A predictive model for canine dilated cardiomyopathy – a meta-analysis of Doberman Pinscher data

Dilated cardiomyopathy is a prevalent and often fatal disease in humans and dogs. Indeed dilated cardiomyopathy is the third most common form of cardiac disease in humans, reported to affect approximately 36 individuals per 100,000 individuals. In dogs, dilated cardiomyopathy is the second most common cardiac disease and is most prevalent in the Irish Wolfhound, Doberman Pinscher and Newfoundland breeds. Dilated cardiomyopathy is characterised by ventricular chamber enlargement and systolic dysfunction which often leads to congestive heart failure. Although multiple human loci have been implicated in the pathogenesis of dilated cardiomyopathy, the identified variants are typically associated with rare monogenic forms of dilated cardiomyopathy. The potential for multigenic interactions contributing to human dilated cardiomyopathy remains poorly understood. Consistent with this, several known human dilated cardiomyopathy loci have been excluded as common causes of canine dilated cardiomyopathy, although canine dilated cardiomyopathy resembles the human disease functionally. This suggests additional genetic factors contribute to the dilated cardiomyopathy phenotype.

This study represents a meta-analysis of available canine dilated cardiomyopathy genetic datasets with the goal of determining potential multigenic interactions relating the sex chromosome genotype (XX vs XY) with known dilated cardiomyopathy associated loci on chromosome 5 and the PDK4 gene in the incidence and progression of dilated cardiomyopathy. The results show an interaction between known canine dilated cardiomyopathy loci and an unknown X-linked locus. Our study is the first to test a multigenic contribution to dilated cardiomyopathy and suggest a genetic basis for the known sex-disparity in dilated cardiomyopathy outcomes.

Original Article

A predictive model for canine dilated cardiomyopathy – a meta-analysis of Doberman Pinscher data

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Introduction

Dilated cardiomyopathy (DCM) is a prevalent and often fatal disease requiring clinical management in humans and dogs (Egenvall, Bonnett & Häggström, 2006; Hershberger, Morales & Siegfried, 2010). DCM is characterised by ventricular chamber enlargement and systolic dysfunction which often leads to congestive heart failure. The aetiology of DCM is complex, genetic factors, myocardial ischemia, hypertension, toxins, infections and metabolic defects have been implicated (McNally, Golbus & Puckelwartz, 2013). To date mutations in over 50 genes have been associated with DCM in humans, however mutations in the most prevalent DCM related genes only account for approximately 50% of patients with DCM (Posafalvi et al., 2012). In human DCM genetic testing where a panel of approximately 50 loci are tested concurrently, often more than one locus can be implicated in the disease (McNally, Golbus & Puckelwartz, 2013), suggesting multiple genetic factors cooperate in DCM aetiology.

Canine DCM is phenotypically similar to human DCM (Shinbane et al., 1997). Yet to date mutations in only two genes (*PDK4* and *STRN*) and a SNP on chromosome 5 have been associated with canine DCM (Mausberg et al., 2011; Meurs et al., 2012, 2013), suggesting additional genetic causes remain unknown. Canine studies have often been impaired by limited sample size (typically less than 10 individuals), however, those studies with larger sample numbers (greater than 50 individuals) have also frequently failed to find a significant association with DCM (e.g. Philipp et al. 2007, 2008; Wiersma et al. 2008). One possible explanation for the challenges in identifying DCM associated loci in humans and dogs is that even within an extended family or breed, variation in no single gene can explain the development of DCM.

There is a well-established sex-disparity in the incidence of cardiovascular disease in humans (Ghali et al., 2003). While X-linked inheritance has been demonstrated in some human DCM families, in most cases the underlying physiological and molecular basis of sex bias in cardiac abnormalities remains poorly understood (Hershberger, Morales & Siegfried, 2010; Diegoli et al., 2011). In canine DCM, Great Danes display X-linked inheritance, but no other dog breed has shown this type of inheritance (Meurs, Miller & Wright, 2001). Despite this, in common with human heart disease, studies of canine heart disease often have an over-representation of male dogs implying that there is also a sex-disparity in the development of canine heart disease (e.g. Distl et al. 2007; Martin et al. 2009).

Dog breeds can be considered as large families, with dogs within a breed more related to each other than dogs of other breeds (Parker et al., 2004). In the same way that some human families are affected by DCM some breeds are more frequently affected by DCM than others (Egenvall, Bonnett & Häggström, 2006). Dobermans Pinschers (hereafter Dobermans) are particularly affected by DCM, with both a high prevalence (58.2% in European Dobermans) and severity, death often occurs within 8 weeks of diagnosis (Calvert et al., 1997; Wess et al., 2010). Median life expectancy of DCM affected European Dobermans is 7.8 years, compared with 11 years for unaffected European Dobermans (Proschowsky, Rugbjerg & Ersbøll, 2003; Egenvall, Bonnett & Häggström, 2006). A deletion in a splice site of the *PDK4* gene (Meurs et al., 2012) and a SNP on chromosome 5 (Mausberg et al., 2011) in Dobermans are two of only three canine DCM mutations identified. While two loci have been identified as associated with Doberman DCM, individually neither locus explains all cases of Doberman DCM (Mausberg et al., 2011; Meurs et al., 2012). Individuals heterozygous at the Chr5 SNP are more likely to develop DCM, but there are many DCM cases that are homozygous for the healthy allele (Mausberg et al., 2011). *PDK4* genotypes are less definite predictors of DCM with both affected and unaffected individuals with all three possible genotypes, however, the 16bp deletion is more frequently found in individuals with DCM than those without DCM (Meurs et al., 2012). Despite the *PDK4* finding in North American Dobermans an analysis of European Dobermans failed to identify an association between the *PDK4* allele and DCM (Owczarek-Lipska et al., 2013), suggesting additional factors influence the effect of *PDK4* in predisposing individuals to DCM. While genome wide association studies (GWAS) are identifying potential causal Single Nucleotide Polymorphisms (SNPs) in this and other highly affected breeds, novel genetic causes of canine DCM remain to be identified (Mausberg et al., 2011; Philipp et al., 2012).

