | 2 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.37 | MEI4 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.24 | LOC482182 |
| Markby et al ., (2020b) | Grade 3 with normal | -3.15 | HIF3A |


| Markby et al ., (2020b) | Grade 3 with normal | -2.99 | $\begin{aligned} & \text { ENSCAFG0000002517 } \\ & 2 \end{aligned}$ |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -2.96 | FREM1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.95 | GRIN2A |
| Markby et al ., (2020b) | Grade 3 with normal | -2.93 | NT5E |
| Markby et al ., (2020b) | Grade 3 with normal | -2.92 | SCN3B |
| Markby et al ., (2020b) | Grade 3 with normal | -2.91 | FSTL4 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.83 | FMO2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.83 | ABCA6 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.83 | ENSCAFG00000002274 <br> 3 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.82 | HAPLN1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.79 | FGL1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.78 | TSHR |
| Markby et al ., (2020b) | Grade 3 with normal | -2.78 | ENSCAFG00000003168 <br> 2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.74 | SLC24A2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.72 | NEBL |
| Markby et al ., (2020b) | Grade 3 with normal | -2.68 | TMEFF2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.66 | LOC479934 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.65 | CDH22 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.61 | ENSCAFG00000002843 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.6 | SLC2A12 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.6 | WNT16 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.6 | ENSCAFG00000002272 <br> 1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.59 | ADRA1A |
| Markby et al ., (2020b) | Grade 3 with normal | -2.59 | ABCC9 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.56 | ADCY2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.56 | ENSCAFG00000002273 <br> 8 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.56 | ENSCAFG00000000144 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.56 | $\begin{aligned} & \text { ENSCAFG0000000144 } \\ & 6 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.56 | ENSCAFG00000000144 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.54 | KCND2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.53 | AMIGO2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.53 | MPZL2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.52 | GFRA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.45 | KCNQ5 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.43 | CCBE1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.43 | KCNJ8 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.42 | CILP2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.42 | TMEM132C |
| Markby et al ., (2020b) | Grade 3 with normal | -2.42 | ANGPTL5 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.4 | TMEFF2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.37 | GPR37 |


| Markby et al ., (2020b) | Grade 3 with normal | -2.36 | TNXB |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -2.35 | PI15 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.35 | PCSK6 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.35 | LAYN |
| Markby et al ., (2020b) | Grade 3 with normal | -2.34 | $\begin{aligned} & \text { ENSCAFG0000003126 } \\ & 4 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.34 | $\begin{aligned} & \text { ENSCAFG0000002271 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.32 | GPM6A |
| Markby et al ., (2020b) | Grade 3 with normal | -2.32 | FAM20A |
| Markby et al ., (2020b) | Grade 3 with normal | -2.31 | FSTL4 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.31 | ADCYAP1R1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.3 | RASGRF2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.29 | MIR218-1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.29 | CDC42EP2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.29 | SCN4B |
| Markby et al ., (2020b) | Grade 3 with normal | -2.29 | $\begin{aligned} & \text { ENSCAFG0000002273 } \\ & 7 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.27 | TSPAN2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.27 | SLC22A23 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.27 | $\begin{aligned} & \text { ENSCAFG0000000144 } \\ & 6 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.27 | ENSCAFG0000000144 $6$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.27 | $\begin{aligned} & \text { ENSCAFG0000000144 } \\ & 6 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.26 | SLC37A1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.24 | ACKR2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.24 | BICD1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.24 | GRIN2A |
| Markby et al ., (2020b) | Grade 3 with normal | -2.23 | F2RL2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.23 | ENSCAFG0000002901 5 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.22 | SLC26A5 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.21 | ADCY2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.2 | WIPF3 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.2 | ETNPPL |
| Markby et al ., (2020b) | Grade 3 with normal | -2.2 | RYR2 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.2 | SDK1 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.19 | LIFR |
| Markby et al ., (2020b) | Grade 3 with normal | -2.18 | KLF9 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.18 | CA3 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.16 | MMP16 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.16 | ENSCAFG0000001823 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.15 | KDR |
| Markby et al ., (2020b) | Grade 3 with normal | -2.15 | RANBP3L |
| Markby et al ., (2020b) | Grade 3 with normal | -2.14 | RNF128 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.12 | $\begin{aligned} & \text { ENSCAFG0000000074 } \\ & 1 \end{aligned}$ |


| Markby et al., (2020b) | Grade 3 with normal | -2.12 | SLIT2 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -2.12 | IGFBP5 |
| Markby et al ., (2020b) | Grade 3 with normal | -2.12 | $\begin{aligned} & \text { ENSCAFG0000001498 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -2.12 | DRP2 |
| Markby et al., (2020b) | Grade 3 with normal | -2.1 | KERA |
| Markby et al., (2020b) | Grade 3 with normal | -2.09 | ALDH1A1 |
| Markby et al., (2020b) | Grade 3 with normal | -2.09 | PTPRB |
| Markby et al., (2020b) | Grade 3 with normal | -2.07 | IGSF3 |
| Markby et al., (2020b) | Grade 3 with normal | -2.07 | TOX |
| Markby et al., (2020b) | Grade 3 with normal | -2.06 | AK5 |
| Markby et al., (2020b) | Grade 3 with normal | -2.05 | WFDC5 |
| Markby et al., (2020b) | Grade 3 with normal | -2.05 | HMCN1 |
| Markby et al., (2020b) | Grade 3 with normal | -2.04 | LAMA1 |
| Markby et al., (2020b) | Grade 3 with normal | -2.03 | SEMA3G |
| Markby et al ., (2020b) | Grade 3 with normal | -2.03 | ENSCAFG0000002363 $7$ |
| Markby et al., (2020b) | Grade 3 with normal | -2.03 | NEGR1 |
| Markby et al., (2020b) | Grade 3 with normal | -2.02 | LHCGR |
| Markby et al., (2020b) | Grade 3 with normal | -2.02 | KCND2 |
| Markby et al., (2020b) | Grade 3 with normal | -2.02 | HCN1 |
| Markby et al., (2020b) | Grade 3 with normal | -2.01 | CCM2L |
| Markby et al., (2020b) | Grade 3 with normal | -2.01 | SLC10A6 |
| Markby et al., (2020b) | Grade 3 with normal | -2.01 | IGF2BP2 |
| Markby et al., (2020b) | Grade 3 with normal | -2 | MSTN |
| Markby et al., (2020b) | Grade 3 with normal | -1.99 | DLL1 |
| Markby et al., (2020b) | Grade 3 with normal | -1.99 | LOC478001 |
| Markby et al., (2020b) | Grade 3 with normal | -1.99 | FAT3 |
| Markby et al., (2020b) | Grade 3 with normal | -1.98 | PDZD2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.98 | WNT9B |
| Markby et al., (2020b) | Grade 3 with normal | -1.97 | KCNJ2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.96 | STC1 |
| Markby et al., (2020b) | Grade 3 with normal | -1.95 | FRMD3 |
| Markby et al., (2020b) | Grade 3 with normal | -1.95 | VWDE |
| Markby et al., (2020b) | Grade 3 with normal | -1.94 | GPLD1 |
| Markby et al., (2020b) | Grade 3 with normal | -1.94 | SLC1A3 |
| Markby et al., (2020b) | Grade 3 with normal | -1.94 | CRISPLD2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.94 | $\begin{aligned} & \text { ENSCAFG0000002272 } \\ & 7 \end{aligned}$ |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | SLC24A2 |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | SLC4A4 |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | LIPC |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | ENSCAFG0000001327 5 |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | PTGFR |
| Markby et al., (2020b) | Grade 3 with normal | -1.93 | ENSCAFG00000001028 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.92 | CAPN6 |


| Markby et al ., (2020b) | Grade 3 with normal | -1.92 | ENSCAFG0000001087 |
| :---: | :---: | :---: | :---: |
|  |  |  | 7 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.91 | SAMD12 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.91 | ENPEP |
| Markby et al ., (2020b) | Grade 3 with normal | -1.9 | RASIP1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.9 | ENSCAFG0000002359 $1$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.9 | VWDE |
| Markby et al ., (2020b) | Grade 3 with normal | -1.9 | FLT1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.89 | PODXL |
| Markby et al ., (2020b) | Grade 3 with normal | -1.89 | PDK4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.89 | COLCA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | ENSCAFG0000002649 $8$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | KIAA1024L |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | ECSCR |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | WNT11 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | RASL10A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | LIX1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | FAM81A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.88 | NTN1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.87 | ENSCAFG00000001292 $7$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.86 | MET |
| Markby et al ., (2020b) | Grade 3 with normal | -1.86 | LOC608987; CCNJL |
| Markby et al ., (2020b) | Grade 3 with normal | -1.86 | ENSCAFG0000002881 $7$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.85 | ANGPTL4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.85 | MN1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.85 | AFF2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | ENSCAFG00000002093 5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | DOK6 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | ADAMTS19 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | KANK3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | PPP1R16B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | ACADM |
| Markby et al ., (2020b) | Grade 3 with normal | -1.84 | ENSCAFG0000002366 $9$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.83 | SLCO5A1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.83 | TMEM52 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.82 | LAPTM4B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.82 | TMCC3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.82 | ENSCAFG0000001025 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.82 | GAS2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.82 | ACKR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | MIR491 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | ADAMTS5 |


| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | TSPAN14 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | ENSCAFG0000001487 <br> 5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | WFIKKN2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.81 | OLFML2A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.8 | LOC486009 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.8 | GDPD2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.79 | VIPR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.79 | NID1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.79 | LHX9 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.79 | $\begin{aligned} & \text { ENSCAFG0000002302 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.78 | FAM171A1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.78 | ILDR2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.77 | MPP6 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.77 | SEMA3D |
| Markby et al ., (2020b) | Grade 3 with normal | -1.77 | CCNA1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.77 | GABRA1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.77 | PROX1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.76 | BCAM |
| Markby et al ., (2020b) | Grade 3 with normal | -1.76 | SOX10 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | SEMA6C |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | SGCG |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | ENSCAFG0000001380 $6$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | ITGA11 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | C37H2orf88 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | ENSCAFG0000002273 <br> 1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | TSPAN7 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.75 | $\begin{aligned} & \text { ENSCAFG0000000274 } \\ & 4 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.74 | GFRA3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.74 | ADGRB3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.74 | P2RY1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.74 | ITGA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.74 | ST8SIA5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.73 | FRMD3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.73 | TRPM3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.73 | PERP |
| Markby et al ., (2020b) | Grade 3 with normal | -1.73 | CNTFR |
| Markby et al ., (2020b) | Grade 3 with normal | -1.73 | PDZD2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | NFATC1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | CACNA2D3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | ADRB1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | FAM13A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | MYOC |
| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | TANC2 |


| Markby et al ., (2020b) | Grade 3 with normal | -1.72 | ENSCAFG0000001545 |
| :---: | :---: | :---: | :---: |
|  |  | -1.72 | 0 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.71 | ANGPTL7 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.71 | DCLK1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.71 | LGI2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.71 | MASP1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.71 | SDK1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.7 | PLLP |
| Markby et al ., (2020b) | Grade 3 with normal | -1.7 | ENSCAFG0000001003 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.69 | COL6A3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.69 | ANO2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.69 | CCDC65 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.68 | ARHGAP32 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.68 | CYGB |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | ELOVL4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | SERINC2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | AS3MT |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | FAM53B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | NID1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.67 | ENSCAFG0000001222 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | SYTL3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | TTYH1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | SHISA3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | APOLD1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | PENK |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | NTN1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | C5H11orf63 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.66 | CACHD1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | AKAP12 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | CBD108 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | EFCC1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | SCARA5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | MOK |
| Markby et al ., (2020b) | Grade 3 with normal | -1.65 | $\begin{aligned} & \text { ENSCAFG0000000293 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | PTPRD |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ENPP2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | FOXP2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | THSD7A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | IGF2; INS |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | SLIT2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ANKRD45 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ADCYAP1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ABCA9 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ENSCAFG0000002273 <br> 9 |


| Markby et al ., (2020b) | Grade 3 with normal | -1.64 | ENSCAFG0000000072 |
| :---: | :---: | :---: | :---: |
|  |  |  | 2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | CD8A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | SEMA3A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | TNFRSF19 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | CIT |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | ERG |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | GRIA3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.63 | ENSCAFG00000000090 <br> 3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.62 | WDR54 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.62 | ENSCAFG0000002169 $3$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.62 | CDH5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | COL14A1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | C1QTNF4 |
| Markby et al., (2020b) | Grade 3 with normal | -1.61 | B4GAT1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | ISM1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | CCND2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | FGF12 |
| Markby et al., (2020b) | Grade 3 with normal | -1.61 | KCNT2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | CFH |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | DSG2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | LHFPL1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | $\begin{aligned} & \text { ENSCAFG0000003218 } \\ & 7 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | ENSCAFG00000001994 $1$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.61 | ENSCAFG00000000768 $6$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | KCNQ5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | CACNA2D1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | IRS2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | CCK |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | ADHFE1 |
| Markby et al., (2020b) | Grade 3 with normal | -1.6 | SCN2B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | S1PR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | CD55 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | F8 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.6 | ENSCAFG00000001295 <br> 9 |
| Markby et al., (2020b) | Grade 3 with normal | -1.59 | TEK |
| Markby et al ., (2020b) | Grade 3 with normal | -1.59 | HSPA12B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.59 | ADCY2 |
| Markby et al., (2020b) | Grade 3 with normal | -1.59 | PRLR |
| Markby et al ., (2020b) | Grade 3 with normal | -1.59 | SSTR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | FBXO10 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | SNTB1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | EDNRA |


| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | GPR171 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | GFRA1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | MAP2K6 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.58 | ABI3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | OGN |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | ADAMTS2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | TMOD1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | LOC102152109 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | LOC487080 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | CNTN4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | DOCK9 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | ZHX3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | DCLK1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | SEMA4B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | ESAM |
| Markby et al ., (2020b) | Grade 3 with normal | -1.57 | ENSCAFG00000001532 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | ENSCAFG00000001209 $2$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | OMD |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | IDNK |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | SNTB1 |
| Markby et al., (2020b) | Grade 3 with normal | -1.56 | MAML3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | CGNL1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.56 | MKL2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | FAM184A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | KIAA0355 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | ENSCAFG0000002365 5 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | CFAP54 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | PDE3B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | ATP9A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | GATSL3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.55 | ENG |
| Markby et al ., (2020b) | Grade 3 with normal | -1.54 | SEMA3C |
| Markby et al ., (2020b) | Grade 3 with normal | -1.54 | SOX7 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.54 | ABHD10 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.54 | $\begin{aligned} & \text { ENSCAFG0000001672 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | -1.54 | ENSCAFG0000001006 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | THBS2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | POU3F1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | LRP1B |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | FAM198A |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | LGI3 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | PHKA2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.53 | ENSCAFG00000000323 <br> 3 |


| Markby et al ., (2020b) | Grade 3 with normal | -1.52 | FIBIN |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | -1.52 | NFIA |
| Markby et al ., (2020b) | Grade 3 with normal | -1.52 | NPR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.52 | TANC2 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | LOC102152842 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | KIAA1462 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | CAMK1D |
| Markby et al., (2020b) | Grade 3 with normal | -1.51 | ARGLU1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | MSI1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | DECR1 |
| Markby et al ., (2020b) | Grade 3 with normal | -1.51 | KCNN3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | CDKN1A |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | CCND3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | SUGCT |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | NUDT22; DNAJC4 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | NXPH2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | EPHA2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | CALCA |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | CD40 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | NGEF |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | TMEM106C |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | ELL2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | ACSBG1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | BECN1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.51 | LOC480571 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | FBXO27 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | PPP1R14B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | STAB1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | TLR1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | NPNT |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | MED10 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | SATB2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | SMIM3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | ENSCAFG0000001672 $2$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.52 | ENSCAFG0000002597 $3$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | NUAK1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | TREML1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | PRR15 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | TBXAS1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | ENSCAFG00000000423 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | P2RX7 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | $\begin{aligned} & \text { ENSCAFG0000001233 } \\ & 1 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | NLRP1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.53 | IGFBP4 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | GLIPR1 |


| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | CSRP2 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | RNASEH2C |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | SEMA6B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | LOC486400 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | LOC610887 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | EGR2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | SAMD11 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | FZD9 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | ENSCAFG0000002558 <br> 9 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.54 | ENSCAFG0000001237 $6$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | ENSCAFG0000001559 $3$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | WISP1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | ADAM22 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | GNG11 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | TNFAIP8L2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | ARMC6 |
| Markby et al ., (2020b) | Grade 3 with normal |  | ENSCAFG0000000423 |
|  |  | 1.55 | 4/ENSCAFG00000025 |
|  |  |  | 939 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | UBTD1 |
| Markby et al ., (2020b) | Grade 3 with normal |  | ENSCAFG0000000423 |
|  |  | 1.55 | 4/ENSCAFG00000025 |
|  |  |  | 939 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.55 | LOC479476 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | ATP8B1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | LOC611446; LOC100688921 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | MGARP |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | COL4A2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | EAF1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | SLC16A12 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.56 | PARP8 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | KCNN4 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | TYROBP |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | PAPPA |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | SYNC |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | LAMTOR2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.57 | UBL4A |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | COX7A1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | MAPK13 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | BCL2A1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | FAM174A |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | ACHE |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | AKAP5 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.58 | ENSCAFG0000003140 |


| Markby et al ., (2020b) | Grade 3 with normal | 1.59 | TAL1 |
| :---: | :---: | :---: | :---: |
| Markby et al., (2020b) | Grade 3 with normal | 1.59 | MSR1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.59 | TWIST2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.59 | $\begin{aligned} & \text { ENSCAFG0000001103 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.59 | $\begin{aligned} & \text { ENSCAFG0000000929 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.59 | TNFRSF14 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.59 | ENSCAFG0000001928 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.6 | CCDC159 |
| Markby et al., (2020b) | Grade 3 with normal | 1.6 | FOLH1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.6 | GREM1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.6 | HSPB2 |
| Markby et al., (2020b) | Grade 3 with normal | 1.61 | WFS1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.61 | $\begin{aligned} & \text { ENSCAFG0000002037 } \\ & 3 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.61 | RTCA |
| Markby et al., (2020b) | Grade 3 with normal | 1.62 | LRRC25 |
| Markby et al., (2020b) | Grade 3 with normal | 1.62 | WSCD2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.62 | $\begin{aligned} & \text { SLC5A3; } \\ & \text { LOC100856716 } \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.62 | STK17B |
| Markby et al., (2020b) | Grade 3 with normal | 1.62 | PHPT1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.62 | $\begin{aligned} & \text { ENSCAFG00000000450 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.63 | ACYP2 |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | BNC2 |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | ARMC2 |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | DDC |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | BAMBI |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | DYNLRB1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | C1RL |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | CYTIP |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | CLCA1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.63 | CYR61 |
| Markby et al., (2020b) | Grade 3 with normal | 1.64 | MYL9 |
| Markby et al., (2020b) | Grade 3 with normal | 1.64 | LMOD1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.64 | BATF |
| Markby et al., (2020b) | Grade 3 with normal | 1.65 | LOC481248 |
| Markby et al., (2020b) | Grade 3 with normal | 1.65 | ENSCAFG00000002755 <br> 2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.65 | C5 |
| Markby et al., (2020b) | Grade 3 with normal | 1.66 | PDE7B |
| Markby et al., (2020b) | Grade 3 with normal | 1.66 | CCDC115 |
| Markby et al., (2020b) | Grade 3 with normal | 1.66 | PDLIM2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.66 | SLC45A1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.66 | $\begin{aligned} & \text { ENSCAFG00000001029 } \\ & 2 \end{aligned}$ |


| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | CFB; C2 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | RNF19B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | MAPKAPK3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | DZIP1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | GRID2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | LPP |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | ENO1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.67 | WFDC1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.68 | SKAP2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.68 | LBH |
| Markby et al ., (2020b) | Grade 3 with normal | 1.68 | C17H1orf54 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.68 | C24H20orf24 |
| Markby et al., (2020b) | Grade 3 with normal | 1.68 | GAP43 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.69 | RHNO1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.7 | HSPH1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.7 | TAGLN |
| Markby et al., (2020b) | Grade 3 with normal | 1.71 | ITGA8 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.71 | GALNT6 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.71 | CD86 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.72 | CNN2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.72 | LAP3 |
| Markby et al., (2020b) | Grade 3 with normal | 1.72 | ALCAM |
| Markby et al ., (2020b) | Grade 3 with normal | 1.72 | ENSCAFG00000001577 <br> 4 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.73 | SEC61B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.73 | ETF1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.73 | $\begin{aligned} & \text { ENSCAFG0000000274 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.73 | $\begin{aligned} & \text { ENSCAFG0000003275 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.74 | CACNA1D |
| Markby et al., (2020b) | Grade 3 with normal | 1.74 | BTK |
| Markby et al ., (2020b) | Grade 3 with normal | 1.75 | ENSCAFG0000000200 $7$ |
| Markby et al ., (2020b) | Grade 3 with normal | 1.76 | BLVRB |
| Markby et al ., (2020b) | Grade 3 with normal | 1.76 | SEC11C |
| Markby et al., (2020b) | Grade 3 with normal | 1.76 | VASH2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.77 | POU2F2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.77 | CAPG |
| Markby et al ., (2020b) | Grade 3 with normal | 1.77 | FOXS1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.77 | LOC100856200 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.78 | VAV1 |
| Markby et al., (2020b) | Grade 3 with normal | 1.78 | HAVCR1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.78 | PNCK |
| Markby et al ., (2020b) | Grade 3 with normal | 1.78 | ENSCAFG0000000208 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.79 | SPI1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.79 | ENSCAFG0000000534 5 |


| Markby et al ., (2020b) | Grade 3 with normal | 1.79 | ENSCAFG0000002924 |
| :---: | :---: | :---: | :---: |
|  |  | 1.79 | 8 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.8 | SLC7A11 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.8 | ARNTL2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.8 | ENSCAFG00000002637 <br> 3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.8 | LTBP2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.81 | LOXL3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.81 | NDNF |
| Markby et al ., (2020b) | Grade 3 with normal | 1.82 | BNC2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.82 | TLR10 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.82 | MYOCD |
| Markby et al ., (2020b) | Grade 3 with normal | 1.83 | LYZF2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.83 | ENSCAFG00000000534 <br> 5 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.84 | TREM2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.85 | DLA-79 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.86 | ENSCAFG00000002552 <br> 9 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.86 | ADAM28 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.86 | C3AR1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.86 | LOC487977 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.87 | LOC102156311 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.87 | NOV |
| Markby et al ., (2020b) | Grade 3 with normal | 1.87 | LOC478984 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.88 | SENP3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.88 | CLEC3A |
| Markby et al ., (2020b) | Grade 3 with normal | 1.89 | BMP6 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.9 | LOC100686271 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | RSAD2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | CGREF1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | SYNDIG1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | CYTL1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | UCHL1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.91 | NPAS3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.92 | ITGA10 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.92 | RGS2 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.93 | SBSPON |
| Markby et al ., (2020b) | Grade 3 with normal | 1.94 | CDKN2B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.94 | NTRK3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.95 | IL18 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.96 | NME1 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.97 | TNFRSF11B |
| Markby et al ., (2020b) | Grade 3 with normal | 1.98 | ID3 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.99 | MIA |
| Markby et al ., (2020b) | Grade 3 with normal | 1.99 | IFITM10 |
| Markby et al ., (2020b) | Grade 3 with normal | 1.99 | SYTL2 |
| Markby et al ., (2020b) | Grade 3 with normal | 2 | LOC612564 |
| Markby et al ., (2020b) | Grade 3 with normal | 2 | GPER1 |


| Markby et al ., (2020b) | Grade 3 with normal | 2 | LOC100856638; UPP1 |
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| Markby et al ., (2020b) | Grade 3 with normal | 2.01 | DAPP1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.01 | VCAM1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.02 | PILRA |
| Markby et al ., (2020b) | Grade 3 with normal | 2.04 | TNFRSF12A |
| Markby et al ., (2020b) | Grade 3 with normal | 2.04 | ENSCAFG0000003322 <br> 8 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.05 | MRVI1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.06 | TMEM61 |
| Markby et al., (2020b) | Grade 3 with normal | 2.08 | DDX60 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.11 | SPN |
| Markby et al ., (2020b) | Grade 3 with normal | 2.11 | TVP23A |
| Markby et al ., (2020b) | Grade 3 with normal | 2.12 | DNAJB1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.12 | HTR2A |
| Markby et al ., (2020b) | Grade 3 with normal | 2.12 | USP18 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.12 | HOXD8 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.12 | HENMT1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.13 | ENSCAFG0000003841 5 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.14 | HTR2B |
| Markby et al., (2020b) | Grade 3 with normal | 2.14 | TYSND1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.15 | PTGS2 |
| Markby et al., (2020b) | Grade 3 with normal | 2.16 | CCL5 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.17 | EVI2B |
| Markby et al ., (2020b) | Grade 3 with normal | 2.19 | ENSCAFG00000001365 $1$ |
| Markby et al ., (2020b) | Grade 3 with normal | 2.22 | C10H2orf40 |
| Markby et al., (2020b) | Grade 3 with normal | 2.24 | ZNF385B |
| Markby et al ., (2020b) | Grade 3 with normal | 2.24 | C6 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.25 | FCGR1A |
| Markby et al ., (2020b) | Grade 3 with normal | 2.26 | RARRES3 |
| Markby et al., (2020b) | Grade 3 with normal | 2.27 | PAPPA2 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.3 | ARAP2 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.33 | RXFP1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.33 | ENSCAFG00000000294 <br> 7 |
| Markby et al., (2020b) | Grade 3 with normal | 2.36 | CXHXorf21 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.38 | CSTA |
| Markby et al ., (2020b) | Grade 3 with normal | 2.39 | NLGN4X |
| Markby et al ., (2020b) | Grade 3 with normal | 2.4 | CASP14 |
| Markby et al., (2020b) | Grade 3 with normal | 2.4 | SLCO2A1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.41 | ENSCAFG00000003248 $3$ |
| Markby et al ., (2020b) | Grade 3 with normal | 2.42 | $\begin{aligned} & \text { ENSCAFG0000001732 } \\ & 6 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 3 with normal | 2.44 | KCNMB1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.46 | ENSCAFG00000000056 <br> 2. |
| Markby et al ., (2020b) | Grade 3 with normal | 2.46 | ABCC4 |


| Markby et al ., (2020b) | Grade 3 with normal | 2.47 | LOC485235 |
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| Markby et al ., (2020b) | Grade 3 with normal | 2.5 | EPHA3 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.63 | ENSCAFG0000000208 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.65 | CLEC5A |
| Markby et al ., (2020b) | Grade 3 with normal | 2.65 | ENSCAFG0000001166 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.69 | KCNK2 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.7 | IL1RL1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.71 | ZNF385B |
| Markby et al ., (2020b) | Grade 3 with normal | 2.74 | ANGPT1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.77 | LRRC3B |
| Markby et al ., (2020b) | Grade 3 with normal | 2.8 | TNFSF15 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.87 | MMP12 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.92 | SERPINA1 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.97 | HSP70 |
| Markby et al ., (2020b) | Grade 3 with normal | 2.98 | PLCXD3 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.01 | LOC100687667 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.03 | CRLF1 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.03 | CLEC7A |
| Markby et al ., (2020b) | Grade 3 with normal | 3.07 | ENSCAFG0000003273 <br> 1 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.09 | HSP70 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.1 | CNN1 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.17 | COL6A5 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.17 | SERPINE1 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.24 | LOC611538 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.31 | CXCL14 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.35 | IGFBP2 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.38 | TUBB3 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.4 | ENSCAFG0000001166 <br> 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.4 | CCL8 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.43 | TPM2 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.66 | ENSCAFG0000000604 6 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.78 | ENSCAFG0000002956 <br> 8 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.87 | CXCL8 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.87 | MYH11 |
| Markby et al ., (2020b) | Grade 3 with normal | 3.92 | ENSCAFG0000000604 $6$ |
| Markby et al ., (2020b) | Grade 3 with normal | 4.07 | FGG |
| Markby et al ., (2020b) | Grade 3 with normal | 4.28 | RGS4 |
| Markby et al ., (2020b) | Grade 3 with normal | 4.43 | ACTA2 |
| Markby et al ., (2020b) | Grade 3 with normal | 4.57 | ACTG2 |
| Markby et al ., (2020b) | Grade 3 with normal | 5.34 | CDKN2A |
| Markby et al ., (2020b) | Grade 3 with normal | 5.42 | SFRP2 |
| Markby et al ., (2020b) | Grade 3 with normal | 6.1 | LRRN1 |


| Markby et al ., (2020b) | Grade 3 with normal | 7.35 | ENSCAFG0000002955 |
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| Markby et al ., (2020b) | Grade 3 with normal | 7.79 | CDKN2A |
| Markby et al., (2020b) | Grade 3 with normal | 11.7 | ENSCAFG0000002274 |
|  |  |  | 3 |
| Markby et al ., (2020b) | Grade 4 with normal | -4.32 | ENSCAFG0000002517 |
|  |  |  | 2 |
| Markby et al ., (2020b) | Grade 4 with normal | -4.04 | RANBP3L |
| Markby et al ., (2020b) | Grade 4 with normal | -3.81 | TNMD |
| Markby et al ., (2020b) | Grade 4 with normal | -3.75 | ENSCAFG0000002363 |
|  |  |  | 7 |
| Markby et al ., (2020b) | Grade 4 with normal | -3.68 | NKAIN2 |
| Markby et al ., (2020b) | Grade 4 with normal | -3.68 | WFDC5 |
| Markby et al ., (2020b) | Grade 4 with normal | -3.65 | MIR99A-1 |
| Markby et al ., (2020b) | Grade 4 with normal | -3.64 | CILP |
| Markby et al ., (2020b) | Grade 4 with normal | -3.55 | LHCGR |
| Markby et al ., (2020b) | Grade 4 with normal | -3.34 | MMRN1 |
| Markby et al ., (2020b) | Grade 4 with normal | -3.32 | ADRA1A |
| Markby et al ., (2020b) | Grade 4 with normal | -3.28 | SLC26A5 |
| Markby et al., (2020b) | Grade 4 with normal | -3.16 | FSHR |
| Markby et al ., (2020b) | Grade 4 with normal | -3.04 | MIRLET7C |
| Markby et al., (2020b) | Grade 4 with normal | -2.99 | NT5E |
| Markby et al., (2020b) | Grade 4 with normal | -2.9 | LOC488818 |
| Markby et al., (2020b) | Grade 4 with normal | -2.89 | NELL2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.77 | AQP4 |
| Markby et al., (2020b) | Grade 4 with normal | -2.73 | TMEFF2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.73 | FSTL4 |
| Markby et al., (2020b) | Grade 4 with normal | -2.73 | MIR214 |
| Markby et al., (2020b) | Grade 4 with normal | -2.68 | SLC24A2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.68 | IGSF10 |
| Markby et al., (2020b) | Grade 4 with normal | -2.65 | ADCYAP1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.63 | ENSCAFG0000001911 |
|  |  |  | 4 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.6 | WIF1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.6 | MIR218-1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.56 | KCND2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.56 | MPZL2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.51 | MMP3 |
| Markby et al., (2020b) | Grade 4 with normal | -2.49 | GRIN2A |
| Markby et al., (2020b) | Grade 4 with normal | -2.48 | ENSCAFG0000002649 |
|  |  |  | 8 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.47 | KCND2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.43 | HAPLN1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.42 | GJB6 |
| Markby et al., (2020b) | Grade 4 with normal | -2.4 | ADCY2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.4 | ACKR1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.39 | MEI4 |
| Markby et al., (2020b) | Grade 4 with normal | -2.39 | GAS2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.37 | FSTL4 |


