Frequency estimation of disease-causing mutations in the Belgian population of some dog breeds - Part 2: retrievers and other breed types

Frequentieschatting van ziekteveroorzakende mutaties in de Belgische populatie van enkele hondenrassen - Deel 2: retrievers en andere rastypes

E. Beckers, M. Van Poucke, L. Ronsyn, L. Peelman

Department of Nutrition, Genetics and Ethology,
Faculty of Veterinary Medicine, Ghent University,
Heidestraat 19, B-9820 Merelbeke, Belgium

luc.peelman@ugent.be

Abstract

A Belgian population of ten breeds with a low to moderately low genetic diversity or which are relatively popular in Belgium, i.e. Bichon frise, Bloodhound, Bouvier des Flandres, Boxer, Cavalier King Charles spaniel, Irish setter, Papillon, Rottweiler, Golden retriever and Labrador retriever, was genotyped for all potentially relevant disease-causing variants known at the start of the study. In this way, the frequency was estimated for 26 variants in order to improve breeding advice. Disorders with a frequency high enough to recommend routine genotyping in breeding programs are (1) degenerative myelopathy for the Bloodhound, (2) arrhythmogenic right ventricular cardiomyopathy and degenerative myelopathy for Boxers, (3) episodic falling syndrome and macrothrombocytopenia for the Cavalier King Charles spaniel, (4) progressive retinal atrophy rod cone dysplasia 4 for the Irish setter (5) Golden retriever progressive retinal atrophy 1 for the Golden retriever and (6) exercise induced collapse and progressive rod-cone degeneration for the Labrador retriever. To the authors' knowledge, in this study, the presence of a causal mutation for a short tail in the Bouvier des Flandres is described for the first time.

SAMENVATTING

De Belgische populatie van tien hondenrassen (de bichonfrisé, sint-hubertushond, Vlaamse koehond, boxer, cavalier-kingcharlesspaniël, Ierse setter, het vlinderhondje, de rottweiler, golden retriever en labrador-retriever), waarvan de genetische diversiteit in België laag tot middelmatig laag is of die relatief populair zijn, werd gegenotypeerd voor ziekteveroorzakende mutaties die potentieel relevant zijn voor deze rassen. Op deze manier werd de frequentie van 26 mutaties geschat om zo gerichter fokadvies te kunnen geven. Aandoeningen waarvan de frequentie hoog genoeg ligt om routine-genotypering aan te raden in fokprogramma's zijn (1) degeneratieve myelopathie voor de sint-hubertushond, (2) "arrhythmogenic right ventricular cardiomyopathy" en degeneratieve myelopathie voor boxers, (3) "episodic falling syndrome" en macrothrombocytopenie voor de cavalier-kingcharlesspaniël (4) progressieve retina-atrofie "rod-cone" dysplasie 4 voor de Ierse setter, (5) golden retriever progressieve retina-atrofie 1 voor de golden retriever en (6) "exercise induced collapse" en progressieve "rod-cone" degeneratie voor de labrador-retriever. De aanwezigheid van de oorzakelijke mutatie voor een korte staart bij de Vlaamse koehond wordt hier volgens de auteurs voor het eerst beschreven.

INTRODUCTION

A low genetic diversity prevails in several dog breeds. Consequently, high prevalences of genetic disorders may occur (Calboli et al., 2008; Leroy et al., 2006). In order to improve this situation, a good knowledge of the disorders in each breed is necessary as well as of the prevalence in a population (McGreevy and Nicholas, 1999).

A total of seventeen dog breeds were involved in the entire study. Each breed population was tested for potentially relevant disease-causing variants, more specifically those that have been described in the corresponding breed and that are available in the public domain. In part 2, the results obtained for two retriever breeds (Golden and Labrador retriever) and eight other breeds (the Bichon frise, Bloodhound, Bouvier des Flandres, Boxer, Cavalier King Charles spaniel,

Table 1. Overview of the performed DNA tests. Ta indicates the annealing temperature, bp indicates base pairs and F/R indicates the forward/reverse primer. TR indicates Texas Red.

Disorder: Arrhythmogenic right ventricular cardiomyopathy (ARVC) **Gene symbol:** STRN **Inheritance:** Autosomal dominant, incomplete penetrance **Assay:** PCR with F/R primers and sequence analysis with R primer **Primers:** F: 5'-acaaaacagtaaaatgcacctatggtt-3'; R: 5'-tgactctcattccttcagtattcttgc-3'	**dbSNP ID:** ss1961068750 **Ta:** 64°C	**OMIA ID:** 000878-9615 **Reference:** Meurs et al., 2010 **Amplicon:** 239 bp
Disorder: Canine leukocyte adhesion deficiency type 1 (CLAD1) **Gene symbol:** ITGB2 **Inheritance:** Autosomal recessive **Assay:** PCR with F/R primers and sequence analysis with F primer **Primers:** F: 5'-ggaccctccttctccaccac-3'; R: 5'-gcctccccggaaacagagt-3'	**dbSNP ID:** ss1961068757 **Ta:** 65°C	**OMIA ID:** 000595-9615 **Reference:** Kijas et al., 1999 **Amplicon:** 344 bp
Disorder: Centronuclear myopathy (CNM) **Gene symbol:** PTPLA **Inheritance:** Autosomal recessive **Assay:** PCR with F/R primers and gel electrophoresis **Primers:** F: 5'- tggttctagctattgccatggtacg -3'; R: 5'- acctcaagcaaggcaaatgtctg -3'	**dbVar ID:** nsv1397888 **Ta:** 64°C	**OMIA ID:** 001374-9615 **Reference:** Pelé et al., 2005 **Amplicon:** Wt: 115 bp; Mt: 364 bp

Disorder: Degenerative myelopathy (DM)
Gene symbol: SOD1   **Inheritance:** Autosomal recessive
Assay: qPCR with dual labeled probes
Primers: F: 5'-cttccactttcttgtgattg-3'
        R: 5'-caccttgtgtattatctccaa-3'
	dbSNP ID:
	ss1961068758
	Ta: 56°C
		OMIA ID: 000263-9615
		Reference: Awano et al., 2009
		Amplicon: 192 bp
		Probes: Wt: HEX-cgccttcagtcagcc-BHQ1
		        Mt: TR-cgcctttagtcagccc-BHQ2

Disorder: Duchenne type muscular dystrophy cavalier (DMD-C) **Gene symbol:** DMD **Inheritance:** X-linked recessive **Assay:** PCR-RFLP with AccI **Primers:** F: 5'-aatatttgtagggtggttggctaaaataat-3' R: 5'-taccttcggccccagaaaag-3'	**dbSNP ID:** ss1961068760 **Ta:** 64°C	**OMIA ID:** 001081-9615 **Reference:** Walmsley et al., 2010 **Amplicon:** 752 bp **Fragment lengths**: Wt: 303/243/206; Mt: 546/206
Disorder: Duchenne type muscular dystrophy Golden retriever (DMD-GR) **Gene symbol:** DMD **Inheritance:** Autosomal recessive **Assay:** PCR with F/R primers and sequence analysis with F primer **Primers:** F: 5'-aatgatgggcatgggttg-3'; R: 5'-ccagaaatgtaccgaccttca-3'	**dbSNP ID:** ss1961068761 **Ta:** 60°C	**OMIA ID:** 001081-9615 **Reference** Sharp et al., 1992 **Amplicon:** 235 bp
Disorder: Duchenne type muscular dystrophy rottweiler (DMD-R) **Gene symbol:** DMD **Inheritance:** X-linked recessive **Assay:** PCR-RFLP with PsiI **Primers:** F: 5'-agcatttcttttcatctcatttcacag-3' R: 5'-agcattccttctcaccaataatctca-3'	**dbSNP ID:** ss1961068762 **Ta:** 64°C	**OMIA ID:** 001081-9615 **Reference:** Winand et al., 1994 **Amplicon:** 434 bp **Fragment lengths**: Wt: 308/126; Mt: 194/126/114