68 There are two genetic variants associated with DCM in Dobermans, a deletion in a *PDK4*
69 splice site and a SNP on Chromosome 5 (Mausberg et al., 2011; Meurs et al., 2012). Individually
70 these variants do not explain all DCM cases, therefore additional factors are likely. In this study
71 we developed genetic models incorporating known Doberman DCM loci with additional, as yet
72 unknown, genetic factors to predict which genotype combinations are likely to develop DCM.
73 Using this method we provide evidence for a sex-linked genetic influence on known DCM loci in
74 the pathogenesis of canine DCM. Our study is the first to propose a multigenic contribution to
75 canine DCM and suggests a genetic basis for the known sex-disparity in canine DCM outcomes.

Materials and Methods

Model development

Only two loci have been identified as associated with DCM in Dobermans. This was established by searching Pubmed and Web of Knowledge with the following search terms: "Doberman DCM loci", "Doberman Dilated Cardiomyopathy loci", "Doberman DCM gene", "Doberman Dilated Cardiomyopathy gene", "Doberman DCM locus", "Doberman Dilated Cardiomyopathy locus". 30 records were identified following removal of duplicates. These were then screened for articles clearly not about Doberman DCM or only available as meeting abstracts and then further screened for articles identifying a genetic variant as associated with Doberman DCM. No negative/non association studies were found in the literature in relation to these two loci, DCM and the Doberman. It is possible that these types of studies have not been published, however other non-association genes have been published in the Doberman in relation to DCM, reducing possible risk of literature bias towards negative results.

By combining the genotypes from the identified Doberman DCM associated loci, and additional putative loci, predictive models were developed and tested against observed DCM incidence data. All genotype combinations for the DCM associated SNP identified on chromosome 5 (TIGRP2P73097:CFA5:g.53,941,386T>C, CanFam2.1) (Mausberg et al., 2011) and the *PDK4* (GeneID:482310) splice site deletion (CFA14:g.20,829,667_20,829,682del, CanFam3.1) (Meurs et al., 2012) were determined. Further analysis determined which genotype combinations were likely to lead to DCM. Some genotypes are definitive; all individuals homozygous for the susceptibility allele at CFA5:g.53,941,386T>C develop DCM (Mausberg et al., 2011).

Determining which genotypes develop DCM

Five genetic models incorporating genotypes at multiple observed and hypothetical loci were developed including: 1. two known DCM loci; 2. two known loci + 50% of the population more susceptible to developing DCM; 3. two known loci + a novel autosomal dominant DCM locus; 4. two known loci + a novel autosomal recessive DCM locus; 5. two known loci + a novel additive DCM locus and 6. two known loci + a novel X-linked DCM locus.

For each model, different biologically feasible phenotype outcomes were tested for each genotype combination to establish the best fit of the model to the observed DCM incidence data. Each model was subject to the following constraints: individuals that are homozygous CC at the Chr5 SNP develop DCM, and individuals with no susceptibility alleles are healthy.

Model testing

For each model the frequency of each genotype combination was calculated by multiplying the genotype frequencies together, with *PDK4* and Chr5 frequencies obtained from Owczarek-Lipska et al. (2013) and Mausberg et al. (2011), see Table 1, and a range of frequencies tested for hypothetical loci. For example, for the model incorporating only *PDK4* and Chr5 variants, one genotype combination is WtWt-TT. The frequency of this genotype combination is the product of the frequency of WtWt and the frequency of TT in the population. From the combined genotype frequencies the expected numbers of individuals with each genotype combination were calculated by multiplying the frequency by the number of individuals in the study to be compared with (182 when compared with Mausberg, et al. (2011) and Owczarek-Lipska et al. (2013)). Thus the numbers of individuals in the model that were, for example, WtWt healthy and WtWt DCM were obtained by summing the numbers in each category. Having obtained the numbers of affected and unaffected individuals that the model

predicts for each genotype, these were tested against the observed data using a χ^2 test. Where additional putative DCM loci were included in a model, several allele frequencies were tested. However, as GWAS studies have previously been carried out (Mausberg et al., 2011; Meurs et al., 2012) it is unlikely that additional DCM alleles are at higher frequencies than those already identified. For this reason DCM allele frequencies over 0.5 were not tested.

The proportion of the population that the model predicts to have DCM was determined by taking the sum of all the genotype combined frequencies that lead to DCM in the model. For example, for the model incorporating just the two known loci this is 0.0144+0.0624+0.0052+0.0048+0.0004 = 0.0872 – see supplementary material, Table 1. This proportion was then compared to the observed DCM frequency of 0.582 (Wess et al., 2010).

Odds ratios of each genotype for each model were obtained by testing each genotype against the other two combined. Odds ratios for each allele were also obtained. The significance of these odds ratios were assessed using χ^2 tests. It is expected that the models will show similar odds ratio patterns and significance levels to those of the reported data. Odds ratios of both genotypes and alleles were obtained from the original studies, Tables 2 and 3.

Results

Following the constraints stated in the methods and using biologically feasible reasoning each model was optimised to minimise the χ^2 test statistic – the closer the fit of the model to the observed data the smaller the χ^2 value. For each model the genotype-phenotype decision descriptions are shown in Table 4. Tables of each model are in supplementary material.

Comparing model predictions with observed data

χ^2 test values comparing predicted numbers with observed numbers of DCM and healthy individuals at each genotype ranged from 4.35 to 7766.06. A χ^2 value of less than 11.07 indicates there is no significant difference between predicted and observed genotype-phenotype data, (5% significance level, with 5 degrees of freedom). Values less than 15.09 represent predictions not significantly different to observed values at the 1% significance level. χ^2 values less than these critical values are indicated in Table 5. These models are those that most accurately match with the observed data.