| Markby et al ., (2020b) | Grade 4 with normal | -2.36 | ENSCAFG0000002272 |
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| Markby et al ., (2020b) | Grade 4 with normal | -2.34 | LOC482182 |
| Markby et al., (2020b) | Grade 4 with normal | -2.34 | SCN3B |
| Markby et al ., (2020b) | Grade 4 with normal | -2.33 | CCBE1 |
| Markby et al., (2020b) | Grade 4 with normal | -2.32 | FMO2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.3 | ENSCAFG0000004045 <br> 1 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.25 | GFRA2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.24 | ENSCAFG0000001025 $6$ |
| Markby et al ., (2020b) | Grade 4 with normal | -2.23 | ADAMTS15 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.21 | GRIN2A |
| Markby et al ., (2020b) | Grade 4 with normal | -2.2 | ENSCAFG0000000074 $1$ |
| Markby et al ., (2020b) | Grade 4 with normal | -2.19 | ENSCAFG0000002491 <br> 6 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.16 | VWDE |
| Markby et al., (2020b) | Grade 4 with normal | -2.15 | SLC24A2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.15 | MOXD1 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.13 | ENSCAFG0000002806 6 |
| Markby et al ., (2020b) | Grade 4 with normal | -2.08 | HIF3A |
| Markby et al., (2020b) | Grade 4 with normal | -2.08 | VWDE |
| Markby et al., (2020b) | Grade 4 with normal | -2.07 | KCNQ5 |
| Markby et al., (2020b) | Grade 4 with normal | -2.05 | SV2B |
| Markby et al ., (2020b) | Grade 4 with normal | -2.04 | PTPRD |
| Markby et al., (2020b) | Grade 4 with normal | -2.03 | FAM20A |
| Markby et al ., (2020b) | Grade 4 with normal | -2.03 | MYOC |
| Markby et al., (2020b) | Grade 4 with normal | -2.02 | CAPN6 |
| Markby et al., (2020b) | Grade 4 with normal | -2.02 | RBPJL |
| Markby et al., (2020b) | Grade 4 with normal | -2.02 | PPP1R1B |
| Markby et al., (2020b) | Grade 4 with normal | -2.01 | COLCA2 |
| Markby et al., (2020b) | Grade 4 with normal | -2.01 | LRP1B |
| Markby et al ., (2020b) | Grade 4 with normal | -2 | ALDH1A1 |
| Markby et al., (2020b) | Grade 4 with normal | -2 | IGF2BP2 |
| Markby et al ., (2020b) | Grade 4 with normal | -2 | $\begin{aligned} & \text { ENSCAFG0000002273 } \\ & 9 \end{aligned}$ |
| Markby et al., (2020b) | Grade 4 with normal | -1.99 | KANK3 |
| Markby et al., (2020b) | Grade 4 with normal | -1.98 | ENSCAFG0000001866 <br> 1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.97 | ANGPTL5 |
| Markby et al., (2020b) | Grade 4 with normal | -1.96 | KDR |
| Markby et al ., (2020b) | Grade 4 with normal | -1.96 | PDZD2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.95 | TMEFF2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.95 | ADCY2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.95 | FAM209B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.94 | ENSCAFG0000002169 $3$ |


| Markby et al ., (2020b) | Grade 4 with normal | -1.94 | ENSCAFG0000001994 $1$ |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 4 with normal | -1.93 | CRISPLD2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.93 | KIAA1024L |
| Markby et al ., (2020b) | Grade 4 with normal | -1.91 | GPR37 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.91 | LOC474938 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.9 | WNT9B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.9 | AFF2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.9 | LGI2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.9 | MASP1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.9 | ADGRB3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.88 | ANGPTL4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.88 | TRPM3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.88 | S100B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.88 | $\begin{aligned} & \text { ENSCAFG00000002824 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.87 | RASGRF2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.87 | SGCG |
| Markby et al ., (2020b) | Grade 4 with normal | -1.87 | ENSCAFG0000001006 <br> 4 |
| Markby et al ., (2020b) | Grade 4 with normal | $-1.86$ | PTGFR |
| Markby et al ., (2020b) | Grade 4 with normal | -1.86 | $\begin{aligned} & \text { ENSCAFG0000002193 } \\ & 1 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.85 | OVGP1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.84 | ENSCAFG0000002359 <br> 1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.84 | ARGLU1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.84 | SCIN |
| Markby et al ., (2020b) | Grade 4 with normal | -1.83 | LAMA2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | ABCA6 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | DRP2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | MIR491 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | ADGRB3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | PDZD2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.82 | MIRLET7D |
| Markby et al ., (2020b) | Grade 4 with normal | -1.81 | CDC42EP2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.81 | LAMA1 |
| Markby et al ., (2020b) | Grade 4 with normal | $-1.81$ | $\begin{aligned} & \text { ENSCAFG00000003706 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.8 | ENSCAFG0000002901 5 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.8 | TNFRSF19 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.8 | ENSCAFG0000000878 <br> 4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.8 | $\begin{aligned} & \text { ENSCAFG00000002624 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.79 | RNF128 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.79 | ADAMTS19 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.79 | FGF12 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.79 | CCK |


| Markby et al ., (2020b) | Grade 4 with normal | -1.79 | MAP2K6 |
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| Markby et al., (2020b) | Grade 4 with normal | -1.77 | TMEM132C |
| Markby et al ., (2020b) | Grade 4 with normal | -1.77 | SLC4A4 |
| Markby et al., (2020b) | Grade 4 with normal | -1.77 | SEMA3D |
| Markby et al., (2020b) | Grade 4 with normal | -1.77 | GJB2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.77 | ENSCAFG0000002498 <br> 5 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.76 | ENSCAFG0000001286 <br> 0 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.75 | KCNJ2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.75 | SHISA3 |
| Markby et al., (2020b) | Grade 4 with normal | -1.74 | NEBL |
| Markby et al., (2020b) | Grade 4 with normal | -1.74 | TSPAN2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.74 | SOX10 |
| Markby et al., (2020b) | Grade 4 with normal | -1.73 | SNTB1 |
| Markby et al., (2020b) | Grade 4 with normal | -1.72 | SLC2A12 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.72 | ENSCAFG0000000274 $4$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.72 | CD8A |
| Markby et al., (2020b) | Grade 4 with normal | -1.71 | CDH22 |
| Markby et al., (2020b) | Grade 4 with normal | -1.71 | KCNQ5 |
| Markby et al., (2020b) | Grade 4 with normal | -1.71 | TTC21A |
| Markby et al., (2020b) | Grade 4 with normal | -1.71 | FLVCR2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.7 | SLC22A23 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.7 | ENSCAFG00000002366 9 |
| Markby et al., (2020b) | Grade 4 with normal | -1.7 | SYT17 |
| Markby et al., (2020b) | Grade 4 with normal | -1.7 | ENSCAFG00000000289 $7$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | CILP2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | $\begin{aligned} & \text { ENSCAFG00000000293 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | ENSCAFG0000002608 <br> 6 |
| Markby et al., (2020b) | Grade 4 with normal | -1.69 | LOC479911 |
| Markby et al., (2020b) | Grade 4 with normal | -1.69 | $\begin{aligned} & \text { ENSCAFG0000003242 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | ENSCAFG0000001929 <br> 4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | ENSCAFG0000001929 <br> 4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.69 | ENSCAFG0000001929 <br> 4 |
| Markby et al., (2020b) | Grade 4 with normal | -1.68 | WNT16 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.68 | $\begin{aligned} & \text { ENSCAFG0000001498 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.68 | HCN1 |
| Markby et al., (2020b) | Grade 4 with normal | -1.68 | ADAMTSL2 |
| Markby et al., (2020b) | Grade 4 with normal | -1.67 | LAMA2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.67 | PI15 |


| Markby et al ., (2020b) | Grade 4 with normal | -1.67 | SLC10A6 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.67 | SLC1A3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.67 | ENSCAFG0000002601 $1$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.66 | $\begin{aligned} & \text { ENSCAFG0000003435 } \\ & 0 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.66 | UBE2QL1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | ```ENSCAFG0000001327 5``` |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | LIX1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | CNTFR |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | WDR54 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | PDGFRL |
| Markby et al ., (2020b) | Grade 4 with normal | -1.65 | ENSCAFG0000000848 $2$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.64 | IGF2; INS |
| Markby et al ., (2020b) | Grade 4 with normal | -1.64 | GLS2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.64 | DLG2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.64 | SEL1L3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.63 | PCSK6 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.63 | TMEM52 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.63 | SNTB1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.63 | ENSCAFG0000002611 <br> 8 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.63 | DDX31 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.62 | F2RL2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.62 | C1QTNF4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.62 | HSPA12B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.62 | PTN |
| Markby et al ., (2020b) | Grade 4 with normal | -1.62 | GCNT4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.61 | ADCY2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.61 | LOC607729 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | GPLD1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | RASIP1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | ITGA2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | CACNA2D1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | PLSCR4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | $\begin{aligned} & \text { ENSCAFG0000000790 } \\ & 9 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | -1.6 | LPAR4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.59 | MPP6 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.59 | SCARA5 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.59 | RHOU |
| Markby et al ., (2020b) | Grade 4 with normal | -1.59 | LOC490151 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | IGSF3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | ENPP2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | ENSCAFG0000001209 $2$ |


| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | ENSCAFG0000002703 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | TYW3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | ACOT6 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.58 | SLC35F4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | FGL1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | ENSCAFG00000001087 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | ENSCAFG0000001087 |
|  |  |  | 7 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | ENSCAFG0000001087 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | KCNT2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | THBS2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | ENSCAFG00000000541 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.57 | KIAA1755 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | ACKR2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | KERA |
| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | ENSCAFG0000001028 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | CNR1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | RAD52 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | ENSCAFG0000001372 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.56 | BCL6B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | FREM1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | LIPC |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | AASS |
| Markby et al., (2020b) | Grade 4 with normal | -1.55 | ENOX1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | NEIL1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | ENSCAFG0000003027 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | ACAP1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.55 | PRMT6 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.54 | GRIA3 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.54 | C17H1orf56 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.54 | LOC100686869 |
| Markby et al., (2020b) | Grade 4 with normal | -1.54 | RAB9B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.53 | RASL10A |
| Markby et al ., (2020b) | Grade 4 with normal | -1.53 | THPO |
| Markby et al ., (2020b) | Grade 4 with normal | -1.53 | SLC16A9 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.53 | ENSCAFG0000002356 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.52 | SLCO5A1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.52 | MOB3B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.52 | NLGN1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.52 | ENSCAFG0000001089 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.52 | LOC489911; ZNF785; LOC100683431 |
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| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | ANKRD45 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | FKBPL |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | PKHD1L1 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | FAM107B |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | ERBB4 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | CABYR |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | SRSF2 |
| Markby et al ., (2020b) | Grade 4 with normal | -1.51 | CHAD |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | FAM174A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | SGK1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | ALK |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | HYOU1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | CDR2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.51 | GNPNAT1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | TYROBP |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | PDE7B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | FAT1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | TPX2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | PLCB1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | RND1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | HN1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | LOC491973 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | ENSCAFG0000001095 <br> 8 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | $\begin{aligned} & \text { ENSCAFG0000001321 } \\ & 7 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | $\begin{aligned} & \text { ENSCAFG0000001321 } \\ & 7 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.52 | $\begin{aligned} & \text { ENSCAFG0000001321 } \\ & 7 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | TBXAS1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | GALNT6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | CACNA1D |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | SPI1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | TMEM200A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | DYNC1I1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | LIF |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | ERAP2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | ITGA1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | ITGAX |
| Markby et al ., (2020b) | Grade 4 with normal | 1.53 | NEXN |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | EVI2B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | SOSTDC1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | KCNQ1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | ACP5 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | LRRC32 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | SNRNP35 |


| Markby et al ., (2020b) | Grade 4 with normal | 1.54 | BANK1 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | SYNDIG1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | ST5 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | $\begin{aligned} & \text { ENSCAFG0000003101 } \\ & 6 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | RAI14 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | IL10RA |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | ENSCAFG00000002005 9 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.55 | KCNA3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.56 | $\begin{aligned} & \text { ENSCAFG0000001907 } \\ & 2 \end{aligned}$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.56 | BLVRB |
| Markby et al ., (2020b) | Grade 4 with normal | 1.56 | NPAS3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.56 | STK32B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.56 | SCG3 |
| Markby et al., (2020b) | Grade 4 with normal | 1.57 | SERPINI1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.57 | PCP4L1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.57 | DDAH1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.58 | CYR61 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.58 | CKAP2L |
| Markby et al ., (2020b) | Grade 4 with normal | 1.58 | EPHX3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.58 | FLNA |
| Markby et al ., (2020b) | Grade 4 with normal | 1.59 | LOC479476 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.59 | LOC100856200 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.59 | FILIP1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.59 | MT2A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.59 | ARL4C |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.6 | MX1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | NUAK1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | MSR1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | KHDRBS3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | C16H8orf4 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | ENTPD3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.61 | CYBB |
| Markby et al ., (2020b) | Grade 4 with normal | 1.62 | CDKN1A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.62 | CCL8 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.62 | ID4 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.62 | SLIT3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.62 | ANXA8L1 |
| Markby et al., (2020b) | Grade 4 with normal | 1.62 | SEPTIN6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | SEMA6B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | MGARP |
| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | ENSCAFG00000002944 <br> 2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | LOXL2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | MX2 |


| Markby et al ., (2020b) | Grade 4 with normal | 1.63 | ETV4 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | Grade 4 with normal | 1.64 | SYTL2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.64 | TNC |
| Markby et al ., (2020b) | Grade 4 with normal | 1.64 | ECE2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.64 | ADAM19 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.64 | DHX58 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.65 | NOV |
| Markby et al ., (2020b) | Grade 4 with normal | 1.65 | NME1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.65 | GCSAM |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | CCL5 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | CLEC7A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | HDAC9 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | GIMAP2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | COL4A1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | OSBPL10 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.66 | PIK3AP1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | SKAP2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | IL18 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | ENSCAFG0000003248 $3$ |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | ANLN |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | TMEM178A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.67 | SIX1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.68 | GRID2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.68 | LOC100856638; UPP1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.68 | ENSCAFG0000003015 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.68 | AHNAK2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.69 | EGR2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.69 | LPP |
| Markby et al ., (2020b) | Grade 4 with normal | 1.69 | MEOX2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.69 | RELN |
| Markby et al ., (2020b) | Grade 4 with normal | 1.7 | BNC2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.7 | DAPP1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.7 | C5AR1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.7 | ADAM 22 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.7 | CACNA1A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.71 | LMOD1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.72 | BNC2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.72 | ABCC4 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.73 | SATB2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.73 | DDC |
| Markby et al ., (2020b) | Grade 4 with normal | 1.73 | VASH2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.73 | VCAM1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.74 | RSAD2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.74 | OSR1 |
| Markby et al., (2020b) | Grade 4 with normal | 1.74 | ENSCAFG0000002534 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.74 | ENSCAFG0000002534 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.74 | ENSCAFG0000002534 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.75 | CYTL1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.75 | THBS 4 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.76 | HAVCR1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.76 | ZNF804B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.77 | ENSCAFG0000002558 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.77 | ENSCAFG0000003100 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.77 | NDNF |
| Markby et al ., (2020b) | Grade 4 with normal | 1.77 | SLCO2A1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.77 | ENSCAFG0000002039 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.78 | GAP43 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.79 | STK17B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.79 | ARNTL2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.79 | MCAM |
| Markby et al ., (2020b) | Grade 4 with normal | 1.8 | CD86 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.8 | SBSPON |
| Markby et al ., (2020b) | Grade 4 with normal | 1.81 | WISP1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.81 | BTK |
| Markby et al ., (2020b) | Grade 4 with normal | 1.81 | LOC612564 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.81 | TMEM236 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.81 | TMEM255A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.82 | ATP8B1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.82 | FOXS1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.82 | TNFRSF11B |
| Markby et al ., (2020b) | Grade 4 with normal | 1.82 | TYSND1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.82 | PXDNL |
| Markby et al ., (2020b) | Grade 4 with normal | 1.83 | ENSCAFG0000001732 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.83 | CLEC4G |
| Markby et al ., (2020b) | Grade 4 with normal | 1.84 | LTBP2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.85 | ADAM28 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.85 | SMPDL3A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.86 | CPNE4 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.86 | EGR3 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.87 | GPER1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.87 | CLEC5A |
| Markby et al ., (2020b) | Grade 4 with normal | 1.87 | EDN1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.87 | IL7R |
| Markby et al ., (2020b) | Grade 4 with normal | 1.88 | SLC7A11 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.88 | CGREF1 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.88 | ARAP2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.88 | CD70 |


| Markby et al ., (2020b) | Grade 4 with normal | 1.89 | ANGPTL1 |
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| Markby et al ., (2020b) | Grade 4 with normal | 1.9 | CAPG |
| Markby et al ., (2020b) | Grade 4 with normal | 1.9 | TNFRSF12A |
| Markby et al., (2020b) | Grade 4 with normal | 1.9 | ENSCAFG0000000604 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.9 | ENSCAFG0000000604 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.9 | ENSCAFG0000000604 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.9 | MFSD2A |
| Markby et al., (2020b) | Grade 4 with normal | 1.9 | ALK |
| Markby et al., (2020b) | Grade 4 with normal | 1.91 | DLA-79 |
| Markby et al., (2020b) | Grade 4 with normal | 1.91 | RGS2 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.91 | MRVI1 |
| Markby et al., (2020b) | Grade 4 with normal | 1.91 | SMOC2 |
| Markby et al., (2020b) | Grade 4 with normal | 1.91 | NID2 |
| Markby et al., (2020b) | Grade 4 with normal | 1.91 | NXPH3 |
| Markby et al., (2020b) | Grade 4 with normal | 1.92 | USP18 |
| Markby et al ., (2020b) | Grade 4 with normal | 1.93 | ENSCAFG0000001904 $8$ |
| Markby et al., (2020b) | Grade 4 with normal | 1.94 | LYZF2 |
| Markby et al., (2020b) | Grade 4 with normal | 1.95 | TREML1 |
| Markby et al., (2020b) | Grade 4 with normal | 1.95 | CLEC3A |
| Markby et al., (2020b) | Grade 4 with normal | 1.95 | DDX60 |
| Markby et al., (2020b) | Grade 4 with normal | 1.97 | DAPK2 |
| Markby et al., (2020b) | Grade 4 with normal | 1.99 | ADAM22 |
| Markby et al., (2020b) | Grade 4 with normal | 1.99 | SLIT3 |
| Markby et al., (2020b) | Grade 4 with normal | 2 | HTR2A |
| Markby et al., (2020b) | Grade 4 with normal | 2 | FNDC1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.01 | AGMO |
| Markby et al., (2020b) | Grade 4 with normal | 2.03 | COL4A2 |
| Markby et al., (2020b) | Grade 4 with normal | 2.03 | PLCXD3 |
| Markby et al., (2020b) | Grade 4 with normal | 2.05 | PAPPA |
| Markby et al., (2020b) | Grade 4 with normal | 2.07 | CYTIP |
| Markby et al., (2020b) | Grade 4 with normal | 2.07 | C6 |
| Markby et al., (2020b) | Grade 4 with normal | 2.07 | CD80 |
| Markby et al., (2020b) | Grade 4 with normal | 2.08 | TREM1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.09 | TREM2 |
| Markby et al., (2020b) | Grade 4 with normal | 2.1 | ENSCAFG0000000208 <br> 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.1 | TNFSF15 |
| Markby et al., (2020b) | Grade 4 with normal | 2.15 | PTGS2 |
| Markby et al., (2020b) | Grade 4 with normal | 2.16 | UCHL1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.16 | FCGR1A |
| Markby et al ., (2020b) | Grade 4 with normal | 2.19 | BMP6 |
| Markby et al., (2020b) | Grade 4 with normal | 2.19 | ELFN1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.2 | ENSCAFG0000000604 6 |


| Markby et al ., (2020b) | Grade 4 with normal | 2.2 | ENSCAFG0000000604 |
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| Markby et al ., (2020b) | Grade 4 with normal | 2.2 | ENSCAFG0000000604 |
|  |  |  | 6 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.22 | CASP14 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.23 | RGS4 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.25 | KCNMB1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.25 | SDK2 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.33 | ANGPT1 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.35 | LOC486400 |
| Markby et al., (2020b) | Grade 4 with normal | 2.35 | PAPPA2 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.35 | LOC100686047 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.38 | SPN |
| Markby et al ., (2020b) | Grade 4 with normal | 2.38 | HOXD8 |
| Markby et al., (2020b) | Grade 4 with normal | 2.4 | NTRK3 |
| Markby et al., (2020b) | Grade 4 with normal | 2.41 | RGS1 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.45 | CLDN1 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.5 | CSTA |
| Markby et al., (2020b) | Grade 4 with normal | 2.51 | NLGN4X |
| Markby et al., (2020b) | Grade 4 with normal | 2.51 | SERPINA1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.52 | LOC611538 |
| Markby et al ., (2020b) | Grade 4 with normal | 2.54 | MYOCD |
| Markby et al., (2020b) | Grade 4 with normal | 2.55 | CNN1 |
| Markby et al., (2020b) | Grade 4 with normal | 2.73 | LRRC3B |
| Markby et al., (2020b) | Grade 4 with normal | 2.76 | IGFBP2 |
| Markby et al., (2020b) | Grade 4 with normal | 2.83 | TPM2 |
| Markby et al., (2020b) | Grade 4 with normal | 2.88 | RXFP1 |
| Markby et al., (2020b) | Grade 4 with normal | 3 | FGG |
| Markby et al., (2020b) | Grade 4 with normal | 3.11 | HTR2B |
| Markby et al., (2020b) | Grade 4 with normal | 3.19 | EPHA3 |
| Markby et al ., (2020b) | Grade 4 with normal | 3.2 | ENSCAFG0000000208 $6$ |
| Markby et al., (2020b) | Grade 4 with normal | 3.23 | CDKN2A |
| Markby et al., (2020b) | Grade 4 with normal | 3.24 | ACTA2 |
| Markby et al ., (2020b) | Grade 4 with normal | 3.44 | ENSCAFG0000000294 7 |
| Markby et al ., (2020b) | Grade 4 with normal | 3.68 | CRLF1 |
| Markby et al., (2020b) | Grade 4 with normal | 3.77 | MMP12 |
| Markby et al., (2020b) | Grade 4 with normal | 3.82 | CCL13 |
| Markby et al ., (2020b) | Grade 4 with normal | 3.89 | LRRN1 |
| Markby et al., (2020b) | Grade 4 with normal | 4.02 | MYH11 |
| Markby et al., (2020b) | Grade 4 with normal | 4.13 | CCL24 |
| Markby et al., (2020b) | Grade 4 with normal | 4.18 | ACTG2 |
| Markby et al ., (2020b) | Grade 4 with normal | 4.34 | SERPINE1 |
| Markby et al., (2020b) | Grade 4 with normal | 4.97 | CEMIP |
| Markby et al., (2020b) | Grade 4 with normal | 5.07 | CDKN2A |
| Markby et al., (2020b) | Grade 4 with normal | 5.63 | CXCL8 |
| Markby et al ., (2020b) | Grade 4 with normal | 5.72 | ENSCAFG0000002956 <br> 8 |

Markby et al ., (2020b) Grade 4 with normal
5.72
5.89
9.57

Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 16.73
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 16.64
Markby et al., (2020b) "disease" dissected with "normal" dissectı - 16.27
Markby et al., (2020b) "disease" dissected with "normal" dissecti - 16.25
Markby et al.,(2020b) "disease" dissected with "normal" dissectı - 12.87
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 12.85
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 9.72
Markby et al ., (2020b) "disease" dissected with "normal" dissecti - 8.95
Markby et al ., (2020b) "disease" dissected with "normal" dissectı -8.91
Markby et al ., (2020b) "disease" dissected with "normal" dissecti - 8.9
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 8.49
Markby et al ., (2020b) "disease" dissected with "normal" dissectı -8.09
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 7.81
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 7.59
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 6.72
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 6.59
Markby et al ., (2020b) "disease" dissected with "normal" dissecti - 5.99
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 5.77
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 5.73
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 5.49
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 5.22
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 5.18
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 4.82
Markby et al ., (2020b) "disease" dissected with "normal" dissect - -4.7
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 4.66
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 4.58
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 4.32
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 4.31
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 4.28
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 4.22
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 4.02
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 4
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 3.79
Markby et al ., (2020b) "disease" dissected with "normal" dissectı -3.78
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 3.71
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 3.69
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 3.51
Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 3.43
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 3.42
Markby et al ., (2020b) "disease" dissected with "normal" dissectı -3.4
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 3.23
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 3.19

ENSCAFG0000002956 8

ENSCAFG0000002956
8
SFRP2
ENSCAFG0000002274
3

ADIPOQ

PCK1
PLIN4
CIDEC
F3
THRSP
MMRN1
FGL1
SLC22A1
DGAT2
SCN7A
LOC479668
GPD1
CIDEA
PLIN1
FFAR4
PHEX
CILP
AGT
TUSC5
EPYC
MGST1
AQP3
LGALS12
CD36
ACVR1C
TSHR
SGK2
PCP4
TNMD
PPARG
ASPA
COMP
SIX1
CXCL12
ZNF385B
OMD
ZNF385B
PROKR1
NRXN1
CLCA2
MAL

Markby et al ., (2020b) "disease" dissected with "normal" dissecti - 3.12

NRXN1
LOC476900
CFH
FMO2
IGF2
CHL1
SDR16C5
CALB2
MAL2
LYZF2
KLKB1
ABCA6
LIPE
ACKR4
WDR88
KCNT2
SLITRK6
ACSM3
GALNT15
NNAT
LGI1
BMP5
ERICH3
ENPEP
ABCD2 LEP LRIG3 AGMO WISP3 CLDN5 Mar-01 FAM213A
NMUR2
PDE8B
MLXIPL
ISM1
F2RL2
CFD
FAT3
ALDH1A3
ANGPTL5
TENM2
PCOLCE2 SDK1 LPAR4
RASGRF2
ANGPTL7 CCK

Markby et al ., (2020b) "disease" dissected with "normal" dissect - 2.02

SCD
OGN MRAP SDK1

KANK3
TLL1
ADIPOR2
LPIN1
TMEM235
ABCA8
HGF
SEMA3D
VEGFC
LAYN
ESR1
INSIG1
UCP1
SYBU
C1QTNF7
GPLD1
ENOX1
CDRT4
ADRA1A
MYBL1
THSD7A
THSD7A
THSD7A
STC2
HMGCS1
COL14A1
LEPR
LIFR
EYA4
MCHR1
ADGRB3
WISP2
GREM2
ADH4
LOC476006
SEMA3C
HCAR1
AMPH
PLA2G16
TENM2
CDKL1
SCNN1B
SGCG
SLC10A6

Markby et al ., (2020b) "disease" dissected with "normal" dissecti - 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.66
Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.65
Markby et al ., (2020b) "disease" dissected with "normal" dissectı -1.64 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.63 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.63 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.62 Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 1.61 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.61 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.59 Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 1.58 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.58 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.57 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.57 Markby et al ., (2020b) "disease" dissected with "normal" dissectı -1.57 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.56 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.56 Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 1.55 Markby et al ., (2020b) "disease" dissected with "normal" dissectı -1.55 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.55 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.54 Markby et al ., (2020b) "disease" dissected with "normal" dissectı -1.54 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı - 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissect - 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.51 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.52 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.53 Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.54

SIDT1
LOC490151
MAP2K6
FMN2
ADGRB3
SLC18A2
SCN9A
GHR
SVEP1
LYVE1
LOC610975
NIPSNAP1
GRIA1
HMGCLL1 EBF2

SMOC1
ADAMTS9
C28H10orf10
AGMO
EFCC1
IGFBP2
SLC4A4
HRH2
MAB21L2 FZD4

IGFBP6
GPR1
PDE3B
EPHX2
SIX4
EBF2
PLCL1
LOC100683099
BMP8B
ANPEP
SCG3
SHC3
PARVG
DLA-DMB
CDK6
CACNA1A
SLC4A8
PIK3AP1
LY9
CAMTA1
LOC484897
BLNK
IKZF1

Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.54
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.54
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.54
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.54
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Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.56
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.57
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.57
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.57
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Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.57
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.57
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.58
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Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.58
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Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.59
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.59
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.59
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.6
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.6
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.6
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.6
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Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.6
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.61
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.61
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.61
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.61
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.62
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.62
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.62
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.63
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.63
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.63
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.64
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.64
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.65
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.65
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.66
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.66
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.67

PTPN5
CD86
LOC478984
CDH23
IL31RA
CSF2RA
CSF3R
FCGR1A
HEPACAM
PHLDA1
CAPG
HTR2B
TMEM229B
GJC1
PCYT1B
ELOF1
PRPH
CD80
HAVCR1
CLEC7A
ENO2
DAPP1
SLC16A6
PLAUR
CYTH4
ALOX5AP
HHEX
DUSP5
IL21R
APCDD1
LOC481722
TBXAS1
FERMT3
ARAP2
KCNN4
LOC482987
LOC100856638
CA12
CLDN1
CYTIP
TMEM59L
RGS10
LY86
HTR4
MRVI1
ASPM
WISP1
LPXN

Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.67
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.7
Markby et al., (2020b) "disease" dissected with "normal" dissect 1.7
Markby et al., (2020b) "disease" dissected with "normal" dissect 1.7
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.71
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.71
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.71
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.71
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.72
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.72
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.74
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.74
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.76
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.76
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.77
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.79
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.79
Markby et al., (2020b) "disease" dissected with "normal" dissect 1.8
Markby et al.,(2020b) "disease" dissected with "normal" dissect 1.8
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.8
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.81
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.83
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.89
Markby et al., (2020b) "disease" dissected with "normal" dissectı 1.9
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.93
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.94
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.95
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.95
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.95
Markby et al ., (2020b) "disease" dissected with "normal" dissect 1.96
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 1.99
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.07
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 2.11
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 2.13
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 2.22
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.25
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.26
Markby et al ., (2020b) "disease" dissected with "normal" dissecti 2.27
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.27
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.33
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 2.38
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.41
Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.49
Markby et al ., (2020b) "disease" dissected with "normal" dissectı 2.59

ADGRG1 CCR5
MMP12
IL10RA
TRIM9
PLAT
ADAM28
CH25H
TNFSF8
UNC5D
CLSTN2
KMO
CTHRC1
LPAR3
LOC102152056
NDP
SMPDL3A
DLA-DMA
DLA-DQB1
ACTA2
ITGAX
CLEC5A
GJB2
CCL24
SALL3
C5AR1
UBE2C
HBEGF
FNDC1
EGR2
TREM1
SLC10A4
SELL
TVP23A
CDKN2A
TUBB3
LOC611538
IL2RA
RGS4
SELE
SERPINA1
LOC481248
SBSPON
CCL3
CSTA
SFRP2
CDKN2A
CNTNAP4

