COMPANION OR PET ANIMALS

Genetic screening and mutation identification in a rare canine breed, the Drentsche patrijshond

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SUMMARY

The Drentsche patrijshond (Drent) is a relatively rare breed that is used as a versatile hunting dog. Although inherited diseases occur in this breed, no genetic mutations were previously known to contribute to these inherited disorders. Thirteen Drents were screened for 142 known disease-associated mutations that occur in domestic dogs. Of these, two mutations were identified to segregate in three pedigrees: mutations for hyperuricosuria and von Willebrand disease type 1. This information can be used to screen Drents before breeding to improve the health of the breed and to avoid producing affected dogs and emphasises the importance of genetic screening for inherited diseases in rare breeds.

BACKGROUND

There are >400 recognised breeds of the domestic dog, *Canis familiaris*, which show some of the greatest morphological differences within a species. These differences, for example, in skull shape, coat colour, leg length and behaviours, likely have significant genetic components and human beings have artificially selected for these genetic traits during breed identification for thousands of years.

Advances in veterinary medicine have increased the longevity of many breeds of domesticated dogs. In doing so, late onset diseases that were previously passed unnoticed from parent to offspring have the opportunity to manifest. Advances in molecular techniques and the sequencing of the canine genome (Lindblad-Toh and others 2005) allow for the identification of causal mutations and for the development of diagnostic tests that can be used to screen dogs before breeding. These advances in veterinary medicine and genomic diagnostics can be used to improve the overall health of a breed and to guide breeders in the choice of mating pairs to avoid producing carriers and affected dogs.

More than 150 mutations have been identified that contribute to clinical diseases of dogs (Nicholas and Hobbs 2014); many of which have been identified in the most popular breeds, such as Labrador retrievers and golden retrievers. Given that all breeds carry recessive mutations, rare breeds, such as the Drentsche patrijshond, have fewer numbers of available breeding dogs and are therefore more susceptible to inheriting genetic conditions simply because of population constraints. Identifying the breed-specific risks for disease is crucial to implementing any breeding programme aimed at controlling inherited disease transmission.

Through the genetic screening of known disease-causing mutations in domestic dogs, the goal of this study was to identify mutations in the Drentsche patrijshond that could be screened for in breeding programmes and used in the development of health guidelines of the Drentsche Patrijshond Club of North America. The Drentsche patrijshond, also known as the Dutch partridge dog or Drent, is a breed originating from the province of Drenthe in the Netherlands. Developed in the 17th century, the closest relatives to the Drents are believed to be the Epagneul Français and the small Münsterländer (O'Connor 2014). The Dutch Kennel Club officially recognised the Drentsche patrijshond as a breed in 1943. There are currently about 5000 Drents worldwide and 120 Drents in North America, with the majority of these imported from the Netherlands (O'Connor 2014).

CASE PRESENTATION

Sample collection and DNA extraction

Buccal samples were collected from 13 dogs attending the annual meeting of the Drentsche Patrijshond Club of North America. All dogs were known to be healthy at the time of sample collection. Three buccal swabs were used for collection on each dog. DNA was extracted using the Archive Pure DNA Tissue Kit (5Prime, Gaithersburg, Maryland, USA) using the 5Prime buccal brush protocol according to the manufacturer's specifications with the only exception to the protocol being that three swabs were used in a single extraction instead of one and that all reagent volumes were doubled. DNA concentrations were determined using a Nanodrop ND-2000 spectrophotometer (NanoDrop, Wilmington, Delaware, USA).

DNA analysis

For each of the 142 potential mutations, the DNA from each dog was analysed either by sequencing or by a PCR-based molecular method. Depending on whether one groups diseases based on clinical presentation, causative gene or that the diseases share the same mutation, these mutations represent approximately 115 different disorders. Of these, eight are autosomal dominant, nine are X-linked recessive, two are mitochondrial and the remaining disorders are autosomal recessive in mode of inheritance. Table 1 shows the routine molecular method used for each of the 142 disease-associated mutations.