Model predicted DCM population frequency

For each model the predicted DCM frequency was calculated to provide an additional way of examining the accuracy of the model. The DCM frequency in the European Doberman population is estimated to be 58.2% (Wess et al., 2010) assuming this estimate is valid, accurate models should predict similar frequencies. The frequencies predicted by each model are displayed in Table 6, with those within 0.1 of the reported 0.582 highlighted as models which predict a similar frequency to that observed.

Odds ratios

For the Chr5 SNP there are no odds ratio for CC as all individuals that are CC develop DCM in both the original study (Mausberg et al., 2011) and models so odds ratios cannot be calculated. Despite this a χ^2 test can be performed on the counts of affected and unaffected individuals observed and predicted with the genotype so the significance of the results was obtained.

For the Chr5 SNP 12 of 18 models the genotypes odds ratios remain in the same direction and significance as the original studies (Table 8), while 15 of the allele odds ratios remain in the same direction and significance (Table 10). The *PDK4* deletion association was identified in the North American Doberman population, in the European population the odds ratios (Tables 7 & 9) are not significantly different from the null result of 1. Once combined with additional loci similar significant likelihood ratios as the North American population are obtained for 13 of 18 models (Tables 7 & 9).

Selecting the most realistic model

For a model to be considered plausible it should predict similar numbers of affected and unaffected individuals at each genotype as observed in Mausberg et al. (2011) and Owczarek-Lipska et al. (2013), predict similar DCM frequency as reported in the population (Wess et al., 2010), and give odds ratios of genotypes and alleles similar to those from the studies which report an association. To assist in determining which models meet these requirements Table 11 shows which conditions each model meets. From this it is possible to see that no model meets all the conditions, but two similar models, the models incorporating the two identified loci and an additional X-linked DCM locus with the novel DCM allele frequency at 0.4 and 0.5, meet all but one condition each. An additional exploration of the additional X-linked DCM allele frequency

179 indicates that an X-linked DCM allele frequency between 0.4 and 0.5 leads to all conditions
180 being met.

Discussion

This study used publicly available data to test the prediction that genetic models incorporating multiple factors can better explain and predict the incidence of canine DCM than those utilising a single factor. Until now, the possibility that multiple genes combine to influence DCM phenotype has been alluded to, but has not yet been investigated, despite the effect of multiple loci in related diseases being identified (Ingles et al., 2005; Xu et al., 2010; Rampersaud et al., 2011; Posafalvi et al., 2012). This is the first study to investigate the combined effect of multiple factors on the predisposition to DCM. Although our models do not explain all cases of canine DCM and there are other possible models that require testing, by combining three factors (*PDK4*, Chr5 TIGRP2P73097 SNP and an X-linked locus) we show that DCM incidence can be more thoroughly explained than by a single locus (Tables 6-11). This result is important because it has implications for successful prediction of canine and human DCM.

193 To assess the accuracy of each model we performed several tests. For a model to be an
194 accurate representation of observed data it should predict similar numbers of affected and
195 unaffected individuals at each genotype as have been reported in the published data. It should
196 also predict a similar DCM frequency to that found in the population. The final test is that the
197 odds ratios of genotypes and alleles are in the expected direction and significance to allow us to
198 conclude that the locus is associated with DCM. The models incorporating the two known DCM
199 loci and an additional X-linked locus with the novel susceptible allele frequency at 0.5 and 0.4
200 satisfy all these tests apart from one. An intermediary allele frequency of 0.46 for the novel
201 susceptible allele allowed all conditions to be met. These susceptible allele frequencies are
202 intriguing as they are quite high so it could be expected that the locus should have been identified
203 via the GWAS studies that have previously been undertaken. The nature of this additional locus
204 and the frequency of the susceptible allele will only be possible to verify once the locus has been
205 identified.

206 Interestingly the *PDK4* splice site deletion is not significantly associated with DCM in the
207 European population, but in the model only incorporating the two known loci, it improves the
208 odds ratio for the Chr5 SNP. This further indicates that models incorporating multiple factors are
209 more effective than those incorporating a single factor.

While the odds of a genotype being associated with a phenotype can be useful in determining an individual's risk of developing disease, incorporating additional factors could lead to accurate prediction of future disease status. Accurate prediction could allow individuals predicted to develop the disease to be closely monitored and medical intervention be administered earlier in disease progression, thus potentially improving the outcome for the affected individual. Most predictive models are based on either knowing individuals genotypes at multiple loci or simulating individuals genotypes at multiple loci (Janssens et al., 2006; Pencina, D'Agostino & Vasan, 2008). They do not account for known effects of genotypes, for example all Chr5 CC individuals have DCM, or allow the inclusion of additional, as yet unknown, loci. Our methodology is unique and useful where there are multiple, but limited, known and unknown factors involved in disease progression. In particular it allows specific gene combinations to lead to disease rather than incremental risk factors as is the case in other predictive models (Janssens et al., 2006; Pencina, D'Agostino & Vasan, 2008). Limitations to our methodology include the limited number of factors that can be modelled given the data available. Despite this our methodology could be used in other situations. While many phenotypes have multiple loci, each of small effect, there can be some loci which have comparatively larger effects (e.g. Strange et al. 2011; Papa et al. 2013). Identifying these larger effect loci can be the first steps in predicting phenotypes (e.g. Hayes et al. 2010; Papa et al. 2013). Following the identification of loci associated with a trait our methodology can be used to indicate what type of additional loci may be influencing the trait of interest, which could make locating additional loci more straightforward.