Disorder: Episodic falling syndrome (EFS)
Gene symbol: BCAN   **Inheritance:** Autosomal recessive
Assay: PCR with F/R$_1$/R$_2$ primers and gel electrophoresis
Primers: F: 5'-aaggtcttacacctgcaatgaatag-3'; R$_1$: 5'-agcaaatgtaaagtcctgtgaccat-3';
      R$_2$: 5'-agttcacattgtgctctctctactg-3'
	dbVar ID:
	nsv1397889
	
	Ta: 64°C
		OMIA ID: 001592-9615
		Reference: Gill et al., 2012
		Amplicon: Wt: 393 bp;
		        Mt: 273 bp

Disorder: Exercise induced collapse (EIC) **Gene symbol:** DNM1 **Inheritance:** Autosomal recessive **Assay:** PCR-RFLP with SmlI **Primers:** F: 5'-ggctggttgcccctgactt-3' R: 5'-ttttgttttccttttccccaggcttgagttccttacctg-3'	**dbSNP ID:** ss1961068747 **Ta:** 64°C	**OMIA ID:** 001466-9615 **Reference:** Patterson et al., 2008 **Amplicon:** 232 bp **Fragment lengths**: Wt: 205/27; Mt: 113/92/27
Disorder: Globoid cell leukodystrophy (GCL) **Gene symbol:** GALC **Inheritance:** Autosomal recessive **Assay:** PCR with F/R primers and gel electrophoresis **Primers:** F: 5'-cacgtctgccttttcctatttcca-3'; R: 5'-ggttcaatatgcggcccaag-3'	**dbVar ID:** nsv1397890 **Ta:** 64°C	**OMIA ID:** 000578-9615 **Reference:** McGraw & Carmichael, 2006 **Amplicon:** Wt: 168 bp; Mt: 246 bp
Disorder: Golden retriever progressive retinal atrophy 1 (GR-PRA 1) **Gene symbol:** SLC4A3 **Inheritance:** Autosomal recessive **Assay:** PCR with F/R primers and sequence analysis with F primer **Primers:** F: F: 5'-gtcgtggaatactgttgcctggt-3'; R: 5'-gccgtgttgggctggttc-3'	**dbSNP ID:** ss1961068759 **Ta:** 64°C	**OMIA ID:** 001572-9615 **Reference:** Downs et al., 2011 **Amplicon:** 264 bp

Disorder: Hyperuricosuria (HUU)
Gene symbol: SLC2A9   **Inheritance:** Autosomal recessive
 	
Assay: qPCR with dual labeled probes
Primers: F: 5'-ccaaggagatccgtggc -3'
        R: 5'-cttccccagcagctcag -3'
	dbSNP ID:
	ss1961068738
	
	Ta: 63°C
		OMIA ID: 001033-9615
		Reference:
		Bannasch et al., 2008
		Amplicon: 101 bp
		Probes: Wt: FAM-ccatcttcatctgcatcggtgt-BHQ1;
		        Mt: TR-catcttcatcttcatcggtgtgttc-BHQ2

Disorder: Mucopolysaccharidosis I (MPS I)	**dbSNP ID:** ss1961068739	**OMIA ID:** 000664-9615
Gene symbol: IDUA **Inheritance:** Autosomal recessive		**Reference:** Menon et al., 1992
Assay: PCR with F/R primers and sequence analysis with R primer	**Ta:** 68°C	**Amplicon:** 433 bp
Primers: F: 5'-gacccggaggcggaacc-3'; R: 5'-ggacggccgaggtcacg-3'		

Disorder: Macrothrombocytopenia (MTC)	**dbSNP ID:** ss1961068754	**OMIA ID:** 001001-9615
Gene symbol: TUBB1 **Inheritance:** Autosomal recessive		**Reference:** Davis et al., 2008
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 60°C	**Amplicon:** 278 bp
Primers: F: 5'-cctggtgtccctgaccatga-3'; R: 5'-ggccccggaagatgcag-3'		

Disorder: Narcolepsy (NA)	**dbSNP ID:** ss1961068748	**OMIA ID:** 000703-9615
Gene symbol: HCRTR2 **Inheritance:** Autosomal recessive		**Reference:** Lin et al., 1999
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 64°C	**Amplicon:** 382 bp
Primers: F: 5'-ccacacggaagacagagagacc-3'; R: 5'-ctccagttcctacaattttcactttcc-3'		

Disorder: Osteogenesis imperfecta (OI)	**dbSNP ID:** ss1961068756	**OMIA ID:** 000754-9615
Gene symbol: COL1A1 **Inheritance:** Autosomal recessive		**Reference:** Campbell et al., 2000
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 64°C	**Amplicon:** 282 bp
Primers: F: 5'- agggcgtgcgtggtga -3'; R: 5'- ccctgtgggaggcagaaca -3'		

Disorder: Progressive retinal atrophy (PRA)	**dbSNP ID:** ss1961068735	**OMIA ID:** 000830-9615
Gene symbol: CNGB1 **Inheritance:** Autosomal recessive		**Reference:** Ahonen et al., 2013
Assay: PCR-RFLP with AluI	**Ta:** 64°C	**Amplicon:** 287 bp
Primers: F: 5'-acgctcggcaaaccgcag-3' R: 5'-acagagaaagcaaagacacccgtga-3'	**Fragment lengths:** Wt: 206/45/36; Mt: 116/95/45/36	

Disorder: Progressive rod-cone degeneration (PRCD)	**dbSNP ID:** ss1961068746	**OMIA ID:** 001298-9615
Gene symbol: PRCD **Inheritance:** Autosomal recessive		**Reference:** Zangerl et al., 2006
Assay: PCR-RFLP with ApaLI	**Ta:** 60°C	**Amplicon:** 129 bp
Primers: F: 5'-aggatgggcagcagtgg-3'; R: 5'-ttttttttttgctgagtacgaagagggtggt -3'	**Fragment lengths:** Wt: 97/32 bp; Mt: 129 bp	

Disorder: Rod-cone dysplasia 1 (RCD1)	**dbSNP ID:** ss1961068741	**OMIA ID:** 000882-9615
Gene symbol: PDE6B **Inheritance:** Autosomal recessive		**Reference:** Suber et al., 1993
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 58°C	**Amplicon:** 122 bp
Primers: F: 5'-ttcccgtttccacgaaga-3'; R: 5'-gctgcttctcctcctccag-3'		

Disorder: Rod-cone dysplasia 4 (RCD4)	**dbSNP ID:** ss1961068751	**OMIA ID:** 001575-9615
Gene symbol: C17H2orf71 **Inheritance:** Autosomal recessive		**Reference:** Downs et al., 2012
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 64°C	**Amplicon:** 358 bp
Primers: F: 5'-acacgccgagcagacaggag-3'; R: 5'-aggacgcgggccagaagag-3'		

Disorder: Recessive dystrophic epidermolysis bullosa (RDEB)	**dbSNP ID:** ss1961068752	**OMIA ID:** 000341-9615
Gene symbol: COL7A1 **Inheritance:** Autosomal recessive		**Reference:** Baldeschi et al., 2003
Assay: PCR-RFLP with HaeIII	**Ta:** 64°C	**Amplicon:** 304 bp
Primers: F: 5'-tggtcccaagggtgaacgtg-3'; R: 5'-aaatgccccagccctgctc-3'	**Fragment lengths:** Wt: 105/66/49/48/18/9/9 bp; Mt: 171/49/48/18/9/9 bp	