INVESTIGATIONS

DNA sequencing

DNA sequence analysis was performed using an Illumina MiSeq DNA Sequencer (Illumina, San Diego,

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TABLE 1: Genotyping methods used for each mutation tested in the Drentsche patrijshond

Disease	Affected gene	Mutation	Sequencing	PCR-based molecular assay
Alaskan husky encephalopathy	SLC19A3	4 bp insertion (ins TTGC); C>A	√	
Alaskan Malamute polyneuropathy	NDRG1	G>T	√	
Amelogenesis imperfecta	ENAM	5 bp deletion (del TTTCC)	√	
Anhidrotic ectodermal dysplasia	EDA	G>A	√	
Benign familial juvenile epilepsy	LGI2	A>T	√	
Canine multiple system degeneration (Kerry blue terrier type)	SERAC1	C>T	√	
Canine multiple system degeneration (Chinese crested type)	SERAC1	4 bp deletion (del TTAC)	√	
Centronuclear myopathy	PTPLA	236 bp insertion; 13 bp duplication (dup CACACAAAGGTTT)		Size analysis
Cerebellar ataxia (Finnish hound type)	SEL1L	T>C	√	
Chondrodysplasia	ITGA10	C>T	√	
Coagulation factor VII deficiency	F7	G>A	√	
Collie eye anomaly	NHEJ1	~7.8 kb deletion	√	
Complement 3 deficiency	C3	1 bp deletion (del C)	√	
Cone degeneration (Australian shepherd/Siberian husky type)	CNGB3	~400 kb deletion	√	
Cone degeneration (German shorthaired pointer type)	CNGB3	G>A	√	
Congenital hypothyroidism with goitre	TPO	C>T	√	
Congenital macrothrombocytopenia	TUBB1	G>A	√	
Congenital myasthenic syndrome	CHAT	G>A	√	
Congenital stationary night blindness	RPE65	4 bp deletion (del AAGA)	√	
Copper storage disease	COMMD1	~39.7 kb deletion	√	
Curly coat dry eye syndrome	FAM83H	1 bp deletion (del C)	√	
Cystinuria (Labrador retriever type)	SLC3A1	1 bp deletion (del G)		Restriction digest
Cystinuria (Australian cattle dog type)	SLC3A1	6 bp deletion (del ACCACC)	√	
Cystinuria (Newfoundland type)	SLC3A1	C>T	√	
Degenerative myelopathy	SOD1	G>A	√	
Dilated cardiomyopathy	PDK4	16 bp deletion (del GTATCCTTTCAACCCA)		Restriction digest
Dilute (D-locus) coat colour	MLPH	G>A	√	
Dystrophic epidermolysis bullosa	COL7A1	G>A	√	
Early retinal degeneration	STK38L	229 bp insertion; 15 bp duplication (dup GGAAACAGAGTTCTT)		Size analysis
Elliptocytosis	SPTB	C>T	√	
Epidermolytic hyperkeratosis	KRT10	G>T	√	
Episodic falling syndrome	BCAN	~15.7 kb deletion, 5 bp insertion (ins AAGGC)	√	
Exercise-induced collapse	DNM1	G>T	√	
Familial nephropathy (Cocker spaniel type)	COL4A4	A>T	√	
Familial nephropathy (English springer spaniel type)	COL4A4	C>T	√	
Fanconi syndrome	FAN1	317 bp deletion	√	
Gall bladder mucoceles	ABCB4	1 bp insertion (ins G)	√	
Glanzmann's thrombasthenia (Great Pyrenees type)	ITGA2B	14 bp duplication (dup GGTGCCACAGACAT)	√	
Glanzmann's thrombasthenia (Otterhound type)	ITGA2B	G>C	√	
Globoid cell leukodystrophy (Irish setter type)	GALC	78 bp insertion; 16 bp duplication (dup GATTTTAGCACTTTAA)	√	
Globoid cell leukodystrophy (terrier type)	GALC	A>C	√	
Glycogen storage disease Ia	G6PC	G>C	√	
Glycogen storage disease VII (Wachtelhund type)	PFKM	C>T	√	
Glycogen storage disease VII (Cocker spaniel/whippet type)	PFKM	G>A	√	
GM1 gangliosidosis (Portuguese water dog type)	GLB1	G>A	√	
GM1 gangliosidosis (Shiba Inu type)	GLB1	1 bp deletion (del C)	√	
GM1 gangliosidosis (Alaskan husky type)	GLB1	19 bp duplication (dup TCCCAGACTTGCCCCAGGA)		Surveyor
GM2 gangliosidosis (Japanese chin type)	HEXA	G>A	√	
GM2 gangliosidosis (Poodle type)	HEXB	1 bp deletion (del G)	√	
Golden retriever muscular dystrophy	DMD	A>G	√	
Harlequin coat colour	PSMB7	T>G	√	
Hemophilia A	F8	G>A	√	
Hemophilia B (Cairn terrier type)	F9	G>A	√	
Hemophilia B (Rhodesian ridgeback type)	F9	G>A	√	