Conclusions

There are many unknown factors involved in the aetiology of canine and human DCM. In Dobermans we have identified novel variables influencing DCM risk – multigenic effects and a possible X-linked locus. While there are many possible explanations for sex-disparity in heart conditions none have been shown to cause the sex bias observed in DCM, our findings indicate that in Dobermans this could be attributable to an X-linked DCM locus. While the PDK4 splice site deletion and the Chr5 SNP have both been assessed for association with DCM in the European population of Dobermans, the combined genotype of individuals has not yet been considered (Mausberg et al., 2011; Owczarek-Lipska et al., 2013). Our model would benefit from further testing by genotyping Dobermans at both the *PDK4* and Chr5 variants to further validate the principle behind the model. Future work is also required to identify X-linked DCM loci if the model is verified for the known loci. If our model is validated, there are implications for current breeding practices and welfare of individuals within the breed. If dogs are screened prior to being included in the breeding population, matings of individuals with a high chance of producing offspring with deleterious combinations of alleles can be prevented, and individuals with allele combinations that are more likely to develop DCM can be monitored more intensely than those with less genetic risk. This will have welfare benefits by reducing the prevalence of DCM-associated alleles within the population and potentially increasing the lifespan and welfare of affected dogs by enabling monitoring and earlier clinical management. By utilising similar methodology equivalent multigenic effects and possible additional loci could be identified in human DCM, giving similar benefits to those described for Dobermans.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Appendix A: Supplementary material

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348 **Table 1.** Genotype frequencies assuming Hardy Weinberg Equilibrium, allele frequencies taken

349 from Mausberg et al. (2011) and Owczarek-Lipska et al. (2013)

PDK4		***Chr5* SNP**	
genotype	**freq**	**genotype**	**freq**
Wt Wt	0.72	TT	0.74
Wt del	0.26	TC	0.24
Del del	0.02	CC	0.02

Table 2. Genotype odds ratios from the original studies reporting an association at the PDK4 locus (Meurs et al., 2012) and Chromosome 5 SNP (Mausberg et al., 2011). The PDK4 χ^2 test results indicate that the WtWt genotype significantly associated with non-DCM and the WtDel genotype significantly associated with DCM at the 0.01 significance level, the DelDel genotype odds ratio whilst different from the null result of 1, is not significantly so. For the chromosome 5 SNP all individuals that are CC in the original study developed DCM, thus and odds ratio and confidence interval cannot be calculated, but χ^2 tests can be performed on the data. TT is significantly associated with non-DCM and the TC and CC genotypes are significantly associated with DCM at the 0.01 significance level.

Genotype	Odds ratio	95% CI
PDK4 WtWt	0.14	0.07, 0.32
PDK4 WtDel	5.21	2.70, 12.09
PDK4 DelDel	1.14	0.41, 3.18
Chr5 TT	0.11	0.05, 0.24
Chr5 TC	6.23	2.78, 14.00
Chr5 CC	NA	NA

359 **Table 3.** Allele odds ratios from the original studies reporting an association at the PDK4 locus
360 (Meurs et al., 2012) and Chromosome 5 SNP (Mausberg et al., 2011). The χ^2 test results indicate
361 that each susceptibility (Del and C respectively) allele is significantly associated with DCM and
362 the alternate allele significantly associated with non-DCM at the 0.01 significance level.

Allele	Odds ratio	95% CI
PDK4 Wt	0.38	0.23, 0.64
PDK4 Del	2.63	1.57, 4.42
Chr5 T	0.12	0.06, 0.26
Chr5 C	8.11	3.85, 17.09

Table 4. Genotype-phenotype decision descriptions for each model 1. the two known DCM loci; 2. two known loci + 50% of the population is more susceptible to developing DCM; 3. two known DCM loci combined with a novel autosomal dominant DCM locus; 4. two known DCM loci combined with an autosomal recessive locus; 5. two known DCM loci combined with a an additional DCM locus that is additive and 6. two known DCM loci combined with an X-linked DCM locus

Model	Genotype – phenotype decision description, in addition to the rules:
1	DCM develops when both the PDK4 locus and Chr5 SNP have at least one DCM susceptibility allele.
2	50% more susceptible only need to have a single DCM susceptibility allele at either locus to develop DCM while the 50% less susceptible to DCM require at least one DCM susceptibility allele at both loci to develop DCM.
3	All individuals that have a susceptibility allele at the additional locus develop DCM. Those individuals with no susceptibility alleles at the additional locus need at least one DCM susceptibility allele at both of the other loci to develop DCM.
4	All homozygous susceptible individuals at the additional locus develop DCM. For individuals that are heterozygous at the additional locus, DCM occurs when combined with another DCM susceptibility allele, while homozygous unsusceptible individuals need at least one DCM susceptibility allele at both of the other loci to develop DCM.
5	All homozygous susceptible individuals at the additional locus develop DCM. Heterozygotes and homozygous unsusceptible individuals need at least one DCM susceptibility allele at both of the other loci to develop DCM.
6	X linked susceptible DCM locus males can either possess a single unsusceptible X (XY) or a single susceptible x (xY), while females can be unsusceptible X homozygotes (XX), heterozygotes (Xx) or susceptible x homozygotes (xx). Unsusceptible X males (XY) are phenotypically identical to unsusceptible X homozygotes (XX) with these individuals requiring at least one DCM susceptibility allele at both of the other loci to develop DCM. All individuals that possess a susceptible X (xY and xx individuals) develop DCM in this model while heterozygotes (Xx) only require a single DCM susceptibility allele at one of the other loci to develop DCM.

369 **Table 5.** χ^2 test statistic results comparing predicted of DCM and healthy individuals at each
370 genotype from each model with observed numbers of DCM and healthy individuals at each
371 genotype from Mausberg et al. (2011) – Chr5 SNP and Owczarek-Lipska et al. (2013) – *PDK4*.
372 ** not significant at 5% significance level, * not significant at 1% significance level.

