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The prevalence of *ABCB1:c.227_230delATAG* mutation in affected dog breeds from European countries

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ABSTRACT

Deletion of 4-base pairs in the canine *ABCB1* (*MDR1*) gene, responsible for encoding P-glycoprotein, leads to nonsense frame-shift mutation, which causes hypersensitivity to macrocyclic lactones drugs (e.g. ivermectin). To date, at least 12 purebred dog breeds have been found to be affected by this mutation. The aim of this study was to update information about the prevalence of *ABCB1* mutation (c.227_230delATAG) in predisposed breeds in multiple European countries. This large scale survey also includes countries which were not involved in previous studies. The samples were collected in the period from 2012 to 2014. The overview is based on genotyping data of 4729 individuals. The observed mutant allele frequencies were 58.5% (Smooth Collie), 48.3% (Rough Collie), 35% (Australian Shepherd), 30.3% (Shetland Sheepdog), 28.1% (Silken Windhound), 26.1% (Miniature Australian Shepherd), 24.3% (Longhaired Whippet), 16.2% (White Swiss Shepherd) and 0% (Border Collie). The possible presence of an *ABCB1* mutant allele in Akita-Inu breed has been investigated with negative results. This information could be helpful for breeders in optimization of their breeding strategy and for veterinarians when prescribing drug therapy for dogs of predisposed breeds.

The canine multidrug resistance gene *ABCB1* codes for the cellular P-glycoprotein (P-gp) which belongs to the superfamily of ABC transporters (Geyer and Janko, 2012; Martinez et al., 2008). P-gp utilizes energy from ATP hydrolysis to transport structurally unrelated drugs, toxins, xenobiotics, and other macromolecules, including drugs used in veterinary medicine out of the cell against the concentration gradient (Geyer and Janko, 2012; Martinez et al., 2008; Merola and Eubig, 2012; Sharom, 2006, 2011). The primary function of P-gp is preventing the uptake of toxic compounds from the gut into the body and expel them in the bile or urine and thus protect some very sensitive organs, such as the brain (Hennessy and Spiers, 2007). Moreover, expression of P-glycoprotein at the luminal membranes of endothelial cells of brain capillaries (the blood-brain barrier) prevents entry of certain drugs into the central nervous system by an efflux based transport mechanism (Fromm, 2004). P-gp structure and localization is highly conserved during evolution (Leslie et al., 2005). The expression was observed in various mammalian organs, such as brain, kidney, intestine, lung, placenta, uterus and testis (Fromm, 2004; Mealey and Fidel, 2015; Thuerauf and Fromm, 2006).

In the 1980′s the new antiparasitic drug ivermectin was first employed in dogs. Ivermectin belongs to the macrocyclic lactone endectocides derived from the bacterium *Streptomyces avermitilis*. Shortly thereafter it became apparent that this new drug might be responsible for neurological problems in certain types of dog (Preston, 1983; Seward, 1983). The ivermectin-sensitivity phenotype was associated with a 4-bp deletion mutation in exon 4 of the *ABCB1* gene (Mealey et al., 2001; Roulet et al., 2003). This mutation causes a frame-shift early in the coding sequence and as a consequence P-gp is truncated to 91 amino acids as opposed to 1281 amino acids of full length protein (Fecht and Distl, 2008; Geyer et al., 2005b; Klintzsch et al., 2010; Mealey et al., 2001; Nelson et al., 2003; Roulet et al., 2003). Genotyping studies indicate that many additional purebred dog breeds are affected, including Australian Shepherd, Miniature Australian Shepherd, Shetland Sheepdog, German Shepherd, White Swiss Shepherd, Old English Sheepdog, English Shepherd, Wäller, Longhaired Whippet, Silken Windhound, Border Collie and McNab (Geyer et al., 2005b; Gramer et al. 2011; Mealey et al. 2005; Neff et al. 2004). The lack of normal P-glycoprotein affects the integrity of the blood-brain barrier, which causes an increase in the drug concentration in the brain (Mealey and Meurs, 2008). Accumulation of ivermectin in the brain causes typical symptoms such as mydriasis, salivation, somnolence, depression, disorientation, ataxia, tremors, coma and can result in death (Fecht et al., 2007; Geyer and Janko, 2012).