Continued

TABLE 1: Continued

Disease	Affected gene	Mutation	Sequencing	PCR-based molecular assay
Hemophilia B (Lhasa Apso type)	F9	5 bp deletion (del GCACC); C>T	√	
Hereditary nasal parakeratosis	SUV39H2	T>G	√	
Hereditary nephritis	COL4A5	G>T	√	
Hyperuricosuria	SLC2A9	G>T	√	
Ichthyosis	PNPLA1	3 bp deletion (del ACC), 8 bp insertion (ins TACTACTA)	√	
Inherited myopathy of great danes	BIN1	A>G	√	
Intestinal cobalamin malabsorption	CUBN	1 bp deletion (del C)	√	
L-2-hydroxyglutaric aciduria	L2HGDH	C>T; T>C	√	
Late onset ataxia	CAPN1	G>A	√	
Leucocyte adhesion deficiency, type I	ITGB2	G>C		Restriction digest
Leucocyte adhesion deficiency, type III	FERMT3	12 bp insertion (ins AAGACGGCTGCC)	√	
May-Hegglin anomaly	MYH9	G>A	√	
Mucopolysaccharidosis I	IDUA	G>A		Restriction digest
Mucopolysaccharidosis IIIA (wiredhaired dachshund type)	SGSH	3 bp deletion (del CCA)	√	
Mucopolysaccharidosis IIIA (New Zealand huntaway type)	SGSH	1 bp insertion (ins A)	√	
Mucopolysaccharidosis VII (Brazilian terrier type)	GUSB	C>T	√	
Mucopolysaccharidosis VII (German shepherd dog type)	GUSB	G>A	√	
Multidrug resistance 1	ABCB1	4 bp deletion (del AGAT)	√	
Multifocal retinopathy 1	BEST1	C>T	√	
Multifocal retinopathy 2	BEST1	G>A	√	
Multifocal retinopathy 3	BEST1	1 bp deletion (del C)	√	
Musladin-Lueke syndrome	ADAMTSL2	C>T	√	
Myotonia congenita (Australian cattle dog type)	CLCN1	1 bp insertion (ins A)	√	
Myotonia congenita (miniature schnauzer type)	CLCN1	C>T	√	
Myotubular myopathy 1	MTM1	C>A	√	
Narcolepsy (dachshund type)	HCRTR2	G>A	√	
Narcolepsy (dobermann type)	HCRTR2	226 bp insertion	√	
Narcolepsy (Labrador retriever type)	HCRTR2	G>A	√	
Neonatal ataxia	GRM1	62 bp insertion (ins AAGACCCGCAACGT$(A)_{48}$); 14 bp duplication (AAGACCCGCAACGT)	√	
Neonatal cerebellar cortical degeneration	SPTBN2	8 bp deletion (del TCAAGGCA)	√	
Neonatal encephalopathy with seizures	ATF2	T>G	√	
Neuroaxonal dystrophy	MFN2	3 bp deletion (del GGA)	√	
Neuronal ceroid lipofuscinosis 1	PPT1	1 bp insertion (ins C)	√	
Neuronal ceroid lipofuscinosis 10	CTSD	G>A	√	
Neuronal ceroid lipofuscinosis 2	TPP1	1 bp deletion (del C)	√	
Neuronal ceroid lipofuscinosis 4A	ARSG	G>A	√	
Neuronal ceroid lipofuscinosis 5	CLN5	C>T	√	
Neuronal ceroid lipofuscinosis 6	CLN6	T>C	√	
Neuronal ceroid lipofuscinosis 8	CLN8	T>C	√	
Osteochondrodysplasia	SLC13A1	~130 kb deletion	√	
Osteogenesis imperfecta (dachshund type)	SERPINH1	T>C	√	
Osteogenesis imperfecta (beagle type)	COL1A2	4 bp deletion (del CTGA), 9 bp insertion (ins TGTCATTGG)	√	
Osteogenesis imperfecta (Golden retriever type)	COL1A1	G>C	√	
P2RY12 receptor platelet disorder	P2RY12	3 bp deletion (del CTC)	√	
Persistent Müllerian duct syndrome	AMHR2	C>T	√	
Polycystic kidney disease	PKD1	G>A		Restriction digest
Polyneuropathy	NDRG1	10 bp deletion (del TCGCCTGGAC)	√	
Pompe disease	GAA	G>A	√	
Prekallikrein deficiency	KLKB1	T>A	√	
Primary ciliary dyskinesia	CCDC39	C>T	√	
Primary hyperoxaluria	AGXT	G>A; G>A	√	
Primary lens luxation	ADAMTS17	G>A	√	
Primary open angle glaucoma	ADAMTS10	G>A	√	
Progressive retinal atrophy (Basenji type)	SAG	T>C	√	
Progressive retinal atrophy (Mastiff type)	RHO	C>G	√	