	χ^2 test statistic for each model							
Model	***PDK4***				**Chr5**			
1.	1269.23				7766.06			
2.	110.45				596.68			
DCM allele freq	**0.5**	**0.4**	**0.3**	**0.2**	**0.5**	**0.4**	**0.3**	**0.2**
3.	32.47	29.25	51.42	113.35	6.58**	7.69**	24.30	69.27
4.	26.24	74.61	171.69	379.06	31.65	67.45	145.76	360.86
5.	88.95	31.36	4.97**	4.36**	114.72	53.10	23.13	17.21
DCM X allele (x) freq	**0.5**	**0.4**	**0.3**	**0.2**	**0.5**	**0.4**	**0.3**	**0.2**
6.	10.57**	10.06**	25.38	71.30	11.32*	9.29**	19.55	52.86

373 **Table 6.** DCM frequency predicted by each model, * indicates frequencies within 0.1 of the

374 reported frequency (0.582 (Wess et al., 2010)) in the European Doberman pincher population.

Model	**DCM freq for each model**			
1.	0.0872			
2.	0.2772			
DCM allele freq	**0.5**	**0.4**	**0.3**	**0.2**
	0.5054			
3.	*	0.415648	0.328952	0.245321
4.	0.3154	0.233248	0.169352	0.123712
5.	0.7718	0.671392*	0.552728*	0.415808
DCM X allele (x) freq	**0.5**	**0.4**	**0.3**	**0.2**
	0.5245			
6.	*	0.433984	0.350432	0.257536

375 **Table 7.** Odds ratios of each PDK4 genotype with χ^2 significnace, ** significant at 1% level, *

376 significant at 5% level

	PDK4 genotype odds ratio											
Model	**wtwt**	**wtdel**	**deldel**	**wtwt**	**wtdel**	**deldel**	**wtwt**	**wtdel**	**deldel**	**wtwt**	**wtdel**	**deldel**
individual loci	0.78	1.29	1.11									
1.	0.06**	12.91**	3.85									
2.	0.1**	9.41**	4.6									
DCM allele freq	**0.5**			**0.4**			**0.3**			**0.2**		
3.	0.14**	6.70**	4.42	0.15**	6.31**	3.98	0.15**	6.21**	3.69	0.14**	6.47**	3.53
4.	0.45*	2.17*	1.76	0.35**	2.73**	2.03	0.25**	3.77**	2.43	0.15**	5.82**	2.98
5.	0.7	1.42	1.31	0.67	1.49	1.36	0.62	1.6	1.43	0.53	1.84	1.58
DCM X allele (x) freq	**0.5**			**0.4**			**0.3**			**0.2**		
6.	0.31**	3.12**	2.4	0.30**	3.23**	2.41	0.28**	3.41**	2.45	0.24**	3.89**	2.59

377 **Table 8.** Odds ratios of each Chr5 SNP genotype with χ^2 significnace, ** significant at 1% level,

378 * significant at 5% level

Model	Chr5 genotype odds ratio											
	TT	**TC**	**CC**	**TT**	**TC**	**CC**	**TT**	**TC**	**CC**	**TT**	**TC**	**CC**
individual loci	0.11**	6.23**	-**									
1.	0.02**	11.37**	-**									
2.	0.09**	9.23**	-**									
DCM allele freq	**0.5**			**0.4**			**0.3**			**0.2**		
3.	0.14**	6.74**	-	0.14**	6.34**	-*	0.13**	6.25**	-**	0.12**	6.56**	-**
4.	0.35**	2.33*	-**	0.25**	2.96**	-**	0.16**	4.13**	-**	0.08**	6.45**	-**
5.	0.67	1.51	-	0.61	1.57	-	0.54	1.7	-	0.44*	1.96	-*
DCM X allele (x) freq	**0.5**			**0.4**			**0.3**			**0.2**		
6.	0.29**	3.22**	-	0.27**	3.34**	-*	0.24**	3.55**	-**	0.19**	4.08**	-**

379 **Table 9.** Odds ratios of each PDK4 allele with χ^2 significnace, ** significant at 1% level, *

380 significant at 5% level

Model	PDK4 allele odds ratio							
	Wt	**Del**	**Wt**	**Del**	**Wt**	**Del**	**Wt**	**Del**
individual loci	0.81	1.23						
	0.17*							
1.	*	5.84**						
	0.16*							
2.	*	6.22**						
DCM allele freq	**0.5**		**0.4**		**0.3**		**0.2**	
	0.19*			4.91*		4.65*	0.22*	
3.	*	5.37**	0.2**	*	0.22**	*	*	4.57**
			0.43*	2.32*		2.94*	0.22*	
4.	0.52*	1.94*	*	*	0.34**	*	*	3.91**
5.	0.74	1.36	0.71	1.36	0.66	1.51	0.59	1.69
DCM X allele (x) freq	**0.5**		**0.4**		**0.3**		**0.2**	
	0.37*		0.36*	2.76*		2.94*	0.32*	
6.	*	2.71**	*	*	0.35**	*	*	3.1**

381 **Table 10.** Odds ratios of each Chr5 SNP allele with χ^2 significnace, ** significant at 1% level, *

382 significant at 5% level

Model	Chr5 allele odds ratio							
	T	**C**	**T**	**C**	**T**	**C**	**T**	**C**
individual loci	0.15**	6.64**						
1.	0.08**	12.33**						
2.	0.13**	7.49**						
DCM allele freq	**0.5**		**0.4**		**0.3**		**0.2**	
3.	0.19**	5.34**	0.19**	5.37**	0.18**	5.55**	0.16**	6.07**
4.	0.36**	2.76**	0.28**	3.62**	0.20**	5.08**	0.16**	7.68**
5.	0.72	1.38	0.64	1.38	0.55	1.82	0.45**	2.23**
DCM X allele (x)								
freq	**0.5**		**0.4**		**0.3**		**0.2**	
6.	0.33**	3.02**	0.3**	3.28**	0.27**	5.08**	0.23**	4.35**

Table 11. Shows if each model (with the new DCM allele frequency indicated) meets each condition, Y the condition is met, x the condition is not met. The number of conditions not met is also indicated.