Markby et al ., (2020b) "disease" dissected with "normal" dissect 2.6

CCL13
CRLF1
SLITRK2 CCL7

OPRD1
LOC612122
LOC608320
HPRT1
GABRG3
CAPN6
KCNK1
TSPAN2
MIRLET7D
FBXO48
CDH2
WDR54
SLC2A12
LOC102156643
CDKN2AIP
NELL2
TMEM55A
TMEM35
ARMT1
C15H12orf29
ATP6V0E2
MCAT
MBLAC2
PURA
ASB7
PSAT1
SEC62
ADCY2
TSPAN12
PSMD9
MTO1
NEU3
PTGS1
SPIN4
CMTM6
GATM
MYCN
SEC22A
LOC102152109
MOB3C
SEH1L
SNAI2
PACRGL
MARS2

| Markby et al ., (2020b) | "normal" dissected with whole valve norn | -1.53 | PMP22 |
| :---: | :---: | :---: | :---: |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | -1.52 | ALOX5AP |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | -1.51 | RMDN3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | -1.51 | PABPC5 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.51 | FBXO46 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.51 | MGARP |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.51 | MEDAG |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.51 | NPAS3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | RABAC1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | AGPAT4 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | DNTTIP1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | TP53RK |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | PSMD1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | TLR1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.52 | CLCN2 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.52 | LMF1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | RNASET2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | NUAK1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | MNF1 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.53 | CPSF1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | INSIG1 |
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| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | SP100 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.53 | DECR1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | MINK1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | SNX29 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.53 | VASH1 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.54 | APBA1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.54 | TYROBP |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.54 | C12H6orf136 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.54 | RGS22 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.54 | NUDT17 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.54 | SLC10A6 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.54 | NEXN |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.55 | SMARCC2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | ZC3H3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | PYCRL |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | TSTA3 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.55 | GSTK1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | SUCLG1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | TAS1R2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.55 | RPL21 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.56 | HIVEP2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | COQ10A |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | LOC102155065 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | NDUFB7 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | LOC100687825 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | PHB |


| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.56 | ADGRG2 |
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| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.57 | RABEP2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | TBC1D10C |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | GTPBP4 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | USE1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | LOC611113 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | DNAJC6 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | STX1B |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.58 | SLC25A29 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.59 | JRK |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.59 | LIMK2 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.6 | ADIPOR2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.6 | GHDC |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.61 | METTL17 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.61 | ANGPT2 |
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| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.61 | NFKB2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.61 | ALCAM |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.61 | PLEKHA6 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.62 | FOXE3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.63 | SLC22A4 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.63 | TRMT112 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.63 | LOC106557821 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.63 | C5H16orf74 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.63 | LPAR3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.64 | RNF32 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.64 | LOC106558651 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.64 | LOC488298 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.64 | DNAJB2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.64 | CLDN3 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.64 | IRAK1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.65 | SYTL3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.65 | UBAC2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.66 | HOXA5 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.66 | RPL23 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.67 | CLEC5A |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.67 | SLC39A12 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.67 | IGFBP6 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.67 | KIAA2012 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.67 | ASPSCR1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.68 | STEAP1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.69 | FAM110A |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.69 | WSCD2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.69 | AAAS |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.69 | LOC607806 |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.7 | METTL24 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.7 | RGS22 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.7 | AMDHD1 |


| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.7 | LOC611835 |
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| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.7 | NAT6 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.7 | GREM2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.71 | RPL7 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.71 | FBXO28 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.72 | CAECAM1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.72 | CMPK1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.72 | LOC102153034 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.72 | TMEM256 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 1.73 | PLA2G16 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 1.73 | GPC5 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.73 | PHF23 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.74 | LOC612471 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.74 | PRR16 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.74 | SNX21 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.76 | PSMC3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.76 | CAPS |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.76 | NFATC4 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.77 | MYBL1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.78 | CHSY3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.79 | DAPK2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.79 | VASH2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.81 | GPIHBP1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.81 | C18H11orf85 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.81 | LOC102151205 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 1.81 | ECI2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.81 | ZC3H12B |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.82 | MFSD2A |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.82 | INPP1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.82 | RPS2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.83 | SYN2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.83 | MRPL48 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.84 | FGF1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.84 | VAT1L |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.87 | TMEM200A |
| Markby et al., (2020b) | "normal" dissected with whole valve norn | 1.88 | RETN |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.92 | MAL |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.93 | WISP1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.93 | GMPPA |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.95 | TREM2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.95 | MX1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.96 | CA3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.97 | UBA52 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.99 | PTPN23 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 1.99 | ADAM33 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 2 | DUSP22 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 2.01 | CMTM8 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.03 | SIT1 |


| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.03 | KRT18 |
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| Markby et al ., (2020b) | "normal" dissected with whole valve norn | 2.07 | LOC106559613 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.11 | GTF2A2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.13 | ANGPTL1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.13 | HLF |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.18 | NMUR2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.18 | CIDEA |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.23 | B3GAT3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.25 | TYSND1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.29 | LEPR |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.42 | TCEANC |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.49 | CALB2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.68 | IL1RL1 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 2.88 | F3 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 3.1 | LOC608162 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 3.5 | DGAT2 |
| Markby et al ., (2020b) | "normal" dissected with whole valve norm | 3.63 | LYZF2 |
| Liet al ., (2015) | LV tissue | -3.81 | A5H028 |
| Liet al ., (2015) | LV tissue | NA | A7XZY9 |
| Liet al., (2015) | LV tissue | -6.45 | AADAC |
| Liet al ., (2015) | LV tissue | -2.9 | ABCA2 |
| Liet al ., (2015) | LV tissue | -3.56 | ABCA3 |
| Liet al., (2015) | LV tissue | 3 | ABCC4 |
| Liet al., (2015) | LV tissue | -5.49 | ABCC8 |
| Li et al ., (2015) | LV tissue | -2.29 | ABHD6 |
| Liet al ., (2015) | LV tissue | 2.27 | ABRA |
| Li et al ., (2015) | LV tissue | 3.64 | ABRACL |
| Li et al ., (2015) | LV tissue | 5.91 | ACER2 |
| Li et al ., (2015) | LV tissue | -3.67 | ACPP |
| Liet al ., (2015) | LV tissue | -2.98 | ACSL1 |
| Liet al ., (2015) | LV tissue | 2.43 | ACTB |
| Li et al ., (2015) | LV tissue | 3.09 | ACTG1 |
| Liet al ., (2015) | LV tissue | 3.71 | ACTN1 |
| Liet al ., (2015) | LV tissue | 4.23 | ADAMTS1 |
| Li et al ., (2015) | LV tissue | 13.36 | ADAMTS4 |
| Li et al ., (2015) | LV tissue | -4.12 | ADAMTS7 |
| Liet al ., (2015) | LV tissue | 13.83 | ADAMTS9 |
| Liet al ., (2015) | LV tissue | -2.74 | ADCY3 |
| Liet al., (2015) | LV tissue | -2.67 | ADCY5 |
| Li et al ., (2015) | LV tissue | -4.65 | ADCYAP1R1 |
| Liet al ., (2015) | LV tissue | 3.29 | ADML |
| Liet al ., (2015) | LV tissue | 2.22 | ADSS |
| Liet al ., (2015) | LV tissue | -3.45 | AFAP1L2 |
| Li et al ., (2015) | LV tissue | -5.02 | AGXT2L1 |
| Liet al., (2015) | LV tissue | 100.55 | AHSP |
| Liet al., (2015) | LV tissue | 5.42 | AKAP12 |
| Liet al., (2015) | LV tissue | 2.38 | ALAS1 |
| Liet al., (2015) | LV tissue | 3.59 | ALAS2 |


| Li et al ., (2015) | LV tissue | 2.79 | AMIGO2 |
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| Li et al ., (2015) | LV tissue | -3.32 | AMOT |
| Liet al ., (2015) | LV tissue | 3.87 | ANF |
| Li et al ., (2015) | LV tissue | -4.22 | ANGEL1 |
| Li et al ., (2015) | LV tissue | -7.2 | ANGPTL1 |
| Li et al ., (2015) | LV tissue | -2.99 | ANK1 |
| Liet al ., (2015) | LV tissue | -2.99 | ANKRD29 |
| Li et al., (2015) | LV tissue | 3.18 | ANKRD37 |
| Liet al ., (2015) | LV tissue | 2.71 | AOFA |
| Liet al., (2015) | LV tissue | 3.4 | AP1S3 |
| Liet al., (2015) | LV tissue | 3.07 | AP3S1 |
| Liet al., (2015) | LV tissue | -9.21 | APLNR |
| Li et al ., (2015) | LV tissue | -2.18 | APOL5 |
| Liet al., (2015) | LV tissue | 7.67 | APOLD1 |
| Liet al., (2015) | LV tissue | 2.38 | AQP1 |
| Li et al ., (2015) | LV tissue | 53.71 | AQP9 |
| Li et al ., (2015) | LV tissue | 2.19 | ARF4 |
| Liet al., (2015) | LV tissue | 7.42 | ARHGAP15 |
| Liet al., (2015) | LV tissue | 3.55 | ARID5A |
| Li et al ., (2015) | LV tissue | 4.41 | ARL4A |
| Li et al ., (2015) | LV tissue | 3.68 | ARNTL |
| Li et al., (2015) | LV tissue | 2.8 | ARPC1B |
| Li et al., (2015) | LV tissue | 2.94 | ARPC3 |
| Liet al ., (2015) | LV tissue | 2.38 | ART3 |
| Liet al., (2015) | LV tissue | -5.58 | ART5 |
| Liet al., (2015) | LV tissue | -2.98 | ASB13 |
| Liet al., (2015) | LV tissue | -3.46 | ASB18 |
| Liet al ., (2015) | LV tissue | 2.98 | ASB9 |
| Liet al ., (2015) | LV tissue | 4.45 | ASNS |
| Liet al., (2015) | LV tissue | -3.06 | ASPA |
| Liet al., (2015) | LV tissue | 2.73 | ATP13A3 |
| Liet al., (2015) | LV tissue | 2.52 | ATP1B3 |
| Liet al ., (2015) | LV tissue | -3.37 | ATP2B4 |
| Li et al ., (2015) | LV tissue | -4.58 | ATXN7L1 |
| Liet al., (2015) | LV tissue | 51.17 | B0FF11 |
| Liet al., (2015) | LV tissue | -4.46 | B2CRU9 |
| Liet al., (2015) | LV tissue | -6.96 | B8PZS8 |
| Li et al ., (2015) | LV tissue | -4.21 | BCAR3 |
| Liet al ., (2015) | LV tissue | 32.42 | BCL2A1 |
| Liet al., (2015) | LV tissue | 5.59 | BCL3 |
| Liet al., (2015) | LV tissue | -2.41 | BCL7A |
| Li et al., (2015) | LV tissue | 13.87 | BDNF |
| Li et al ., (2015) | LV tissue | 3.2 | BHLHE40 |
| Liet al., (2015) | LV tissue | -7.47 | BMF |
| Liet al ., (2015) | LV tissue | 6.33 | BSPRY |
| Liet al., (2015) | LV tissue | 2.59 | BTG2 |
| Liet al., (2015) | LV tissue | 2.29 | BZW1 |
| Liet al ., (2015) | LV tissue | 3.53 | C10orf10 |


| Li et al ., (2015) | LV tissue | -3.3 | C12orf52 |
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| Li et al ., (2015) | LV tissue | 5 | C13orf33 |
| Liet al., (2015) | LV tissue | 3.93 | C14orf37 |
| Liet al., (2015) | LV tissue | 20.59 | C15orf48 |
| Li et al ., (2015) | LV tissue | -2.49 | C17orf28 |
| Li et al ., (2015) | LV tissue | 6.54 | C17orf64 |
| Liet al., (2015) | LV tissue | 16.02 | C19orf59 |
| Liet al., (2015) | LV tissue | -3.63 | C19orf68 |
| Li et al ., (2015) | LV tissue | -3.64 | C1orf192 |
| Li et al ., (2015) | LV tissue | 2.57 | C1QA |
| Liet al., (2015) | LV tissue | 2.97 | C1QB |
| Liet al., (2015) | LV tissue | 2.31 | C1QC |
| Li et al ., (2015) | LV tissue | -3.39 | C1QTNF2 |
| Li et al ., (2015) | LV tissue | -2.94 | C1QTNF7 |
| Li et al ., (2015) | LV tissue | 13.47 | C5AR1 |
| Li et al., (2015) | LV tissue | -2.3 | C5orf4 |
| Li et al ., (2015) | LV tissue | 6.86 | C5orf62 |
| Li et al ., (2015) | LV tissue | -3.08 | C5orf65 |
| Li et al ., (2015) | LV tissue | 2.86 | C9orf153 |
| Li et al., (2015) | LV tissue | NA | CA3 |
| Li et al ., (2015) | LV tissue | 5.5 | CA4 |
| Liet al., (2015) | LV tissue | -5.3 | CA8 |
| Liet al ., (2015) | LV tissue | -8.98 | CAMKV |
| Li et al., (2015) | LV tissue | -9.3 | CAPN11 |
| Liet al., (2015) | LV tissue | -2.55 | CASZ1 |
| Liet al., (2015) | LV tissue | 10.92 | CCBP2 |
| Liet al., (2015) | LV tissue | 4.89 | CCDC172 |
| Li et al ., (2015) | LV tissue | -4.55 | CCDC68 |
| Liet al., (2015) | LV tissue | -5.76 | CCDC8 |
| Liet al., (2015) | LV tissue | -21.24 | CCDC85C |
| Liet al., (2015) | LV tissue | 19.56 | CCL2 |
| Liet al., (2015) | LV tissue | -9.34 | CCL24 |
| Li et al ., (2015) | LV tissue | -2.73 | CCND1 |
| Li et al., (2015) | LV tissue | 2.39 | CCNL1 |
| Li et al ., (2015) | LV tissue | 2.72 | CD163 |
| Li et al ., (2015) | LV tissue | 2.46 | CD22 |
| Li et al ., (2015) | LV tissue | -2.67 | CD248 |
| Liet al., (2015) | LV tissue | 12.83 | CD274 |
| Li et al., (2015) | LV tissue | 4.77 | CD300C |
| Li et al., (2015) | LV tissue | -10.19 | CD300LG |
| Li et al ., (2015) | LV tissue | -7.32 | CDH15 |
| Liet al., (2015) | LV tissue | 10.44 | CH25H |
| Li et al., (2015) | LV tissue | -3.21 | CIDEA |
| Liet al., (2015) | LV tissue | -7.25 | CIDEC |
| Liet al., (2015) | LV tissue | 9.45 | CLDN1 |
| Liet al., (2015) | LV tissue | -3.38 | CLDN4 |
| Liet al., (2015) | LV tissue | -3.31 | CLEC3B |
| Liet al ., (2015) | LV tissue | 2.45 | CLIC1 |


| Li et al., (2015) | LV tissue | 4.35 | CLIC2 |
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| Li et al., (2015) | LV tissue | 2.34 | CLK1 |
| Liet al., (2015) | LV tissue | -2.59 | CLN6 |
| Liet al., (2015) | LV tissue | -7.93 | CMA1 |
| Liet al., (2015) | LV tissue | 2.48 | CNN2 |
| Liet al., (2015) | LV tissue | -2.67 | COL14A1 |
| Liet al., (2015) | LV tissue | 2.7 | COL16A1 |
| Liet al., (2015) | LV tissue | 2.72 | CORO1A |
| Liet al., (2015) | LV tissue | 2.83 | COTL1 |
| Liet al., (2015) | LV tissue | -5.86 | CPA3 |
| Liet al., (2015) | LV tissue | 3.54 | CPS1 |
| Li et al., (2015) | LV tissue | NA | CRHR1 |
| Li et al., (2015) | LV tissue | 2.93 | CRISPLD2 |
| Li et al., (2015) | LV tissue | -4.59 | CSAD |
| Li et al., (2015) | LV tissue | 3.95 | CSF1 |
| Liet al., (2015) | LV tissue | 10.55 | CSF2RA |
| Li et al., (2015) | LV tissue | 10.57 | CSF3R |
| Liet al., (2015) | LV tissue | -3.9 | CSGALNACT1 |
| Liet al., (2015) | LV tissue | 7.11 | CSRNP1 |
| Liet al., (2015) | LV tissue | 6.85 | CTGF |
| Liet al., (2015) | LV tissue | 3.77 | CTHRC1 |
| Li et al., (2015) | LV tissue | 2.61 | CTTNBP2NL |
| Liet al., (2015) | LV tissue | 8.09 | CXCL14 |
| Li et al., (2015) | LV tissue | 3.33 | CXCL16 |
| Liet al., (2015) | LV tissue | 2.28 | CXorf36 |
| Li et al., (2015) | LV tissue | -3.69 | CYB561 |
| Li et al., (2015) | LV tissue | -13.74 | CYP1A1 |
| Li et al., (2015) | LV tissue | 3.5 | CYP1B1 |
| Liet al., (2015) | LV tissue | 3.19 | CYR61 |
| Li et al., (2015) | LV tissue | -6.2 | MMP11 |
| Li et al., (2015) | LV tissue | -3.69 | DACT1 |
| Li et al., (2015) | LV tissue | -8.37 | DAO |
| Liet al., (2015) | LV tissue | 14.6 | DARC |
| Liet al., (2015) | LV tissue | 11.55 | DDIT4 |
| Li et al., (2015) | LV tissue | -7.03 | DDN |
| Li et al., (2015) | LV tissue | 3.44 | DEF6 |
| Liet al., (2015) | LV tissue | 2.57 | DEGS1 |
| Li et al., (2015) | LV tissue | -2.44 | DGCR2 |
| Li et al., (2015) | LV tissue | 6.4 | DKK2 |
| Li et al., (2015) | LV tissue | 3.77 | DLL1 |
| Li et al., (2015) | LV tissue | 3.13 | DOK2 |
| Liet al., (2015) | LV tissue | -18.13 | DPP10 |
| Li et al., (2015) | LV tissue | 5.95 | DRAM1 |
| Liet al., (2015) | LV tissue | 18.3 | DUSP5 |
| Li et al., (2015) | LV tissue | 2.9 | DUSP6 |
| Liet al., (2015) | LV tissue | 4.62 | E5D812 |
| Li et al., (2015) | LV tissue | -3.47 | E5G722 |
| Liet al., (2015) | LV tissue | 5.43 | E7ECW0 |


| Li et al ., (2015) | LV tissue | 3.66 | EDNRB |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | LV tissue | 5.27 | EGR1 |
| Li et al ., (2015) | LV tissue | -2.43 | EHMT2 |
| Li et al ., (2015) | LV tissue | 2.34 | ELL |
| Li et al ., (2015) | LV tissue | 3.55 | ELOVL7 |
| Li et al., (2015) | LV tissue | 4.78 | EMB |
| Li et al ., (2015) | LV tissue | 2.51 | EMCN |
| Li et al ., (2015) | LV tissue | 7.26 | EMP1 |
| Liet al., (2015) | LV tissue | 3.16 | EMR1 |
| Liet al ., (2015) | LV tissue | 4.16 | ENTPD3 |
| Liet al., (2015) | LV tissue | 2.46 | EPHA2 |
| Li et al ., (2015) | LV tissue | -3.41 | ERBB3 |
| Li et al ., (2015) | LV tissue | -2.36 | ERMP1 |
| Liet al., (2015) | LV tissue | 3.48 | ERO1L |
| Liet al., (2015) | LV tissue | 3.81 | ERRFI1 |
| Li et al ., (2015) | LV tissue | 2.89 | ESM1 |
| Li et al ., (2015) | LV tissue | 3.59 | ETS2 |
| Liet al., (2015) | LV tissue | -7.61 | EXPH5 |
| Liet al., (2015) | LV tissue | -4.11 | EXTL1 |
| Li et al ., (2015) | LV tissue | -2.85 | EYA1 |
| Li et al ., (2015) | LV tissue | -11.29 | F1SX83 |
| Li et al ., (2015) | LV tissue | -2.87 | FADS1 |
| Li et al., (2015) | LV tissue | -4.03 | FAM110B |
| Li et al ., (2015) | LV tissue | 3.19 | FAM110D |
| Liet al ., (2015) | LV tissue | -2.42 | FAM115A |
| Liet al., (2015) | LV tissue | -9.08 | FAM166B |
| Li et al., (2015) | LV tissue | 7.34 | FAM176C |
| Liet al., (2015) | LV tissue | -3.32 | FAM180B |
| Li et al ., (2015) | LV tissue | 2.85 | FAM188A |
| Liet al., (2015) | LV tissue | -4.36 | FAM198B |
| Liet al ., (2015) | LV tissue | -4.93 | FAM212A |
| Liet al., (2015) | LV tissue | -3.9 | FAM26F |
| Liet al., (2015) | LV tissue | 2.53 | FAM43A |
| Liet al., (2015) | LV tissue | -3.14 | FAM78A |
| Liet al., (2015) | LV tissue | -3.38 | FAT1 |
| Liet al., (2015) | LV tissue | -4.48 | FBLN7 |
| Li et al ., (2015) | LV tissue | -4.13 | FBN2 |
| Li et al., (2015) | LV tissue | -2.72 | FBXO40 |
| Li et al ., (2015) | LV tissue | 7.13 | FCGR1A |
| Li et al ., (2015) | LV tissue | -3.65 | FCHO1 |
| Liet al., (2015) | LV tissue | 2.2 | FDX1 |
| Li et al., (2015) | LV tissue | 20.22 | FFAR2 |
| Li et al ., (2015) | LV tissue | 5.21 | FGF7 |
| Li et al ., (2015) | LV tissue | -3.54 | FGFBP1 |
| Li et al ., (2015) | LV tissue | 5.68 | FGL2 |
| Li et al., (2015) | LV tissue | 4.05 | FGR |
| Liet al., (2015) | LV tissue | 2.76 | FHL1 |
| Liet al ., (2015) | LV tissue | -2.83 | FITM2 |


| Li et al ., (2015) | LV tissue | 3.76 | FKBP5 |
| :---: | :---: | :---: | :---: |
| Liet al ., (2015) | LV tissue | 2.69 | FLNB |
| Li et al ., (2015) | LV tissue | -3.18 | FLYWCH2 |
| Liet al., (2015) | LV tissue | -5.61 | FMO2 |
| Li et al ., (2015) | LV tissue | -3.73 | FMO3 |
| Liet al ., (2015) | LV tissue | -4.29 | FNDC1 |
| Liet al ., (2015) | LV tissue | 9.93 | FOS |
| Liet al., (2015) | LV tissue | NA | FOSL1 |
| Li et al ., (2015) | LV tissue | 8.08 | FOSL2 |
| Li et al ., (2015) | LV tissue | -19.56 | FOXR1 |
| Liet al., (2015) | LV tissue | -5.23 | FREM2 |
| Liet al., (2015) | LV tissue | 2.28 | FRIL |
| Li et al ., (2015) | LV tissue | 2.32 | FRIL |
| Li et al ., (2015) | LV tissue | 2.85 | FRMD8 |
| Liet al ., (2015) | LV tissue | 2.32 | FSTL3 |
| Liet al ., (2015) | LV tissue | -3 | FYCO1 |
| Li et al ., (2015) | LV tissue | -5.38 | FZD8 |
| Li et al ., (2015) | LV tissue | 5.39 | G0ZS87 |
| Liet al ., (2015) | LV tissue | -4.04 | GAB3 |
| Liet al ., (2015) | LV tissue | 6.4 | GADD45B |
| Liet al ., (2015) | LV tissue | -4.67 | GALNTL1 |
| Liet al., (2015) | LV tissue | -2.23 | GATA4 |
| Li et al ., (2015) | LV tissue | -4.63 | GBP6 |
| Liet al., (2015) | LV tissue | 3.17 | GEM |
| Liet al ., (2015) | LV tissue | 5.08 | GFPT2 |
| Liet al., (2015) | LV tissue | -5.99 | GFRA2 |
| Liet al., (2015) | LV tissue | -3.9 | GJA1 |
| Liet al., (2015) | LV tissue | -3.33 | GLE1 |
| Liet al., (2015) | LV tissue | 2.94 | GLIPR2 |
| Liet al., (2015) | LV tissue | -17.35 | GLT25D2 |
| Liet al., (2015) | LV tissue | -3.43 | GLTPD1 |
| Liet al., (2015) | LV tissue | -2.57 | GM2A |
| Liet al., (2015) | LV tissue | 41.66 | GNAT1 |
| Liet al., (2015) | LV tissue | -2.42 | GNB3 |
| Li et al ., (2015) | LV tissue | 2.91 | GNE |
| Li et al., (2015) | LV tissue | 13.94 | GNRH1 |
| Liet al ., (2015) | LV tissue | -4.53 | GPD1 |
| Liet al., (2015) | LV tissue | -3.08 | GPR162 |
| Liet al ., (2015) | LV tissue | 2.93 | GPR4 |
| Liet al., (2015) | LV tissue | -2.79 | GPT |
| Li et al ., (2015) | LV tissue | -3.23 | GPT2 |
| Liet al ., (2015) | LV tissue | 2.36 | GRAMD3 |
| Liet al., (2015) | LV tissue | -4.49 | GRIA3 |
| Liet al., (2015) | LV tissue | 2.56 | GSTM3 |
| Liet al., (2015) | LV tissue | 3.82 | HAPLN3 |
| Liet al., (2015) | LV tissue | 37.31 | HAS1 |
| Liet al., (2015) | LV tissue | 58.03 | HBA |
| Li et al ., (2015) | LV tissue | 84.56 | HBM |


| Li et al., (2015) | LV tissue | 5.31 | HGF |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | LV tissue | 6.43 | HIVEP3 |
| Liet al., (2015) | LV tissue | 5.76 | HK2 |
| Li et al., (2015) | LV tissue | -5.12 | GDA |
| Liet al., (2015) | LV tissue | 9.35 | ENSCAFG0000000740 <br> 1 |
| Li et al., (2015) | LV tissue | 10.16 | ENSCAFG0000002340 <br> 1 |
| Liet al., (2015) | LV tissue | 11.1 | ENSCAFG0000003273 <br> 1 |
| Liet al., (2015) | LV tissue | 11.21 | ENSCAFG0000000715 <br> 4 |
| Liet al., (2015) | LV tissue | 13.63 | $\begin{aligned} & \text { ENSCAFG0000001126 } \\ & 3 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 15.77 | $\begin{aligned} & \text { ENSCAFG0000003217 } \\ & 3 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 28.57 | ENSCAFG0000000719 <br> 9 |
| Liet al., (2015) | LV tissue | 33.62 | $\begin{aligned} & \text { ENSCAFG0000001495 } \\ & 0 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 50.49 | ENSCAFG0000003261 <br> 5 |
| Liet al., (2015) | LV tissue | 53.57 | ENSCAFG0000000039 9 |
| Liet al., (2015) | LV tissue | 2.19 | $\begin{aligned} & \text { ENSCAFG0000000251 } \\ & 7 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.22 | ENSCAFG0000000274 <br> 8 |
| Liet al., (2015) | LV tissue | 2.23 | ENSCAFG0000000874 <br> 1 |
| Liet al., (2015) | LV tissue | 2.24 | $\begin{aligned} & \text { ENSCAFG0000003155 } \\ & 7 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.24 | H3F3A |
| Liet al., (2015) | LV tissue | 2.26 | $\begin{aligned} & \text { ENSCAFG0000001722 } \\ & 1 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.26 | ENSCAFG0000000657 <br> 7 |
| Liet al., (2015) | LV tissue | 2.28 | $\begin{aligned} & \text { ENSCAFG0000001590 } \\ & 3 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.3 | ENSCAFG0000001195 <br> 4 |
| Liet al., (2015) | LV tissue | 2.31 | $\begin{aligned} & \text { ENSCAFG0000000711 } \\ & 2 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.31 | $\begin{aligned} & \text { ENSCAFG0000001222 } \\ & 2 \end{aligned}$ |
| Liet al., (2015) | LV tissue | 2.32 | ENSCAFG0000002495 5 |
| Liet al., (2015) | LV tissue | 2.32 |  |
| Liet al., (2015) | LV tissue | 2.33 | ENSCAFG0000001496 $8$ |


| Li et al ., (2015) | LV tissue | 2.35 | ENSCAFG0000001399 |
| :---: | :---: | :---: | :---: |
|  |  |  | 0 |
| Liet al ., (2015) | LV tissue | 2.36 | ENSCAFG0000001613 |
|  |  |  | 1 |
| Li et al ., (2015) | LV tissue | 2.38 | ENSCAFG0000000136 |
|  |  |  | 9 |
| Liet al ., (2015) | LV tissue | 2.41 | ENSCAFG0000000056 |
|  |  |  | 5 |
| Liet al ., (2015) | LV tissue | 2.41 | ENSCAFG0000000233 |
|  |  |  | 6 |
| Li et al ., (2015) | LV tissue | 2.42 | ENSCAFG0000000458 |
|  |  |  | 9 |
| Liet al ., (2015) | LV tissue | 2.42 | ENSCAFG0000003253 |
|  |  |  | 7 |
| Liet al ., (2015) | LV tissue | 2.43 | ENSCAFG0000001967 |
|  |  |  | 7 |
| Li et al ., (2015) | LV tissue | 2.43 | ENSCAFG0000000208 |
|  |  |  | 4 |
| Li et al ., (2015) | LV tissue | 2.5 | ENSCAFG0000002874 |
|  |  |  | 4 |
| Liet al ., (2015) | LV tissue | 2.51 | ENSCAFG0000000845 |
|  |  |  | 6 |
| Li et al ., (2015) | LV tissue | 2.54 | ENSCAFG0000001479 |
|  |  |  | 0 |
| Liet al ., (2015) | LV tissue | 2.55 | ENSCAFG0000001914 |
|  |  |  | 1 |
| Liet al ., (2015) | LV tissue | 2.6 | ENSCAFG0000000640 |
|  |  |  | 3 |
| Liet al ., (2015) | LV tissue | 2.6 | ENSCAFG0000003094 |
|  |  |  | 2 |
| Liet al ., (2015) | LV tissue | 2.61 | ENSCAFG0000003094 |
|  |  |  | 3 |
| Liet al ., (2015) | LV tissue | 2.64 | ENSCAFG0000003129 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 2.66 | ENSCAFG0000002311 |
|  |  |  | 1 |
| Li et al ., (2015) | LV tissue | 2.66 | ENSCAFG0000000906 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | 2.77 | ENSCAFG0000000786 |
|  |  |  | 5 |
| Liet al ., (2015) | LV tissue | 2.8 | ENSCAFG0000000223 |
|  |  |  | 7 |
| Liet al ., (2015) | LV tissue | 2.85 | ENSCAFG0000002506 |
|  |  |  | 3 |
| Liet al ., (2015) | LV tissue | 2.93 | ENSCAFG0000001111 |
|  |  |  | 9 |
| Liet al ., (2015) | LV tissue | 2.95 | ENSCAFG0000000388 |
|  |  |  | 0 |
| Li et al ., (2015) | LV tissue | 3.08 | ENSCAFG0000003049 |
|  |  |  | 8 |
| Liet al ., (2015) | LV tissue | 3.1 | ENSCAFG0000003018 |
|  |  |  | 7 |


| Li et al ., (2015) | LV tissue | 3.19 | ENSCAFG0000001223 |
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|  |  |  | 3 |
| Li et al ., (2015) | LV tissue | 3.31 | ENSCAFG0000001468 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 3.36 | ENSCAFG0000001915 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 3.58 | ENSCAFG0000001844 |
|  |  |  | 0 |
| Liet al ., (2015) | LV tissue | 3.61 | ENSCAFG0000000449 |
|  |  |  | 6 |
| Liet al ., (2015) | LV tissue | 3.61 | ENSCAFG0000001425 |
|  |  |  | 6 |
| Liet al ., (2015) | LV tissue | 3.63 | ENSCAFG0000000898 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 3.91 | ENSCAFG0000002494 |
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| Li et al ., (2015) | LV tissue | 4 | ENSCAFG0000000585 |
|  |  |  | 2 |
| Li et al ., (2015) | LV tissue | 4.26 | ENSCAFG0000003154 |
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| Liet al ., (2015) | LV tissue | 4.72 | ENSCAFG0000003074 |
|  |  |  | 6 |
| Liet al ., (2015) | LV tissue | 4.74 | ENSCAFG0000000062 |
|  |  |  | 4 |
| Li et al ., (2015) | LV tissue | 5.06 | ENSCAFG0000000726 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 5.1 | ENSCAFG0000000864 |
|  |  |  | 8 |
| Li et al ., (2015) | LV tissue | 5.48 | ENSCAFG0000000557 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | 5.7 | ENSCAFG0000001380 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | 6.25 | ENSCAFG0000000730 |
|  |  |  | 7 |
| Li et al ., (2015) | LV tissue | 6.45 | ENSCAFG0000001919 |
|  |  |  | 8 |
| Liet al ., (2015) | LV tissue | 6.76 | ENSCAFG0000002893 |
|  |  |  | 2 |
| Li et al ., (2015) | LV tissue | 6.94 | ENSCAFG0000003186 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | 7.78 | ENSCAFG0000003184 |
|  |  |  |  |
| Li et al ., (2015) | LV tissue | 8.89 | ENSCAFG0000003130 |
|  |  |  | 6 |
| Li et al ., (2015) | LV tissue | -10.77 | ENSCAFG0000000564 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | -10.71 | ENSCAFG0000000240 |
|  |  |  | 9 |
| Liet al ., (2015) | LV tissue | -9.47 | ENSCAFG0000002271 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | -9.12 | ENSCAFG0000002272 |
|  |  |  | 1 |


| Li et al ., (2015) | LV tissue | -8.8 | ENSCAFG0000002272 |
| :---: | :---: | :---: | :---: |
|  |  |  | 2 |
| Li et al ., (2015) | LV tissue | -8.19 | ENSCAFG0000002272 |
|  |  |  | 0 |
| Li et al ., (2015) | LV tissue | -7.76 | ENSCAFG0000000712 |
|  |  |  | 0 |
| Li et al ., (2015) | LV tissue | -6.71 | ENSCAFG0000002882 |
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| Liet al ., (2015) | LV tissue | -6.56 | ENSCAFG0000002864 |
|  |  |  | 2 |
| Liet al ., (2015) | LV tissue | -6.32 | ENSCAFG0000002272 |
|  |  |  | 5 |
| Liet al ., (2015) | LV tissue | -6.02 | ENSCAFG0000000901 |
|  |  |  | 4 |
| Liet al ., (2015) | LV tissue | -5.86 | ENSCAFG0000003008 |
|  |  |  | 9 |
| Li et al ., (2015) | LV tissue | -5.58 | ENSCAFG0000002932 |
|  |  |  | 4 |
| Li et al ., (2015) | LV tissue | -5.4 | ENSCAFG0000001684 |
|  |  |  | 8 |
| Liet al ., (2015) | LV tissue | -5.16 | ENSCAFG0000000674 |
|  |  |  | 5 |
| Liet al ., (2015) | LV tissue | -5.07 | ENSCAFG0000002464 |
|  |  |  | 1 |
| Li et al ., (2015) | LV tissue | -4.99 | ENSCAFG0000003066 |
|  |  |  | 2 |
| Li et al ., (2015) | LV tissue | -4.41 | ENSCAFG0000000567 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | -4.39 | ENSCAFG0000003184 |
|  |  |  | 8 |
| Li et al ., (2015) | LV tissue | -4.27 | ENSCAFG0000000049 |
|  |  |  | 7 |
| Li et al ., (2015) | LV tissue | -4.01 | ENSCAFG0000001161 |
|  |  |  | 6 |
| Li et al ., (2015) | LV tissue | -3.81 | ENSCAFG0000002271 |
|  |  |  | 2 |
| Liet al ., (2015) | LV tissue | -3.73 | ENSCAFG0000003014 |
|  |  |  | 0 |
| Li et al ., (2015) | LV tissue | -3.57 | ENSCAFG0000000381 |
|  |  |  | 8 |
| Li et al ., (2015) | LV tissue | -3.31 | ENSCAFG0000001682 |
|  |  |  |  |
| Li et al ., (2015) | LV tissue | -3.19 | ENSCAFG0000003133 |
|  |  |  | 7 |
| Li et al ., (2015) | LV tissue | -3.12 | ENSCAFG0000002271 |
|  |  |  | 6 |
| Li et al ., (2015) | LV tissue | -3.05 | ENSCAFG0000002273 |
|  |  |  | 1 |
| Li et al ., (2015) | LV tissue | -2.86 | ENSCAFG0000001995 |
|  |  |  | 0 |
| Li et al ., (2015) | LV tissue | -2.38 | ENSCAFG0000001603 |
|  |  |  | 1 |