Continued

TABLE 1: Continued

Disease	Affected gene	Mutation	Sequencing	PCR-based molecular assay
Progressive retinal atrophy (Irish setter type)	PDE6B	G>A	√	
Progressive retinal atrophy (Sloughi type)	PDE6B	8 bp insertion (ins TGAAGTCC)	√	
Progressive retinal atrophy (corgi/Chinese crested type)	PDE6A	1 bp deletion (del A)	√	
Progressive retinal atrophy, cone-rod dystrophy	NPHP4	180 bp deletion	√	
Progressive retinal atrophy, cone-rod dystrophy 3	ADAM9	40 kb deletion	√	
Progressive retinal atrophy, cone-rod dystrophy 4	RPGRIP1	44 bp insertion (ins $(A)_{29}$GGAAGCAACAGGATG) or 59 bp insertion (ins $(A)_{44}$GGAAGCAACAGGATG)		Size Analysis
Progressive retinal atrophy, PRA1	CNGB1	1 bp deletion (del A); 6 bp insertion (ins AGCTAC)	√	
Progressive retinal atrophy, progressive rod-cone degeneration	PRCD	G>A	√	
Pyruvate dehydrogenase deficiency	PDP1	C>T	√	
Pyruvate kinase deficiency (Basenji type)	PKLR	1 bp deletion (del C)	√	
Pyruvate kinase deficiency (Labrador retriever type)	PKLR	C>T	√	
Pyruvate kinase deficiency (pug type)	PKLR	T>C	√	
Pyruvate kinase deficiency (terrier type)	PKLR	6 bp insertion (ins AAGACC or ACCAAG)	√	
Renal cystadenocarcinoma and nodular dermatofibrosis	FLCN	A>G	√	
Sensory ataxic neuropathy	tRNA-Tyr	1 bp deletion (del A)	√	
Severe combined immunodeficiency disease (terrier type)	PRKDC	G>T	√	
Severe combined immunodeficiency disease (Wetterhoun type)	RAG1	G>T	√	
Severe combined immunodeficiency disease, X-linked (Basset hound type)	IL2RG	4 bp deletion (del CCTC)	√	
Severe combined immunodeficiency disease, X-linked (corgi type)	IL2RG	1 bp insertion (ins C)	√	
Spinocerebellar ataxia	KCNJ10	C>G	√	
Spongiform leucoencephalomyelopathy	CYTB	G>A	√	
Startle disease	SLC6A5	~4.2 kb deletion	√	
Subvalvular aortic stenosis	PICALM	3 bp insertion (ins CTC)	√	
Thrombopathia (American Eskimo dog type)	RASGRP1	1 bp insertion (ins A)	√	
Thrombopathia (Basset hound type)	RASGRP1	3 bp deletion (del TCT)	√	
Thrombopathia (Newfoundland type)	RASGRP1	C>T	√	
Trapped neutrophil syndrome	VPS13B	4 bp deletion (del GTTT)	√	
Von Willebrand disease type 1	VWF	G>A	√	
Von Willebrand disease type 2	VWF	A>G	√	
Von Willebrand disease type 3 (Kooikerhondje type)	VWF	G>A	√	