	χ^2			OR genotype		OR allele		number of
		Chr5			Chr5		Chr5	
model	PDK4	SNP	DCM freq	PDK4	SNP	PDK4	SNP	conditions not met
individual	-	-	-	x	Y	x	x	3
1.	x	x	x	Y	Y	Y	x	4
2.	x	x	x	Y	Y	Y	x	4
3.								
0.5	x	x	Y	Y	x	Y	Y	3
0.4	x	x	x	Y	Y	Y	Y	3
0.3	x	x	x	Y	Y	Y	Y	3
0.2	x	x	x	Y	Y	Y	Y	3
4.								
0.5	x	x	x	Y	Y	Y	Y	3
0.4	x	x	x	Y	Y	Y	Y	3
0.3	x	x	x	Y	Y	Y	Y	3
0.2	x	x	x	Y	Y	Y	Y	3
5.								
0.5	x	x	x	x	x	x	x	7
0.4	x	x	Y	x	x	x	x	6
0.3	Y	x	Y	x	x	x	x	5
0.2	Y	x	x	x	x	x	Y	5
6.								
0.5	Y	Y	Y	Y	x	Y	Y	1
0.4	Y	Y	x	Y	Y	Y	Y	1
0.3	x	x	x	Y	Y	Y	Y	3
0.2	x	x	x	Y	Y	Y	Y	3

Table 1(on next page)

Suplementary tables

Supplementary Table 1. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci.

PDK4		Chr5 SNP		Combined	Predicted	Phenotype
genotype	freq	genotype	freq	genotype freq	number of individuals	
Wt Wt	0.72	TT	0.74	0.5328	96.9696	Healthy
Wt Wt	0.72	TC	0.24	0.1728	31.4496	Healthy
Wt Wt	0.72	CC	0.02	0.0144	2.6208	DCM
Wt del	0.26	TT	0.74	0.1924	35.0168	Healthy
Wt del	0.26	TC	0.24	0.0624	11.3568	DCM
Wt del	0.26	CC	0.02	0.0052	0.9464	DCM
Del del	0.02	TT	0.74	0.0148	2.6936	Healthy
Del del	0.02	TC	0.24	0.0048	0.8736	DCM
Del del	0.02	CC	0.02	0.0004	0.0728	DCM

Supplementary Table 2. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci + 50% of the population more susceptible to DCM (in this case males more susceptible than females).

sex		PDK4		Chr5 SNP		combined	Predicted	
sex	freq	genotype	freq	genotype	freq	genotype freq	number of individuals	Phenotype
female	0.5	Wt Wt	0.72	TT	0.74	0.2664	48.4848	Healthy
female	0.5	Wt Wt	0.72	TC	0.24	0.0864	15.7248	Healthy
female	0.5	Wt Wt	0.72	CC	0.02	0.0072	1.3104	DCM
female	0.5	Wt del	0.26	TT	0.74	0.0962	17.5084	Healthy
female	0.5	Wt del	0.26	TC	0.24	0.0312	5.6784	DCM
female	0.5	Wt del	0.26	CC	0.02	0.0026	0.4732	DCM
female	0.5	Del del	0.02	TT	0.74	0.0074	1.3468	Healthy
female	0.5	Del del	0.02	TC	0.24	0.0024	0.4368	DCM
female	0.5	Del del	0.02	CC	0.02	0.0002	0.0364	DCM
male	0.5	Wt Wt	0.72	TT	0.74	0.2664	48.4848	Healthy
male	0.5	Wt Wt	0.72	TC	0.24	0.0864	15.7248	DCM
male	0.5	Wt Wt	0.72	CC	0.02	0.0072	1.3104	DCM
male	0.5	Wt del	0.26	TT	0.74	0.0962	17.5084	DCM
male	0.5	Wt del	0.26	TC	0.24	0.0312	5.6784	DCM
male	0.5	Wt del	0.26	CC	0.02	0.0026	0.4732	DCM
male	0.5	Del del	0.02	TT	0.74	0.0074	1.3468	DCM
male	0.5	Del del	0.02	TC	0.24	0.0024	0.4368	DCM
male	0.5	Del del	0.02	CC	0.02	0.0002	0.0364	DCM

Supplementary Table 3. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci + an additional autosomal dominant DCM susceptibility locus.

additional autosomal dominant locus		PDK4		Chr5 SNP		combined genotype freq	Predicted number of individuals	Phenotype
genotype	freq	genotype	freq	genotype	freq			
good good	0.490	Wt Wt	0.72	TT	0.74	0.261072	47.515104	Healthy
good good	0.490	Wt Wt	0.72	TC	0.24	0.084672	15.410304	Healthy
good good	0.490	Wt Wt	0.72	CC	0.02	0.007056	1.284192	DCM
good good	0.490	Wt del	0.26	TT	0.74	0.094276	17.158232	Healthy
good good	0.490	Wt del	0.26	TC	0.24	0.030576	5.564832	DCM
good good	0.490	Wt del	0.26	CC	0.02	0.002548	0.463736	DCM