Disorder: Short tail (ST)	**dbSNP ID:** ss1961068734	**OMIA ID:** 000975-9615
Gene symbol: T **Inheritance:** Autosomal dominant		**Reference:** Haworth et al., 2001
Assay: PCR-RFLP with BstEII	**Ta:** 64°C	**Amplicon:** 343 bp
Primers: F: 5'-tgagcgccgtggagagcg-3' R: 5'-cccagaaaacccagagagtgacga-3'	**Fragment lengths:** Wt: 184/159; Mt: 184/128/31	

Disorder: von Willebrandt disease type 1 (VWD1)	**dbSNP ID:** ss1961068765	**OMIA ID:** 001057-9615
Gene symbol: VWF **Inheritance:** Autosomal recessive		**Reference:** Brewer et al., 1998
Assay: PCR with F/R primers and sequence analysis with F primer	**Ta:** 65°C	**Amplicon:** 287 bp
Primers: F: 5'-cgaggcaccatctaccctgtg-3'; R: 5'-tcacccaacctcagtcctctcc-3'		

Disorder: X-linked hypohydrotic ectodermal dysplasia (XHED)	**dbSNP ID:** ss1961068763	**OMIA ID:** 000543-9615
Gene symbol: EDA **Inheritance:** X-linked recessive		**Reference:** Casal et al., 2005
Assay: PCR-RFLP with ScrFI	**Ta:** 64°C	**Amplicon:** 194 bp
Primers: F: 5'-tcccttcttgttgcctctcacc-3' R: 5'-ccatcttcaccgcaatcttctg-3'	**Fragment lengths:** Wt: 146/25/23; Mt: 169/25	

Disorder: X-linked myotubular myopathy (XLMTM)	**dbSNP ID:** ss1961068764	**OMIA ID:** 001508-9615
Gene symbol: MTM1 **Inheritance:** X-linked recessive		**Reference:** Beggs et al., 2010
Assay: qPCR with dual labeled probes	**Ta:** 57°C	**Amplicon:** 181 bp
Primers: F: 5'- tgcggaggacatagta -3' R: 5'- gcggcttactgactta -3'	**Probes:** Wt: FAM- aactttcttcgtttagaaatgc -BHQ1; Mt: TR- aactttcttctttagaaatgca -BHQ2	

Irish setter, Papillon and Rottweiler) are highlighted. The results of seven shepherd breeds were discussed in part 1.

MATERIALS AND METHODS

Breed selection

The chosen breeds for this research were based on a study examining the genetic diversity of 23 breeds (Wijnrocx et al., 2012). The more popular breeds and breeds with the lowest genetic diversity, for which at least two DNA tests were available at the start of the study, were chosen for inclusion in this frequency study. One exception was the Bloodhound, for which only the presence of degenerative myelopathy was tested.

Samples and DNA extraction

Whole blood samples collected in EDTA-tubes were used. They originated from a pool of samples (stored at -20°C) delivered by veterinarians, animal clinics, independent breeders and breeding associations all over Belgium for routine genotyping, making the sample as representative as possible for the Belgian population. To the best of the authors' ability, closely related animals (relation >12.5%) were excluded in order to keep the bias as low as possible, but in some cases sufficient information was lacking or the population size was too small. The aimed minimum of 50 samples per breed was not reached for the Bichon frise (n = 27), for the Bloodhound (n = 27) and for the Papillon (n = 39).

One hundred µl of each sample was washed with 500 µl Tris-HCl-EDTA until a clean pellet of white blood cells remained. The cells were resuspended in a lysis buffer with proteinase K, and DNA was released during an incubation kept at 56 °C for 45 minutes. Afterwards, the enzyme was inactivated at 95 °C during a ten-minutes incubation.

Disorders and DNA tests

In total, 26 different tests were performed. The assays were validated by sequencing, which is the golden standard. When applicable, the assays were later performed with quicker, cheaper and/or easier techniques (PCR(-RFLP) followed by gel electrophoresis or qPCR with dual labeled probes). Details of the mutations and the performed (alternative) tests can be found in Table 1. The Hardy-Weinberg (H-W) equilibrium was calculated for each disorder, as well as the allele frequencies.

PCR-sequencing

All assays were first validated through PCR followed by sequencing. This was done in the same manner as explained in part 1.

PCR-gel electrophoresis

For the detection, if INDELs (insertions and/or deletions) longer than 20 bp, a PCR was performed

Table 2. All DNA tests with at least one mutation found in the tested population (+) and with no mutation in the corresponding breed (-). Disorders indicated with a "*" should be routinely tested in the corresponding breed.

Breed	Outcome	Test(s)
Bichon frise	+	Degenerative myelopathy (DM)
	-	X-linked hypohydrotic ectodermal dysplasia (XHED)
Bloodhound	+	DM*
Bouvier des Flandres	+	Exercise induced collapse (EIC), short tail (ST)
Boxer	+	Arrhythmogenic right ventricular cardiomyopathy* (ARVC), DM*
Cavalier King	+	Episodic falling syndrome* (EFS), macrothrombocytopenia* (MTC)
Charles spaniel	-	Duchenne muscular dystrophy (DMD-C)
Golden retriever	+	Golden retriever progressive retinal atrophy 1* (GR-PRA), progressive rod-cone degeneration (PRCD)
	-	DM, Duchenne muscular dystrophy (DMD-GR), osteogenesis imperfecta (OI), recessive dystrophic epidermolysis bullosa (RDEB)
Irish setter	+	Progressive retinal atrophy rod cone dysplasia 4* (RCD4)
	-	Canine leukocyte adhesion deficiency type 1 (CLAD1), DM, globoid cell leukodystrophy (GCL), RCD1
Labrador retriever	+	Exercise induced collapse* (EIC), PRCD*, centronuclear myopathy (CNM)
	-	HUU, narcolepsy (NA), X-linked myotubular myopathy (XLMTM)
Papillon	+	Progressive retinal atrophy (PRA), von Willebrandt disease type 1 (VWD1)
Rottweiler	-	DMD-R, mucopolysaccharidosis I (MPS I)

Table 3. Overview of the DNA tests per breed, for which at least one mutant allele was found with the total number of dogs tested (Total), the number of homozygous normal (Wt/Wt), heterozygous (Wt/Mt) and affected (Mt/Mt) individuals and the corresponding mutant allele frequency (q).

Breed	Test	Total	Wt/Wt	Wt/Mt	Mt/Mt	q (%)
Bichon frise	DM	27	25	2	0	3.7
Bloodhound	DM	27	14	13	0	24.1
Bouvier des Flandres	EIC	101	94	7	0	3.5
	ST	102	97	5	0	2.5
Boxer	ARVC	50	41	8	1	10
	DM	51	44	7	0	6.9
Cavalier King Charles spaniel	EFS	57	50	7	0	6.1
	MTC	58	25	24	15	46.6
Golden retriever	GR-PRA1	85	73	12	0	7.1
	PRCD	92	85	5	2	4.9
Irish setter	RCD4	86	52	27	7	23.8
Labrador retriever	CNM	143	141	2	0	0.7
	EIC	143	103	32	8	16.8
	PRCD	134	103	26	5	13.4
Papillon	PRA	39	36	3	0	3.8
	VWD1	39	37	2	0	2.6

followed by gel electrophoresis. The same PCR mix as described for the PCR-sequencing was made. After the PCR, the product was loaded on a 2%-agarose gel. The fragment lengths can be viewed in Table 1.