Von Willebrand disease type 3 (Shetland sheepdog type)	VWF	1 bp deletion (del T)	√	
Von Willebrand disease type 3 (Scottish terrier type)	VWF	1 bp deletion (del C)	√	

California, USA). Target enrichment and bar code library construction were performed as described by Bourgon and others (2014) using the Fluidigm Access Array system (Fluidigm Corporation, San Francisco, California, USA). Briefly, 100 ng of sample gDNA was preamplified in a multiplexed reaction with 351 primer pairs to enrich for the target regions. Each sample was subsequently amplified in a 48.48 Access Array Integrated Fluidic Circuit with target-specific primer pairs tagged with common sequence tags. PCR products from each sample were then harvested using the Access Array platform. A final PCR was carried out to introduce sample-specific bar codes and Illumina sequencing adaptors. The 48 sample bar coded amplicon libraries were pooled, purified and quantified. Paired-end sequencing (2×250 bp) of 7.6 pM pooled libraries with 1 per cent PhiX control library was performed on an Illumina MiSeq DNA Sequencer. MiSeq sequencing reads were processed by MiSeq Reporter (V.2.2.29) which uses the Burrows-Wheeler Alignment (BWA) to align the reads to the Canine Genome (CanFam3.1/canFam3) and the Genome Analysis Toolkit for variant calling. FASTA Quality file format files were processed in a second alignment using the BWA Tool, BWA-MEM (Li 2014) for the analysis of larger indels and unaligned reads.

PCR-based size analysis and enzymatic digestion

In some cases, additional PCR-based assays were employed to detect wild-type versus mutant alleles by size analysis, restriction enzymes or Surveyor enzymatic digestion assays. PCR primers were designed to flank each mutation and produce an amplicon of ~100–600 bp. Standard PCR was performed using the Qiagen Multiplex PCR Kit (Qiagen, Valencia, California, USA) according to the manufacturer's protocol, except denaturation was performed for 15 seconds at 95°C and annealing at 60°C.

PCR products were visualised on 2 or 4 per cent agarose gels or on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, USA).