The aim of this study was to determine the frequency of the mutant allele in 6 breeds (Rough Collie, Smooth Collie, Border Collie, Shetland Sheepdog, Australian Shepherd, White Swiss Shepherd) known to be affected by *ABCB1* mutation. In addition the Akita-Inu dog breed was analyzed for the presence of the deletion mutation in the *ABCB1*gene. The overview is based on genotyping data from 13 European countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Netherlands, Poland, Slovakia, Spain and United Kingdom).

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DNA samples were obtained from owners and breeders, in cooperation with veterinarians and kindly provided from the Slovgen dog genomic library. Blood samples (K3EDTA), buccal swab samples and cytological brush samples were collected from 4729 purebred dogs during the period 2012–2014. DNA extraction from blood and buccal brush was tested from a small group of samples of different breeds in parallel to establish the PCR method that could be used as a diagnostic screening method. The results were identical and reproducible for all tested samples. To date, various genotyping methods have been developed for the detection of the 4-bp deletion mutation (Baars et al., 2008; Geyer et al., 2005b; Hugnet et al., 2004; Kawabata et al., 2005; Klintzsch et al., 2010; Mizukami et al., 2012; Neff et al., 2004; Roulet et al., 2003). These methods are mainly based on PCR amplification with subsequent length polymorphism analysis. In addition to genotype survey we have evaluated four PCR-based methods (Geyer et al., 2005b; Hugnet et al., 2004; Klintzsch et al., 2010; Roulet et al., 2003) and their reliability was subsequently verified with Sanger DNA sequencing.

During the diagnostic research survey of the c.227_230delATAG mutation in *ABCB1* we have collected genotyping data from purebred dogs from various countries. Since the majority of samples (except Silken Windhound) were obtained from European countries, we expect that the data (Table 1) represents the situation in the European population. All genotyping results were stored in a relational database of genetic analyses and were used to extract frequency of mutant alleles within the most affected breeds and in Akita-Inu (Table 1). Blood relations among analyzed individuals were unknown, however due to breeding methods (selection, limiting mating population, etc.) it can be assumed that the Hardy-Weinberg principle does not apply to this population. The representativeness of allelic frequencies is probably influenced by common bias in submitting samples. Breeders and owners tend to offer for test dogs with unclear *ABCB1* genotype rather than animals that were already tested or those with genotypes which could be deduced from their parents.

The frequencies of the mutant allele for selected European countries with reasonable numbers of individuals from each breed were compared (Table 2). The distribution of the *ABCB1* genotypes in European population of Collies was similar to large-scale surveys in United States (Mealey and Meurs, 2008) and Germany (Gramer et al., 2011). The distribution of genotypes appears to be stable in Collie populations over the last years. This is true for all included countries with reasonable amount of samples (Table 2) except Germany where allelic frequency of *ABCB1* mutation has apparently decreased from 55 to 59% to 35.1% (Geyer et al., 2005a; Gramer et al., 2011). This may be caused by breeding strategy or by the smaller size of the analyzed population. Another possible reason is that defect in *ABCB1* is not considered as the most important disorder by breeders of Collies. Collies are affected by several other more severe genetic disorders like Collie Eye Anomaly (CEA) and Progressive Retinal Atrophy (PRA) (Kukekova et al., 2009; Lowe et al., 2003). Observed allelic frequencies for Shetland Sheepdog (30.3%), Australian Shepherd (35%), Miniature Australian Shepherd (26.1%), Silken Windhound (28.1%) and Longhaired Whippet (25.3%) are in concordance with previous *ABCB1* genotyping studies (Geyer et al., 2005a; Gramer et al., 2011; Mealey and Meurs, 2008; Neff et al., 2004; Tappin et al., 2012). Although these breeds also suffer from severe hereditary diseases (Mellersh et al., 2009; Parker et al., 2007; Zangerl et al., 2006), the allelic frequencies of *ABCB1* mutations and the portion of homozygous mutants are apparently lower in comparison with Collies. Surprisingly the allelic frequency of mutant *ABCB1* in Shetland Sheepdog samples from Germany (Table 2) was lower (17.6%) than 30% observed in the study by Geyer (Geyer et al., 2005a) or by Gramer (Gramer et al., 2011). Moreover no homozygous mutant genotypes were detected. As for the situation in Collies, we can only deduce probable causes of this decline in Germany. The opposite situation was in the Australian Shepherd where the 34.5% in this survey is higher than 20% and 22% observed by others in Germany (Geyer et al., 2005a; Gramer et al., 2011). The occurrence of *ABCB1* mutant alleles in European Shetland Sheepdog population (30.3%) differs from that reported in US (7–8%) (Mealey and Meurs, 2008; Neff et al., 2004). No mutant *ABCB1* alleles were detected in Border Collies, in concordance with very low frequencies observed by others in this breed (Geyer et al., 2005a; Gramer et al., 2011; Mealey and Meurs, 2008; Neff et al., 2004). Our survey confirmed the prevalence of the mutant allele for White Swiss Shepherd observed in Germany (Gramer et al., 2011). Lower occurrence in the US is probably caused by the fact the White Swiss Shepherd is not considered as an independent breed there, but rather as a color variety of the German Shepherd.