PCR-RFLP

For mutations creating or destroying a restriction enzyme recognition site, a PCR-restriction fragment length polymorphism (PCR-RFLP) was performed. The same PCR mix was made as described for PCR-sequencing. Subsequently, the product was digested by a restriction enzyme, cutting either the Wt or the Mt allele. The restriction digest was done overnight with 5 U of enzyme according to the instructions of Bioké, Leiden, the Netherlands. The end product was loaded onto a 3%-agarose gel, and the fragment lengths can be viewed in Table 1.

qPCR with dual labeled probes

A mix was made according to the descriptions in part 1. The mix was put in the Bio-Rad CFX96 C100 Touch™ Thermal Cycler real-time PCR, resulting in an amplification curve and a melting curve. The annealing temperature for each test is mentioned in Table 1.

RESULTS AND DISCUSSION

In total, 26 tests were performed. Three different breed specific tests were used for Duchenne type muscular dystrophy (DMD) in the Cavalier King Charles spaniel, the Golden retriever and the Rottweiler. In Table 2, the tested disorders for each breed are shown. An overview of all tests, for which at least one mutant allele was found, can be viewed in Table 3. In case no mutant allele or a low mutant allele frequency was found, routine genotyping for this disorder is not advised by the authors. It should be noted that in the cases where the mutant allele in the population studied was not found, the allele might however be present in the Belgian population, especially in the breeds, of which only a relatively small number of animals were sampled. In case of precedents in a family or line, it should be considered to test for the disorder anyway. By making a deliberate partner choice, based on the genotyping results, creation of affected animals can be prevented and the mutant allele frequency can be reduced, without (further) endangering of the genetic diversity.

Bichon frise

The Bichon frise (n = 27) was genotyped for two disorders. X-linked hypohydrotic ectodermal dysplasia (XHED), an X-linked trait of the ectoderm, usually characterized by the absence of hair follicles and skin glands, has been described previously in the Bichon frise in only one case report (Moura and Cirio, 2004). No mutant allele was found in the tested population. No other cases have been reported as of yet and no frequency data is available. It can be assumed that the mutant allele frequency is low to very low and maybe even absent in the Belgian population. However, the results should be interpreted with care given the small sample size.

Degenerative myelopathy (DM) is a late-onset progressive neurodegenerative disease with an autosomal recessive mode of inheritance (Zeng et al., 2014). Two carriers (Wt/Mt) were found in the sample, which indicates a mutant allele frequency of 3.7% (Table 3). The Orthopedic Foundation for Animals (OFA) geno-

typed six Bichon frise dogs and found one homozygous mutant (Mt/Mt) animal. The Bloodhound and Boxer were also genotyped for DM (see below).

Bloodhound

For the Bloodhound population (n = 27), only DM was tested. A mutant allele frequency of 24.1% was found (Table 3). No homozygous mutant and 13 heterozygous animals were found, creating a disequilibrium in the H-W equilibrium. Fifteen point six normal (Wt/Wt) dogs, 9.8 carriers (Wt/Mt) and 1.6 affected (Mt/Mt) animals are expected in an ideally balanced population. The disequilibrium is probably due to the small sample size and not due to (sample) selection bias as DM is a late-onset disorder. Frequency measurements have been performed in the USA. Zeng et al. (2014) found a mutant allele frequency of 30.0%, which is in line with the findings of the present study, and the OFA found an even higher mutant allele frequency of 44.0% (n = 311). However, the latter is probably an overestimation due to a selection bias of the sample tested. Despite the H-W disequilibrium, results of the present study indicate a very high frequency in the Belgian population, as was the case in the USA population. Due to the high frequency and the severity of the disorder, routine testing should be encouraged.

Bouvier des Flandres

The Belgian population of the Bouvier des Flandres was tested for the presence of the mutation causing exercise induced collapse (EIC) and that of a short tail (ST) (n = 101 and n = 102, respectively). The former is an autosomal recessive neuromuscular disorder, first discovered in the Labrador retriever (see also part 1) (Minor et al., 2011). A mutant allele frequency of 3.5% was found in this study for the Bouvier des Flandres (Table 3). No information on frequency elsewhere is available as of yet.

The first causative mutation for "short tail" was found in the Pembroke Welsh corgi. This mutation is autosomal dominant and the mutation is lethal in the homozygous form (Haworth et al., 2001). An innate short tail occurs in many dog breeds and this same mutation was found in several of these breeds (Hytönen et al., 2009). In the present study, it was tested if this mutation is also present in the Bouvier des Flandres. Out of 102 dogs, the five dogs that were born with a short tail, were genotyped as Wt/Mt (Table 3), indicating this mutation is also responsible for or at least one of the causal mutations for the short tail phenotype in the Bouvier des Flandres. None of the other dogs, which had a normal tail length, carried the mutation. A short tail is neither a desirable nor an unattractive property according to the breed standards of the Bouvier des Flandres, so Belgian breeders generally do not breed in favor of this trait. Genotyping

for ST is uncalled-for, since heterozygotes are phenotypically identifiable.

Boxer

Two tests were performed for the Boxer, namely arrhythmogenic right ventricular cardiomyopathy (ARVC) and degenerative myelopathy (DM). The presence of a mutant allele was confirmed in the studied population for both disorders.

Arrhythmogenic right ventricular cardiomyopathy is a serious and lethal heart condition that is associated with a dominant mutation in the *STRN* gene. The penetrance (the percentage of individuals carrying the mutation that also expresses the associated phenotype) of this mutation was estimated to be 100% for homozygotes and 82% for heterozygotes (Meurs et al., 2013). A far lower penetrance (although it could not be reliably calculated) was recently estimated in the UK by Cattanach et al. (2015). They suggested that the *STRN* mutation is not the cause, but is closely linked to the causal mutation. Pedigree analysis pinpointed the source of ARVC in the UK to a small number of breeding animals imported from America, but the *STRN* mutation had already been present in the UK population. The indigenous population, with no ancestry to the USA, is largely free of the disease. There may be a link between *STRN* homozygosity and more severe symptoms (Cattanach et al., 2015). A mutant allele frequency of 10.0% was found in the Belgian population of Boxers (n = 50) (Table 3). No frequency data is available from other countries. Given the high frequency, routine genotyping in breeding dogs is recommended and given the severity of this disease, non-breeding dogs should also be considered for testing. However, it should be stressed that due to the incomplete penetrance, not all animals carrying the mutation, will develop the disease.

Fifty-one Boxers were tested for DM and 13.7% were found to be heterozygous dogs (Table 3). These results are similar to the ones of a recent study, which reported 13.0% carriers out of 15 tested Boxers for DM (Broeckx et al., 2013). The OFA tested 2987 Boxers for DM and found a much higher percentage of 35.9% carriers (Wt/Mt) and 45.9% affected (Mt/Mt) animals. This is however not a randomized study, since the samples are derived from animals presented in the clinic, though the percentage is still a high one. It is advised for the Boxer to be routinely genotyped for DM.

Cavalier King Charles spaniel

The Cavalier King Charles spaniel was genotyped for three disorders. Duchenne muscular dystrophy (DMD-C) is a lethal X-linked recessive muscle disorder (Walmsley et al., 2010). The causal mutation (Walmsley et al., 2010) is different from the one found in the Rottweiler (Winand et al., 1994) and in

the Golden retriever (Sharp et al., 1992), which will both be discussed later. No mutant allele was found in the tested population (n = 58) and no frequency determinations have been performed in other populations as of yet.

The autosomal recessive disorder episodic falling syndrome (EFS) causes neurological episodes often triggered by stress, excitement or exercise. Symptoms occur at the age of three months to four years and become progressively worse. A frequency of 12.3% (Wt/Mt) carriers was found in 57 genotyped animals, which is very similar to the estimate of 12.9% carriers in a USA population (Gill et al., 2011). No homozygous mutant individuals were encountered (Table 3). The authors encourage routine genotyping in breeding dogs because of the high frequency.