Size analysis allowed for the detection of insertions or deletions of 4 to ~500 bp through standard PCR amplification and gel electrophoresis techniques. Restriction enzymes were used to digest PCR products where a mutation, typically a single nucleotide polymorphism (SNP) or small insertion, created or abolished a restriction enzyme recognition site. In these instances, PCR-amplified products were digested with a restriction enzyme specific to either the wild-type or mutant alleles, resulting in differing fragment patterns when visualised on a gel or on the Agilent 2100 Bioanalyzer. Finally, some genotypes were determined using the Surveyor endonuclease (Transgenomic, Gaithersburg, Maryland, USA) that specifically cleaves mismatched bp in DNA heteroduplexes, including single-base substitutions, deletions and insertions (Shi and others 2007). Surveyor was used to detect SNPs and small insertions or deletions where a restriction enzyme could not be employed. PCR for Surveyor was performed using the Roche FastStart High Fidelity PCR System (Roche, Basel, Switzerland) with the following reagent concentrations: 1x FastStart High Fidelity Reaction Buffer with $MgCl_2$, 5 per cent dimethyl sulfoxide, 200 µM PCR Grade Nucleotide Mix, and 0.1 U/µl FastStart High Fidelity Enzyme Blend. Surveyor hybridisation and digestion were performed according to the manufacturer's protocol, except that 2 µl of endonuclease was used during digestion. Surveyor products were visualised using an Agilent 2100 Bioanalyzer.

Von Willebrand factor (VWF) analysis

Blood was drawn and plasma was prepared on dogs 617 and 665 according to instructions provided by the Animal Health Diagnostic Center (Cornell University, Ithaca, New York, USA). Plasma was shipped to the diagnostic laboratory and VWF antigen was measured using an ELISA.

Pedigree analysis

Pedigree analysis was used to identify obligate carriers that were not available for testing.

OUTCOME AND FOLLOW-UP

Mutation analysis results

Each of the 13 dogs was screened for 142 disease-associated mutations known to occur in various breeds of domesticated dogs (Table 1). Of the 142 mutations screened, mutations for hyperuricosuria and von Willebrand disease type 1 were identified in multiple dogs studied (Table 2). By mutation analysis, three dogs carried one copy of the mutation in *SLC2A9* associated with hyperuricosuria and four dogs carried one copy of the mutation in *VWF* associated with von Willebrand disease 1 (vWD1) while three dogs were homozygous for the mutant allele and at-risk for vWD1. All dogs were homozygous for the wild-type allele in the remaining 140 mutations.

VWF analysis results

Dogs 617 and 665 showed abnormally low results (21 and 19 per cent as compared with a 100 per cent standard, respectively), consistent with the homozygous mutation of VWF found in these dogs. The normal reference interval for this assay is 70–180 per cent.

TABLE 2: Genotyping results for the 13 Drentsche patrijshond studied for von Willebrand disease and hyperuricosuria

Sample number	Hyperuricosuria	von Willebrand disease, type 1	Interpretation
617	WT/WT	M/M	At risk for vWD1
618	WT/M	WT/WT	Carrier for HU
619	WT/M	WT/M	Carrier for both HU and vWD1
620	WT/WT	WT/M	Carrier for vWD1
621	WT/WT	WT/WT	
622	WT/WT	WT/WT	
623	WT/WT	WT/M	Carrier for vWD1
624	WT/WT	WT/WT	
625	WT/WT	WT/WT	
626	WT/WT	WT/WT	
627	WT/WT	M/M	At risk for vWD1
628	WT/M	WT/M	Carrier for both HU and vWD1
665	WT/WT	M/M	At risk for vWD1

HU, hyperuricosuria; M, mutant; vWD1, von Willebrand disease type 1; WT, wild type

Pedigree analysis results

Pedigree analysis can be used to identify obligate carriers that are not available for testing. An obligate carrier is defined as an individual who must carry the mutation based on examination of the pedigree for which offspring are available and have been studied. In certain cases, offspring that have not been tested may also be deemed obligate carriers if they are offspring of an at-risk individual (who possesses two copies of the mutation). In clinical practice, pedigree analysis can also be used to identify dogs that are at an increased risk of being a carrier, based on their parents' status and should consider molecular testing to determine their genotype.