| Li et al ., (2015) | LV tissue | 2.12 | ENSCAFG0000001607 |
| :---: | :---: | :---: | :---: |
|  |  |  | 2 |
| Liet al ., (2015) | LV tissue | -9.36 | ENSCAFG0000000359 |
|  |  |  | 5 |
| Li et al ., (2015) | LV tissue | 3.45 | STAT3 |
| Liet al., (2015) | LV tissue | 41.8 | STC1 |
| Liet al., (2015) | LV tissue | -5.54 | SV2B |
| Li et al ., (2015) | LV tissue | 2.65 | SWAP70 |
| Li et al ., (2015) | LV tissue | -4.07 | SYNPO2L |
| Liet al., (2015) | LV tissue | -8.15 | SYT7 |
| Liet al., (2015) | LV tissue | -2.63 | TAB1 |
| Li et al ., (2015) | LV tissue | -2.3 | TACO1 |
| Li et al ., (2015) | LV tissue | 3.17 | TAF7L |
| Li et al ., (2015) | LV tissue | 2.42 | TAGLN |
| Li et al., (2015) | LV tissue | 2.85 | TAGLN2 |
| Li et al ., (2015) | LV tissue | -2.61 | TBC1D2B |
| Li et al ., (2015) | LV tissue | -10.56 | TCEAL7 |
| Li et al ., (2015) | LV tissue | -4.72 | TCF15 |
| Liet al., (2015) | LV tissue | -5.74 | TDRD1 |
| Li et al ., (2015) | LV tissue | 3.81 | TEAD4 |
| Liet al., (2015) | LV tissue | -2.84 | TEF |
| Liet al., (2015) | LV tissue | 24.88 | TESPA1 |
| Li et al., (2015) | LV tissue | 2.58 | TGFBR2 |
| Liet al., (2015) | LV tissue | 2.75 | THBD |
| Liet al., (2015) | LV tissue | 17.77 | THBS1 |
| Liet al., (2015) | LV tissue | 5.45 | THBS4 |
| Li et al ., (2015) | LV tissue | -2.83 | THNSL2 |
| Liet al., (2015) | LV tissue | -3.29 | THSD1 |
| Li et al ., (2015) | LV tissue | -3.81 | TK1 |
| Liet al., (2015) | LV tissue | 6.51 | TKTL1 |
| Li et al ., (2015) | LV tissue | 2.68 | TLR4 |
| Li et al ., (2015) | LV tissue | -5.23 | TMC5 |
| Li et al ., (2015) | LV tissue | -2.44 | TMC6 |
| Li et al., (2015) | LV tissue | -3.91 | TMEM164 |
| Li et al ., (2015) | LV tissue | 3.18 | TMEM176A |
| Li et al ., (2015) | LV tissue | 2.65 | TMEM181 |
| Liet al., (2015) | LV tissue | 3.69 | TMEM182 |
| Li et al., (2015) | LV tissue | 2.3 | TMEM2 |
| Liet al., (2015) | LV tissue | -2.78 | TMEM205 |
| Li et al ., (2015) | LV tissue | -3.52 | TMEM97 |
| Li et al ., (2015) | LV tissue | 2.43 | TMM47 |
| Li et al., (2015) | LV tissue | -11.61 | TMOD4 |
| Liet al., (2015) | LV tissue | 11.98 | TNC |
| Li et al ., (2015) | LV tissue | 3.45 | TNFAIP3 |
| Liet al., (2015) | LV tissue | -4.06 | TNFSF10 |
| Liet al., (2015) | LV tissue | 9.35 | TNFSF9 |
| Li et al ., (2015) | LV tissue | -2.41 | TOM1 |
| Li et al ., (2015) | LV tissue | 4.15 | TPM4 |
| Li et al ., (2015) | LV tissue | 6.15 | TREM2 |


| Li et al., (2015) | LV tissue |
| :---: | :---: |
| Li et al., (2015) | LV tissue |
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| Li et al., (2015) | LV tissue |
| Li et al., (2015) | LV tissue |
| Li et al., (2015) | LV tissue |
| Liet al., (2015) | LV tissue |
| Li et al., (2015) | LV tissue |
| Liet al., (2015) | LV tissue |


| 3.28 | TRIB1 |
| :---: | :---: |
| 2.98 | TRMT11 |
| -4.37 | TRYT |
| 3.41 | TSPAN19 |
| -3.12 | TSPAN9 |
| -3.22 | TTC28 |
| -3.82 | TTC34 |
| -6.55 | TTYH2 |
| 2.68 | TUBA1B |
| 2.31 | TUBA1C |
| 7.55 | TUBB6 |
| NA | TUSC5 |
| 2.57 | TXN |
| 3.44 | TYROBP |
| 11.52 | U3 |
| -2.46 | UACA |
| 2.4 | UAP1 |
| 4.15 | UGCG |
| 2.92 | UGDH |
| -2.13 | UNC45B |
| -2.65 | USP9X |
| 4.9 | VCAN |
| -3.76 | VIPR1 |
| 4.99 | VIPR2 |
| -3.07 | VPS13D |
| -44.28 | VWCE |
| 3.45 | VWF |
| 6.17 | WDR89 |
| 15.05 | WFDC1 |
| 13.34 | WNT9B |
| -3.21 | WSCD1 |
| -2.95 | XIRP2 |
| NA | Xist_exon4 |
| -3.26 | ZBTB12 |
| -5.21 | ZBTB20 |
| -3.73 | ZBTB40 |
| 7.81 | ZFP36 |
| -5.27 | ZNF446 |
| -2.13 | ZNF532 |
| -16.8 | ZNF835 |
| 19.74 | ZP2 |
| 9.51 | ZPLD1 |
| NA | ENSCAFG0000003029 |
|  | 9 |
| -17.68 | ENSCAFG0000001712 |
|  | 5 |
| -16.91 | ENSCAFG0000000050 |
|  | 2 |


| Li et al ., (2015) | LV tissue | -12.31 | ENSCAFG0000002848 |
| :---: | :---: | :---: | :---: |
|  |  |  | 2 |
| Li et al ., (2015) | LV tissue | -12.11 | ENSCAFG0000001265 |
|  |  |  | 7 |
| Liet al ., (2015) | LV tissue | -60.39 | MYH13 |
| Li et al ., (2015) | LV tissue | -48.34 | MYH4 |
| Li et al ., (2015) | LV tissue | -3.13 | MYH7B |
| Li et al ., (2015) | LV tissue | -2.43 | MYH8 |
| Li et al ., (2015) | LV tissue | 11.51 | MYL1 |
| Li et al ., (2015) | LV tissue | 5.55 | MYOC |
| Liet al ., (2015) | LV tissue | 5.85 | MYOF |
| Li et al ., (2015) | LV tissue | -4.05 | N4BP3 |
| Liet al ., (2015) | LV tissue | -4.42 | NAALAD2 |
| Li et al ., (2015) | LV tissue | 3.45 | NABP1 |
| Li et al ., (2015) | LV tissue | -3.39 | NAV3 |
| Li et al ., (2015) | LV tissue | 2.91 | NDRG1 |
| Li et al ., (2015) | LV tissue | -2.53 | NDST1 |
| Li et al ., (2015) | LV tissue | -12.29 | NDST3 |
| Li et al ., (2015) | LV tissue | -5.35 | NEURL1B |
| Liet al ., (2015) | LV tissue | 5.19 | NFE2 |
| Li et al ., (2015) | LV tissue | 2.15 | NFIL3 |
| Li et al ., (2015) | LV tissue | -2.84 | NFIX |
| Li et al ., (2015) | LV tissue | 4.25 | NFKBIA |
| Li et al ., (2015) | LV tissue | 4.98 | NFKBIZ |
| Li et al ., (2015) | LV tissue | -9.89 | NGFR |
| Liet al ., (2015) | LV tissue | -2.29 | NID1 |
| Li et al ., (2015) | LV tissue | -6.74 | NIPAL1 |
| Li et al ., (2015) | LV tissue | -3.94 | NLRX1 |
| Li et al ., (2015) | LV tissue | -21.84 | NOS2 |
| Li et al ., (2015) | LV tissue | -2.74 | NOTCH3 |
| Liet al ., (2015) | LV tissue | 2.44 | NOV |
| Liet al ., (2015) | LV tissue | -4.48 | NOX5 |
| Liet al ., (2015) | LV tissue | 2.7 | NPNT |
| Liet al ., (2015) | LV tissue | 3.08 | NPPB |
| Liet al ., (2015) | LV tissue | -2.7 | NPR3 |
| Li et al ., (2015) | LV tissue | -4.21 | NPTXR |
| Li et al ., (2015) | LV tissue | -2.24 | NR1D2 |
| Li et al ., (2015) | LV tissue | -2.48 | NR2F6 |
| Li et al ., (2015) | LV tissue | 3.04 | NR4A1 |
| Li et al ., (2015) | LV tissue | -5.81 | NRIP2 |
| Li et al ., (2015) | LV tissue | -2.53 | NRP1 |
| Li et al ., (2015) | LV tissue | -3.34 | NT5C1A |
| Li et al ., (2015) | LV tissue | -4.12 | NTHL1 |
| Li et al ., (2015) | LV tissue | 2.51 | 097530 |
| Li et al ., (2015) | LV tissue | 4.41 | 097702 |
| Li et al ., (2015) | LV tissue | -3.26 | OBSCN |
| Liet al ., (2015) | LV tissue | 2.16 | ODC1 |
| Li et al ., (2015) | LV tissue | -3.2 | OLFML1 |
| Li et al ., (2015) | LV tissue | -5.36 | OLFML2A |


| Li et al., (2015) | LV tissue | 2.45 | OR51E2 |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | LV tissue | 41.64 | OSM |
| Li et al ., (2015) | LV tissue | 5.4 | OSMR |
| Lietal., (2015) | LV tissue | -4.39 | OSR2 |
| Li et al ., (2015) | LV tissue | 3.2 | OTUD1 |
| Li et al., (2015) | LV tissue | -3.21 | P2RY1 |
| Liet al., (2015) | LV tissue | -3.19 | PABPC1L |
| Li et al ., (2015) | LV tissue | 6.71 | PADI4 |
| Li et al ., (2015) | LV tissue | -2.47 | PAK6 |
| Li et al ., (2015) | LV tissue | -7.97 | PCDH12 |
| Li et al ., (2015) | LV tissue | -48.44 | PCP2 |
| Li et al., (2015) | LV tissue | 2.44 | PDE4B |
| Li et al ., (2015) | LV tissue | -2.34 | PDGFB |
| Li et al ., (2015) | LV tissue | -2.3 | PDIA2 |
| Li et al ., (2015) | LV tissue | 2.54 | PDXK |
| Li et al., (2015) | LV tissue | -2.36 | PDZRN3 |
| Li et al ., (2015) | LV tissue | -3.75 | PER3 |
| Li et al., (2015) | LV tissue | 2.26 | PFN2 |
| Li et al ., (2015) | LV tissue | -4.32 | PGBD5 |
| Li et al., (2015) | LV tissue | 4.86 | PGF |
| Li et al., (2015) | LV tissue | -3.41 | PHACTR3 |
| Liet al., (2015) | LV tissue | 3.27 | PIK3R5 |
| Liet al., (2015) | LV tissue | -2.29 | PLA2G4A |
| Li et al ., (2015) | LV tissue | -4.89 | PLA2G5 |
| Liet al., (2015) | LV tissue | 6.45 | PLAC8 |
| Li et al., (2015) | LV tissue | 2.84 | PLAGL1 |
| Li et al., (2015) | LV tissue | 17.27 | PLAUR |
| Li et al ., (2015) | LV tissue | 6.02 | PLBD1 |
| Li et al ., (2015) | LV tissue | -2.93 | PLCD1 |
| Liet al., (2015) | LV tissue | -2.59 | PLCE1 |
| Li et al., (2015) | LV tissue | 4.77 | PLEK |
| Li et al ., (2015) | LV tissue | -22.27 | PLIN1 |
| Li et al ., (2015) | LV tissue | 2.23 | PLK2 |
| Liet al., (2015) | LV tissue | 3.26 | PLK3 |
| Li et al., (2015) | LV tissue | -4.09 | PLP1 |
| Li et al., (2015) | LV tissue | 2.99 | PLP2 |
| Li et al., (2015) | LV tissue | -4.07 | PLSCR4 |
| Liet al., (2015) | LV tissue | -5.03 | PLXDC1 |
| Liet al., (2015) | LV tissue | -2.31 | PLXNA1 |
| Liet al., (2015) | LV tissue | -3.74 | PLXNB1 |
| Li et al., (2015) | LV tissue | -2.5 | PLXND1 |
| Li et al., (2015) | LV tissue | 2.88 | PM20D2 |
| Liet al., (2015) | LV tissue | 4.52 | PMEPA1 |
| Liet al., (2015) | LV tissue | 2.55 | PNPLA8 |
| Liet al., (2015) | LV tissue | 12.43 | POSTN |
| Liet al., (2015) | LV tissue | -10.36 | PPP1R16B |
| Li et al ., (2015) | LV tissue | -6.23 | PPP1R1B |
| Liet al., (2015) | LV tissue | -4.87 | PPP1R1C |


| Liet al., (2015) | LV tissue | -2.3 | PPP1R3A |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | LV tissue | -2.7 | PRICKLE1 |
| Liet al., (2015) | LV tissue | -2.55 | PRKAR2B |
| Li et al., (2015) | LV tissue | -5.3 | PROM1 |
| Li et al., (2015) | LV tissue | 7.17 | PRPH |
| Li et al., (2015) | LV tissue | -2.55 | PRR12 |
| Li et al., (2015) | LV tissue | 4.39 | PRSS23 |
| Li et al., (2015) | LV tissue | 3.81 | PTGIR |
| Li et al., (2015) | LV tissue | 8.99 | PTHR |
| Liet al., (2015) | LV tissue | -2.53 | PTPRE |
| Liet al., (2015) | LV tissue | 58.92 | PTX3 |
| Li et al., (2015) | LV tissue | 5.29 | PVR |
| Li et al., (2015) | LV tissue | 3.39 | PXDC1 |
| Liet al., (2015) | LV tissue | -2.52 | Q19KA9 |
| Liet al., (2015) | LV tissue | 42.54 | Q1ERY9 |
| Li et al., (2015) | LV tissue | 23.73 | Q2EG92 |
| Li et al., (2015) | LV tissue | 54.31 | Q2LC20 |
| Liet al., (2015) | LV tissue | -10.62 | Q3HTT6 |
| Liet al., (2015) | LV tissue | -3.51 | Q3HTT9 |
| Li et al., (2015) | LV tissue | -4.18 | Q4PLA8 |
| Li et al., (2015) | LV tissue | -2.74 | Q5BMM8 |
| Li et al., (2015) | LV tissue | -2.37 | Q5J2F2 |
| Li et al., (2015) | LV tissue | -3.11 | Q5SBJ3 |
| Liet al., (2015) | LV tissue | -3.03 | Q5YLN6 |
| Liet al., (2015) | LV tissue | 5.66 | Q683K8 |
| Liet al., (2015) | LV tissue | 47.68 | TIMP1 |
| Li et al., (2015) | LV tissue | 5.72 | NOS3 |
| Liet al., (2015) | LV tissue | 2.43 | Q6SLL2 |
| Li et al., (2015) | LV tissue | NA | Q6TN20 |
| Liet al., (2015) | LV tissue | 29.66 | Q7YSA1 |
| Li et al., (2015) | LV tissue | 2.22 | Q866G8 |
| Liet al., (2015) | LV tissue | 2.23 | Q866G8 |
| Liet al., (2015) | LV tissue | -3.22 | Q8HYR4 |
| Liet al., (2015) | LV tissue | 2.28 | Q8SPM0 |
| Li et al., (2015) | LV tissue | 12.51 | Q8SPQ9 |
| Li et al., (2015) | LV tissue | -2.74 | Q8WMS5 |
| Liet al., (2015) | LV tissue | -31.79 | Q95159 |
| Li et al., (2015) | LV tissue | -23.23 | Q95J95 |
| Liet al., (2015) | LV tissue | 584.84 | IL6 |
| Liet al., (2015) | LV tissue | -6.5 | Q9GK59 |
| Li et al., (2015) | LV tissue | NA | MMP9 |
| Li et al., (2015) | LV tissue | -4.3 | RAB33A |
| Liet al., (2015) | LV tissue | 3.62 | RAI14 |
| Liet al., (2015) | LV tissue | 3.22 | RALB |
| Liet al., (2015) | LV tissue | 2.6 | RAP1B |
| Li et al., (2015) | LV tissue | -2.24 | RAPSN |
| Liet al., (2015) | LV tissue | -5.03 | RARG |
| Liet al., (2015) | LV tissue | 2.64 | RASA2 |


| Li et al., (2015) | LV tissue | -7.37 | RASD2 |
| :---: | :---: | :---: | :---: |
| Liet al., (2015) | LV tissue | -2.37 | RASGRP3 |
| Li et al., (2015) | LV tissue | 3.44 | RASSF1 |
| Li et al., (2015) | LV tissue | 2.97 | RASSF5 |
| Liet al., (2015) | LV tissue | 2.74 | RBM3 |
| Liet al., (2015) | LV tissue | -14.32 | RCOR2 |
| Li et al., (2015) | LV tissue | 7.83 | RDH10 |
| Li et al., (2015) | LV tissue | 2.23 | RELL1 |
| Liet al., (2015) | LV tissue | 5.9 | RETN |
| Liet al., (2015) | LV tissue | 2.81 | RFX2 |
| Liet al., (2015) | LV tissue | 21.69 | RGS1 |
| Li et al., (2015) | LV tissue | 9.68 | RGS2 |
| Liet al., (2015) | LV tissue | 2.36 | RHOB |
| Li et al., (2015) | LV tissue | 3.5 | RHOJ |
| Li et al., (2015) | LV tissue | 2.85 | RHOU |
| Liet al., (2015) | LV tissue | 2.1 | RIOK3 |
| Liet al., (2015) | LV tissue | 2.99 | RND3 |
| Liet al., (2015) | LV tissue | -2.58 | RNF128 |
| Li et al., (2015) | LV tissue | 3.2 | ROBO4 |
| Liet al., (2015) | LV tissue | 3.26 | ROR1 |
| Li et al., (2015) | LV tissue | 2.48 | RPF2 |
| Liet al., (2015) | LV tissue | 2.29 | RPL21 |
| Li et al., (2015) | LV tissue | 2.26 | RPS6KA2 |
| Liet al., (2015) | LV tissue | -2.98 | RSG1 |
| Liet al., (2015) | LV tissue | 2.57 | S100A11 |
| Li et al., (2015) | LV tissue | 29.89 | S100A12 |
| Liet al., (2015) | LV tissue | 2.31 | S100A6 |
| Li et al., (2015) | LV tissue | 25.07 | S100A8 |
| Liet al., (2015) | LV tissue | 19.31 | S100A9 |
| Li et al., (2015) | LV tissue | 10.8 | S100P |
| Liet al., (2015) | LV tissue | 3.07 | S1PR1 |
| Li et al., (2015) | LV tissue | 2.24 | SAMD8 |
| Liet al., (2015) | LV tissue | -2.86 | SARDH |
| Li et al., (2015) | LV tissue | -3.31 | SCN2B |
| Liet al., (2015) | LV tissue | -4.35 | SDC1 |
| Li et al., (2015) | LV tissue | 2.23 | SDC4 |
| Liet al., (2015) | LV tissue | 3.11 | SDF2L1 |
| Li et al., (2015) | LV tissue | -15.18 | SDSL |
| Liet al., (2015) | LV tissue | 9.94 | SELE |
| Li et al., (2015) | LV tissue | 10.52 | SELL |
| Liet al., (2015) | LV tissue | 8.54 | SELP |
| Li et al., (2015) | LV tissue | 5.85 | SEMA3F |
| Liet al., (2015) | LV tissue | -4.67 | SEMA3G |
| Liet al., (2015) | LV tissue | -3.57 | SEMA5B |
| Li et al., (2015) | LV tissue | -3.21 | SEMA6C |
| Li et al., (2015) | LV tissue | 4.09 | HK3 |
| Liet al., (2015) | LV tissue | -2.68 | HMCN1 |
| Li et al., (2015) | LV tissue | -4.53 | HMGCLL1 |


| Li et al ., (2015) | LV tissue | 10.53 | HSD17B13 |
| :---: | :---: | :---: | :---: |
| Li et al ., (2015) | LV tissue | 5.22 | ICAM1 |
| Liet al., (2015) | LV tissue | 5.3 | ICOSLG |
| Li et al ., (2015) | LV tissue | -2.52 | IDH1 |
| Li et al ., (2015) | LV tissue | 3.14 | IER3 |
| Li et al ., (2015) | LV tissue | 4.57 | IER5L |
| Liet al ., (2015) | LV tissue | -3.17 | IFI35 |
| Li et al., (2015) | LV tissue | -5.34 | IFIT2 |
| Liet al ., (2015) | LV tissue | 2.63 | IFRD1 |
| Liet al., (2015) | LV tissue | 6.28 | IGFBP2 |
| Liet al., (2015) | LV tissue | -5.49 | IGSF11 |
| Liet al., (2015) | LV tissue | 5.04 | IL15 |
| Li et al ., (2015) | LV tissue | 8.3 | IL18RAP |
| Liet al., (2015) | LV tissue | 16.57 | IL1B |
| Liet al ., (2015) | LV tissue | 4.28 | IL1R2 |
| Li et al ., (2015) | LV tissue | 10.38 | IL1RL1 |
| Liet al., (2015) | LV tissue | 2.87 | IL33 |
| Liet al., (2015) | LV tissue | 2.66 | IL4R |
| Liet al ., (2015) | LV tissue | 135.16 | IL8 |
| Li et al ., (2015) | LV tissue | -5.14 | INHA |
| Li et al ., (2015) | LV tissue | 23.44 | INHBB |
| Liet al., (2015) | LV tissue | -3.35 | INPP55 |
| Li et al., (2015) | LV tissue | 6.26 | IRF4 |
| Liet al ., (2015) | LV tissue | 3.17 | IRS2 |
| Liet al., (2015) | LV tissue | -3.18 | ITGA11 |
| Liet al., (2015) | LV tissue | -2.5 | ITIH5 |
| Liet al., (2015) | LV tissue | -5.69 | ITPKB |
| Liet al., (2015) | LV tissue | -2.7 | JPH2 |
| Li et al., (2015) | LV tissue | 6.07 | JUNB |
| Li et al ., (2015) | LV tissue | -3.88 | KANK4 |
| Liet al., (2015) | LV tissue | -4.67 | KCNK13 |
| Li et al ., (2015) | LV tissue | 3.22 | KDM6B |
| Li et al ., (2015) | LV tissue | 3.96 | KIAA0556 |
| Li et al ., (2015) | LV tissue | -3.03 | KIAA1161 |
| Liet al., (2015) | LV tissue | -2.21 | KIAA1462 |
| Liet al., (2015) | LV tissue | -2.43 | KIAA1467 |
| Liet al., (2015) | LV tissue | -3.85 | KIF26A |
| Li et al., (2015) | LV tissue | -2.46 | KLF11 |
| Li et al., (2015) | LV tissue | 3.4 | KLF4 |
| Li et al., (2015) | LV tissue | 3.54 | KLF5 |
| Liet al., (2015) | LV tissue | 2.39 | KLHL2 |
| Li et al., (2015) | LV tissue | 3.61 | KLHL29 |
| Li et al., (2015) | LV tissue | 4.05 | KRT80 |
| Li et al., (2015) | LV tissue | -4.79 | LAMA3 |
| Liet al., (2015) | LV tissue | -4.33 | LAMC3 |
| Liet al., (2015) | LV tissue | -2.56 | LARS2 |
| Liet al., (2015) | LV tissue | -2.85 | LGALS9 |
| Liet al ., (2015) | LV tissue | -4.98 | LGR6 |


| Li et al., (2015) | LV tissue | 5.61 | LOX |
| :---: | :---: | :---: | :---: |
| Li et al ., (2015) | LV tissue | -2.8 | LRRC10 |
| Li et al ., (2015) | LV tissue | -7.9 | LRRC14B |
| Liet al., (2015) | LV tissue | 6.55 | LRRC25 |
| Li et al ., (2015) | LV tissue | 4.09 | LRRC32 |
| Li et al., (2015) | LV tissue | -5.94 | LRRC38 |
| Liet al., (2015) | LV tissue | 3.32 | LRRC8C |
| Liet al., (2015) | LV tissue | 5.85 | LY86 |
| Li et al ., (2015) | LV tissue | 3.8 | LY9 |
| Li et al ., (2015) | LV tissue | 21.5 | LYSC1 |
| Li et al ., (2015) | LV tissue | 4.23 | LYVE1 |
| Li et al., (2015) | LV tissue | -2.42 | MACROD1 |
| Li et al ., (2015) | LV tissue | 11.63 | MAFF |
| Li et al ., (2015) | LV tissue | -4.44 | MAL |
| Li et al ., (2015) | LV tissue | 2.29 | MALL |
| Li et al ., (2015) | LV tissue | -2.66 | MAP4K2 |
| Li et al ., (2015) | LV tissue | -2.54 | MAPK12 |
| Li et al., (2015) | LV tissue | 6.71 | MAPK13 |
| Li et al ., (2015) | LV tissue | -4.28 | MAPT |
| Li et al., (2015) | LV tissue | -2.16 | MCAM |
| Li et al., (2015) | LV tissue | 2.45 | MCL1 |
| Liet al., (2015) | LV tissue | -2.36 | MEOX2 |
| Liet al., (2015) | LV tissue | -2.78 | MGAT5 |
| Li et al ., (2015) | LV tissue | 35.1 | MIOX |
| Li et al., (2015) | LV tissue | -23.52 | MKRN2-AS1 |
| Li et al., (2015) | LV tissue | -2.68 | MLYCD |
| Li et al., (2015) | LV tissue | 2.56 | MMD |
| Li et al ., (2015) | LV tissue | -3.39 | MMP15 |
| Li et al ., (2015) | LV tissue | 162.28 | MMP8 |
| Liet al., (2015) | LV tissue | -2.93 | MOCS1 |
| Li et al., (2015) | LV tissue | -3.89 | MOGAT1 |
| Li et al ., (2015) | LV tissue | -2.52 | MPI |
| Li et al ., (2015) | LV tissue | -3.15 | MPP2 |
| Liet al., (2015) | LV tissue | 2.87 | MSX1 |
| Li et al ., (2015) | LV tissue | 24.13 | MT1 |
| Li et al., (2015) | LV tissue | 134.14 | MT2 |
| Li et al., (2015) | LV tissue | 4.79 | MTHFD1L |
| Liet al., (2015) | LV tissue | 3.72 | MTHFD2 |
| Liet al., (2015) | LV tissue | 5.02 | MUC20 |
| Liet al., (2015) | LV tissue | 5.24 | MYC |
| Li et al., (2015) | LV tissue | -4.13 | MYH1 |
| Li et al., (2015) | LV tissue | 3.97 | SEMA7A |
| Liet al., (2015) | LV tissue | 8.31 | SERPINA1 |
| Liet al., (2015) | LV tissue | 6.78 | SERPINA3 |
| Li et al ., (2015) | LV tissue | 8.59 | SERPINB10 |
| Liet al., (2015) | LV tissue | 6.02 | SERPINE2 |
| Li et al., (2015) | LV tissue | -2.17 | SERPINF1 |
| Li et al ., (2015) | LV tissue | -2.92 | SETD7 |


| Li et al., (2015) | LV tissue | 4.68 | SGK1 |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | LV tissue | 2.17 | SGMS1 |
| Li et al ., (2015) | LV tissue | 2.56 | SH2D3C |
| Liet al., (2015) | LV tissue | -2.61 | SH3BP5 |
| Li et al ., (2015) | LV tissue | 4.2 | SIGIRR |
| Li et al., (2015) | LV tissue | 7.17 | SIK1 |
| Liet al., (2015) | LV tissue | 2.65 | SKIL |
| Liet al., (2015) | LV tissue | 7.6 | SLC11A1 |
| Li et al ., (2015) | LV tissue | -3.82 | SLC12A7 |
| Li et al ., (2015) | LV tissue | 4.84 | SLC16A3 |
| Li et al ., (2015) | LV tissue | 2.94 | SLC1A5 |
| Li et al., (2015) | LV tissue | 4.18 | SLC20A1 |
| Li et al ., (2015) | LV tissue | 2.76 | SLC25A25 |
| Li et al ., (2015) | LV tissue | 2.21 | SLC25A33 |
| Li et al ., (2015) | LV tissue | -2.55 | SLC25A34 |
| Li et al., (2015) | LV tissue | -2.99 | SLC25A42 |
| Li et al ., (2015) | LV tissue | 7.16 | SLC26A7 |
| Li et al., (2015) | LV tissue | 16.51 | GLUT3 |
| Li et al., (2015) | LV tissue | -4.16 | GLUT5 |
| Li et al., (2015) | LV tissue | 11.72 | GLUT6 |
| Li et al., (2015) | LV tissue | 2.71 | SLC35D1 |
| Liet al., (2015) | LV tissue | 2.83 | SLC3A1 |
| Liet al., (2015) | LV tissue | -2.47 | SLC40A1 |
| Li et al ., (2015) | LV tissue | -2.18 | SLC41A1 |
| Liet al., (2015) | LV tissue | -3.4 | SLC4A5 |
| Li et al., (2015) | LV tissue | 48.85 | SLC7A5 |
| Li et al., (2015) | LV tissue | -4.13 | SLC8A3 |
| Li et al ., (2015) | LV tissue | 3.26 | SLCO1C1 |
| Lietal., (2015) | LV tissue | 15.34 | SLCO2A1 |
| Liet al., (2015) | LV tissue | 50.74 | SLCO4A1 |
| Li et al., (2015) | LV tissue | 4.85 | SLCO6A1 |
| Li et al ., (2015) | LV tissue | -5.99 | SLITRK6 |
| Li et al., (2015) | LV tissue | -2.44 | SLX4 |
| Liet al., (2015) | LV tissue | 2.94 | SMAD6 |
| Li et al., (2015) | LV tissue | 4.99 | SNAI1 |
| Li et al., (2015) | LV tissue | 6.25 | SNORA25 |
| Li et al., (2015) | LV tissue | NA | SNORD113 |
| Liet al., (2015) | LV tissue | 2.94 | SOAT1 |
| Liet al., (2015) | LV tissue | -5.92 | SORCS1 |
| Liet al., (2015) | LV tissue | 2.78 | SORL1 |
| Li et al., (2015) | LV tissue | -4.04 | SOX12 |
| Li et al., (2015) | LV tissue | 4.86 | SOX17 |
| Liet al., (2015) | LV tissue | 3.02 | SOX9 |
| Liet al., (2015) | LV tissue | -3.29 | SPESP1 |
| Liet al., (2015) | LV tissue | -2.4 | SPG7 |
| Liet al., (2015) | LV tissue | 4.3 | SPINK4 |
| Li et al ., (2015) | LV tissue | -3.53 | SPOCK2 |
| Liet al., (2015) | LV tissue | 5.04 | SPP1 |