Macrothrombocytopenia (MTC) is an autosomal intermediate inheriting trait in the Cavalier King Charles spaniel. Animals with this mutation have a shortage of platelets and may potentially develop problems in the primary hemostasis. Heterozygotes have less thrombocytes than homozygous normal animals, but homozygous mutant animals have an even bigger shortage. This disorder is not a disease per se, since affected animals do not usually have bleeding tendencies. The main problem is situated in unnecessary treatments with antibiotics or corticosteroids, which are often given because of the abnormal blood parameters (Davis et al., 2008). In the genotyped population of the Cavalier King Charles spaniel of this study, a large mutant allele frequency of 46.6% was estimated (15.9% homozygous mutant and 41.4% heterozygous dogs) (Table 3). This is similar to the one found by Davis et al. (2008). They genotyped a Dublin population (n = 40) and found 12.5% homozygous mutants and 52.5% heterozygotes. In the same study, an even larger percentage (47.0% homozygous mutant and 45.0% heterozygous dogs) was found in a USA population (n = 60). In view of the very high percentage, the authors recommend routine genotyping in all dogs in order to prevent wrongful medicinal use.

Irish setter

Of the five disorders the Irish setter was genotyped for, only progressive retinal atrophy rod-cone dysplasia 4 (RCD4) was present in the tested Belgian population. Canine leukocyte adhesion deficiency type 1 (CLAD1) is an autosomal recessive disease of the immune system, giving animals a low immune response against all sorts of infections, often leading to death (Kijas et al., 1999). Eighty-five Irish setters were tested for this disorder and no mutant allele was found. When the causal mutation was described for the first time, a frequency of 4.5% was found (Kijas et al., 1999). A follow-up study was performed, in which they found a mutant allele frequency of 3.2% in the Belgian population of Irish setters. The mutation was

found in all ten countries included in the study (Kijas et al., 2000). Other studies estimated mutant allele frequencies between 7.6% and 13.0% in the USA (Foureman et al., 2002), 7.6% in Australia (Jobling et al., 2003) and 11.0% in Germany (Pfeiffer and Brenig, 2005). The frequency of the CLAD-mutation in the Belgian population has apparently been reduced to near zero during the last 15 years, which is proof of the efficiency of using DNA-testing to decrease genetic disorders.

Eighty-six dogs were genotyped for DM, but no mutant allele was found in the Belgian population (n = 86). According to the OFA DM is present in the Irish setter. They estimated a mutant allele frequency of 14.3% in the USA population (n = 14). However, the selection bias (animals presented in the animal clinic and small population size) should be taken into account.

Globoid cell leukodystrophy (GCL) is a fatal autosomal recessive lysosomal storage disease. The causal mutation was not found in the tested population (n = 86). Three carriers were found in a sample of 24 Irish setters in the USA (McGraw and Carmichael, 2006).

Progressive retinal atrophy rod cone dysplasia 1 and 4 (RCD1 and 4) are autosomal recessive disorders resulting in a degeneration of the retina. The former is an early-onset disorder (Suber et al., 1993), while the latter is a late-onset trait (Downs et al., 2013). The causal mutation for RCD1 was not found in any of the 86 genotyped Irish setters. A frequency determination for RCD1 was performed in the USA, where they found 7.8% heterozygotes (Aguirre et al., 1999). This disorder was tested on Belgian Irish setters in the past years to try and decrease the mutant allele frequency, which was effectively obtained. For RCD4, 31.4% carriers, 8.1% affected animals and a mutant allele frequency of 23.8% were found (Table 3). The mutation causing this disorder is spread worldwide in Irish setters at a high frequency. In the UK and the USA, a study found 41.0% carriers and 8.3% affected animals (Downs et al., 2013). The results of the present study are well in line with these data. The high frequency might be explained by the fact that RCD4 is a late-onset disease. The authors advise to routinely test for this disorder in animals used for breeding.

Papillon

The Papillon was genotyped for progressive retinal atrophy (PRA) and von Willebrandt disease type 1 (VWD1). The mutant allele was found in the tested population (n = 39) for both disorders. Progressive retinal atrophy is a degenerative eye condition that may lead to blindness and is transmitted in an autosomal recessive way (Ahonen et al., 2013). Three carriers (Wt/Mt) and no homozygous mutant individuals were found (Table 3). No frequency data is available as of yet in other populations. von Willebrandt disease type 1 is an autosomal recessive disorder of the

platelets that causes mild bleedings. These bleedings are the result of a decrease of von Willebrandt factor multimers, which are important for the adhesion of platelets to the endothelium (Boudreaux, 2012). A mutant allele frequency of 2.6% was found in the studied population (Table 3). No frequency determination is available in the literature for this condition. The results should be interpreted with care, given the small sample size. However,it can be concluded that both disorders are present in the Belgian population of the Papillon.

Rottweiler

Two DNA tests were performed for the Rottweiler, i.e. for genotyping DMD-R and mucopolysaccharidosis I (MPS I). Ninety-one dogs were genotyped 91 for DMD-R, but the mutation was not found. There is no frequency data available as of yet in other populations. The lysosomal storage disease MPS I has an autosomal recessive mode of inheritance (Menon et al., 1992). The mutation was not detected in the genotyped population (n = 85) and no frequency data is available from other populations. It can be assumed that the prevalence of these two disorders in the Belgian Rottweiler population is probably very low, and the mutation may even be absent.

Golden retriever

The Golden retriever was genotyped for a total of six disorders. No mutant allele was found in the Golden retriever sample (n = 91) for DM, indicating that the frequency in the Belgian population lies much lower and differs significantly from the one found by the OFA in the USA population. Three carriers and four Golden retrievers homozygous for the DM mutation out of a population counting 185 dogs have been reported by OFA. A mutant allele frequency of 3.3% was calculated in another American population, including 334 samples from a variety of sources (Zeng et al., 2014). However, there is a deviation of the Hardy-Weinberg equilibrium in both these studies.

Duchenne muscular dystrophy (DMD-GR), a degenerative muscle disease, has an X-chromosome linked recessive mode of inheritance (Sharp et al., 1992). Other causal mutations have been described for the Cavalier King Charles spaniel (Walmsley et al., 2010) and the Rottweiler (Winand et al., 1994). The mutation was not found in the tested samples (n = 90) (Table 2). Nor did Broeckx et al. (2013) find the mutation in their population. No other frequency determinations have been performed as of yet.

Osteogenesis imperfecta (OI) probably has an autosomal dominant mode of inheritance and is caused by a defective collagen type 1 fiber (Campbell et al., 2000). In the Beagle, a mutation in another gene was found, resulting in the same defect, which implies that OI is genetically heterogeneous (Campbell et al.,

2001). The mutation was not found in the Belgian population (n = 90) and a frequency determination has not yet been performed elsewhere (Table 2).

For recessive dystrophic epidermolysis bullosa (RDEB), an autosomal recessive skin disorder caused by a defect in collagen type VII (Palazzi et al., 2000), the mutation was not found in the population of Golden retrievers (n = 90), and no frequency data is available in other countries (Table 2).