			6		2			
good good	0.490	Del del	0.02	TT	0.74	0.007252	1.319864	Healthy
good good	0.490	Del del	0.02	TC	0.24	0.002352	0.428064	DCM
good good	0.490	Del del	0.02	CC	0.02	0.000196	0.035672	DCM
good bad	0.420	Wt Wt	0.72	TT	0.74	0.223776	40.727232	DCM
good bad	0.420	Wt Wt	0.72	TC	0.24	0.072576	13.208832	DCM
good bad	0.420	Wt Wt	0.72	CC	0.02	0.006048	1.100736	DCM
good bad	0.420	Wt del	0.26	TT	0.74	0.080808	14.707056	DCM
good bad	0.420	Wt del	0.26	TC	0.24	0.026208	4.769856	DCM
good bad	0.420	Wt del	0.2	CC	0.0	0.002184	0.397488	DCM

good bad	0.420	Del del	0.02	TT	0.74	0.006216	1.131312	DCM
good bad	0.420	Del del	0.02	TC	0.24	0.002016	0.366912	DCM
good bad	0.420	Del del	0.02	CC	0.02	0.000168	0.030576	DCM
bad bad	0.090	Wt Wt	0.72	TT	0.74	0.047952	8.727264	DCM
bad bad	0.090	Wt Wt	0.72	TC	0.24	0.015552	2.830464	DCM
bad bad	0.090	Wt Wt	0.72	CC	0.02	0.001296	0.235872	DCM
bad bad	0.090	Wt del	0.26	TT	0.74	0.017316	3.151512	DCM
bad bad	0.090	Wt del	0.26	TC	0.24	0.005616	1.022112	DCM
bad bad	0.090	Wt del	0.2	CC	0.0	0.000468	0.085176	DCM

bad bad	0.090	Del del	0.02	TT	0.74	0.001332	0.242424	DCM
bad bad	0.090	Del del	0.02	TC	0.24	0.000432	0.078624	DCM
bad bad	0.090	Del del	0.02	CC	0.02	0.000036	0.006552	DCM

Supplementary Table 4. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci + an additional autosomal additive DCM susceptibility locus.

additional autosomal additive locus		PDK4		Chr5 SNP		combined genotype	Predicted number of	Phenotype
genotype	freq	genotype	freq	genotype	freq	freq	individuals	
good good	0.490	Wt Wt	0.72	TT	0.74	0.261072	47.515104	Healthy
good good	0.490	Wt Wt	0.72	TC	0.24	0.084672	15.410304	Healthy
good good	0.490	Wt Wt	0.72	CC	0.02	0.007056	1.284192	DCM
good good	0.490	Wt del	0.26	TT	0.74	0.094276	17.158232	Healthy
good good	0.490	Wt del	0.26	TC	0.24	0.030576	5.564832	DCM
good good	0.490	Wt del	0.26	CC	0.02	0.002548	0.463736	DCM
good good	0.490	Del del	0.02	TT	0.74	0.007252	1.319864	Healthy
good good	0.490	Del del	0.02	TC	0.24	0.002352	0.428064	DCM
good good	0.490	Del del	0.02	CC	0.02	0.000196	0.035672	DCM
good bad	0.420	Wt Wt	0.72	TT	0.74	0.223776	40.727232	Healthy
good bad	0.420	Wt Wt	0.72	TC	0.24	0.072576	13.208832	DCM
good bad	0.420	Wt Wt	0.72	CC	0.02	0.006048	1.100736	DCM

good bad	0.420	Wt del	0.26	TT	0.74	0.080808	14.707056	DCM
good bad	0.420	Wt del	0.26	TC	0.24	0.026208	4.769856	DCM
good bad	0.420	Wt del	0.26	CC	0.02	0.002184	0.397488	DCM
good bad	0.420	Del del	0.02	TT	0.74	0.006216	1.131312	DCM
good bad	0.420	Del del	0.02	TC	0.24	0.002016	0.366912	DCM
good bad	0.420	Del del	0.02	CC	0.02	0.000168	0.030576	DCM
bad bad	0.090	Wt Wt	0.72	TT	0.74	0.047952	8.727264	DCM
bad bad	0.090	Wt Wt	0.72	TC	0.24	0.015552	2.830464	DCM
bad bad	0.090	Wt Wt	0.72	CC	0.02	0.001296	0.235872	DCM
bad bad	0.090	Wt del	0.26	TT	0.74	0.017316	3.151512	DCM
bad bad	0.090	Wt del	0.26	TC	0.24	0.005616	1.022112	DCM
bad bad	0.090	Wt del	0.26	CC	0.02	0.000468	0.085176	DCM
bad bad	0.090	Del del	0.02	TT	0.74	0.001332	0.242424	DCM
bad bad	0.090	Del del	0.02	TC	0.24	0.000432	0.078624	DCM
bad bad	0.090	Del del	0.02	CC	0.02	0.000036	0.006552	DCM

Supplementary Table 5. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci + an additional autosomal recessive DCM susceptibility locus.

additional autosomal recessive locus		PDK4		Chr5 SNP		combined genotype freq	Predicted number of individuals	Phenotype
genotype	freq	genotype	freq	genotype	freq			
good good	0.490	Wt Wt	0.72	TT	0.74	0.261072	47.515104	Healthy
good good	0.490	Wt Wt	0.72	TC	0.24	0.084672	15.410304	Healthy
good good	0.490	Wt Wt	0.72	CC	0.02	0.007056	1.284192	DCM
good good	0.490	Wt del	0.26	TT	0.74	0.094276	17.158232	Healthy
good good	0.490	Wt del	0.26	TC	0.24	0.030576	5.564832	DCM
good good	0.490	Wt del	0.26	CC	0.02	0.002548	0.463736	DCM
good good	0.490	Del del	0.02	TT	0.74	0.007252	1.319864	Healthy
good good	0.490	Del del	0.02	TC	0.24	0.002352	0.428064	DCM
good good	0.490	Del del	0.02	CC	0.02	0.000196	0.035672	DCM
good bad	0.420	Wt Wt	0.72	TT	0.74	0.223776	40.727232	Healthy
good bad	0.420	Wt Wt	0.72	TC	0.24	0.072576	13.208832	Healthy
good bad	0.420	Wt Wt	0.72	CC	0.02	0.006048	1.100736	DCM