| Li et al ., (2015) | LV tissue |
| :---: | :---: |
| Li et al ., (2015) | LV tissue |
| Liet al ., (2015) | LV tissue |
| Li et al ., (2015) | LV tissue |
| Li et al ., (2015) | LV tissue |
| Li et al ., (2015) | LV tissue |
| Liet al ., (2015) | LV tissue |
| Li et al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |
| Liet al ., (2015) | MV tissue |
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| Liet al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |
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| Li et al ., (2015) | MV tissue |
| Liet al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |
| Li et al ., (2015) | MV tissue |


| 2.41 | SPSB1 |
| :---: | :---: |
| -4.58 | SPTBN2 |
| -2.33 | SREBF2 |
| 5.99 | SRGN |
| 2.47 | SRSF3 |
| -3.06 | ST3GAL2 |
| 3.94 | ST6GALNAC3 |
| 16.57 | 5_8S_r |
| 9.21 | 7SK |
| 3.12 | A7E3K |
| 28.56 | A7XZY9 |
| -3.33 | ACOT6 |
| -2.57 | ACSL1 |
| 4.07 | ACTG2 |
| 3.15 | ADAM2 |
| 7.45 | ADAMT |
| -4.8 | ADIPO |
| -3.11 | ADSSL1 |
| -8.07 | AGT |
| 4.06 | AMPN |
| -8.39 | ASB18 |
| 2.51 | ATP8B |
| 2.48 | BOFF1 |
| 3.67 | BCL3 |
| 3.53 | BPI |
| 2.6 | C13o |
| 2.64 | C1QA |
| 4.35 | C1QB |
| 3.21 | C1QC |
| -11.06 | C3orf43 |
| 4.42 | C5AR1 |
| -7.13 | CA3 |
| -5.1 | CAPN6 |
| 3.14 | CCL2 |
| 5.52 | CCL24 |
| 3.98 | CD163 |
| 4.22 | CD53 |
| 3.48 | CD55 |
| 4.25 | CD74 |
| 3.44 | CDH11 |
| 2.75 | CGNL1 |
| 4.6 | CH25 |
| 13.38 | CHGB |
| -4.07 | CIDEA |
| 6.14 | CLDN1 |
| 2.56 | CLIC2 |
| -4.61 | CNKSR2 |
| 25.76 | CNTNAP |


| Li et al., (2015) | MV tissue | 10.18 | COL13A |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | MV tissue | 5.18 | COL23 |
| Liet al., (2015) | MV tissue | 8.79 | COL6A |
| Li et al., (2015) | MV tissue | 2.76 | CSF1 |
| Li et al., (2015) | MV tissue | 6.47 | CSF2R |
| Li et al., (2015) | MV tissue | 11.45 | CSF3R |
| Liet al., (2015) | MV tissue | 2.46 | CSGAL |
| Li et al., (2015) | MV tissue | 3.4 | CTSC |
| Li et al., (2015) | MV tissue | 2.31 | CTSH |
| Li et al., (2015) | MV tissue | 3.07 | CTSS |
| Li et al., (2015) | MV tissue | -6.85 | CYP1A1 |
| Li et al., (2015) | MV tissue | 2.79 | CYTL1 |
| Liet al., (2015) | MV tissue | 8.85 | DDIT4 |
| Liet al., (2015) | MV tissue | 3.92 | DLA-D |
| Liet al., (2015) | MV tissue | 3.64 | DLL1 |
| Li et al., (2015) | MV tissue | -7.82 | DPP6 |
| Liet al., (2015) | MV tissue | 3.39 | EDN1 |
| Liet al., (2015) | MV tissue | 2.38 | ELN |
| Liet al., (2015) | MV tissue | 5.17 | EMCN |
| Li et al., (2015) | MV tissue | -3.6 | ENO3 |
| Liet al., (2015) | MV tissue | 2.95 | ENPP6 |
| Liet al., (2015) | MV tissue | 6.82 | ENTPD |
| Liet al., (2015) | MV tissue | 4.6 | ESPN |
| Li et al., (2015) | MV tissue | -7.29 | EXTL1 |
| Li et al., (2015) | MV tissue | -2.81 | EYA1 |
| Liet al., (2015) | MV tissue | 2.94 | F13A1 |
| Li et al., (2015) | MV tissue | 9.96 | F5 |
| Liet al., (2015) | MV tissue | -2.91 | FABP4 |
| Liet al., (2015) | MV tissue | 3.53 | FAM17 |
| Liet al., (2015) | MV tissue | -4.3 | FGF13 |
| Li et al., (2015) | MV tissue | -2.51 | FHL2 |
| Liet al., (2015) | MV tissue | 2.69 | FNDC1 |
| Li et al., (2015) | MV tissue | 5.14 | FOSL2 |
| Li et al., (2015) | MV tissue | 3.84 | FOXC2 |
| Li et al., (2015) | MV tissue | 4.14 | GADD4 |
| Liet al., (2015) | MV tissue | 3.64 | GALNT |
| Liet al., (2015) | MV tissue | 4.12 | GATA3 |
| Liet al., (2015) | MV tissue | -2.26 | GMPR |
| Liet al., (2015) | MV tissue | -4.25 | GPD1 |
| Liet al., (2015) | MV tissue | -11.89 | GPR162 |
| Liet al., (2015) | MV tissue | -2.97 | GPR98 |
| Liet al., (2015) | MV tissue | -3.2 | GPT |
| Li et al., (2015) | MV tissue | -2.56 | GSTP1 |
| Liet al., (2015) | MV tissue | -4.46 | HAND1 |
| Li et al., (2015) | MV tissue | 17.56 | HBA |
| Li et al., (2015) | MV tissue | 3.81 | HLA-D |
| Li et al., (2015) | MV tissue | 3.93 | HPSE |
| Liet al., (2015) | MV tissue | -6.95 | HPT |


| Li et al ., (2015) | MV tissue | 6.79 | HSD17 |
| :---: | :---: | :---: | :---: |
| Li et al ., (2015) | MV tissue | -2.64 | HSPB6 |
| Liet al ., (2015) | MV tissue | 3.17 | ICAM1 |
| Li et al ., (2015) | MV tissue | 3.45 | ICOSL |
| Li et al ., (2015) | MV tissue | 2.59 | IER3 |
| Li et al ., (2015) | MV tissue | 4.25 | IGFBP |
| Liet al ., (2015) | MV tissue | 2.95 | IGFBP |
| Li et al ., (2015) | MV tissue | 3.98 | IL10R |
| Liet al ., (2015) | MV tissue | 17.14 | IL1RL1 |
| Liet al., (2015) | MV tissue | 14.89 | IL6 |
| Liet al., (2015) | MV tissue | 18.31 | IL8 |
| Li et al ., (2015) | MV tissue | 5.7 | INHB |
| Li et al ., (2015) | MV tissue | -2.62 | INHBE |
| Liet al., (2015) | MV tissue | 2.75 | INPP5 |
| Liet al., (2015) | MV tissue | -26.71 | IRK2 |
| Li et al ., (2015) | MV tissue | -13.42 | IRX3 |
| Li et al ., (2015) | MV tissue | -137.71 | IRX4 |
| Liet al., (2015) | MV tissue | 4.25 | ITGA1 |
| Liet al., (2015) | MV tissue | 5.94 | ITGA8 |
| Li et al ., (2015) | MV tissue | -2.78 | IVNS1A |
| Li et al ., (2015) | MV tissue | -3.34 | KCNJ4 |
| Liet al ., (2015) | MV tissue | -5.2 | KCNK1 |
| Li et al., (2015) | MV tissue | 4.23 | KCNMB |
| Liet al ., (2015) | MV tissue | -6.63 | LAMB4 |
| Liet al., (2015) | MV tissue | 2.95 | LAPTM |
| Liet al., (2015) | MV tissue | 4.01 | LCP1 |
| Liet al., (2015) | MV tissue | -2.53 | LRRC2 |
| Liet al ., (2015) | MV tissue | -12.64 | LRRC38 |
| Liet al ., (2015) | MV tissue | -8.5 | LRRC3 |
| Liet al., (2015) | MV tissue | 2.41 | LRRC8 |
| Liet al., (2015) | MV tissue | 4.79 | LYPD6 |
| Liet al., (2015) | MV tissue | 3.44 | LYVE1 |
| Liet al ., (2015) | MV tissue | -2.43 | MACROD |
| Liet al., (2015) | MV tissue | 2.96 | MAFF |
| Li et al ., (2015) | MV tissue | -3.01 | MAPK12 |
| Li et al ., (2015) | MV tissue | -3.7 | MAPT |
| Liet al., (2015) | MV tissue | 3.12 | MEGF6 |
| Li et al ., (2015) | MV tissue | 3.23 | MESDC |
| Liet al ., (2015) | MV tissue | -3.26 | MGST1 |
| Liet al., (2015) | MV tissue | 2.81 | MMRN2 |
| Liet al., (2015) | MV tissue | 5.34 | MPEG1 |
| Li et al., (2015) | MV tissue | -2.62 | MRPS6 |
| Li et al ., (2015) | MV tissue | 3.87 | MT1 |
| Liet al., (2015) | MV tissue | 7.24 | MT2 |
| Liet al ., (2015) | MV tissue | 2.38 | MTHFD |
| Liet al., (2015) | MV tissue | 90.16 | MUC16 |
| Liet al., (2015) | MV tissue | 2.67 | MYC |
| Liet al ., (2015) | MV tissue | -5.08 | MYH8 |


| Li et al ., (2015) | MV tissue | -36.45 | MYL3 |
| :---: | :---: | :---: | :---: |
| Li et al ., (2015) | MV tissue | -11.25 | MYOT |
| Li et al ., (2015) | MV tissue | 2.63 | NID2 |
| Li et al ., (2015) | MV tissue | 4.62 | NOS3 |
| Liet al ., (2015) | MV tissue | 2.74 | ENSCAFG0000000049 |
|  |  |  | 2 |
| Liet al ., (2015) | MV tissue | 3.39 | ENSCAFG0000000438 |
|  |  |  | 4 |
| Li et al ., (2015) | MV tissue | 4.15 | ENSCAFG0000000449 |
|  |  |  | 6 |
| Liet al ., (2015) | MV tissue | 4.43 | ENSCAFG0000000557 |
|  |  |  | 5 |
| Liet al ., (2015) | MV tissue | 3.93 | ENSCAFG0000000585 |
|  |  |  | 2 |
| Li et al ., (2015) | MV tissue | 5.84 | ENSCAFG0000000715 |
|  |  |  | 4 |
| Liet al ., (2015) | MV tissue | 14.56 | ENSCAFG0000000719 |
|  |  |  | 9 |
| Li et al ., (2015) | MV tissue | 3.38 | ENSCAFG0000000874 |
|  |  |  | 1 |
| Liet al ., (2015) | MV tissue | 5.62 | ENSCAFG0000000901 |
|  |  |  | 4 |
| Li et al ., (2015) | MV tissue | 3.91 | ENSCAFG0000001301 |
|  |  |  | 5 |
| Liet al ., (2015) | MV tissue | 27.07 | ENSCAFG0000001378 |
|  |  |  | 1 |
| Liet al ., (2015) | MV tissue | 2.75 | ENSCAFG0000001732 |
|  |  |  | 6 |
| Liet al ., (2015) | MV tissue | $-2.33$ | ENSCAFG0000001782 |
|  |  |  | 4 |
| Liet al ., (2015) | MV tissue | -6.4 | ENSCAFG0000002271 |
|  |  |  | 6 |
| Liet al ., (2015) | MV tissue | -2.96 | ENSCAFG0000002271 |
|  |  |  | 9 |
| Li et al ., (2015) | MV tissue | -3.01 | ENSCAFG0000002272 |
|  |  |  | 1 |
| Liet al ., (2015) | MV tissue | -4.12 | ENSCAFG0000002272 |
|  |  |  | 7 |
| Li et al ., (2015) | MV tissue | -3.01 | PHYH |
| Liet al ., (2015) | MV tissue | 17.68 | ENSCAFG0000002340 |
|  |  |  | 1 |
| Li et al ., (2015) | MV tissue | 53.64 | ENSCAFG0000002520 |
|  |  |  |  |
| Li et al ., (2015) | MV tissue | 3.73 | ENSCAFG0000002845 |
|  |  |  | 3 |
| Li et al ., (2015) | MV tissue | 2.8 | ENSCAFG0000002934 |
|  |  |  | 6 |
| Li et al ., (2015) | MV tissue | 3.58 | ENSCAFG0000002955 |
|  |  |  | 3 |
| Li et al ., (2015) | MV tissue | 7.18 | ENSCAFG0000003065 |
|  |  |  | 5 |


| Li et al ., (2015) | MV tissue | 24.55 | ENSCAFG0000003093 |
| :---: | :---: | :---: | :---: |
|  |  |  | 5 |
| Li et al ., (2015) | MV tissue | 4.22 | ENSCAFG0000003178 |
|  |  |  | 6 |
| Li et al ., (2015) | MV tissue | 3.67 | ENSCAFG0000003180 |
|  |  |  | 6 |
| Li et al ., (2015) | MV tissue | 4.4 | ENSCAFG0000003186 |
|  |  |  | 9 |
| Li et al ., (2015) | MV tissue | 7.18 | ENSCAFG0000003216 |
|  |  |  | 3 |
| Liet al ., (2015) | MV tissue | 4.24 | ENSCAFG0000003217 |
|  |  |  | 3 |
| Liet al ., (2015) | MV tissue | 11.19 | ENSCAFG0000003232 |
|  |  |  | 8 |
| Li et al ., (2015) | MV tissue | 4.96 | ENSCAFG0000003235 |
|  |  |  | 8 |
| Li et al ., (2015) | MV tissue | 17.82 | ENSCAFG0000003261 |
|  |  |  | 5 |
| Liet al ., (2015) | MV tissue | 7.18 | ENSCAFG0000003269 |
|  |  |  | 6 |
| Li et al ., (2015) | MV tissue | 151.37 | ENSCAFG0000003270 |
|  |  |  | 6 |
| Li et al ., (2015) | MV tissue | 4.37 | ENSCAFG0000003273 |
|  |  |  | 1 |
| Liet al ., (2015) | MV tissue | 4.17 | NPTX2 |
| Li et al., (2015) | MV tissue | -9.27 | O3FAR1 |
| Li et al ., (2015) | MV tissue | 2.56 | 09770 |
| Li et al., (2015) | MV tissue | -2.54 | OBSCN |
| Li et al., (2015) | MV tissue | 4.24 | OLFML |
| Li et al., (2015) | MV tissue | 13.39 | OLR1 |
| Liet al ., (2015) | MV tissue | -2.5 | OXCT1 |
| Li et al ., (2015) | MV tissue | -19.27 | PABPC1L |
| Li et al ., (2015) | MV tissue | -4.5 | PAK6 |
| Li et al., (2015) | MV tissue | -2.69 | PDIA2 |
| Liet al ., (2015) | MV tissue | 4.12 | PDPN |
| Li et al ., (2015) | MV tissue | 3.76 | PIK3R |
| Liet al ., (2015) | MV tissue | 3.16 | PKIB |
| Li et al ., (2015) | MV tissue | -3.91 | PLA2G7 |
| Liet al ., (2015) | MV tissue | 4.68 | PLEK |
| Liet al ., (2015) | MV tissue | -4.39 | PLIN1 |
| Liet al ., (2015) | MV tissue | 10.49 | PLSCR5 |
| Li et al ., (2015) | MV tissue | 2.49 | PMEPA |
| Li et al., (2015) | MV tissue | 6.06 | PPBP |
| Li et al., (2015) | MV tissue | -3.4 | PPP1R |
| Liet al., (2015) | MV tissue | -2.64 | PPP1R3 |
| Li et al ., (2015) | MV tissue | 4.45 | PROCR |
| Li et al., (2015) | MV tissue | 2.86 | PTGIS |
| Li et al ., (2015) | MV tissue | 2.99 | PTK2B |
| Li et al ., (2015) | MV tissue | 3.68 | PTPN6 |
| Li et al ., (2015) | MV tissue | 4.02 | PTPRC |


| Li et al., (2015) | MV tissue | 10.63 | PTX3 |
| :---: | :---: | :---: | :---: |
| Li et al., (2015) | MV tissue | 20.95 | Q1ERY9 |
| Li et al., (2015) | MV tissue | 4.78 | Q3042 |
| Li et al., (2015) | MV tissue | 5.54 | Q4ZHP |
| Li et al., (2015) | MV tissue | -4.5 | Q8MIM |
| Li et al., (2015) | MV tissue | 9.99 | Q8SPQ |
| Li et al., (2015) | MV tissue | 4.18 | Q8SPY |
| Li et al., (2015) | MV tissue | -122.02 | MYL2 |
| Li et al., (2015) | MV tissue | 4.24 | Q9TTF |
| Li et al., (2015) | MV tissue | 13.95 | REG3A |
| Li et al., (2015) | MV tissue | 19.76 | REG3A |
| Li et al., (2015) | MV tissue | 4.62 | RELN |
| Li et al., (2015) | MV tissue | 3.33 | RGS1 |
| Li et al., (2015) | MV tissue | -7.52 | RPS11 |
| Li et al., (2015) | MV tissue | -4.81 | RSPO2 |
| Li et al., (2015) | MV tissue | -3.84 | RXRG |
| Li et al., (2015) | MV tissue | 12.26 | S100A9 |
| Li et al., (2015) | MV tissue | 2.74 | S1PR1 |
| Li et al., (2015) | MV tissue | 12.74 | SELP |
| Li et al., (2015) | MV tissue | 5.38 | SERPI |
| Li et al., (2015) | MV tissue | 5.25 | PAI-1 |
| Li et al., (2015) | MV tissue | -2.31 | SIR5 |
| Li et al., (2015) | MV tissue | 2.71 | SKAP2 |
| Li et al., (2015) | MV tissue | -2.94 | SLC12A |
| Li et al., (2015) | MV tissue | 4.52 | SLC16 |
| Li et al., (2015) | MV tissue | 4.01 | FATP6 |
| Li et al., (2015) | MV tissue | 7.49 | GLUT3 |
| Li et al., (2015) | MV tissue | 6.87 | SLCO2 |
| Li et al., (2015) | MV tissue | 11.29 | SLCO4A |
| Li et al., (2015) | MV tissue | 2.84 | SMOC1 |
| Li et al., (2015) | MV tissue | 4.03 | SMPDL |
| Li et al., (2015) | MV tissue | -3.58 | SMYD2 |
| Li et al., (2015) | MV tissue | 2.98 | SNAI1 |
| Li et al., (2015) | MV tissue | 8.44 | SNORD |
| Lietal., (2015) | MV tissue | 7.17 | SNORD |
| Li et al., (2015) | MV tissue | 5.23 | SNORD |
| Li et al., (2015) | MV tissue | 71.14 | SNORD4 |
| Li et al., (2015) | MV tissue | 3.1 | ST6G |
| Li et al., (2015) | MV tissue | 4.13 | ST8SI |
| Li et al., (2015) | MV tissue | 3.1 | STAB |
| Li et al., (2015) | MV tissue | 4.44 | STC1 |
| Li et al., (2015) | MV tissue | 7.23 | STOX1 |
| Li et al., (2015) | MV tissue | -5.97 | SV2B |
| Li et al., (2015) | MV tissue | -12.53 | TDRD1 |
| Li et al., (2015) | MV tissue | 2.61 | TEK |
| Li et al., (2015) | MV tissue | 4.81 | TFPI2 |
| Li et al., (2015) | MV tissue | 5.05 | THBD |
| Li et al., (2015) | MV tissue | 2.87 | THBS 1 |


| Liet al ., (2015) | MV tissue | 3.17 | TNFRS |
| :--- | :--- | :--- | :--- |
| Liet al ., (2015) | MV tissue | -2.83 | TNNT1 |
| Liet al ., (2015) | MV tissue | 40.42 | UPK1B |
| Liet al ., (2015) | MV tissue | -4.54 | VWCE |
| Liet al ., (2015) | MV tissue | 4.4 | WFDC |
| Liet al ., (2015) | MV tissue | 3.17 | WNT11 |
| Liet al ., (2015) | MV tissue | 5.76 | WNT2 |
| Liet al ., (2015) | MV tissue | 22.14 | WNT9B |
| Liet al ., (2015) | MV tissue | -4.72 | XIRP2 |
| Liet al ., (2015) | MV tissue | 37.34 | Y_RNA |
| Lietal ., (2015) | MV tissue | 39.6 | Y_RNA |
| Lietal ., (2015) | MV tissue | 37.59 | Y_RNA |

Table S3. Final input parameters for the primaryand validation runs of homozygosity analyses.Determined to maximise genome coverage usingprocess decribed by Meyermans et al., (2020)
Initial Validation
homozygsnp ..... 102 ..... 94
homozygdensity ..... 15 ..... 18
homozyggap ..... 500 ..... 720
homozyghet ..... 1 ..... 1
homozygwindowsnp ..... 102 ..... 94
homozygwindowhet ..... 1 ..... 1
homozygwindowmissing ..... 1 ..... 1
homozygwindowthreshold ..... 0.05 ..... 0.05

Table S4. location and frequency of genomic markers within the CFA12 ROH hotspot

| CHR | SNP | BP | A1 | A2 | MAF | Freq |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 12 BICF2P1424C | 33673559 | G | C | 0.001799 | 99.998201 |
|  | 12 BICF2S23521 | 33676653 | G | A | 0 | 100 |
|  | 12 BICF2P60322 | 33687831 | G | A | 0.007491 | 99.992509 |
|  | 12 chr12_33690 | 33690693 | G | A | 0 | 100 |
|  | 12 BICF2S23453 | 33702526 | A | G | 0.001792 | 99.998208 |
|  | 12 TIGRP2P163: | 33711198 | G | A | 0.001799 | 99.998201 |
|  | 12 BICF2P22312 | 33722785 |  | 0 A | 0 | 100 |
|  | 12 BICF2S23423 | 33733595 | G | A | 0 | 100 |
|  | 12 BICF2P1218S | 33750613 | A | T | 0.001786 | 99.998214 |
|  | 12 BICF2P90927 | 33756605 | A | G | 0 | 100 |
|  | 12 TIGRP2P163: | 33770958 | A | C | 0.001786 | 99.998214 |
|  | 12 BICF2P14228 | 33787258 | T | A | 0.005357 | 99.994643 |
|  | 12 BICF2P31531 | 33790732 | C | A | 0.007143 | 99.992857 |
|  | 12 BICF2P3722E | 33806410 | A | G | 0.007143 | 99.992857 |
|  | 12 BICF2P2711E | 33823270 | C | A | 0.01015 | 99.98985 |
|  | 12 BICF2P21645 | 33827121 | G | A | 0.003623 | 99.996377 |
|  | 12 BICF2S23131 | 33852536 | A | G | 0.003584 | 99.996416 |
|  | 12 BICF2S2361S | 33861688 | G | A | 0 | 100 |
|  | 12 BICF2P1708S | 33880377 | C | A | 0.001792 | 99.998208 |
|  | 12 BICF2S23234 | 33909719 | G | A | 0.005376 | 99.994624 |
|  | 12 BICF2P3645¢ | 33917041 | G | A | 0 | 100 |
|  | 12 chr12_33928 | 33928877 | A | G | 0 | 100 |
|  | 12 chr12_33940 | 33940008 | G | A | 0 | 100 |
|  | 12 chr12_33947 | 33947104 | A | C | 0 | 100 |
|  | 12 chr12_33958 | 33958989 | A | G | 0.002717 | 99.997283 |
|  | 12 chr12_33967 | 33967036 | A | G | 0 | 100 |
|  | $12 \mathrm{G742f37S17t}$ | 33970263 | A | C | 0 | 100 |
|  | $12 \mathrm{G743f37S} 25$ ! | 34013054 | A | C | 0.001792 | 99.998208 |
|  | 12 BICF2P23321 | 34033916 | C | A | 0 | 100 |
|  | 12 TIGRP2P163: | 34057309 | G | A | 0.001799 | 99.998201 |
|  | 12 BICF2P1304S | 34079620 | A | G | 0.003571 | 99.996429 |
|  | 12 TIGRP2P163: | 34095587 | T | A | 0.001792 | 99.998208 |
|  | 12 BICF2P21164 | 34100160 | C | A | 0.005415 | 99.994585 |
|  | 12 BICF2P9795C | 34119473 | A | G | 0.003571 | 99.996429 |
|  | 12 BICF2P1617\% | 34123601 | C | A | 0.003584 | 99.996416 |
|  | 12 BICF2P8258C | 34135038 | C | A | 0.001799 | 99.998201 |
|  | 12 BICF2P39264 | 34152723 | A | C | 0.003584 | 99.996416 |
|  | 12 BICF2S24411 | 34177615 | G | A | 0.001792 | 99.998208 |
|  | 12 BICF2P82578 | 34181708 | G | A | 0.001805 | 99.998195 |
|  | 12 BICF2P5980¢ | 34192767 | G | A | 0.001792 | 99.998208 |
|  | 12 BICF2P83012 | 34216705 | G | C | 0.001799 | 99.998201 |
|  | 12 BICF2P12087 | 34247982 |  | 0 A | 0 | 100 |
|  | 12 BICF2S24118 | 34255708 | A | G | 0 | 100 |
|  | 12 TIGRP2P163: | 34265433 | A | G | 0.001799 | 99.998201 |
|  | 12 BICF2S23654 | 34274295 | A | C | 0.003584 | 99.996416 |
|  | 12 BICF2S23675 | 34293219 |  | A | 0.003597 | 99.996403 |


| 12 BICF2P11027 | 34310132 A | G |
| :---: | :---: | :---: |
| 12 BICF2P3212\& | 34324308 C | A |
| 12 BICF2P13455 | 34331512 C | A |
| 12 BICF2P2623\& | 34356834 A | G |
| 12 BICF2P1230¢ | 34379906 C | A |
| 12 BICF2P53494 | 34409595 C | A |
| 12 BICF2S23522 | 34413811 G |  |
| 12 BICF2P8009€ | 34453457 G |  |
| 12 BICF2S23644 | 34474554 A | G |
| 12 BICF2S23242 | 34481365 A | C |
| 12 BICF2S2324C | 34495251 G | A |
| 12 BICF2P32038 | 34506664 T | A |
| 12 BICF2P1435E | 34529726 A |  |
| 12 BICF2P9674E | 34537479 C | A |
| 12 TIGRP2P163: | 34557559 A | G |
| 12 BICF2P1000C | 34566189 A | C |
| 12 BICF2P14835 | 34575510 G | A |
| 12 BICF2P5568 | 34588382 A | G |
| 12 BICF2P13992 | 34596593 A |  |
| 12 BICF2P1450¢ | 34607921 C |  |
| 12 BICF2S2311C | 34632402 A |  |
| 12 BICF2S23631 | 34643172 A | G |
| 12 BICF2S2353C | 34677845 G |  |
| 12 BICF2S23331 | 34705441 A |  |
| 12 BICF2S23023 | 34710479 A |  |
| 12 BICF2S23043 | 34734003 G |  |
| 12 chr12_34743 | 34743193 C |  |
| 12 chr12_34754 | 34754272 G |  |
| 12 G744f31S91 | 34755669 A | C |
| 12 chr12_34766 | 34766791 G |  |
| 12 chr12_34784 | 34784001 A |  |
| 12 BICF2P12511 | 34794096 G |  |
| 12 G745f34S15 | 34807019 A | G |
| 12 BICF2P71772 | 34826720 C |  |
| 12 BICF2P11972 | 34847628 A | G |
| 12 TIGRP2P163: | 34859802 A | G |
| 12 BICF2P2625き | 34878649 G | A |
| 12 BICF2S23632 | 34899565 G | A |
| 12 BICF2S24578 | 34911197 | 0 A |
| 12 BICF2P8637C | 34922296 A | G |
| 12 BICF2P1511C | 34936264 A | C |
| 12 TIGRP2P163: | 34944473 G | A |
| 12 BICF2P1304C | 34959290 C |  |
| 12 BICF2P1349き | 34964695 A | G |
| 12 TIGRP2P163، | 34981336 C | A |
| 12 BICF2P47865 | 35003527 A | G |
| 12 BICF2P3714C | 35015908 G | A |
| 12 BICF2P31931 | 35022785 A | C |


| 0.001792 | 99.998208 |
| :---: | :---: |
| 0.001799 | 99.998201 |
| 0.001805 | 99.998195 |
| 0 | 100 |
| 0.001799 | 99.998201 |
| 0 | 100 |
| 0.001792 | 99.998208 |
| 0.001792 | 99.998208 |
| 0.001786 | 99.998214 |
| 0.005376 | 99.994624 |
| 0.001984 | 99.998016 |
| 0.003663 | 99.996337 |
| 0.001786 | 99.998214 |
| 0.002174 | 99.997826 |
| 0 | 100 |
| 0.003571 | 99.996429 |
| 0.001805 | 99.998195 |
| 0.003953 | 99.996047 |
| 0.003597 | 99.996403 |
| 0.007722 | 99.992278 |
| 0 | 100 |
| 0.001786 | 99.998214 |
| 0 | 100 |
| 0.007326 | 99.992674 |
| 0.005396 | 99.994604 |
| 0.005396 | 99.994604 |
| 0 | 100 |
| 0 | 100 |
| 0 | 100 |
| 0 | 100 |
| 0 | 100 |
| 0 | 100 |
| 0.008929 | 99.991071 |
| 0.005376 | 99.994624 |
| 0.003597 | 99.996403 |
| 0.00722 | 99.99278 |
| 0.001792 | 99.998208 |
| 0.003597 | 99.996403 |
| 0 | 100 |
| 0.003597 | 99.996403 |
| 0.001805 | 99.998195 |
| 0.005357 | 99.994643 |
| 0.005357 | 99.994643 |
| 0.001786 | 99.998214 |
| 0.003597 | 99.996403 |
| 0.003571 | 99.996429 |
| 0.003584 | 99.996416 |
| 0.005396 | 99.994604 |


| 12 TIGRP2P163، | 35032540 A | G | 0.003584 | 99.996416 |
| :---: | :---: | :---: | :---: | :---: |
| 12 BICF2S22962 | 35043281 G | A | 0.003584 | 99.996416 |
| 12 TIGRP2P163، | 35050172 T | A | 0.001792 | 99.998208 |
| 12 BICF2P46204 | 35064873 A | G | 0.01661 | 99.98339 |
| 12 BICF2P13094 | 35073109 G | A | 0.001792 | 99.998208 |
| 12 BICF2P11473 | 35080194 C | A | 0.003623 | 99.996377 |
| 12 BICF2P2477E | 35093467 C | A | 0.003717 | 99.996283 |
| 12 BICF2S2418¢ | 35119760 A | G | 0.001792 | 99.998208 |
| 12 BICF2P96547 | 35123671 C | A | 0.003663 | 99.996337 |
| 12 BICF2S23543 | 35150380 A | G | 0.00361 | 99.99639 |
| 12 TIGRP2P163، | 35160151 A | G | 0.001786 | 99.998214 |
| 12 BICF2P7885ミ | 35178202 A | G | 0.001786 | 99.998214 |
| 12 BICF2P1354¢ | 35183147 A | G | 0.001792 | 99.998208 |
| 12 BICF2P3204¢ | 35203261 A | G | 0.001786 | 99.998214 |
| 12 TIGRP2P163، | 35205435 G | A | 0.001799 | 99.998201 |
| 12 BICF2S2343C | 35215025 G | A | 0.001792 | 99.998208 |
| 12 BICF2P10897 | 35229941 A | T | 0.001805 | 99.998195 |
| 12 BICF2P12754 | 35240215 | 0 A | 0 | 100 |
| 12 BICF2P7119C | 35250221 A | G | 0 | 100 |
| 12 TIGRP2P163، | 35264527 G | A | 0.001799 | 99.998201 |
| 12 BICF2P13898 | 35273672 C | A | 0.005357 | 99.994643 |
| 12 BICF2P2824¢ | 35296744 A | C | 0.003584 | 99.996416 |

Table S5. Overlap of genes in ROH with MMVD GO terms identified in transcriptomic GO_term_ID GO_GO_term_def Nol Genes