In this study, pedigree analysis was used to identify obligate carriers among the dogs not available for testing. In this process, the authors identified the following dogs as obligate carriers: dogs I.1, I.2, II.1 and II.4 for vWD1 (Fig 1a) and dog II.5 for hyperuricosuria (Fig 1a). In addition, both the dam and sire to dogs 665 and 627 carry at least one copy of the vWD1 mutation and at least the dam or sire of 620 and 623 carry one or two copies of the vWD1 mutation (Fig 1b, c). Pedigree analysis does not allow one to distinguish carriers from affected individuals.

DISCUSSION

The Drents have been documented to be predisposed to a few hereditary diseases, some of which occur in other breeds with known mutations, including progressive retinal atrophy, distichiasis, entropion and ectropion, persistent pupillary membranes, retinal dysplasia and von Willebrand disease (O'Connor 2014). Familial stomatocytosis-hypertrophic gastritis, has also been identified in a group of closely related Drents (Slappendel and others 1991). In addition, several disorders that likely have a genetic component are found in Drents including hip dysplasia, hypothyroidism, absent teeth and cryptorchidism (O'Connor 2014). Genetic mutations have not been published for any of these conditions in Drents.

The authors screened 13 Drents for 142 mutations in genes known to cause disease in other breeds. The authors identified five carriers (dogs 618, 619, 620, 623 and 628) by mutation analysis and at least five carriers by pedigree analysis (dogs I.1,

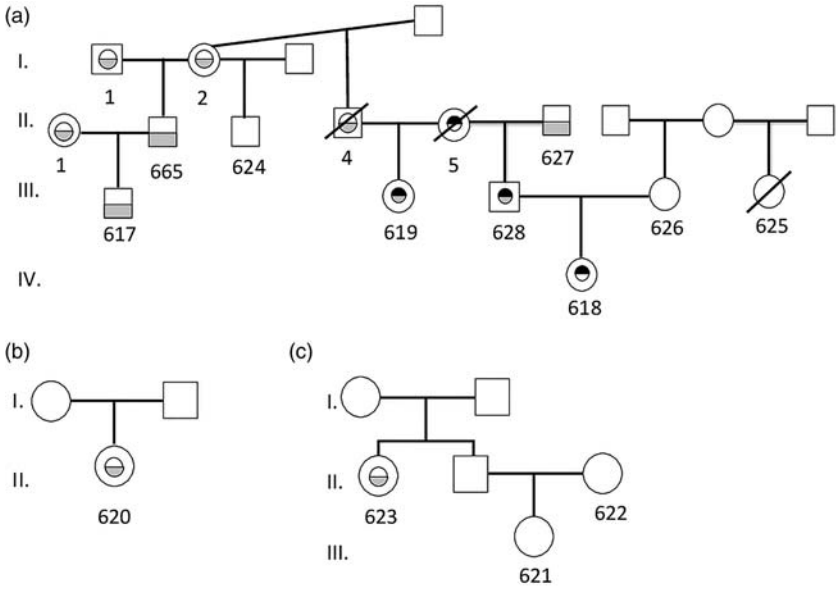

FIG 1: Black shaded areas indicate one or two copies of the mutation associated with hyperuricosuria. Grey shaded areas indicate one or two copies of the mutation associated with von Willebrand disease type 1. Internal circles indicate carrier dog