Golden retriever progressive retinal atrophy 1 (GR-PRA 1) is an autosomal recessive disorder caused by degeneration of photoreceptor cells in the retina (Downs et al, 2011). Being a genetic heterogenic trait in the Golden retriever, this mutation is only responsible for part of the PRA cases in this breed, with an estimate of about 56.0% and varying from country to country. Twelve carriers were detected in the available samples (n = 85, q = 7.1%), giving a result in line with the frequency in the rest of Europe. Frequencies of 0.0% (USA), 2.0% (France), 4.0% (UK) and 6.0% (Sweden) have been described. The absence of the mutation in the USA suggests that the mutation originated in Europe (Downs et al., 2011). The authors advise to routinely genotype Golden retrievers used for breeding purposes for GR-PRA 1.

Progressive rod cone degeneration (PRCD) is an autosomal recessive and late-onset photoreceptor degeneration. The mutation is present in many breeds, including the Golden retriever and Labrador retriever (Zangerl et al., 2006). A total of 92 Golden retrievers were genotyped and a mutant allele frequency of 4.9% was found. The percentage found in the Golden retriever was much lower than for the Labrador retriever (see below). However, a mutant allele frequency of 4.9% is not low enough to be ignored. PCRD is preferably to be tested simultaneously with GR-PRA1. No frequency measurements have been performed as of yet in other countries.

Labrador retriever

Six different tests were performed on the available Labrador retriever samples. No mutant allele was found in the available samples (n = 143) for HUU, narcolepsy (NA) and X-linked myotubular myopathy (XLMTM) (Table 2). Hyperuricosuria is present in the Labrador retriever, though at a very low prevalence (Karmi et al., 2010). For NA, an autosomal recessive sleeping disorder (Lin et al., 1999), no frequency data is available as of yet. Also for XLMTM, an X-linked recessive muscular defect, no frequency information is available so far (Beggs et al., 2010).

Centronuclear myopathy (CNM) is an autosomal recessive muscle disorder causing muscle weakness (Pelé et al., 2005). One hundred and forty-three Labrador retrievers were genotyped in this study and a low mutant allele frequency of 0.7% was calculated (Table 3). Maurer et al. (2012) genotyped over 7000 Labrador retrievers in 13 countries. Mutant allele fre-

quencies of 6.9% (Canada), 8.9% (USA) and 10.2% (UK and Ireland; Continental Europe) were found. It was also proven that some of the affected dogs in Germany and France were directly related to popular sires in the UK. The mutation probably originated in the UK and got spread to many other countries in the world (Maurer et al., 2012); however, as the results of the present study indicate, the mutation spread much less or only recently to the Belgian population. In line with the present results, Gentilini et al. (2011) estimated a low frequency of 0.5% in Italy. Despite the low mutant allele frequency in Belgium, the authors advise to test Labrador retrievers for CNM, especially when they have ties to the UK population.

Exercise induced collapse (EIC) is an autosomal recessive neuromuscular disorder characterized by exercise intolerance in otherwise healthy young adult dogs. Clinical signs are precipitated by heavy exercise (Minor et al., 2011). A percentage of 5.6% affected dogs and a high mutant allele frequency of 16.8% were found in the available samples (n = 143) for EIC (Table 3). Two independent studies in the USA estimated a frequency of 3.0% affected dogs and 37.0% carriers (Patterson et al., 2008), and 9.9% affected dogs and 37.2% carriers (Minor et al. 2011), respectively, which is in line with the results of the present study. Broeckx et al. (2013) found 29.0% affected animals and 25.0% carriers in a population of Labrador retrievers of Belgium, the Netherlands and Germany. This is a much higher percentage than the percentage of the present study and the one described by Minor et al. (2011). This huge difference in frequency and the deviation in the Hardy-Weinberg equilibrium in the study of Broeckx et al. (2013) may be explained by a bias caused by the fact that most samples were selected for a study involving hip dysplasia and mostly concerned show animals. The highest frequency of EIC in Labrador retrievers is indeed present in show/conformation dogs (Minor et al., 2011). The authors recommend to routinely genotype Labrador retrievers used for breeding for EIC.

The causal mutation of PRCD has been identified in many breeds, including the Labrador retriever (Zangerl et al., 2006). A mutant allele frequency of 13.4% was found in the Belgian population tested (n = 134) (Table 3). There is no frequency data available in the literature as of yet. The high mutant allele frequency in the Labrador retriever may be explained by the late-onset of the defect, precluding selection against it. The authors encourage testing in order to (slowly) decrease the frequency.

GENERAL DISCUSSION AND CONCLUSION

No mutant allele was found for the X-linked traits (DMD-C, DMD-GR, DMD-R, XHED and XLMTM) which, as explained in part 1, may be due to their mode of inheritance.

The same logic should apply to dominant traits. Indeed, a similar conclusion may be reached for OI in the Golden retriever, an early-onset disorder with full penetrance. For the previously assumed partially dominant ARVC (Meurs et al., 2013) on the other hand, a high mutant allele frequency of 10.0% was found. However, Cattanach et al. (2015) recently showed that the *STRN* mutation is not the causal mutation, but is only linked to the disorder. They also suggested a very low penetrance, close to the 20.0-30.0% estimate seen in humans. This may partly explain the high frequency of the mutation.

The effectiveness of using a DNA-test to decrease the prevalence of hereditary disorders has been well demonstrated by CLAD and RCD1 in the Irish setter. About 15 years ago, a mutant allele frequency of 3.2% was found for CLAD in Belgium (Kijas et al., 2000) and 7.8% heterozygotes were found in the USA for RCD1 (Aguirre et al., 1999). The routine genotyping for these disorders has probably led to the very low frequency in Belgium (no mutant alleles were found in this study).

A significant deviation was found to frequencies described in other populations for some disorders. The mutant DM allele was not found in the Golden retriever, while it was found in two USA populations at a frequency of 3.0-3.3% (OFA; Zeng et al., 2014). The mutation causing GR-PRA 1 in the Golden retriever was found at a frequency of 7.1% in the Belgian population and at similar frequencies in other countries in Europe, but was not found in a USA population (Downs et al., 2011), suggesting the mutation originated in Europe. The mutant allele frequency of CNM in the Golden retriever was calculated at 0.7%, while larger percentages were previously found in Canada (q = 6.9%), the USA (q = 8.9%), the UK and Ireland and Continental Europe (q = 10.2%) (Maurer et al., 2012), proving that even within the same continent, there may be significant differences in occurrence.

Degenerative myelopathy was tested in the Bichon frise (q = 3.7%), the Bloodhound (q = 24.1%), the Boxer (q = 6.9%), the Irish setter (q = 0.0%) and the Labrador retriever (q = 0.0%) and RCD4 was tested in the Irish setter (q = 23.8%). These (fairly) high frequencies (except for DM in the Irish setter and Golden retriever) may be explained by the fact that both disorders are late onset. Often, affected animals have already sired offspring before they show clinical signs, thus spreading the mutation. Degenerative myelopathy is present in a lot of breeds (see part 1). The OFA has already found the causal mutation in 88 different dog breeds, one of which was a wolf hybrid.

A very high frequency was found for MTC in the Cavalier King Charles spaniel (q = 46.6%). The lack of symptoms may partly explain the high frequency. Another hypothesis is that the causal mutation is closely linked to a positive quality, thus selecting in favor of the MTC mutation, but this has yet to be tested.

For five disorders (DM in the Bloodhound, EFS and MTC in the Cavalier King Charles spaniel, RCD4 in the Irish setter and GR-PRA 1 in the Golden retriever), the results are in line with the frequency data reported in the literature. However, for the majority of the genotyped disorders, insufficient (DM in the Bichon frise), contradicting (EIC in the Labrador Retriever) or no frequency data (CN, EIC, ARVC, DMD-C, PRA, VWD1, DMD-R, DMD-GR, MPS I, OI, RDEB, PRCD, NA, XLMTM and HUU) was available, or the results did not correspond with the data found in the literature. This underscores the need of this type of studies to assist breeders in their endeavors to reduce the genetic load put on the populations by genetic diseases. Every year, more and more causal mutations are described. Frequency estimations for these new variants as well as follow-up studies on the frequency of variants already described are necessary in order to be able to further decrease the prevalence of genetic disorders.