ADCYAP1|CLUL1|CNTLN|COL11A
1|COLEC12|OLFM3|USP14|VCA

| GO:0005615 | CP | extracellular space | 9 M 1 YEES1 |
| :---: | :---: | :---: | :---: |
|  |  |  | GPR88\|KCNQ5|OLFM3|PLPPR4| |
| GO:0005887 | CP | integral component of plasma $n$ | 6 PLPPR5\|VCAM1 |
|  |  |  | NTNG1\|PARD3|ROCK1|S1PR1|V |
| GO:0007155 | BP | cell adhesion | 5 CAM1 |
|  |  |  | NTNG1\|PARD3|ROCK1|S1PR1|V |
| GO:0022610 | BP | biological adhesion | 5 CAM1 |
|  |  |  | AGL\|COLEC12|ROCK1|USP14|VC |
| GO:0006955 | BP | immune response | 5 AM1 |
| GO:0008284 | BP | positive regulation of cell popula | 4 ADCYAP1\|FGF4|S1PR1|VCAM1 |
| GO:0007267 | BP | cell-cell signaling | 4 ADCYAP1\|FGF4|RIMS1|USP14 |
| GO:0072359 | BP | circulatory system development | 4 COL11A1\|ROCK1|S1PR1|VCAM1 |
| GO:0000166 | MF | nucleotide binding | 4 ROCK1\|RTCA|TYMS|YES1 |
| GO:0051270 | BP | regulation of cellular component | 4 FGF4\|PHACTR1|ROCK1|S1PR1 |
| GO:2000145 | BP | regulation of cell motility | 4 FGF4\|PHACTR1|ROCK1|S1PR1 |
| GO:0045087 | BP | innate immune response | 3 COLEC12\|USP14|VCAM1 |
| GO:0006886 | BP | intracellular protein transport | 3 PARD3\|RIMS1|THOC1 |
| GO:0043066 | BP | negative regulation of apoptotic | 3 ADCYAP1\|FGF4|ROCK1 |
| GO:0043547 | BP | positive regulation of GTPase ac | 3 ADCYAP1\|S1PR1|TBC1D7 |
| GO:0002684 | BP | positive regulation of immune s) | 3 COLEC12\|VCAM1|YES1 |
| GO:0030334 | BP | regulation of cell migration | 3 FGF4\|PHACTR1|S1PR1 |
| GO:0003013 | BP | circulatory system process | 3 ADCYAP1\|ROCK1|YES1 |
| GO:0045785 | BP | positive regulation of cell adhesi | 3 ROCK1\|VCAM1|YES1 |
| GO:0060548 | BP | negative regulation of cell death | 3 ADCYAP1\|FGF4|ROCK1 |
| GO:0001775 | BP | cell activation | 3 AGL\|ROCK1|VCAM1 |
| GO:0009986 | CP | cell surface | 3 S1PR1\|USP14|VCAM1 |
| GO:0045321 | BP | leukocyte activation | 3 AGL\|ROCK1|VCAM1 |
| GO:0008201 | MF | heparin binding | 2 COL11A1\|FGF4 |
| GO:0051493 | BP | regulation of cytoskeleton organ | 2 ROCK1\|S1PR1 |
| GO:0030198 | BP | extracellular matrix organizatior | 2 COL11A1\|VCAM1 |
| GO:0043062 | BP | extracellular structure organizati | 2 Col11A1\|VCAM1 |
| GO:0031012 | CP | extracellular matrix | 2 COL11A1\|COLEC12 |
| GO:0009897 | CP | external side of plasma membra | 2 S1PR1\|VCAM1 |
| GO:0070374 | BP | positive regulation of ERK1 and 1 | 2 ADCYAP1\|FGF4 |
| GO:0050867 | BP | positive regulation of cell activat | 2 VCAM1\|YES1 |
| GO:0009142 | BP | nucleoside triphosphate biosyntr | 2 ATP5PD\|TYMS |
| GO:0005884 | CP | actin filament | 1 YES1 |
| GO:0016529 | CP | sarcoplasmic reticulum | 1 AGL |
| GO:0051451 | BP | myoblast migration | 1 ROCK1 |
| GO:0007189 | BP | adenylate cyclase-activating G p | 1 ADCYAP1 |


| GO:0005509 | MF | calcium ion binding | 1 CETN1 |
| :---: | :---: | :---: | :---: |
| GO:0055010 | BP | ventricular cardiac muscle tissue | 1 COL11A1 |
| GO:0016525 | BP | negative regulation of angiogent | 1 ROCK1 |
| GO:0001558 | BP | regulation of cell growth | 1 RIMS1 |
| GO:0005044 | MF | scavenger receptor activity | 1 COLEC12 |
| GO:0051272 | BP | positive regulation of cellular co | 1 S1PR1 |
| GO:0032870 | BP | cellular response to hormone sti | 1 ADCYAP1 |
| GO:0030016 | CP | myofibril | 1 LRRC39 |
| GO:0008021 | CP | synaptic vesicle | 1 SH3GL2 |
| GO:2000147 | BP | positive regulation of cell motilit | 1 S1PR1 |
| GO:0050728 | BP | negative regulation of inflamma | 1 ADCYAP1 |
| GO:0008528 | MF | G protein-coupled peptide recep | 1 OGFRL1 |
| GO:0040017 | BP | positive regulation of locomotior | 1 S1PR1 |
| GO:0006954 | BP | inflammatory response | 1 VCAM1 |
| GO:0005178 | MF | integrin binding | 1 VCAM1 |
| GO:0001666 | BP | response to hypoxia | 1 VCAM1 |
| GO:0001503 | BP | ossification | 1 COL11A1 |
| GO:0007160 | BP | cell-matrix adhesion | 1 VCAM1 |
| GO:0070482 | BP | response to oxygen levels | 1 VCAM1 |
| GO:0071356 | BP | cellular response to tumor necro | 1 VCAM1 |
| GO:0007200 | BP | phospholipase C-activating G prc | 1 S1PR1 |
| GO:0030335 | BP | positive regulation of cell migrat | 1 S1PR1 |
| GO:0030175 | CP | filopodium | 1 VCAM1 |
| GO:0043235 | CP | receptor complex | 1 OLFM3 |
| GO:0048514 | BP | blood vessel morphogenesis | 1 S1PR1 |
| GO:0001525 | BP | angiogenesis | 1 S1PR1 |
| GO:0001568 | BP | blood vessel development | 1 S1PR1 |
| GO:0072358 | BP | cardiovascular system developm | 1 S1PR1 |
| GO:0060349 | BP | bone morphogenesis | 1 FGF4 |
| GO:0001944 | BP | vasculature development | 1 S1PR1 |
| GO:0001725 | CP | stress fiber | 0 |
| GO:0051898 | BP | negative regulation of protein kil | 0 |
| GO:0030193 | BP | regulation of blood coagulation | 0 |
| GO:0008009 | MF | chemokine activity | 0 |
| GO:0004222 | MF | metalloendopeptidase activity | 0 |
| GO:0002548 | BP | monocyte chemotaxis | 0 |
| GO:0009143 | BP | nucleoside triphosphate cataboli | 0 |
| GO:0048701 | BP | embryonic cranial skeleton morr | 0 |
| GO:0045056 | BP | transcytosis | 0 |
| GO:0051607 | BP | defense response to virus | 0 |
| GO:0016941 | MF | natriuretic peptide receptor acti। | 0 |
| GO:0048703 | BP | embryonic viscerocranium morp | 0 |
| GO:2000352 | BP | negative regulation of endothelii | 0 |
| GO:2000721 | BP | positive regulation of transcripti | 0 |
| GO:0030240 | BP | skeletal muscle thin filament as: | 0 |
| GO:2000772 |  | regulation of cellular senescence | 0 |


| GO:0014704 | CP | in |
| :---: | :---: | :---: |
| GO:0060231 | BP | mesenchymal to epithelial trans |
| GO:0030018 | CP | Z disc |
| GO:0019915 | BP | lipid storage |
| GO:0002687 | BP | positive regulation of leukocyte r |
| GO:0071526 | BP | semaphorin-plexin signaling pat |
| GO:0001658 | BP | branching involved in ureteric bu |
| GO:0034383 | BP | low-density lipoprotein particle ( |
| GO:0001938 | BP | positive regulation of endothelia |
| GO:0022625 | CP | cytosolic large ribosomal subuni* |
| GO:0002027 | BP | regulation of heart rate |
| GO:0048843 | BP | negative regulation of axon exte |
| GO:0097120 | BP | receptor localization to synapse |
| GO:0050729 | BP | positive regulation of inflammat |
| GO:0016323 | CP | basolateral plasma membrane |
| GO:0086091 | BP | regulation of heart rate by cardic |
| GO:0033089 | BP | positive regulation of T cell diffe |
| GO:0032868 | BP | response to insulin |
| GO:0030593 | BP | neutrophil chemotaxis |
| GO:0001837 | BP | epithelial to mesenchymal trans |
| GO:0044319 | BP | wound healing, spreading of cell |
| GO:0071222 | BP | cellular response to lipopolysacc |
| GO:0060048 | BP | cardiac muscle contraction |
| GO:0048245 | BP | eosinophil chemotaxis |
| GO:0030308 | BP | negative regulation of cell growt |
| GO:0070098 | BP | chemokine-mediated signaling F |
| GO:0045669 | BP | positive regulation of osteoblast |
| GO:0009611 | BP | response to wounding |
| GO:0032963 | BP | collagen metabolic process |
| GO:0006879 | BP | cellular iron ion homeostasis |
| GO:0048247 | BP | lymphocyte chemotaxis |
| GO:0048020 | MF | CCR chemokine receptor binding |
| GO:0005003 | MF | ephrin receptor activity |
| GO:0048863 | BP | stem cell differentiation |
| GO:0005587 | CP | collagen type IV trimer |
| GO:0098911 | BP | regulation of ventricular cardiac |
| GO:0045926 | BP | negative regulation of growth |
| GO:0002026 | BP | regulation of the force of heart c |
| GO:0001755 | BP | neural crest cell migration |
| GO:0071265 | BP | L-methionine biosynthetic proce: |
| GO:0071456 | BP | cellular response to hypoxia |
| GO:0045499 | MF | chemorepellent activity |
| GO:0048285 | BP | organelle fission |
| GO:0045766 | BP | positive regulation of angiogene |
| GO:0003735 | MF | structural constituent of ribosor |
| GO:0030215 | MF | semaphorin receptor binding |
| GO:0050678 | BP | regulation of epithelial cell proli |
| GO:0042593 | BP | glucose homeostasis |

GO:0033017 CP sarcoplasmic reticulum membra 0
GO:0090131 BP mesenchyme migration 0
GO:0051495 BP positive regulation of cytoskelet، 0
GO:0010881 BP regulation of cardiac muscle con
GO:0050795 BP regulation of behavior 0

Table S6. Genes in CKCS ROH hotspots that are differentially expressed in dogs with MMVD

| Reference | Method | Model | $\sum_{\underline{\sim}}^{\substack{n}}$ | $\sum_{\substack{\infty}}^{\stackrel{\circ}{\gtrless}}$ | S | - | $\sum_{\substack{4}}^{\substack{\text { d }}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Markvy et al (2020) | Microarray | CKCS vs unaffected dogs | $\downarrow$ |  | $\uparrow$ |  | $\uparrow$ |
| Markvy et al (2020) | Microarray | All diseased valves and normal valves | $\downarrow$ |  |  |  |  |
| Markvy et al (2020) | Microarray | CKCS vs NON-CKCS with MMVD |  |  |  |  |  |
| Markby et al ., (2020b) | Microarray | Grade 2 with normal |  |  |  |  |  |
| Markby et al ., (2020b) | Microarray | Grade 3 with normal | $\downarrow$ |  | $\uparrow$ | $\downarrow$ | $\uparrow$ |
| Markby et al ., (2020b) | Microarray | Grade 4 with normal | $\downarrow$ | $\downarrow$ |  |  | $\uparrow$ |
| Markby et al ., (2020b) | Microarray | "disease" dissected with "normal" dissected |  |  |  |  |  |
| Markby et al ., (2020b) | Microarray | "normal" dissected with whole valve normal |  |  |  |  |  |
| Lu et al., (2015) | Microarray | MMVD in CKCS compared to normal dogs (non-CKCS) | $\downarrow$ |  |  |  |  |
| Li et al., (2015) | RNA-seq | LV tissue |  |  |  | $\uparrow$ |  |
| Li et al., (2015) | RNA-seq | MV tissue |  |  |  | $\uparrow$ |  |

Table S7. summary of variants annotated across consensus ROH, before and after biallelic MAF-filtering

|  | ALL | AF>0.8 |
| :--- | ---: | ---: |
| Variants processed | 27274 | 21082 |
| Novel variants [Number (\%)] | $15458(56.7)$ | 1317 (53.7) |
| Existing variants [Number (\%)] | $11816(43.3)$ | $9765(46.3)$ |
| Overlapping genes | 69 | 66 |
| Overlapping transcripts | 164 | 159 |
| Consequence |  |  |
| intron_variant | 29124 | 21148 |
| intergenic_variant | 18222 | 13521 |
| intron_variant,non_coding_transcript_variant | 2001 | 1229 |
| synonymous_variant | 86 | 85 |
| 3_prime_UTR_variant | 76 | 45 |
| 5_prime_UTR_variant | 68 | 44 |
| non_coding_transcript_exon_variant | 68 | 42 |
| missense_variant | 55 | 55 |
| splice_region_variant,intron_variant | 40 | 21 |
| frameshift_variant | 13 | 10 |
| inframe_insertion | 5 | 5 |
| inframe_deletion | 4 | 4 |
| splice_acceptor_variant | 3 | 0 |
| splice_region_variant,synonymous_variant | 2 | 2 |
| splice_acceptor_variant,non_coding_transcript_variant | 2 | 0 |
| deleterious |  | 9 |

Table S8. List of breeds and village dogs used for detection of rare CKCS variants within ROH

Name_ID
AfghanHound01
AfghanHound04
AfghanHound03
AiredaleTerrier02
TA001
AiredaleTerrier05
AiredaleTerrier01
AlaskanHusky01
AlaskanHusky02
AlaskanMalamute01
AM007
AlaskanMalamute02
Bern_AlpineDachsbrack
AustralianCattleDog01
AC023
AustralianCattleDog03
AustralianCattleDog02

CFA. 118003
Basenji03
Basenji02
Basenji01
BG064
Beagle01
Beagle02
CFA. 117995
Beagle04
BeardedCollie02
BeardedCollie01
BeardedCollie04
BeardedCollie03
MA142
MA0163
BelgianMalinois02
BelgianMalinois03
BelgianSheepdog01
BelgianSheepdog06
BelgianSheepdog03
BelgianSheepdog05
BelgianTervuren11
BelgianTervuren01
BelgianTervuren06
BelgianTervuren08
BergerBlancSuisse01
CFA. 109670
BergerPicard03
BergerPicard02

Breed/CommonName
BioProject
BioSample
Sex
<9kg (Y/N)

| Afghan Hound |  |
| :---: | :---: |
|  | Afghan Hound |
|  | Afghan Hound |
|  | Airedale Terrier |
|  | Airedale Terrier |
|  | Airedale Terrier |
|  | Airedale Terrier |
|  | Alaskan Husky |
|  | Alaskan Husky |
|  | Alaskan Malamute |
|  | Alaskan Malamute |
|  | Alaskan Malamute |
|  | Alpine Dachsbracke |
|  | Australian Cattle Dog |
|  | Australian Cattle Dog |
|  | Australian Cattle Dog |
|  | Australian Cattle Dog |
|  | Basenji |
|  | Basenji |
|  | Basenji |
|  | Basenji |
| Bavarian Hound (Bayerisch P |  |
|  | Beagle |
|  | Beagle |

PRJNA266585

PRJNA232497
PRJNA263947
PRJEB16012
PRJNA263947
PRJNA263947
PRJEB9590
PRJEB9591
n/a
PRJEB16012
PRJNA266585
PRJEB14840
PRJNA263947
PRJEB16012
PRJEB16012
PRJEB13468
PRJNA263947
PRJNA263947
PRJNA263947
PRJNA274504
n/a
PRJNA176193
PRJNA263947
n/a
PRJEB16012
PRJEB13468
PRJEB16012
PRJEB16012
PRJEB16012
PRJEB16012
PRJEB16012
PRJEB16012
n/a
n/a
n/a
n/a
n/a
n/a
n/a
n/a
PRJEB16012
PRJNA263947
PRJNA263947
PRJNA263947

| SAMNO2194722 | M | N |
| :--- | :---: | :---: |
| SAMN03168377 | M | N |
| SAMN02485564 | F | N |
| SAMN03580390 | F | N |
| SAMEA4506896 | M | N |
| SAMN04196850 | M | N |
| SAMN03580381 | M | N |
| SAMEA3449656 | M | N |
| SAMEA3449657 | M | N |
| n/a | F | N |


| SAMN06159678 | M | N |
| :---: | :---: | :---: |
| SAMN04196860 | F | N |

SAMN04196849 F N

| BergerPicard01 | Berger Picard | PRJNA263947 | SAMN03580405 | F | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| BerneseMountainDog02 | Bernese Mountain Dog | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| BerneseMountainDog01 | Bernese Mountain Dog | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| BerneseMountainDog11 | Bernese Mountain Dog | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| BerneseMountainDog04 | Bernese Mountain Dog | $\mathrm{n} / \mathrm{a}$ | n/a | M | N |
| Coonhound01 | Black and Tan Coonhound | PRJNA263947 | SAMN04196853 | F | N |
| BlackRussianTerrier01 | Black Russian Terrier | PRJNA263947 | SAMN03323668 | F | N |
| Bloodhound01 | Bloodhound | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| BC0480 | Border Collie | PRJEB16012 | SAMEA104091558 | F | N |
| BorderCollie08 | Border Collie | PRJEB12337 | SAMEA3724571 | F | N |
| BorderCollie03 | Border Collie | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| Helsinki_BC1028 | Border Collie | PRJNA319610 | SAMN04908310 | M | N |
| Borzoi01 | Borzoi | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| BouvierDesFlandres01 | Bouvier des Flandres | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| CFA. 107836 | Bouvier des Flandres | PRJNA263947 | SAMN06159671 | M | N |
| BouvierDesFlandres02 | Bouvier des Flandres | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| Boxer01 | Boxer | PRJNA255370 | SAMN02921305 | F | N |
| BrittanySpaniel01 | Brittany | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| Bullerrier06 | Bull Terrier | n/a | n/a | F | N |
| Bullerrier05 | Bull Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| Bullerrier03 | Bull Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| Bullerrier01 | Bull Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| Bulldog01 | Bulldog | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| BU002 | Bullmastiff | PRJEB16012 | SAMEA103949042 | M | N |
| CaneCorso01 | Cane Corso | PRJNA263947 | SAMN04196864 | F | N |
| CarolinaDog01 | Carolina Dog | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| CaucasianOvcharka01 | Caucasian Ovcharka | PRJNA232497 | SAMN02485585 | F | N |
| CFA. 109671 | Chesapeake Bay Retriever | PRJNA263947 | SAMN06159679 | M | N |
| NGSDOG025 | Chinese Shar-Pei | PRJNA327712 | SAMN05356427 | M | N |
| NGSDOGO24 | Chinese Shar-Pei | PRJNA327712 | SAMN05356426 | M | N |
| Chinook01 | Chinook | PRJNA263947 | SAMN03580382 | F | N |
| ChongqingDog01 | Chongqing Dog | PRJNA232497 | SAMN02485578 | M | N |
| ChowChow02 | Chow Chow | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| CW011 | Chow Chow | PRJEB16012 | SAMEA104091566 | F | N |
| ChowChow03 | Chow Chow | PRJNA232497 | SAMN02485574 | M | N |
| ChowChow01 | Chow Chow | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| ClumberSpaniel01 | Clumber Spaniel | $n / a$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| CockerSpanielAmerican06 | Cocker Spaniel (American) |  | n/a | M | N |
| CockerSpanielAmerican04 | Cocker Spaniel (American) |  | n/a | F | N |
| CockerSpanielAmerican01 | Cocker Spaniel (American) | $n / a$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| CockerSpanielAmerican03 | Cocker Spaniel (American) | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| CR039 | Curly-Coated Retriever | PRJEB16012 | SAMEA104091556 | F | N |
| Dalmatian01 | Dalmatian | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| DAL162K1 | Dalmatian | PRJNA360671 | SAMN06214558 | M | N |
| DO242 | Doberman Pinscher | PRJEB16012 | SAMEA4505489 | F | N |
| Doberman01 | Doberman Pinscher | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| Doberman02 | Doberman Pinscher | PRJNA263947 | SAMN03580409 | M | N |
| Doberman04 | Doberman Pinscher | PRJEB16012 | SAMEA4509492 | M | N |


| EastSiberianLaika01 | East Siberian Laika | PRJNA266585 | SAMN03168390 | F | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Elo01 | Elo | PRJEB16012 | SAMEA4506890 | M | N |
| CockerSpanielEnglish05 | English Cocker Spaniel | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| CP003 | English Cocker Spaniel | PRJEB16012 | SAMEA4506900 | M | N |
| CockerSpanielEnglish01 | English Cocker Spaniel | PRJEB2162 | SAMEA1521941 | M | N |
| CockerSpanielEnglish03 | English Cocker Spaniel | PRJNA263947 | SAMN03323673 | F | N |
| CFA. 117996 | English Setter | PRJNA263947 | SAMN06159682 | M | N |
| CFA. 105990 | English Setter | PRJNA263947 | SAMN06159667 | F | N |
| EnglishSetter01 | English Setter | PRJNA263947 | SAMN04196858 | M | N |
| EnglishSpringerSpaniel01 | English Springer Spaniel | PRJNA263947 | SAMN03580391 | M | N |
| EnglishSpringerSpaniel03 | English Springer Spaniel | PRJNA263947 | SAMN04196857 | M | N |
| EntlebucherSennenhundO: | Entlebucher Sennenhund | PRJEB16012 | SAMEA4505501 | M | N |
| EntlebucherSennenhund0 | Entlebucher Sennenhund | PRJEB16012 | SAMEA4505499 | M | N |
| EntlebucherSennenhund0: | Entlebucher Sennenhund | PRJEB16012 | SAMEA4504828 | M | N |
| EntlebucherSennenhund0: | : Entlebucher Sennenhund | PRJEB16012 | SAMEA4505497 | M | $N$ |
| Eurasier02 | Eurasier | PRJEB16012 | SAMEA4506889 | M | N |
| Eurasier01 | Eurasier | PRJEB6079 | SAMEA2446720 | F | N |
| CFA. 107833 | Field Spaniel | PRJNA263947 | SAMN06159668 | M | N |
| FinnishLapphund01 | Finnish Lapphund | PRJNA266585 | SAMN03168391 | M | $N$ |
| FlatcoatedRetriever01 | Flat-Coated Retriever | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| FlatcoatedRetriever02 | Flat-Coated Retriever | n/a | n/a | F | N |
| FlatcoatedRetriever03 | Flat-Coated Retriever | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| FonniDog01 | Fonni's Dog | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| FrenchBulldog01 | French Bulldog | PRJEB13468 | SAMEA3928146 | M | N |
| FB065 | French Bulldog | PRJEB16012 | SAMEA4504835 | M | N |
| JT007 | German Hunting Terrier | PRJEB16012 | SAMEA104125120 | M | N |
| DS043 | German Shepherd Dog | PRJEB16012 | SAMEA4506895 | F | N |
| GermanShepherd12 | German Shepherd Dog | PRJNA263947 | SAMN03580392 | M | N |
| DS051 | German Shepherd Dog | PRJEB16012 | SAMEA72802168 | F | N |
| DS053 | German Shepherd Dog | PRJEB16012 | SAMEA72802918 | M | N |
| GermanWirehairedPointer | German Wirehaired Pointe | PRJEB13468 | SAMEA3928144 | F | N |
| GoldenJackal01 | Golden Jackal | PRJNA274504 | SAMN03366713 | F | N |
| GoldenRetriever11 | Golden Retriever | PRJNA247491 | SAMN03067876 | M | N |
| GoldenRetriever04 | Golden Retriever | PRJNA247491 | SAMN03067893 | M | N |
| 173006_S10 | Golden Retriever | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| 172384_S14 | Golden Retriever | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| CFA. 109672 | Gordon Setter | PRJNA263947 | SAMN06159680 | F | N |
| GordonSetter01 | Gordon Setter | PRJNA263947 | SAMN04196859 | F | N |
| GreatDane01 | Great Dane | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| CFA. 117997 | Great Dane | PRJNA263947 | SAMN06159683 | F | N |
| CFA. 107837 | Great Dane | PRJNA263947 | SAMN06159672 | M | N |
| DD116 | Great Dane | PRJEB16012 | SAMEA104091557 | M | N |
| GreatPyrenees01 | Great Pyrenees | n/a | n/a | M | N |
| GreaterSwissMountainDo§ | Greater Swiss Mountain Dı | n/a | n/a | M | N |
| CFA. 107841 | Greater Swiss Mountain DI | PRJNA263947 | SAMN06159674 | M | N |
| GreenlandDog01 | Greenland Dog | PRJNA266585 | SAMN03168381 | F | N |
| Greyhound06 | Greyhound | PRJNA247491 | SAMN03067874 | M | N |
| Greyhound08 | Greyhound | PRJNA247491 | SAMN03068228 | F | N |


| Greyhound03 | Greyhound | PRJNA247491 | SAMN03067873 | M | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Greyhound07 | Greyhound | PRJNA247491 | SAMN03067890 | F | N |
| GS104 | Grossspitz | PRJEB16012 | SAMEA104105252 | F | N |
| Heideterrier01 | Heideterrier | PRJEB16012 | SAMEA103135918 | F | N |
| HW1706 | Hovawart | PRJEB16012 | SAMEA4506894 | F | N |
| IrishSetter01 | Irish Setter | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| CFA. 117998 | Irish Terrier | PRJNA263947 | SAMN06159684 | M | N |
| IrishTerrier01 | Irish Terrier | PRJEB13468 | SAMEA3928141 | M | N |
| IrishWaterSpaniel01 | Irish Water Spaniel | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| IrishWaterSpaniel02 | Irish Water Spaniel | $\mathrm{n} / \mathrm{a}$ | n/a | M | N |
| IrishWaterSpaniel03 | Irish Water Spaniel | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| IrishWolfhound01 | Irish Wolfhound | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| IstrianShorthairedHound0: | Istrian Shorthaired Hound | PRJNA232497 | SAMN02485584 | F | N |
| Jamthund01 | Jamthund | PRJNA266585 | SAMN03168383 | M | N |
| Jindo01 | Jindo | PRJDB2266 | SAMD00009664 | M | N |
| Keeshond01 | Keeshond | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| KerryBlueTerrier02 | Kerry Blue Terrier | PRJNA263947 | SAMN03580387 | F | N |
| Komondor01 | Komondor | $\mathrm{n} / \mathrm{a}$ | n/a | M | N |
| Kromfohrlander01 | Kromfohrländer | PRJEB6076 | SAMEA2446055 | M | N |
| VillDog_China16 | Kunming Dog | PRJNA233638 | SAMN02585197 | M | N |
| VillDog_China11 | Kunming Dog | PRJNA233638 | SAMN02585192 | F | N |
| VillDog_China13 | Kunming Dog | PRJNA233638 | SAMN02585194 | F | N |
| VillDog_China15 | Kunming Dog | PRJNA233638 | SAMN02585196 | M | N |
| LabradorRetriever01 | Labrador Retriever | PRJEB5874 | SAMEA2417015 | M | N |
| LabradorRetriever06 | Labrador Retriever | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| LabradorRetriever04 | Labrador Retriever | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | N |
| LabradorRetriever07 | Labrador Retriever | PRJNA263947 | SAMN03580399 | F | N |
| LagottoRomagnolo02 | Lagotto Romagnolo | PRJEB16012 | SAMEA4505491 | M | N |
| LR1030 | Lagotto Romagnolo | PRJEB16012 | SAMEA4509490 | M | N |
| LagottoRomagnolo03 | Lagotto Romagnolo | PRJEB16012 | SAMEA4505494 | F | N |
| LagottoRomagnolo04 | Lagotto Romagnolo | PRJEB16012 | SAMEA4504833 | M | N |
| Landseer01 | Landseer | PRJEB7734 | SAMEA3121328 | M | N |
| LapponianHerder01 | Lapponian Herder | PRJNA266585 | SAMN03168384 | M | N |
| Leonberger01 | Leonberger | PRJEB16012 | SAMEA103935360 | F | N |
| EnglishMastiff01 | Mastiff (English) | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| EnglishMastiff02 | Mastiff (English) | PRJNA263947 | SAMN03580412 | M | N |
| BT007 | Miniature Bull Terrier | PRJEB16012 | SAMEA4506897 | M | N |
| NewGuineaSingingDog01 | New Guinea Singing Dog | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | N |
| NGSD2 | New Guinea Singing Dog | PRJNA263947 | SAMN06608434 | M | N |
| NGSD1 | New Guinea Singing Dog | PRJNA263947 | SAMN06608432 | M | N |
| NGSD3 | New Guinea Singing Dog | PRJNA232497 | SAMN06608444 | M | N |
| NorwegianElkhound02 | Norwegian Elkhound | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| NorwegianElkhound01 | Norwegian Elkhound | PRJNA266585 | SAMN03168382 | M | N |
| NovaScotiaDuckTollingRetı | Nova Scotia Duck Tolling R | PRJNA263947 | SAMN03323675 | M | N |
| PembrokeWelshCorgi02 | Pembroke Welsh Corgi | PRJNA263947 | SAMN03145703 | F | N |
| PembrokeWelshCorgi03 | Pembroke Welsh Corgi | PRJNA263947 | SAMN03145704 | F | N |
| PembrokeWelshCorgi01 | Pembroke Welsh Corgi | PRJNA263947 | SAMN03145702 | M | N |
| PeruvianHairless01 | Peruvian Inca Orchid | PRJNA266585 | SAMN03168386 | M | N |


| PetitBassetGriffonVendeeı | ו Petit Basset Griffon Vende | PRJEB11835 | SAMEA3723573 | M | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| EnglishPointer01 | Pointer (English) | n/a | n/a | M | N |
| EnglishPointer02 | Pointer (English) | PRJNA263947 | SAMN03580383 | F | N |
| PL116 | Poodle unspecified variety | PRJEB16012 | SAMEA4506891 | F | N |
| PortuguesePodengo01 | Portuguese Podengo | PRJNA263947 | SAMN03580388 | M | N |
| PortugueseWaterDog02 | Portuguese Water Dog | n/a | n/a | F | N |
| PortugueseWaterDog01 | Portuguese Water Dog | n/a | n/a | F | N |
| PortugueseWaterDog09 | Portuguese Water Dog | n/a | n/a | M | N |
| PortugueseWaterDog05 | Portuguese Water Dog | n/a | n/a | M | N |
| RR098 | Rhodesian Ridgeback | PRJEB16012 | SAMEA104091554 | F | N |
| RhodesianRidgeback04 | Rhodesian Ridgeback | PRJNA357866 | SAMN06161404 | F | N |
| RhodesianRidgeback01 | Rhodesian Ridgeback | n/a | n/a | F | N |
| RhodesianRidgeback02 | Rhodesian Ridgeback | PRJEB16012 | SAMEA4504822 | F | N |
| Rottweiler04 | Rottweiler | $\mathrm{n} / \mathrm{a}$ | n/a | F | N |
| Rottweiler01 | Rottweiler | n/a | n/a | F | N |
| 168979_S18 | Rottweiler | n/a | n/a | M | N |
| 171515_S19 | Rottweiler | n/a | n/a | M | N |
| SaintBernard01 | Saint Bernard | n/a | n/a | F | N |
| SaintBernard02 | Saint Bernard | PRJNA263947 | SAMN03580386 | M | N |
| Saluki01 | Saluki | n/a | n/a | F | N |
| Saluki02 | Saluki | n/a | n/a | M | N |
| Saluki03 | Saluki | PRJEB16012 | SAMEA4504825 | M | N |
| Samoyed01 | Samoyed | n/a | n/a | F | N |
| Samoyed02 | Samoyed | PRJNA266585 | SAMN03168388 | F | N |
| ScottishDeerhound01 | Scottish Deerhound | PRJNA263947 | SAMN03580401 | M | N |
| ScottishTerrier01 | Scottish Terrier | n/a | n/a | F | N |
| ScottishTerrier02 | Scottish Terrier | n/a | n/a | M | N |
| ScottishTerrier03 | Scottish Terrier | PRJNA263947 | SAMN03580394 | F | N |
| ScottishTerrier04 | Scottish Terrier | n/a | n/a | F | N |
| ShetlandSheepdog01 | Shetland Sheepdog | n/a | n/a | F | N |
| SS004 | Shetland Sheepdog | PRJEB16012 | SAMEA104091573 | F | N |
| ShetlandSheepdog02 | Shetland Sheepdog | PRJNA263947 | SAMN03580413 | M | N |
| CFA. 107839 | Shiba Inu | PRJNA263947 | SAMN05770194 | F | N |
| Shibalnu01 | Shiba Inu | PRJNA263947 | SAMN04196861 | M | N |
| SiberianHusky01 | Siberian Husky | n/a | $\mathrm{n} / \mathrm{a}$ | F | N |
| SY046 | Siberian Husky | PRJEB16012 | SAMEA104091559 | M | N |
| SiberianHusky02 | Siberian Husky | PRJEB10823 | SAMEA3539249 | M | N |
| SiberianHusky04 | Siberian Husky | PRJNA266585 | SAMN03168389 | M | N |
| Sloughi02 | Sloughi | PRJEB16012 | SAMEA4506885 | M | N |
| Sloughi01 | Sloughi | PRJEB13468 | SAMEA3928143 | F | N |
| Sloughi04 | Sloughi | PRJNA266585 | SAMN03168392 | M | N |
| Sloughi03 | Sloughi | PRJEB16012 | SAMEA4506888 | M | N |
| SoftCoatedWheatenTerrie | Soft Coated Wheaten Terr | PRJNA263947 | SAMN03323670 | F | N |
| SoftCoatedWheatenTerrie | Soft Coated Wheaten Terr | PRJNA263947 | SAMN03580410 | F | N |
| SoftCoatedWheatenTerrie | Soft Coated Wheaten Terr | PRJNA263947 | SAMN03580411 | F | N |
| SoftCoatedWheatenTerrie | Soft Coated Wheaten Terr | PRJNA263947 | SAMN03323671 | M | N |
| SpanishGalgo01 | Spanish Galgo | PRJNA266585 | SAMN03168380 | F | N |
| SpanishWaterDog01 | Spanish Water Dog | PRJEB7903 | SAMEA3164479 | M | N |