I.2, II.1, II.4 and II.5). Among these, four dogs from a related pedigree were identified to be carriers of hyperuricosuria (Fig 1a) and eight dogs from three pedigrees were found to be carriers of von Willebrand disease type 1 (Fig 1a–c). Two dogs (619 and 628) were carriers of both diseases. In addition, three dogs (617, 627 and 665), from one pedigree, were found to have two copies of the mutation for von Willebrand disease type 1 and are considered at-risk and expected to have low amounts of VWF as typically associated with this disease. Analysis of plasma from dogs 617 and 665 showed abnormally low VWF antigens (21 and 19 per cent as compared with a 100 per cent standard, respectively; normal reference interval 70–180 per cent). Dogs with antigen measurements of <50 per cent are considered affected and may show bleeding tendencies. The clinical history on dog 665, as reported by his owner, has not shown any abnormal bleeding episodes. Dog 627 was not available for additional analyses of the VWF antigen. No dogs were found with two copies of the hyperuricosuria mutation.

The mutation in the gene *SLC2A9* associated with hyperuricosuria has been reported in a large number of breeds (Karmi and others 2010), but not previously reported in the Drents. The *SLC2A9* gene codes for a protein that allows the kidneys to reabsorb uric acid from the glomerular filtrate. Dogs with mutations in both copies of the *SLC2A9* gene are predisposed to having elevated levels of uric acid in the urine (Bannasch and others 2008). The disease is characterised by the formation of urate crystals and uroliths in the urinary tract.

Because uroliths in the bladder predispose dogs to cystitis, dogs with hyperuricosuria most commonly present with clinical signs of recurrent urinary tract inflammation. They may also have non-specific clinical signs related to renal insufficiency or cystitis. More seriously, uroliths can cause blockage of the urethra especially in males. When urethral blockage occurs, it is an emergency situation. Dog owners who have screened for the genetic mutation associated with hyperuricosuria and know that their dog is at risk for urolith formation are more prepared to recognise signs of cystitis or urethral blockage and take appropriate measures. Dogs with hyperuricosuria can also be placed on a diet low in protein and purines to help prevent stone formation.

The various types of vWD are the most common inherited bleeding disorders of dogs. Three types have been identified based on quantitative and structural defects in VWF: type 1, type 2 and type 3. In addition to having different mutations in VWF, these three types vary in severity with type 1 being the mildest and type 3 being the most severe. In this study, the Drents were found to carry the mutation for type 1 vWD, previously identified in several breeds of dog (Brooks and others 1992, 2001). Type 1 vWD is characterised by abnormally low amounts of VWF protein which is necessary for primary hemostasis. Dogs with vWD1 are at risk for prolonged bleeding, which is most commonly noticed as minor bleeding of the gums when puppies lose their primary teeth, but can be severe after trauma or surgery. Knowing that a dog is at risk for bleeding diathesis allows the owner and veterinarian to recognise the risk of excessive bleeding during routine surgery or after traumatic events and to prepare appropriately.

Limitations

The Drent is a very rare breed in North America with only about 120 dogs known. The authors recognise that the sample size of 13 related dogs is small (roughly 10 per cent of the available population) and that rare mutations will not be identified. In addition, although 142 mutations were tested, mutations not screened for will not be uncovered. Nonetheless, using this small sample size, the authors were able to identify two common mutations in the breed that can be used for further screening and breeding strategies.

Conclusions

Drent breeders can use the information gained in this study to direct their genetic testing and choose appropriate breeding pairs. Results of such genetic screening will inform breeders of the Drentsche patrijshond about the risks of either hyperuricosuria or vWD1 in their dams, sires and puppies. In some breeding programmes, breeders conduct line breeding: mating parents to siblings, grandfather to granddaughter, half brother to half or full sister or uncle to niece. As the pedigree in Fig 1a demonstrates, line breeding of certain dogs within this pedigree is likely to produce dogs affected with hyperuricosuria and/or vWD1. Knowing the genotypes for specific mutations in potential breeding dogs allows for informed decisions, and avoidance of producing affected puppies, within a breeding programme. This study also illustrates the use of high throughput screening

methodologies in the identification of mutations in breeds not previously shown to carry specific diseases and that might not have been considered at risk for certain diseases based on breed history alone.

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