Disorders with a frequency high enough to warrant routine genotyping in breeding programs are DM for the Bloodhound and Boxer, ARVC for Boxers, EFS for the Cavalier King Charles spaniel, RCD4 for the Irish setter, GR-PRA1 in the Golden retriever and EIC and PRCD in the Labrador retriever. Macrothrombocytopenia should be tested in all Cavalier King Charles spaniels. Heterozygotes for these mutations and even homozygotes for the less severe disorders should not be routinely excluded from breeding programs, since this may lead to an even more important decrease in genetic diversity of breeds with an already (relatively) small genetic basis. Instead, the results of the genotyping should be used to make a well-reasoned partner choice.
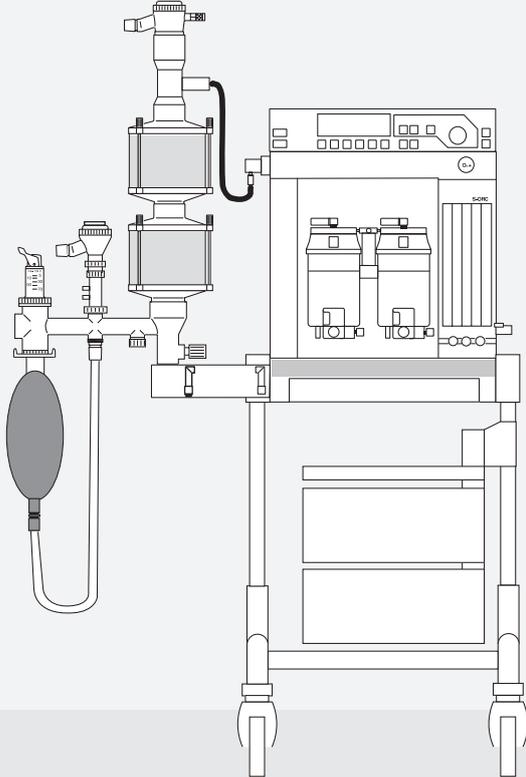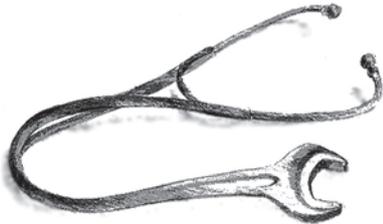
ACKNOWLEDGEMENTS

REFERENCES

Aguirre G.D., Baldwin V., Weeks K.M., Acland G.M., Ray K. (1999). Frequency of the codon 807 mutation in the cGMP phosphodiesterase beta-subunit gene in Irish setters and other dog breeds with hereditary retinal degeneration. *Journal of Heredity 90*, 143-147.

Ahonen S.J., Arumilli M., Lohi H. (2013). A CNGB1 Frameshift mutation in Papillon and Phalène dogs with progressive retinal atrophy. *Public Library of Science One 8(8)*, e72122.

Baldeschi C., Gache Y., Rattenholl A., Bouillé P., Danos O., Ortonne J.P., Bruckner-Tuderman L., Meneguzzi G. (2003). Genetic correction of canine dystrophic epider-molysis bullosa mediated by retroviral vectors. *Human Molecular Genetics 12(15)*, 1897-1905.

Beggs A.H., Böhm J., Snead E., Kozlowski M., Maurer M., Minor K., Childers M.K., Taylor S.M., Hitte C., Mickelson J.R., Guo L.T., Mizisin A.P., Buj-Bello A., Tiret L., Laporte J., Shelton G.D. (2010). MTM1 mutation associated with X-linked myotubular myopathy in Labrador retrievers. In: *Proceedings of the National Academy of Science of the United States of America 107*, 14697-14702.

Boudreaux M.K. (2012). Inherited platelet disorders. *Journal of Veterinary Emergency and Critical Care 22(1)*, 30-41.

Broeckx B.J., Coopman F., Verhoeven G.E., Van Haeringen W., van de Goor L., Bosmans T., Gielen I., Saunders J.H., Soetaert S.S., Van Bree H., Van Neste C., Van Nieuwerburgh F., Van Ryssen B., Verelst E., Van Steendam K., Deforce D. (2013). The prevalence of nine genetic disorders in a dog population from Belgium, the Netherlands and Germany. *Public Library of Science One 8*, e74811.

Calboli F.C., Sampson J., Fretwell N., Balding D.J. (2008). Population structure and inbreeding from pedigree analysis of purebred dogs. *Genetics 179*, 593–601.

Campbell B.G., Wootton J.A.M., MacLeod J.N., Minor R.R. (2000). Sequence of normal canine COL1A1 cDNA and identification of a heterozygous alpha 1(I) collagen Gly208Ala mutation in a severe case of canine osteogenesis imperfect. *Archives of Biochemistry and Biophysics 384*, 37-46.

Campbell B.G., Wootton J.A.M., Macleod J.N., Minor R.R. (2001). Canine COL1A2 mutation resulting in C-terminal truncation of pro-alpha 2(I) and severe osteogenesis imperfect. *Journal of Bone and Mineral Research 16*, 1147-1153.

Cattanach B.M., Dukes-McEwan J., Wotton P.R., Stephenson H.M., Hamilton R.M. (2015). A pedigree-based genetic appraisal of Boxer ARVC and the role of the Striatin mutation. *Veterinary Record 176(19)*, doi: 10.1136/vr.102821.

Dale D.C., Alling D.W., Wolff S.M. (1972). Cyclic hematopoiesis: the mechanism of cyclic neutropenia in Grey collie dogs. *Journal of Clinical Investigation 51*, 2197-2204.

Davis B., Toivio-Kinnucan M., Schuller S., Boudreaux M.K. (2008). Mutation in beta1-tubulin correlates with macrothrombocytopenia in Cavalier King Charles spaniels. *Journal of Veterinary Internal Medicine 22*, 540-545.

Downs L.M., Wallin-Håkansson B., Boursnell M., Marklund S., Hedhammar,Å., Truvé K., Hübinette L., Lindblad-Toh K., Bergström T., Mellersh C.S. (2011). A frameshift mutation in golden retriever dogs with progressive retinal atrophy endorses SLC4A3 as a candidate gene for human retinal degenerations. *Public Library of Science One 6*, e21452.

Downs L.M., Bell J.S., Freeman J., Hartley C., Hayward L.J., Mellersh C.S. (2013). Late-onset progressive retinal atrophy in the Gordon and Irish setter breeds is associated with a frameshift mutation in C2orf71. *Animal Genetics 44*, 169-177.

Foureman P., Whiteley M., Giger U. (2002). Canine leukocyte adhesion deficiency: Presence of the Cys36Ser beta-2 integrin mutation in an affected US Irish setter cross-breed dog and in US Irish red and white setters. *Journal of Veterinary Internal Medicine 16*, 518-523.

Gentilini F., Zambon E., Gandini G., Rosati M., Spadari A., Romagnoli N., Turba M.E., Gernone F. (2011). Frequency of the allelic variant of the PTPLA gene responsible for centronuclear myopathy in Labrador retriever dogs as assessed in Italy. *Journal of Veterinary Diagnostic Investigation 23*, 124-126.

Gill J.L., Tsai K.L., Krey C., Noorai R.E., Vanbellinghen J-F., Garosi L.S., Shelton G.D., Clark L.A., Harvey R.J. (2011). A canine BCAN microdeletion associated with episodic falling syndrome. *Neurobiology of Disease 45(1)*, 130-136.