| CFA. 109669 | Spinone Italiano | PRJNA263947 | SAMN06159677 | F | N |
| :---: | :---: | :---: | :---: | :---: | :---: |
| CFA. 107842 | Standard Poodle | PRJNA263947 | SAMN06159675 | F | N |
| StandardPoodle02 | Standard Poodle | PRJNA263947 | SAMN03580380 | M | N |
| StandardPoodle03 | Standard Poodle | PRJNA263947 | SAMN03580408 | M | N |
| StandardPoodle01 | Standard Poodle | n/a | $\mathrm{n} / \mathrm{a}$ | F | N |
| CFA. 107834 | Standard Schnauzer | PRJNA263947 | SAMN06159669 | M | N |
| CFA. 118002 | Standard Schnauzer | PRJNA263947 | SAMN06159688 | F | N |
| CFA. 118001 | Standard Schnauzer | PRJNA263947 | SAMN06159687 | M | N |
| StandardSchnauzer01 | Standard Schnauzer | PRJNA263947 | SAMN03323676 | M | N |
| SwedishLapphund01 | Swedish Lapphund | PRJNA266585 | SAMN03168387 | F | N |
| TibetanMastiff11 | Tibetan Mastiff - China | PRJNA233638 | SAMN02585160 | F | N |
| TibetanMastiff10 | Tibetan Mastiff - China | PRJNA233638 | SAMN02585159 | F | N |
| TibetanMastiff02 | Tibetan Mastiff - China | PRJNA233638 | SAMN02570458 | M | N |
| TibetanMastiff07 | Tibetan Mastiff - China | PRJNA233638 | SAMN02585156 | M | N |
| TibetanTerrier01 | Tibetan Terrier | PRJNA263947 | SAMN03580403 | M | N |
| TibetanTerrier02 | Tibetan Terrier | PRJNA263947 | SAMN03580406 | M | N |
| Tornjak01 | Tornjak | PRJNA232497 | SAMN02485567 | M | N |
| Vizsla01 | Vizsla | PRJEB12339 | SAMEA3724570 | F | N |
| WE006 | Weimaraner | PRJEB16012 | SAMEA4506902 | F | N |
| Whippet01 | Whippet | PRJEB16012 | SAMEA4506886 | M | N |
| XiasiDog01 | Xiasi Dog | PRJNA232497 | SAMN02485580 | M | N |
| MexicanHairless01 | Xoloitzcuintli | PRJNA266585 | SAMN03168385 | M | N |
| Xoloitzcuintlio1 | Xoloitzcuintli | PRJNA232497 | SAMN02485566 | M | N |
| AmericanHairlessTerrier01 | American Hairless Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| AR001 | Australian Terrier | PRJEB16012 | SAMEA4504840 | M | Y |
| CFA. 107838 | Border Terrier | PRJNA263947 | SAMN06159673 | M | Y |
| BorderTerrier02 | Border Terrier | PRJNA263947 | SAMN04196855 | F | Y |
| BorderTerrier03 | Border Terrier | PRJNA263947 | SAMN04196856 | M | Y |
| BorderTerrier01 | Border Terrier | PRJNA263947 | SAMN03580407 | F | Y |
| BostonTerrier01 | Boston Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| CE073 | Cairn Terrier | PRJEB16012 | SAMEA104091555 | F | Y |
| Chihuahua01 | Chihuahua | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| Chihuahua05 | Chihuahua | PRJEB13139 | SAMEA3905753 | M | Y |
| Chihuahua03 | Chihuahua | PRJNA266585 | SAMN03168379 | M | Y |
| ChineseCrested01 | Chinese Crested | PRJNA261736 | SAMN03075611 | M | Y |
| ChineseCrested05 | Chinese Crested | PRJNA255370 | SAMN02921308 | M | Y |
| CFA. 107835 | Dachshund | PRJNA263947 | SAMN06159670 | M | Y |
| CFA. 109668 | Dachshund | PRJNA263947 | SAMN06159676 | M | Y |
| DH0117 | Dachshund | PRJEB16012 | SAMEA104091567 | M | Y |
| DH126 | Dachshund | PRJEB16012 | SAMEA104125117 | F | Y |
| Dachshund01 | Dachshund | PRJEB7736 | SAMEA3121338 | M | Y |
| ItalianGreyhound02 | Italian Greyhound | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| ItalianGreyhound01 | Italian Greyhound | n/a | n/a | M | Y |
| JackRussellTerrier03 | Jack Russell Terrier | PRJNA263947 | SAMN03580400 | F | Y |
| JackRussellTerrier02 | Jack Russell Terrier | PRJNA263947 | SAMN03580384 | M | Y |
| JackRussellTerrier05 | Jack Russell Terrier | PRJNA263947 | SAMN04196852 | M | Y |
| JackRussellTerrier04 | Jack Russell Terrier | PRJNA263947 | SAMN03580404 | F | Y |
| Lowchen01 | Lowchen | PRJNA263947 | SAMN04196863 | M | Y |


| MiniaturePoodle01 | Miniature Poodle | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| :---: | :---: | :---: | :---: | :---: | :---: |
| BAN00024 | Miniature Poodle | $\mathrm{n} / \mathrm{a}$ | n/a | M | Y |
| MS04593 | Miniature Schnauzer | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| MS04563 | Miniature Schnauzer | $\mathrm{n} / \mathrm{a}$ | n/a | F | Y |
| MiniatureSchnauzer01 | Miniature Schnauzer | PRJNA263947 | SAMN04196847 | F | Y |
| NorwegianLundehund01 | Norwegian Lundehund | PRJNA186960 | SAMN01893932 | M | Y |
| NorwegianLundehund02 | Norwegian Lundehund | PRJNA309755 | SAMN04440505 | F | Y |
| NorwegianLundehund03 | Norwegian Lundehund | PRJNA309755 | SAMN04440506 | F | Y |
| NW062 | Norwich Terrier | PRJEB16012 | SAMEA104091550 | F | Y |
| NW255 | Norwich Terrier | PRJEB16012 | SAMEA104091553 | M | Y |
| NW152 | Norwich Terrier | PRJEB16012 | SAMEA104091551 | F | Y |
| NW206 | Norwich Terrier | PRJEB16012 | SAMEA104091552 | F | Y |
| Pekingese01 | Pekingese | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| Pomeranian01 | Pomeranian | PRJEB16012 | SAMEA4506892 | M | Y |
| Pug05 | Pug | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | Y |
| Pug03 | Pug | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | Y |
| Pug04 | Pug | $\mathrm{n} / \mathrm{a}$ | n/a | F | Y |
| Pug02 | Pug | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | Y |
| PER00751 | Shih Tzu | $\mathrm{n} / \mathrm{a}$ | n/a | M | Y |
| ToyPoodle01 | Toy Poodle | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | F | Y |
| WestHighlandWhiteTerrO; | West Highland White Te | $\mathrm{n} / \mathrm{a}$ | n/a | F | Y |
| WestHighlandWhiteTerro: | West Highland White Te | PRJNA263947 | SAMN03580395 | M | Y |
| WestHighlandWhiteTerrOt | West Highland White Te | PRJNA263947 | SAMN03580398 | F | Y |
| WW362 | West Highland White Te | PRJEB16012 | SAMEA4509489 | M | Y |
| YorkshireTerrier02 | Yorkshire Terrier | PRJNA299099 | SAMN04195509 | M | Y |
| PER00075 | Yorkshire Terrier | n/a | $\mathrm{n} / \mathrm{a}$ | F | Y |
| PER00204 | Yorkshire Terrier | $\mathrm{n} / \mathrm{a}$ | n/a | F | Y |
| BAN00041 | Yorkshire Terrier | $\mathrm{n} / \mathrm{a}$ | $\mathrm{n} / \mathrm{a}$ | M | Y |

Table S9. List of Haplotypes identified in homozygous dogs from 722 WGS catalogue. Haplotype block overlaps COL11a1. Haplotypes Alt1 and Alt 6 represent the overrepresented and rare haplotypes in the CKCS respectively. Highlighted in grey are haplotypes unique to nondomesticated canids.


Canid species and dog breeds With Samples homozygous for the observed haplotype

REF


Labrador Retriever ,Australian Cattle Dog ,UNKNOWN ,Australian Terrier ,Yorkshire Terrier Basenji ,Alpine Dachsbracke ,Brittany ,MIX: American Cocker Spaniel and Beagle ,English Setter ,Bouvier des Flandres ,Irish Terrier ,MIX: Dachsund ,Chihuahua ,Chinese Crested ,Chinese Indigenous Dog ,Cocker Spaniel (American) ,English Cocker Spaniel ,Dachshund ,Doberman Pinscher ,German Shepherd Dog ,Pointer (English) ,English Springer Spaniel ,Fonni's Dog German Wirehaired Pointer ,Golden Retriever ,Nigerian Indigenous Dog ,Irish Setter ,Irish Water Spaniel ,Istrian Shorthaired Hound ,Jack Russell Terrier ,Komondor ,Lapponian Herder Leonberger ,Rottweiler ,MIX: Kerry Blue Terrier and Beagle ,Chinese Shar-Pei ,Portuguese Water Dog ,Soft Coated Wheaten Terrier ,Tibetan Terrier ,Village Dog - China ,Village Dog India ,Village Dog - Namibia ,Village Dog - Qatar ,West Highland White Terrier ,American Hairless Terrier ,Belgian Sheepdog ,Belgian Tervuren ,Boxer ,Miniature Bull Terrier ,Bull Terrier ,Bullmastiff ,Mastiff (English) ,Flat-Coated Retriever ,Greyhound ,Irish Wolfhound ,Landseer ,Pembroke Welsh Corgi ,Pug ,Rhodesian Ridgeback ,Shetland Sheepdog ,Standard Poodle ,Swedish Lapphund ,Weimaraner ,Bloodhound ,Boston Terrier ,Cane Corso ,Great Dane ,Standard Schnauzer ,Curly-Coated Retriever ,Hovawart ,Kerry Blue Terrier

Cavalier King Charle Spaniel, Rottweiler, Border Collie, Belgian Malinois, Belgian Tervuren, Labrador Retriever, Jack Russell Terrier, Keeshond, Lagotto Romagnolo, Norwegian Lundehund, Yorkshire Terrier, Saluki, Samoyed, Soft Coated Wheaten Terrier, UNKNOWN, Village Dog China, Village Dog - India, Village Dog - Portugal, Village Dog - Papua New Guinea, Standard Poodle, Chinese Indigenous Dog
Coyote, Wolf
Bernese Mountain Dog, Entlebucher Sennenhund, Saint Bernard
Golden Retriever, Bull Terrier, Entlebucher Sennenhund, French Bulldog, German Hunting Terrier, Kromfohrländer, Labrador Retriever, Lagotto Romagnolo, Miniature Poodle, Norwich Terrier, Petit Basset Griffon Vendeen, Scottish Terrier, MIX: Miniature Schnauzer and Beagle Shiba Inu, Great Pyrenees, Greyhound, New Guinea Singing Dog, Standard Poodle

| ALT6 | T | T | G | G | A | A | C | A | T | C | Afghan Hound, Airedale Terrier, Yorkshire Terrier, Beagle, Bearded Collie, Bernese Mountain |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| ALT7 | C | C | T | G | T | A | C | G | C | C | Grey Wolf, Grey Wolf (Canis Lupus chanco), Alaskan Husky, UNKNOWN |
| ALT8 | T | T | G | G | A | T | A | G | C | T | Great Dane |
| ALT9 | T | T | G | G | A | A | C | G | T | T | Standard Schnauzer |
| ALT10 | C | C | T | G | A | A | C | G | T | T | Chinese Indigenous Dog, Chow Chow, MIX: Siberian Husky, Siberian Husky, Tibetan Mastiff, |
| ALT11 | C | C | T | G | A | A | C | G | T | C | Qingchuan Dog, Village Dog - China, Village Dog - Borneo |
| ALT12 | C | C | T | G | T | A | C | G | T | C | Grey Wolf, Wolf |
| ALT13 | C | C | T | G | T | A | C | G | T | T | Grey Wolf, Grey Wolf (Canis Lupus chanco) |
| ALT14 | C | C | T | T | T | A | C | G | T | C | Elo, Italian Greyhound, Village Dog - India |
| ALT15 | C | C | T | T | T | A | C | G | T | T | Chongqing Dog |
| ALT16 | C | C | T | G | T | A | C | G | C | C | Golden Jackal |
| ALT17 | C | C | T | G | T | A | A | G | T | C | Andean Fox, Dhole |

Table S10. Dogs from the $\mathbf{7 2 2}$ catalogue homozygous for the haplotype block overlapping COL11a1. Haplotypes Alt1 and Alt6 represent the frequent and rare CKCS haplotypes rspectively. Genomic coordinates for CANFAM3. Variants have been partitioned into Haplotype and splice variants.

|  |  | $\begin{aligned} & \mathrm{N} \\ & \underset{\sim}{\prime} \\ & \underset{N}{\mathrm{~N}} \\ & \underset{\sim}{2} \end{aligned}$ | $\begin{aligned} & \underset{N}{N} \\ & \underset{\sim}{\infty} \\ & \underset{\sim}{+} \end{aligned}$ | $\ddagger$ <br>  <br>  <br>  <br>  | ELSSOSLも | $\begin{aligned} & \underset{\sim}{-} \\ & \underset{-}{-} \\ & \underset{\sim}{\top} \end{aligned}$ | $\begin{aligned} & N \\ & N \\ & \underset{\sim}{N} \\ & N \\ & N \end{aligned}$ | $\begin{aligned} & \underset{m}{m} \\ & \underset{\sim}{\oplus} \\ & \underset{\sim}{\dagger} \end{aligned}$ | $\begin{aligned} & \bullet \\ & \underset{\sim}{-} \\ & \underset{\sim}{\sim} \\ & \underset{\sim}{\top} \end{aligned}$ | $\begin{aligned} & \underset{\sim}{N} \\ & \infty \\ & \underset{\sim}{n} \\ & \underset{\sim}{N} \end{aligned}$ | $47573151$ | $\begin{aligned} & \infty \\ & n^{n} \\ & \underset{\sim}{n} \\ & \infty \\ & \stackrel{\infty}{N} \end{aligned}$ |  | CFA6:g.47507204C>T |  | $\begin{aligned} & \text { 6:4750557 } \\ & 3 \text { tag for } \\ & \text { CFA6:g. } 47 \\ & 507204 C> \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| REF | REFERENCE | T T | T T | G G | G G | A A | A A | C C | G G | A A | T T | C C | T T | A A | G G |  |
| REF | Labrador Retriel 152721_S5 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Labrador Retriel 172281_S2 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | $Y$ |
| REF | Labrador Retriel 87125_S1 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Labrador Retriel 91317_S7 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | CC | T T | A A | G G | $Y$ |
| REF | Australian Cattl AC023 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | $Y$ |
| REF | UNKNOWN AF14-297_S10 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | UNKNOWN AF15-074_S4 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | 00 | Y |
| REF | Australian Terri AR001 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Australian Cattl AustralianCattl | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Yorkshire Terrie BAN00127 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Yorkshire Terrie BAN00150 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | 00 | Y |
| REF | Basenji Basenji03 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Alpine Dachsbra Bern_AlpineDad | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Brittany BrittanySpaniel | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | MIX: American (C750 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | English Setter CFA. 105990 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Bouvier des Flar CFA. 107836 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | English Setter CFA. 117996 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Irish Terrier CFA. 117998 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | MIX: Dachsund CFA. 118000 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Chihuahua Chihuahua03 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Chinese Crested ChineseCrested | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Chinese Crested ChineseCrested | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Chinese Indigenı ChineseIndigend | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | G A | G G | Y |
| REF | Cocker Spaniel ( CockerSpanielA | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | English Cocker S CockerSpanielE | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Dachshund DH0117 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Doberman Pinsc DO242 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Doberman Pinsc Doberman02 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | German Shephe DS043 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | German Shephe DS051 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | German Shephe DS053 | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | Pointer (English) EnglishPointer0 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | $Y$ |
| REF | English Setter EnglishSetter01 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | C C | T T | A A | G G | Y |
| REF | English Springer EnglishSpringer | T T | T T | G G | G G | A A | A A | C C | G G | 00 | T T | C C | T T | A A | G G | $Y$ |

 \begin{tabular}{l|lllllllll}
Nigerian Indiger IndigenousDog \& T T \& TT \& G G G G \& A A \& A A \& C C \& G G \& 00 <br>
Nigerian Indiger IndigenousDog \& T T \& T T \& G G G G G \& A A \& A A \& C C \& G G \& 00

 Nigerian Indiger IndigenousDog $\mid$ TT T T GG GG AA AA CC GG 00 

Irish Setter IrishSetter01 \& TT T T GGGGGAA A A \& CC GG \& 00
\end{tabular} Irish Water SpaıIrishWaterSpan TT TT GG GG AA A A CC GG 00 Irish Water Spaı IrishWaterSpan TT T T GG GG AA AA CC GG 00 Irish Water Spaı IrishWaterSpan T T T T G G GG AA AA CC GG 00 Istrian Shorthaiı IstrianShorthain T T T T G G GG AA AA CC GG 00 Jack Russell Terı JackRussellTerr Komondor Komondor01 TT TTAGGGGAA A A CC G G 00 Labrador Retrie LabradorRetrie 1 T T T T GGGGGAA AA CC GG 00 Lapponian Herdı LapponianHerde Leonberger Leonberger01 Rottweiler LEW02122013_ MIX: Kerry Blue MIX_KerryBlueTTT TT GG GG AA AA CC GG 00 Chinese Shar-Pe NGSDOGO24 Yorkshire Terrie PER00204 Yorkshire Terrie PER00344 Yorkshire Terrie PER00361 Yorkshire Terrie PER00409 Yorkshire Terrie PER00462 Yorkshire Terrie PER00465 Yorkshire Terrie PER00484 Yorkshire Terrie PER00602 Yorkshire Terrie PER00606 Yorkshire Terrie PER00642 Yorkshire Terrie PER00806 Portuguese Wat PortugueseWat Rottweiler Rottweiler01 TT TT GG GG AA AA CC GG 00 Rottweiler Rottweiler02

Tibetan Terrier TibetanTerrierd T Village Dog-Ch VillDog_China2 TT TT GG GG AA AA CC GG 00 Village Dog - Ch VillDog_China3 TT TT GG GG AA AA CC GG 00 Village Dog - Ch VillDog_China5 TT TT GG GG AA AA CC GG 00 Village Dog-Inc VillDog_India04 T T TT GG GG AA A A CC GG 00 Village Dog-NaVillDog_Namibi TT TT GG GG AA A A CC GG 00

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Belgian Sheepdc BelgianSheepdd Belgian Tervure BelgianTervure Belgian Tervure BelgianTervure

Bouvier des Flar BouvierDesFlan

## Miniature Bull T BT007

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Golden Retrieve 164612_S9 TT TT GG GG AA AA CC GG 00


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TT TT GG GG AA AA CC GG 00
TT TT GG GG AA AA CC GG 00


Bull Terrier BullTerrier03 Bull Terrier
Dachshund DH126
.

Doberman Pinsc Doberman04
Mastiff (English) EnglishMastiff0
Mastiff (English) EnglishMastiffo $T$
Flat-Coated Retı FlatcoatedRetri
Golden Retrieve GoldenRetrieve
Golden Retrieve GR0892
Greyhound Greyhound02
Irish Wolfhound IrishWolfhoundd
Landseer Landseer01
Pembroke Welsl PembrokeWelst

Labrador Retriel PERO Yorkshire Terrie PER00321 Yorkshire Terrie PER00408

## Yorkshire Terrie PER00605

## Yorkshire Terrie PER00626

 Yorkshire Terrie PER00777Portuguese Wat PortugueseWat
Pug Pug02 T
Pug Pug05
Rhodesian Ridge RR098
Shetland Sheepı ShetlandSheep
Standard Poodle StandardPoodle
Swedish Lapphu SwedishLapphu
Tibetan Terrier TibetanTerrierd
Village Dog - Qa VillDog_Qatar0
Weimaraner WE006


West Highland IWestHighlandW T T T T GG G G A A A A CC G G 00 West Highland IWestHighlandW T T TT GG GG AA AA CC GG 00 Labrador Retrie 149323_S6

## Rottweiler 165414_S20

## Golden Retrieve 173006_S10

Yorkshire Terrie BAN00032 UNKNOWN BAN0014 Yorkshire Terrie BAN00183 Yorkshire Terrie BAN00235 Yorkshire Terrie BAN00315 Belgian Sheepdc BelgianSheepdd $T$

Bouvier des Flar BouvierDesFlan
Cane Corso CaneCorso01

## Dachshund CFA. 109668

## Great Dane

CFA. 117997

Cocker Spaniel ( CockerSpanielA

## Curly-Coated Re CRO39

Doberman Pinsc Doberman01
Doberman Pinsc Doberman03

German Shephe GermanShephe
Golden Retrieve GoldenRetrieve

Golden Retrieve GoldenRetrieve

Kerry Blue Terri KerryBlueTerrie T
Labrador Retriel PER00177 Yorkshire Terrie PER00226 Yorkshire Terrie PER00324

## Yorkshire Terrie PER00438

## Yorkshire Terrie PER00622

 Yorkshire Terrie PER00650 Portuguese Wat PortugueseWat| REF | Nigerian Indiger IndigenousDog | T T | T T | 00 | G G | A A | A A | CC | G G | 00 | T T | C C | T | A A | G G |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ALT1 | Rottweiler 171515_ | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | CC | G G | A A |
| ALT1 | Border Collie BC485 | CC | CC | T | T T | T T | T T | A A | G G | 00 | CC | T T | CC | G G | A A |
| ALT1 | Border Collie BC518 | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | CC | G G | A A |
| ALT1 | Borde | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | CC | G G | A A |
| ALT1 | Be | C C | C C | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Belgian Tervure B | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Be | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Border Collie BorderCollie02 | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Border Collie BorderCollie03 | C C | CC | T T | T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| T1 | Bo | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Border | C C | CC | T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| AL | La | C C | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T | C C | G G | A A |
| AL | B | C C | CC | T T | T T | T T | T T | A | G G | 00 | CC | T | C C | G G | A A |
| AL | B | C C | CC | T T | T T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Jack Russell Terı Jac | CC | CC | T T | T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| AL | Keeshond Keesh | C C | CC | T | T T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| T1 | Labrador Retrie L | C C | CC | T T | T T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | La | C C | CC | T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | N | C C | CC | T T | T |  | T T | A A | G G | 00 | CC | T T | C | G G | A A |
| ALT | N | C C | C | T T | T T | TT | T T | A A | G G | 0 | CC | T T | C C | G G | A A |
| ALT | N | C C | CC | T | T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| AL | Yo | C C | C | T | T | T T | T T | A | G | 00 | CC | T T | C C | G G | A A |
| AL | Sa | C C | CC | T | T T | T T | T | A | G G | 00 | CC | T T | C C | G G | A A |
| AL | Sa | C C | CC | T T | T T | T T | T | A | G | 00 | CC | T | C C | G G | A A |
| ALT1 | Sa | CC | CC | T T | T T | T T | T T | A | G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Sa | C C | C C | T T | T T | T T | T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT1 | Soft Coated Whi So | C C | CC | T T | T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | UNKNOWN | C C | CC | T | T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| AL | Villag | C C | 00 | T T | T | T T | T T | 00 | G G | 00 | CC | T T | C C | 00 | A A |
| AL | Vi | C C | CC | T T | T | T T | T T | A | G G | 00 | C | T | C C | G G | A A |
| AL |  | C C | CC | T | T | T T | T T | A | G G | 00 | C | T | C C | G G | A A |
| ALT1 | Vi | C C | C C | T T | T T | T T | T T | A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Villag | C C | CC | T T | T T |  | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Villag | C C | CC | T T | T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT1 | Village Dog - Po VillDog_Portug | CC | CC | T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT1 | Villag | C C | CC | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT1 | Stand | C C | C C | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT1 | Chinese | C C | 00 | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT2 | Coyote Co | C C | CC | T T | G G | T T | A | A A | G G | 00 | CC | T T | C C | G G | G G |
| ALT2 | Wolf Wolf | C C | CC | T T | G G | T T | A A | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT3 | Bernese Mountć Bernese | T T | C C | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT3 | Bernese Mount ${ }^{\text {B BerneseMounta }}$ | T T | CC | TT | T T | T T | TT | A A | G G | 00 | CC | T T | CC | G G | A A |
| ALT3 | Bernese Mountc BerneseMounta | T T | CC | T T | TT | TT | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT3 | Bernese Mounta BerneseMou | T T | CC | TT | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT3 | Bernese Mounta BerneseMounta | T T | CC | TT | TT | TT | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT3 | Bernese Mountc BerneseMounta | T T | CC | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT3 | Bernese Mountá BerneseMounta | T T | CC | T T | T T | T T | T T | A A | G G | 00 | CC | T T | C C | G G | A A |


| ALT3 | Bernese Mountç BerneseMounta | T T | CC | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AlT3 | Bernese Mountç BerneseMounta | T T | CC | TT | T T | T T | T T | A A | G G | 00 | CC | TT | C | G G | A A |
| ALT3 | Bernese Mountç BerneseMounta | T | C C | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT3 | Bernese Mountç BerneseMounta | T | CC | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT3 | Entlebucher Sen EntlebucherSen | T | CC | T T | T T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT3 | lebucher Sen EntlebucherSen | T | C C | T T | T T | TT | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT3 | Saint Bernard SaintBernard02 | T T | CC | T T | T | T T | T T | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT4 | Golden Retrieve GoldenRetrieve | T T | CC | TT | T T | T T | T T | A A | G G | 00 | T T | C C | C C | G A | A G |
| ALT4 | Bull Terrier BullTerrier04 | T T | CC | T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT4 | Bull Terrier BullTerrier05 | T T | CC | T | T T | T T | T T | A A | G G | 00 | T T | C C | C | G G | A A |
| ALT4 | Entlebucher Sen EntlebucherSen | T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C | A A | G G |
| ALT4 | French Bulldog FB065 | T | C C | TT | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A |
| ALT4 | Golden Retrieve GoldenRetrieve | T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C | G G | A A |
| ALT4 | German Huntin§ JT007 | T T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT4 | Kromfohrländer Kromfohrlander | T T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | G G | A A |
| ALT4 | Labrador Retrie LabradorRetrie | T T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | CC | G G | A A |
| ALT4 | Lagotto Romagn LagottoRomagn | T T | C C | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | G G | A A |
| ALT4 | Miniature Poodl MiniaturePoodl | T T | CC | T T | TT | TT | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT4 | Norwich Terrier NW062 | T | CC | T | T T | T T | T T | A A | G G | 00 | T T | CC | CC | A A | G G |
| ALT4 | Norwich Terrier NW152 | T T | CC | T | T T | T T | T T | A A | G G | 00 | T T | CC | C | A A | G G |
| ALT4 | Norwich Terrier NW255 | T T | C C | T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | A A | G G |
| ALT4 | Pe | T T | C C | T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | G G | A A |
| ALT4 | Scottish Terrier ScottishTerrierd | T T | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | A A | G G |
| ALT4 | Scottish Terrier ScottishTerrierd | T T | CC | T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | A A | G G |
| T4 | Scottish Terrier ScottishTerrierd | T T | C C | T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | A A | G G |
| ALT4 | MIX: Miniature A168 | T | CC | T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | A A | G G |
| ALT5 | Shiba Inu CFA. 107839 | C C | CC | T T | T T | T | T T | A A | G G | 00 | T T | C C | CC | G G | A A |
| ALT5 | Great Pyrenees GreatPyrenees | C C | CC | T T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | A A | G G |
| ALT5 | Greyhound Grey | C C | CC | T T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT5 | Greyhound | C C | CC | T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT5 | N | C C | CC | T T | T T | T | T | A A | G G | 00 | T T | CC | C C | G G | A A |
| A | Standard Poodle StandardPoodle | C C | C | T T | T T | T T | T T | A A | G G | 00 | T T | CC | C C | G G | A A |
| ALT5 | Village Dog - Ch VillDog_China3 | C C | CC | T T | T T | T T | T T | A A | G G | 00 | T T | C C | C C | G G | A A |
| ALT6 | Afghan Hound AfghanHound01 | T T | T T | G G | G G | A | A A | CC | A A | 00 | T T | C C | T T | A A | G G |
| ALT6 | Aireda | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Airedale Terrier AiredaleTerrier | T T | TT | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Yorkshire Terrie BAN00368 | T T | TT | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| Alt6 | Yorkshire Terrie BAN00437 | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Beagle Beagle02 | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Bearded Collie BeardedCollieOf | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| AlT6 | Bearded Collie BeardedCollie0 | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Bernese Mountá Bern | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Border Terrier BorderTerrierO2 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Spinone Italianc CFA. 109669 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Chinook Chinook01 | T T | TT | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |
| ALT6 | Clumber Spaniel ClumberSpaniel | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | C C | T T | A A | G G |
| ALT6 | Yorkshire Terrie PER00138 | T T | T T | G G | G G | A A | A A | C C | A A | 00 | T T | CC | T T | G A | G G |
| ALT6 | Yorkshire Terrie PER00738 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | CC | T T | A A | G G |


| ALT6 | Yorkshire Terrie | PER00750 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | C C | T T | A A | G G |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ALT6 | Spanish Galgo | SpanishGalgo01 | T T | TT | G G | G G | A A | A A | CC | A A | 00 | T T | C C | T T | G A | A G |
| ALT6 | Kunming Dog | VillDog_China1 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | C C | T T | A A | G G |
| ALT6 | Kunming Dog | VillDog_China1 | T T | T T | G G | G G | A A | A A | CC | A A | 00 | T T | C C | T T | A A | G G |
| ALT7 | Grey Wolf | Wolf01 | C C | CC | T T | G G | TT | A A | CC | G G | 00 | CC | C C | 00 | A A | G G |
| ALT7 | Grey Wolf | Wolf06 | C C | C C | T T | G G | T T | A A | CC | G G | 00 | CC | C C | C C | A A | G G |
| ALT7 | Grey Wolf (Cani؛ | WolfTibetan05 | C C | CC | T T | G G | TT | A A | CC | G G | 00 | C C | C C | C C | G G | A A |
| ALT7 | Alaskan Husk | AlaskanHusky0 | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT7 | UNKNOWN | BH003 | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT8 | Great Dane | CFA. 107837 | T T | T T | G G | G G | A A | TT | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT8 | Great Dane | DD116 | TT | T T | G G | G G | A A | TT | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT8 | Great Dane | GreatDane01 | TT | T T | G G | G G | A A | T T | A A | G G | 00 | CC | T T | C C | G G | A A |
| ALT8 | Great Dane | GreatDane02 | T T | T T | G G | G G | A A | TT | A A | G G | 00 | C C | T T | C C | G G | A A |
| ALT9 | Standard Schnaı | ICFA. 118001 | T T | T T | G G | G G | A A | A A | CC | G G | 00 | T T | T T | T T | A A | G G |
| ALT10 | Chinese Indigenı | ChineseIndigend | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | C C | 00 | G G |
| ALT10 | Chow Chow | CW011 | CC | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | CC | A A | G G |
| ALT10 | MIX: Siberian HI | MixedBreed07 | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT10 | Siberian Husk | SY046 | CC | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT10 | Tibetan Mast | etanMastiffd | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT10 | Tibetan Mast | Mastiffd | CC | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | CC | A A | G G |
| ALT10 | Village Dog - Ch | VillDog_China4 | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT11 | Qingchuan Dog | QingchuanDog0 | C C | C C | T T | G G | A A | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT11 | Village Dog - Ch | VillDog_China4 | C C | CC | T T | G G | A A | A A | CC | G G | 00 | T T | CC | C C | G A | A G |
| ALT11 | Village Dog - Ch | VillDog_China0 | C C | 00 | T T | G G | A A | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT11 | Village Dog | VillDog_Borneo | C C | 00 | T T | G G | A A | A A | C C | G G | 00 | T T | C C | C C | 00 | G G |
| ALT12 | Grey Wolf | Wolf23 | CC | CC | T T | G G | T T | A A | CC | G G | 00 | T T | C C | CC | G G | A A |
| ALT12 | Wolf | W0002_732 | C C | CC | T T | G G | T T | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT12 | Grey Wolf | Wolf05 | CC | CC | T T | G G | T T | A A | CC | G G | 00 | T T | C C | C C | G A | A G |
| ALT12 | Grey Wolf | Wolf22 | C C | CC | TT | G G | T T | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT12 | Red Wolf | Wolf25 | C C | CC | T T | G G | T T | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT13 | Grey Wolf | Wolf08 | C C | CC | T T | G G | T T | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT13 | Grey Wolf (Cani؛ | Wolftibetan01 | C C | CC | T T | G G | T T | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT13 | Grey Wolf (Cani: | : WolfTibetan02 | C C | CC | T T | G G | T T | A A | CC | G G | 00 | T T | T T | C C | A A | G G |
| ALT14 | Elo | Elo01 | C C | CC | T T | T T | T T | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT14 | Italian Greyhour | ItalianGrey hour | CC | CC | T T | T T | T T | A A | CC | G G | 00 | T T | C C | T T | A A | G G |
| ALT14 | Village Dog - Inc | V VillDog_India02 | C C | 00 | T T | TT | TT | A A | CC | G G | 00 | T T | C C | C C | G G | A A |
| ALT15 | Chongqing Dog | ChongqingDog0 | C C | CC | TT | T T | TT | A A | CC | G G | 00 | T T | T T | T T | A A | G G |
| ALT16 | Golden Jackal | GoldenJackal01 | C C | CC | TT | G G | T T | A A | CC | G G | 00 | CC | CC | CC | A A | G G |
| ALT17 | Andean Fox | AndeanFox01 | CC | CC | TT | G G | T T | A A | A A | G G | 00 | T T | C C | C C | A A | A A |
| ALT17 | Dhole | Dhole01 | CC | CC | T T | G G | T T | A A | A A | G G | 00 | T T | C C | C C | A A | A A |

CFA12:33,710,170 bp


Figure S1. Insertion of FGF4-retrogene identified on CFA12. Screenshot of Integrative Genomics Viewer (IGV- Broad Institute). IGV uses colour coding to flag reads that have an atypical alignment such as insertions, deletions and inter-chromosomal rearrangements. Here IGV shows Cavalier King Charles Spaniel whole genome sequence data (WGS; paired-end reads) aligned to CanFam3.1 centred at CFA12:33,710,170 (red dashed line). Black boarder divides the reads from five CKCS WGS samples. Reads coloured in green support insertion of an FGF4-retrogene, with read mates mapping to the original location of the FGF4 gene at CFA18:48.4Mb. Read mates in blue map to CFA7:68.3. PCR investigation of blue reads suggest a genome assembly error (Brown et al., 2017).