good bad	0.420	Wt del	0.26	TT	0.74	0.080808	14.707056	Healthy
good bad	0.420	Wt del	0.26	TC	0.24	0.026208	4.769856	DCM
good bad	0.420	Wt del	0.26	CC	0.02	0.002184	0.397488	DCM
good bad	0.420	Del del	0.02	TT	0.74	0.006216	1.131312	Healthy
good bad	0.420	Del del	0.02	TC	0.24	0.002016	0.366912	DCM
good bad	0.420	Del del	0.02	CC	0.02	0.000168	0.030576	DCM
bad bad	0.090	Wt Wt	0.72	TT	0.74	0.047952	8.727264	DCM
bad bad	0.090	Wt Wt	0.72	TC	0.24	0.015552	2.830464	DCM
bad bad	0.090	Wt Wt	0.72	CC	0.02	0.001296	0.235872	DCM
bad bad	0.090	Wt del	0.26	TT	0.74	0.017316	3.151512	DCM
bad bad	0.090	Wt del	0.26	TC	0.24	0.005616	1.022112	DCM
bad bad	0.090	Wt del	0.26	CC	0.02	0.000468	0.085176	DCM
bad bad	0.090	Del del	0.02	TT	0.74	0.001332	0.242424	DCM
bad bad	0.090	Del del	0.02	TC	0.24	0.000432	0.078624	DCM
bad bad	0.090	Del del	0.02	CC	0.02	0.000036	0.006552	DCM

Supplementary Table 6. The phenotype decisions, combined genotype frequencies and predicted number of individuals for each genotype combination from the model incorporating the two known DCM loci + an additional X-linked DCM susceptibility locus, where X is normal and x is susceptible.

additional X locus		PDK4		Chr5 SNP		combined genotype freq	Predicted number of individuals	Phenotype
genotype	freq	genotype	freq	genotype	freq			
XY	0.350	Wt Wt	0.72	TT	0.74	0.18648	33.93936	Healthy
XY	0.350	Wt Wt	0.72	TC	0.24	0.06048	11.00736	Healthy
XY	0.350	Wt Wt	0.72	CC	0.02	0.00504	0.91728	DCM
XY	0.350	Wt del	0.26	TT	0.74	0.06734	12.25588	Healthy
XY	0.350	Wt del	0.26	TC	0.24	0.02184	3.97488	DCM
XY	0.350	Wt del	0.26	CC	0.02	0.00182	0.33124	DCM
XY	0.350	Del del	0.02	TT	0.74	0.00518	0.94276	Healthy
XY	0.350	Del del	0.02	TC	0.24	0.00168	0.30576	DCM
XY	0.350	Del del	0.02	CC	0.02	0.00014	0.02548	DCM
xY	0.150	Wt Wt	0.72	TT	0.74	0.07992	14.54544	DCM
xY	0.150	Wt Wt	0.72	TC	0.24	0.02592	4.71744	DCM

xY	0.150	Wt Wt	0.72	CC	0.02	0.00216	0.39312	DCM
xY	0.150	Wt del	0.26	TT	0.74	0.02886	5.25252	DCM
xY	0.150	Wt del	0.26	TC	0.24	0.00936	1.70352	DCM
xY	0.150	Wt del	0.26	CC	0.02	0.00078	0.14196	DCM
xY	0.150	Del del	0.02	TT	0.74	0.00222	0.40404	DCM
xY	0.150	Del del	0.02	TC	0.24	0.00072	0.13104	DCM
xY	0.150	Del del	0.02	CC	0.02	0.00006	0.01092	DCM
Females (XX)		**PDK4**		**Chr5 SNP**				**Phenotype**
XX	0.25	Wt Wt	0.72	TT	0.74	0.1332	24.2424	Healthy
XX	0.25	Wt Wt	0.72	TC	0.24	0.0432	7.8624	Healthy
XX	0.25	Wt Wt	0.72	CC	0.02	0.0036	0.6552	DCM
XX	0.25	Wt del	0.26	TT	0.74	0.0481	8.7542	Healthy
XX	0.25	Wt del	0.26	TC	0.24	0.0156	2.8392	DCM
XX	0.25	Wt del	0.26	CC	0.02	0.0013	0.2366	DCM
XX	0.25	Del del	0.02	TT	0.74	0.0037	0.6734	Healthy
XX	0.25	Del del	0.02	TC	0.24	0.0012	0.2184	DCM
XX	0.25	Del del	0.02	CC	0.02	0.0001	0.0182	DCM

Xx	0.210	Wt Wt	0.72	TT	0.74	0.111888	20.363616	Healthy
Xx	0.210	Wt Wt	0.72	TC	0.24	0.036288	6.604416	DCM
Xx	0.210	Wt Wt	0.72	CC	0.02	0.003024	0.550368	DCM
Xx	0.210	Wt del	0.26	TT	0.74	0.040404	7.353528	DCM
Xx	0.210	Wt del	0.26	TC	0.24	0.013104	2.384928	DCM
Xx	0.210	Wt del	0.26	CC	0.02	0.001092	0.198744	DCM
Xx	0.210	Del del	0.02	TT	0.74	0.003108	0.565656	DCM
Xx	0.210	Del del	0.02	TC	0.24	0.001008	0.183456	DCM
Xx	0.210	Del del	0.02	CC	0.02	0.000084	0.015288	DCM
xx	0.050	Wt Wt	0.72	TT	0.74	0.02664	4.84848	DCM
xx	0.050	Wt Wt	0.72	TC	0.24	0.00864	1.57248	DCM
xx	0.050	Wt Wt	0.72	CC	0.02	0.00072	0.13104	DCM
xx	0.050	Wt del	0.26	TT	0.74	0.00962	1.75084	DCM
xx	0.050	Wt del	0.26	TC	0.24	0.00312	0.56784	DCM
xx	0.050	Wt del	0.26	CC	0.02	0.00026	0.04732	DCM
xx	0.050	Del del	0.02	TT	0.74	0.00074	0.13468	DCM
xx	0.050	Del del	0.02	TC	0.24	0.00024	0.04368	DCM
xx	0.050	Del del	0.02	CC	0.02	0.00002	0.00364	DCM