Haworth K., Putt W., Cattanach B., Breen M., Binns M., Lingaas F., Edwards Y.H. (2001). Canine homolog of the T-box transcription factor T; failure of the protein to bind to its DNA target leads to a short-tail phenotype. *Mammalian Genome 12*, 212-218.

Hytönen M.K., Grall A., Hédan B., Dréano S., Seguin S.J., Delattre D., Thomas A., Galibert F., Paulin L., Lohi H., Sainio K., André C. (2009). Ancestral T-box mutation is present in many, but not all, short-tailed dog breeds. *Journal of Heredity 100(2)*, 236-240.

Jobling A.I., Ryan J., Augusteyn R.C. (2003). The frequency of the canine leukocyte adhesion deficiency (CLAD) allele within the Irish Setter population of Australia. *Australian Veterinary Journal 81*, 763-765.

Karmi N., Brown E.A., Hughes S.S., Mclaughlin B., Mellersh C.S., Biourge V., Bannasch D.L. (2010). Estimated frequency of the canine hyperuricosuria mutation in different dog breeds. *Journal of Veterinary Internal Medicine 24*, 1337-1342.

Kijas J.M.H., Bauer T.R., Gafvert S., Marklund S., Trowald-Wigh G., Johannisson A., Hedhammar A., Binns M., Juneja R.K., Hickstein D.D., Andersson L. (1999). A missense mutation in the beta-2 integrin gene (ITGB2) causes canine leukocyte adhesion deficiency. *Genomics 61*, 101-107.

Kijas J.M.H., Juneja R.K., Gafvert S., Andersson L. (2000). Detection of the causal mutation for canine leukocyte adhesion deficiency (CLAD) using pyrosequencing. *Animal Genetics 31*, 326-328.

Leroy G., Rognon X., Varlet A., Joffrin C., Verrier E. (2006). Genetic variability in French dog breeds assessed by pedigree data. *Journal of Animal Breeding and Genetics 123*, 1-9.

Lin L., Faraco J., Li R., Kadotani H., Rogers W., Lin X.Y., Qiu X.H., de Jong P.J., Nishino S., Mignot E. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell 98*, 365-376.

Maurer M., Mary J., Guillaud L., Fender M., Pelé M., Bilzer T., Olby N., Penderis J., Shelton G.D., Panthier J.J., Thibaud J.L., Barthélémy I., Aubin-Houzelstein G., Blot S., Hitte C., Tiret L., (2012). Centronuclear myopathy in Labrador retrievers: a recent founder mutation in the PTPLA gene has rapidly disseminated worldwide. *Public Library of Science One 7*, e46408.

McGraw R.A., Carmichael K.P. (2006). Molecular basis of globoid cell leukodystrophy in Irish setters. *The Veterinary Journal 171(2)*, 370-372.

McGreevy P.D., Nicholas F.M. (1999). Some practical solutions to welfare problems in dog breeding. *Animal Welfare 8*, 329-341.

Menon K.P., Tieu P.T., Neufeld E.F. (1992). Architecture of the canine IDUA gene and mutation underlying canine mucopolysaccharidosis I. *Genomics 14(3)*, 763-768.

Meurs K.M., Stern J.A., Sisson D.D., Kittleson M.D., Cunningham S.M., Ames M.K., Atkins C.E., Defrancesco T., Hodge T.E., Keene B.W., Reina Doreste Y., Leuthy M., Motsinger-Reif A.A., Tou S.P. (2013). Association of dilated cardiomyopathy with the striatin mutation genotype in Boxer dogs. *Journal of Veterinary Internal Medicine 27*, 1437-1440.

Minor K.M., Patterson E.E., Keating M.K., Gross S.D., Ekenstedt K.J., Taylor S.M., Mickelson J.R. (2011). Presence and impact of the exercise-induced collapse associated DNM1 mutation in Labrador Retrievers and other breeds. *The Veterinary Journal 189*, 214-219.

Moura E., Cirio M. (2004). Clinical and genetic aspect of X-linked ectodermal dysplasia in the dog – a review including three new spontaneous cases. *Veterinary Dermatology 15*, 269-277.

Orthopedic Foundation for Animals (s.d.). Statistics and data. Available at: http://www.offa.org/stats.html#breed (Accessed 08 September 2015).

Palazzi X., Marchal T., Chabanne L., Spadafora A., Magnol J.P., Meneguzzi G. (2000). Inherited dystrophic epidermolysis bullosa in inbred dogs: a spontaneous animal model for somatic gene therapy. *Journal of Investigative Dermatology 115*, 135-137.

Patterson E.E., Minor K.M., Tchernatynskaia A.V., Taylor S.M., Shelton G.D., Ekenstedt K.J. Mickelson J.R. (2008). A canine DNM1 mutation is highly associated with the syndrome of exercise-induced collapse. *Nature Genetics 40*, 1235-1239.

Pelé M., Tiret L., Kessler J.L., Blot S., Panthier J.J. (2005). SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. *Human Molecular Genetics 14*, 1417-1427.

Pfeiffer I., Brenig B. (2005). Frequency of the canine leucocyte adhesion deficiency (CLAD) mutation among Irish red setters in Germany. *Journal of Animal Breeding and Genetics 122*, 140-142.

Sharp N.J.H., Kornegay J.N., Vancamp S.D., Herbstreith M.H., Secore S.L., Kettle S., Hung W.Y., Constantinou C.D., Dykstra M.J., Roses A.D., Bartlett R.J. (1992). An error in dystrophin messenger rna processing in Golden retriever Muscular dystrophy, an animal homologue of Duchenne muscular dystrophy. *Genomics 13*, 115-121.

Suber M.L., Pittler S.J., Qin N., Wright G.C., Holcombe V., Lee R.H., Craft C.M., Lolley R.N., Baehr W., Hurwitz R.L. (1993). Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta- subunit gene. In: *Proceedings of the National Academy of Sciences 90*, 3968-3972.

Walmsley G.L., Arechavala-Gomeza V., Fernandez-Fuente M., Burke M.M., Nagel N., Holder A., Stanley R., Chandler K., Marks S.L., Muntoni F., Shelton G.D., Piercy R.J. (2010). A duchenne muscular dystrophy gene hot spot mutation in dystrophin-deficient Cavalier King Charles spaniels is amenable to exon 51 skipping. *Public Library of Science One 5*, e8647.

Winand N.J. (1994). *Molecular genetic characterization of spontaneously occurring animal models of Duchenne muscular dystrophy*. PhD dissertation.

Wijnrocx K., Janssens S., Buys N. (2012). Inteelt en genetische diversiteit van 23 populaties van honden in belgië op basis van afstammingsgegevens van de KMSH. https://lirias.kuleuven.be/bitstream/123456789/358026/3/RapportInteeltGenetischeDiversiteitHondenBelgië.pdf

Zangerl B., Goldstein O., Philp A.R., Lindauer S.J., Pearce-Kelling S.E., Mullins R.F., Graphodatsky A.S., Ripoll D., Felix J.S., Stone E.M., Acland G.M., Aguirre G.D. (2006). Identical mutation in a novel retinal gene causes progressive rod-cone degeneration in dogs and retinitis pigmentosa in humans. *Genomics 88*, 551-563.

Zeng R., Coates J.R., Johnson G.C., Hansen L., Awano T., Kolicheski A., Ivansson E., Perloski M., Lindblad-Toh K., O'Brien D.P., Guo J., Katz M.L., Johnson G.S. (2014). Breed distribution of SOD1 alleles previously associated with canine degenerative myelopathy. *Journal of Veterinary Internal Medicine 28*, 515-521.