# A large deletion on CFA28 omitting ACSL5 gene is associated with intestinal lipid malabsorption in the Australian Kelpie dog breed 


#### Abstract

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Inborn errors of metabolism are genetic conditions that can disrupt intermediary metabolic pathways and cause defective absorption and metabolism of dietary nutrients. In an Australian Kelpie breeding population, 17 puppies presented with intestinal lipid malabsorption. Juvenile dogs exhibited stunted postnatal growth, steatorrhea, abdominal distension and a wiry coat. Using genome-wide association analysis, an associated locus on CFA28 ( $\mathrm{P}_{\mathrm{raw}}=2.87 \mathrm{E}^{-06}$ ) was discovered and validated in a closely related population ( $\mathrm{P}_{\mathrm{raw}}=1.75 \mathrm{E}^{-45}$ ). A 103.3 kb deletion NC_006610.3CFA28:g.23380074_2 3483377del, containing genes Acyl-CoA Synthetase Long Chain Family Member 5 (ACSL5) and Zinc Finger DHHC-Type Containing 6 (ZDHHC6), was characterised using whole transcriptomic data. Whole transcriptomic sequencing revealed no expression of ACSL5 and disrupted splicing of ZDHHC6 in jejunal tissue of affected Kelpies. The ACSL5 gene plays a key role in long chain fatty acid absorption, a phenotype similar to that of our affected Kelpies has been observed in a knockout mouse model. A PCR-based diagnostic test was developed and confirmed fully penetrant autosomal recessive mode of inheritance. We conclude the structural variant causing a deletion of the ACSL5 gene is the most likely cause for intestinal lipid malabsorption in the Australian Kelpie.


[^6]

Figure 1. Simplified pedigree of an AK family showing links among affected individuals with hereditary intestinal lipid malabsorption. Females and males are indicated by circles and squares, respectively. Filled symbols indicate affected samples, half-filled symbols represent carriers of the disease allele based on autosomal recessive inheritance. Offspring from a single litter are represented by a line descending from a horizontal connection between parent symbols. A triangle has been used to designate multiple samples ( N ) from a single litter that are not affected or suspected to be carriers based on recessive inheritance. Litters that included zero affected samples have not been included. Affected samples highlighted in blue were included in the study. Diagnostic testing found all samples to be homozygous for the disease-associated variant.
is a fully penetrant autosomal recessive inherited metabolic disorder. The aim of the current study is to provide insight into the molecular genetic aetiology of intestinal lipid malabsorption in the AK.

## Materials and methods

Ethics. The research described conforms to the recommendations from the Australian Code for the Care and Use of Animals for Scientific Purposes. Animal ethics approval was granted by the Animal Ethics Committee at the University of Sydney (approval numbers 2015/902 and 2018/1449).

Animal selection and phenotype selection. Related juvenile AK dogs were observed to exhibit stunted postnatal growth and intestinal lipid malabsorption. Affected individuals remain a third to one half the size of their littermates during development and mature so that adult dogs are smaller in stature and exhibit persistent intolerance to a fatty diet. Starting in 2011, 17 of 319 puppies, from 45 litters, were born at one Australian kennel presenting with identical clinical features (Fig. 1). As neonates, affected puppies are indistinguishable from their littermates, but rapidly show clinical signs of polyphagia, failure to thrive, stunted growth (around one-third to one-half of the size of their siblings-Fig. 2a), yellowish poorly digested loose and pulpy faeces (Fig. 2b), increased faecal volume, and frequent defecation. Once affected puppies are transferred to a solid diet with digestive enzyme supplementation, faecal consistency normalises. From around six months of age, most affected


Figure 2. Side by side comparisons of affected Kelpie and unaffected littermate size and faecal matter. (a) Affected Kelpie at 10 weeks (right) with his littermate. Size and musculature of the affected pup is in stark contrast. (b) Pale poorly digested faeces from an affected Kelpie (right) in comparison with an unaffected littermate (left). There is a significant difference in colour and consistency between the two samples.

Kelpies appear to outgrow the characteristic clinical presentation. However, the dogs remain smaller in stature than their siblings, consistently produce more voluminous faeces than age-matched dogs, and their intolerance to high-fat foods persists throughout their lives.

This study involved 265 Kelpies. Samples were made up of 35 AK ( 10 cases and 25 controls), 225 AWK ( 225 controls), and 5 international Kelpies (one case and four controls). Cases in this study represent dogs that adhered to the described clinical presentation. Cases were easily recognised through signs of ill thrift, faecal appearance (steatorrhea), and stunted growth when compared to littermates. Seven cases from the originally described kennel have been included in this study. Two samples from separate kennels were reported as dams of affected pups. They were included as control samples and treated as obligate carriers when observing results.

Biological samples were collected as whole blood in EDTA tubes or buccal cells using cheek swabs. Genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) or submitted as EDTA blood to the genotyping service provider on Whatman Flinders Technology Associates (FTA) cards, supplied by the genotyping service. Genotyping array data for 255 samples was obtained from the CanineHD BeadChip (Illumina, San Diego, CA, USA) by Neogen (Lincoln, NE, USA).

A full post-mortem was conducted at the Veterinary Pathology Diagnostic Services (University of Sydney, Camperdown, NSW, Australia) on a 17 -week-old affected AK pup that was euthanized with approval by the owner on welfare grounds. A thorough examination was conducted on tissue of the lung, spleen, liver, heart, major cardiac vessels, lymph nodes, thyroid gland, kidney, bone marrow, pancreas, small intestine (duodenum, jejunum and ileum), brain and spinal cord.

Genome-wide association study (GWAS). To detect and validate signals associated with malabsorption in the Kelpie population two case-control GWAS were performed using Plink 1.9 (--assoc) ${ }^{12}$. Quality control of genotypic data was conducted on 25 AK, five internationally bred Kelpies, and 225 AWK. Single Nucleotide variants (SNVs) were excluded if they exhibited a call rate of less than $90 \%$ (--geno) or a low minor allele frequency $<10 \%$ (--maf). Pairwise identity by decent was calculated (--genome) to detect and remove duplicated or highly related individuals. Population stratification was visualised using a multidimensional scaling (MDS) plot with two dimensions (--mds). One sample from each pair with a pairwise identity by decent $>0.7$ was excluded. This was done to control for inflation resulting from cryptic relatedness and population stratification. Population stratification in the preliminary GWAS was determined by the genomic inflation factor based on the median Chi-squared statistic. The primary GWAS was conducted using 30 Kelpies, including 25 AK and five internationally bred Kelpies. Both groups show evidence of carrying the studied trait; reflected in our dataset. To control for the testing of multiple hypotheses, genome-wide significant and suggestive thresholds were Bon-ferroni-corrected, $5 \times 10^{-7}$ (Bonferroni cut-off of $\alpha=0.05, \mathrm{n}=99,326$ ) and $1 \times 10^{-5}$ (Bonferroni cut-off of $\alpha=1.0$, $\mathrm{n}=99,326$ ), respectively. Reported $P$-values are chi-square allelic test $P$-values as calculated in Plink. The 200 most associated markers from the unstratified preliminary GWAS were taken forward to a second analysis that added 225 control dogs from the closely related population of AWK.

Confirmation of deletion by polymerase chain reaction (PCR). A large segment of consecutive uncalled array markers was observed only in cases suggesting the presence of a large deletion in these animals. Primers designed using primer $3^{13-15}$ were used to detect the presence of the deletion. The novel deletion was confirmed through amplification of the last coding exons in impacted genes by PCR. Where no amplification was observed, to gauge the size of the deletion, further primers were designed to amplify the preceding exon. Alternatively, where amplification was witnessed, we designed primers in the gene's untranslated region (UTR). A total of seven primers were designed (Table S1). PCR was carried out in total volume of $20 \mu \mathrm{l}$ using AmpliTaq

Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Fragments were evaluated on a $1.5 \%$ agarose gel. Briefly, the PCR conditions were heat activation for 10 min (mins) at 95 degrees Celsius ( ${ }^{\circ} \mathrm{C}$ ); and 30 cycles of $30 \mathrm{~s}(\mathrm{~s})$ at $95^{\circ} \mathrm{C}, 58^{\circ} \mathrm{C}$ and $72^{\circ} \mathrm{C}$ for denaturation, annealing and extension respectively. The process concluded with a final elongation step at $72^{\circ} \mathrm{C}$ for 10 min .

RNA sequencing, alignment and variant detection. In order to gauge if mapped candidate genes were influencing the observed phenotype, whole transcriptomic sequencing was conducted on whole tissue of the jejunum collected during post-mortem. Using Invitrogen TRIzol Reagent, RNA was extracted according to the manufacturer's protocol. Total RNA sequencing (RNAseq) was performed on Illumina NovaSeq S1 using the TrueSeq Stranded RNA RiboZero Gold ( $\mathrm{h} / \mathrm{m} / \mathrm{r}$ ) kit at the Ramaciotti Centre for Genomics (University of New South Wales, Kensington, NSW, Australia). A total of 142,658,896, 100 base pair (bp) paired end reads were generated. Tissue matched transcriptomic sequence data for the Labrador retriever JEJUNUM_LABR (Accession identifier: SRR3727723) were obtained from the Sequence Read Archive (SRA) in Genbank (https://www. ncbi.nlm.nih.gov/sra/).

Quality control for the raw paired-end reads was performed with FastQC v0.11.8 (https://www.bioinforma tics.babraham.ac.uk/projects/fastqc/) and visualised using MultiQC ${ }^{16}$. Raw RNAseq reads were mapped to the canine reference genome (CanFam3.1) using STAR aligner v2.7.0e basic options ${ }^{17}$. Reads surrounding the candidate region were visualised and extracted for a genome-guided de novo transcriptome assembly using Trinity $\mathrm{v} 2.8 .3^{18}$. The distribution of read data was calculated across known gene features using RSeQC v3.0.1 ${ }^{19}$.

STAR aligned bam files and Trinity constructed fasta sequences were visualised in Integrative Genomics Viewer v2.8.2 ${ }^{20}$. Variants in the affected Kelpie transcript were compared with those of the tissue matched Labrador retriever. Alternate transcript splicing was visualised using a sashimi plot created using ggsashimi ${ }^{21}$. The minimum read coverage at splice junctions was set to 15 reads to reduce background hybridisation signals.

Multiplex PCR assay design for deletion. Custom primers were designed to capture a disease-associated variant identified in the sequenced AK using primer3. A three-primer multiplex PCR was designed to detect the structural variant (Table S1). The multiplex PCR includes a forward primer, 37 bp upstream of the disease-associated variant, and two reverse primers, one 140 bp downstream from the start of the variant and another $\sim 103.7 \mathrm{~kb}$ downstream of the forward primer. PCR was carried out in total volume of $20 \mu \mathrm{l}$ as previously described. A total of 19 Kelpie samples from affected populations were assessed using this method, including nine cases and ten controls. This encompassed two cases and eight controls also utilised in the GWAS analysis. Six of the controls were known to come from families that have produced affected puppies.

Equipment and settings. All images have been formatted for publishing using Adobe Photoshop 2020 (v21.1.1). Images that have been cropped have been done so to improve clarity and conciseness. As such, all images correctly represent the original data. If an electrophoretic gel images has been cropped, it is stated in the figure legend and an original image has been provided in "Supplementary materials".

## Results

Post-mortem results. Post-mortem was conducted on a young, 17 -week old, female Kelpie. The dog was received in excellent post-mortem condition immediately following euthanasia. The affected Kelpie pup showed signs of malnutrition including decreased body condition (body condition score $2 / 5$ ), atrophied musculature and depleted subcutaneous adipose tissue stores. Histological examination of the small intestine showed evidence of mild non-specific chronic enteritis including focal ileal ulceration, rare crypt abscesses in the ileum and colon, and possible crypt fusion in the jejunum.

Genome-wide association study (GWAS). After frequency and genotype pruning, 99,326 SNVs remained in the analysis. Three cases and 27 control Kelpies were available for the primary analysis. Of these, four controls and one case were bred outside Australia. By MDS the AK and International populations clustered closely and so were treated analytically as one population (Fig. S1a). When AWK were included in the MDS the principal Kelpie population and AWK clustered separately (Fig. S1b).

A preliminary GWAS was performed in the closely clustered Kelpie populations with affected samples (AK and internationally bred Kelpies). The quantile-quantile plot shows limited inflation and the genomic inflation factor was 1.23 (Fig. 3a). GWAS revealed a suggestive association with intestinal lipid malabsorption on canine chromosome 28 (CFA28) (best $\mathrm{P}_{\text {raw }}=2.87 \mathrm{E}^{-6}$ ) (Fig. 3b). Six SNVs within a three megabase ( Mb ) region ( $28: 24,521,377-26,556,336 ; 2.03 \mathrm{Mb}$ ) passed the suggestive genome-wide significance threshold and were in strong linkage with the index SNV ( $\mathrm{r}^{2}>0.93$ ). In the validation analysis, 225 AWK controls were added to the leading dataset. When analysing the top 200 associated SNVs in the primary GWAS in the extended cohort, 52 SNVs passed genome-wide significance (Fig. 3c). Of these SNVs, 21 (40.3\%) were located on CFA28: 20 that clustered within a four Mb region ( $28: 24,030,090-27,194,500 ; 3.16 \mathrm{Mb}$ ) including $16(30.7 \%)$ that matched the expected GT frequency for a recessively inherited trait (Table S2). The top SNV from the preliminary analysis remained the strongest in the validation set (best $\mathrm{P}_{\text {raw }}=1.75 \mathrm{E}^{-45}$ ).

Confirmation of deletion by PCR. Within the associated locus, we identified a region of nine consecutive SNVs spanning marker CFA28:23,370,822 (BICF2P674000) to CFA28:23,493,334 (BICF2P338375) 122.5 kb , where all but two SNVs were consistently uncalled in cases but not controls (Table S3), suggesting the presence of a deleted segment. Within the putatively deleted segment, three genes and one pseudogene were identified,
b



Figure 3. QQ-plot and Manhattan plots from intestinal lipid malabsorption case-control GWAS. (a) QQ-plot of Data shown in the Manhattan plot. The observed distribution of the test statistic closely follows the expected. (b) Manhattan plot showing the negative $\log$ P-value between individual markers. 6 SNVs highlighted in red on CFA28 passed a suggestive threshold ( $1 \times 10^{-5}$, dashed line). No SNVs achieved a Genome wide significant score ( $5 \times 10^{-7}$, solid line). The top 200 SNVs from this association were were taken forward to a second analysis. (c) Manhattan plot demonstrating genotype association to intestinal lipid malabsorption. 52 SNVs highlighted in red pass the significance threshold of $\mathrm{P}<5 \times 10^{-7}$ (solid line), while 13 SNV in yellow passed a suggestive threshold of $\mathrm{P}<1 \times 10^{-5}$ (dashed line). The most significant region of interest is noted on CFA28, where the strongest SNV from the preliminary analysis persisted in the validation set ( $28: 24521377$ best $\mathrm{P}_{\text {raw }}=1.75 \mathrm{E}^{-45}$ ).
being Tectorin beta (TECTB), Acyl-CoA Synthetase Long Chain Family Member 5 (ACSL5), Zinc Finger DHHC Domain-Containing Protein 6 (ZDHHC6) and pseudogene Guanylate Cyclase 2G (GUCY2GP). The gene ACSL5 represented a strong regional candidate for disease. The orientation of the genes within the region of uncalled markers were positioned so that the last coding exon of each gene aligned with the edges of the putative deletion (Fig. S2a). Using seven primer pairs, we confirmed the presence of a deletion in the affected Kelpies between 101.6 kb and 105.2 kb (Fig. S2b). The PCR confirmed a complete loss of GUCY2GP and ACSL5 and partial loss of ZDHHC6. RNAseq data was used to validate this result.

Variant detection and RNA expression. RNAseq data were inspected in Integrative Genomics Viewer. Read distributions indicated RNAseq data had underlying DNA contamination with an equal portion of reads aligning to introns or intergenic regions compared to exons, $34.9 \%$ and $38.1 \%$ respectively. DNA from the AK with intestinal lipid malabsorption harboured a 103.3 kb deletion, NC_006610.3CFA28:g.23380074_234 83377del (CanFam 3.1; Fig. S3), involving the complete loss of ACSL5, pseudogene GUCY2GP and omitting exons 7-10 of ZDHHC6 (Fig. 4). RNAseq data demonstrated no detectable expression of GUCY2GP, ACSL5 and ZDHHC6 exons beyond the breakpoint of the observed deletion. A further gene, TECTB, located outside the deleted region had no observable expression compared with that of the Labrador retriever jejunum. Gene expression of ZDHHC6 and ACSL5 in the control jejunum was consistent with the reference transcript. GUCY2GP and TECTB were not expressed in either case or control. In the AK, expression of novel exons as a result of cryptic splicing were observed 148.4 kb downstream from the $Z D H H C 6$ gene. The alternate splicing event was captured by 152 reads. A consensus sequence was produced using a genome guided de-novo assembly with Trinity (Data S1).

Multiplex PCR assay for deletion. A multiplex PCR test was customised to implicate the associated variant in affected individuals as well as detect carriers of the variant through allele specific amplification. Primer 1 and 3 were designed to amplify the region spanning the disease-associated variant. The variant removes $103,303 \mathrm{bp}$ between the primers and results in a 414 bp fragment in carrier and affected individuals. Primer 2 anneals shortly downstream from the start of the deleted region so that wild type dogs produce a 177 bp fragment while dogs homozygous for the variant show no amplification. Animals that are heterozygous for the dis-


Figure 4. Sashimi plot of RNAseq data for CanFam3 genomic coordinates CFA28:23320000-23500000. The coverage for each alignment track is plotted as a bar graph, the Y axis represents read counts. Arcs are supported exon junctions and reads split across the junction (junction depth). Below the plots are the gene annotations for corresponding genomic coordinates. The figure illustrates RNAseq data from the jejunum of two samples; A case sample (AK Australian Kelpie) and control (LR Labrador retriever). Underlying DNA contamination can be seen in the AK highlighted by the low read count across the genomic region. A 103.3 kb deletion is seen in the AK, illustrated with a transparent box. The gap in the AK includes GUCY2GP, ACSL5, ZDHHC6 and a Long non-coding RNA (lncRNA). In the AK, expression of novel exons can be seen 148.4 kb downstream from the ZDHHC6 gene, the junction is supported by 152 reads. A consensus sequence produced using a genome guided de-novo assembly with Trinity is included in the gene track as ZDHHC6_AK_DEL.
ease-associated variant produce both fragments. Of 19 samples tested, nine were homozygous for the deletion, all of which exhibited signs of disease (Fig. S4). In the controls, six samples were homozygous wild type and four were heterozygous for the variant. Dogs heterozygous for the variant were asymptomatic but came from families known to produce offspring with the disease phenotype.

## Discussion

Inborn errors of metabolism (IEM) are genetic disorders resulting from defects in biochemical pathways that can have a profound effect on an animal's overall health ${ }^{22,23}$. IEM affecting intermediary metabolic pathways are often recognised through clinical signs such as failure to thrive, hypotonia and functional decompensation ${ }^{22,23}$. Increased prevalence of IEM among specific breeds has previously been observed ${ }^{22-24}$. Frequently reported metabolic disorders clinically similar to intestinal lipid malabsorption are hereditary selective ileal cobalamin malabsorption and exocrine pancreatic insufficiency. Both are IEM that present with failure to thrive and persistent diarrhea ${ }^{11,25,26}$, however the AK presents earlier (before six weeks of age), show no signs of lethargy and have clear evidence of fat in faeces (steatorrhea). Here we present an IEM affecting lipid absorption in the AK resulting from the deletion of ACSL5 and partial loss of ZDHHC6.

Characterisation of the genetic factors associated with IEM is of strong interest for improving canine welfare and improving our understanding of the genomic control of metabolism. Genes influencing the phenotype described in this study, ACSL5 and ZDHHC6, have not been previously implicated in naturally occurring disease models. Long chain acyl-CoA synthetases are major enzymes in fatty acid metabolism ${ }^{27-32}$. In human and rodent studies, variation in the ACSL gene family are often associated with diet induced metabolic and body composition phenotypes ${ }^{27,31,33-38}$. ACSL5, essential for lipid metabolism and fat deposition in carnivores ${ }^{39}$, is a principal candidate for the observed phenotype in the AK. ACSL genes have already been implicated in canine body composition phenotypes, with variation in ACSL4 associated with heavy weight dogs ${ }^{40}$.

The clinical phenotype associated with absent expression of ACSL5 in the jejunal tissue of affected AK puppy is consistent with a knockout (KO) mouse model, including delayed fat absorption and a reduced fat mass ${ }^{27}$. KO mice exhibited additional increased lean mass and energy expenditure, as well as improved insulin sensitivity; traits not observed or tested in our cases. The results of the mouse KO study contradicted an earlier ACSL5 knockout study, which showed little effect on long-term dietary LCFA absorption and weight gain, likely
compensated by residual ACSL activity ${ }^{41}$. Long chain fatty acid absorption occurs largely through the jejunum where LCFA are absorbed across the brush border of jejunal enterocytes. ACSL5 is expressed in brown adipose tissue, small intestine, liver ${ }^{27,28,42-44}$ and is the primary activator of dietary LCFA in the jejunum ${ }^{41}$. Expression, synthesis and activity of ACSL5 is connected to the state of villus architecture, epithelial homeostasis and enterocyte apoptosis ${ }^{45-47}$. The relatively improved health status of affected AK at maturity may imply an important role of ACSL5 during early development. The extreme effects identified in immature AK may be partially offset by other ACSL genes as they reach full size or may be linked with a transition to a solid diet.

Following absorption of LCFA in enterocytes, they undergo re-esterification before transportation and storage is possible. Previous research in rodent studies has implicated ACSL5 in fat absorption during the re-esterification of dietary fats ${ }^{3,27,28,48}$. In the present study it has been noted that once affected Kelpies are on a solid diet with enzyme supplementation, dogs continue to present with a low body condition score. While AK display ongoing sensitivity to dietary lipids into adulthood it remains unconfirmed if their smaller size is a result of persistent intestinal lipid malabsorption or stunted early development.

Further to the complete loss of ACSL5, the genomic deletion resulted in the partial deletion and cryptic splicing event downstream of the last translated exon of ZDHHC6. ZDHHC6 plays a role in posttranslational modification (palmitoylation) of proteins, which can contribute to protein function and regulation beyond underlying genomic architecture. Differences in the palmitoylation of proteins involved in fat and carbohydrate transport and signalling may compromise digestion. Articles reviewing the biological effects of protein palmitoylation have anticipated a functional role in lipid and glucose metabolism ${ }^{49,50}$, though ZDHHC6 is not currently implicated. ZDHHC6 localises in the endoplasmic reticulum and is reported to be involved in the palmitoylation of five protein targets ${ }^{51-56}$. Within the context of existing research neither ZDHHC6 nor proteins palmitoylated by ZDHHC6 are expected to play a major role in lipid digestion. However, novel roles and targets of palmitoylation are frequently reported and the list of proteins that undergo palmitoylation is constantly growing ${ }^{57}$ (https:// swisspalm.epfl.ch/). It is possible that other key substrates influencing the observed phenotype in the AK are not yet reported and AK harbouring the disease-associated variant may be a unique tool in furthering our current understanding of post-translational modification.

TECTB and GUCY2GP were not expressed in either the case or control RNAseq samples. The genomic region containing the TECTB transcript falls outside the observed variant. It is unlikely that gene expression is altered in appropriate tissue samples. Mice studies indicated that GUCY2G plays a role in jejunal integrity ${ }^{58}$. However, GUCY2G is a known pseudogene in humans and was suggested to be under purifying selection in the dog ${ }^{59}$. Conversely, Ensembl genebuild predicts the transcript is non-protein coding (Gene identifier: ENSCAFG00000010908), and recent canine gene catalogue observing ten tissue types reported no expression across all samples and replicates ${ }^{60}$. The gastrointestinal tract was not reported in the catalogue but a lack of expression in the Labrador retriever control supports the concept of GUCY2GP as a pseudogene, indicating no involvement in the observed phenotype.

Therapies to overcome deficit in ACSL5 function are currently unknown and were not assessed in this research. In humans, therapies for disorders disrupting lipid digestion and absorption, involve removing lipids from the diet or replacing them with those that bypass the genetic block ${ }^{61,62}$. The disorder described in this study chiefly impacts the metabolism of LCFA. Some human studies have demonstrated positive effects of mediumchain triglyceride formulation (MCT) on individuals suffering from long chain fatty acid disorders ${ }^{63-65}$, however the use of MCT in canine research is restricted ${ }^{66-69}$. Auxiliary research into therapeutic options especially during early development is necessary.

Results of the multiplex-PCR were consistent with a fully-penetrant autosomal recessive disorder. Results reported here are not indicative of breed-wide prevalence rates as dogs included in this study originated from a small group of Australian kennels. However, the presence of the deletion in international samples suggests that the variant allele is globally dispersed. To obtain comprehensive prevalence parameters, randomised and wide scale testing is required.

In conclusion we presented a novel deletion of ACSL5, causing hereditary intestinal lipid malabsorption in the Australian Kelpie dog breed. ACSL5 plays an important role in long chain fatty acid storage and metabolism. The improved health of affected individuals with age implies that genetic compensation of this gene beyond neonatal development is possible. This research identifies the first spontaneous animal model to validate key mouse knockout model findings previously reported. The AK model presents a unique opportunity to improve gaps in our understanding of ACSL5. A simple genetic test has been developed and validated to identify dogs harbouring the described variant. International testing of Australian Kelpies is warranted to obtain better estimates of global prevalence. At this time the disorder is presumed to be restricted to a single breed.

## Data availability

The dataset used in the current study is available at Figshare https://doi.org/10.6084/m9.figshare.12380564.
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## Author contributions

All authors contributed to the conceptualisation of the research and progression of the overall aims. The primary investigation, including experimental work, data analysis, validation and visualisation, was conducted by M.J.O. under the supervision of C.M.W. and N.J.B. T.C. provided valuable advice on the data analysis. C.M.W., N.J.B. and M.S. were involved in the provision of resources. M.J.O. drafted the original manuscript. All authors were involved in editing and refining the manuscript. The final product has been approved by all authors.

## Competing interests

The authors declare no competing interests.

## Additional information

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[^1]:    ${ }^{\text {a }}$ American College of Veterinary Internal Medicine
    ${ }^{\text {b }}$ Left atrium to aortic root ratio
    ${ }^{c}$ Weight normalised left ventricular end diastolic diameter

[^2]:    ${ }^{a}$ Left atrium to aortic root ratio
    ${ }^{b}$ Weight normalised left ventricular end diastolic diameter

[^3]:    E Ensembl
    ${ }^{\mathrm{N}}$ National Center for Biotechnology Information (NCBI)

    - UCSC

[^4]:    ${ }^{\text {a }}$ Echocardiographic
    ${ }^{\text {b }}$ Weight normalised left ventricular end diastolic diameter
    c Left atrium to aortic root ratio

[^5]:    ${ }^{\text {E Ensembl }}$
    ${ }^{\text {n N National Center for Biotechnology Information (NCBI) }}$
    U UCSC

[^6]:    Long chain fatty acids (LCFA) are the most abundant fats in mammals and play a key role in the canine (Canis lupus familiaris) diet. Pancreatic lipases are largely responsible for the hydrolysis of triglycerides into glycerol and fatty acids, which are absorbed across the brush border of jejunal enterocytes. Activation of fatty acids is the first step in intracellular metabolism of LCFA. The process involves the conjugation of fatty acids with coenzyme-A (CoA) and is catalysed by a group of enzymes called Acyl-CoA synthetases (ACS) ${ }^{1-3}$. Thirteen homologous ACS genes that activate LCFA have been annotated in mammals and cluster in three different gene families: acylCoA synthetase long chain (ACSL), acyl-CoA synthetase bubblegum (ACSBG) and fatty acid transport proteins (FATP) ${ }^{1-3}$. The full extent of each gene on normal intestinal absorption of LCFA is unknown. Animal models with heritable phenotypes of intestinal lipid malabsorption provide an opportunity to elucidate this.

    The Kelpie is an iconic dog (Canis lupus familiaris) breed established in the late nineteenth century for its natural working ability and resilience in the extreme weather conditions of Australia ${ }^{4}$. Since inception, the breed has become divided into two separate breeding populations maintained by different pedigree registries ${ }^{5}$. Dogs selected primarily for strong working ability are known as the Australian Working Kelpie (AWK). The Australian Kelpie (AK) is selected according to a conformation breed standard and is registered by the Australian National Kennel Council. The Federation Cytological International recognizes both breed varieties enabling the populations to co-mingle but, in Australia, the two are maintained as separate breeding populations and are genetically distinct; most notably in genes influencing morphology and behaviour ${ }^{4,5}$.

    We describe an inherited intestinal lipid malabsorption (OMIA 002226-9615) in the AK. Unlike cases of similar disorder hereditary selective ileal cobalamin malabsorption ${ }^{6-11}$, AK show no signs of lethargy and have clear evidence of fat in faeces (steatorrhea). Based on the observed familial segregation the observed phenotype
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