To our knowledge there is no information in the literature about the presence of *ABCB1* mutations in the Akita-Inu breed. Nevertheless some breeders and veterinarians recommend *ABCB1*-testing for Akita-Inu dogs. Akita-Inu is an ancient Japanese dog breed. During World War II, dogs in Japan were confiscated by the government as a source of fur for military garments. German Shepherds were the only breed exempt from the cull as they were used as military dogs. For that reason many owners bred their Akitas to German Shepherds in order to escape the cull. This is how the mutation could have been introduced to this breed. To confirm this hypothesis, 38 Akitas were evaluated for the presence of *ABCB1* mutation (c.227_230delATAG). All tested Akita-Inu dogs were homozygous for the wild-type *ABCB1* allele. In 2006 the breed has been officially split by the Fédération Cynologique Internationale (FCI) into American Akita (Great Japanese Dog, FCI number 344) and the Akita (Japanese Akita, FCI number 255). All tested dogs belong to Akita (Japanese Akita) breed. Further research is necessary to rule out the presence of *ABCB1* mutation in American Akitas which were potentially more influenced by crossing with German Shepherds.

In conclusion, our large scale survey of affected breeds in the European population revealed that *ABCB1* (c.227_230delATAG) mutation prevalence is relatively constant and extended the knowledge about genotype distribution in some countries which were not included in previous studies (Czech Republic, Finland, Hungary, Poland, Austria, Spain and Netherlands). Affected breeds suffer from other more severe

Table 1
The distribution of *ABCB1* genotypes in the tested populations.

Dog breed	Number of analyzed samples	Frequency of mutant allele (%)	Genotype (%)		
			ABCB1 (wt/wt)	*ABCB1* (wt/del)	*ABCB1* (del/del)
Rough Collie	1310	48.3	28.3	46.9	24.8
Smooth Collie	389	58.5	20.6	41.9	37.5
Shetland Sheepdog	1400	30.3	48.6	42.4	9
Australian Shepherd	907	35	41.2	47.5	11.3
Miniature Australian Shepherd	92	26.1	53.9	40	6.1
Silken Windhound	105	28.1	47.6	48.6	3.8
Longhaired Whippet	138	24.3	52.2	47.1	0.7
White Swiss Shepherd	234	16.2	68.4	30.8	0.8
Border Collie	116	0	100	0	0
Akita-Inu	38	0	100	0	0

Table 2
Frequency of *ABCB1* alleles of four breeds known to be affected by the c.227_230delATAG mutation in selected European countries. (Collie = Rough Collie + Smooth Collie).

Dog breed	Origin (state)	Number of analyzed samples	Frequency of mutant allele (%)	Genotype (%)		
				ABCB1 (wt/wt)	*ABCB1* (wt/del)	*ABCB1* (del/del)
Collie	Czech Republic	346	50	24	46.8	29.2
	Germany	258	35.1	43	43.8	13.2
	Finland	214	60.7	17.8	43	39.2
	U.K.	184	50.5	27.2	44.6	28.2
	France	182	53.6	22	48.9	29.1
	Hungary	98	61.2	16.3	44.9	38.8
	Poland	79	55	21.5	46.8	31.7
Shetland Sheepdog	France	511	34.7	42.7	45.2	12.1
	Czech Republic	247	25.9	51.4	45.3	3.3
	Poland	136	39.3	56.6	8.1	35.3
	Germany	91	17.6	64.8	35.2	0
	Spain	86	30.8	46.5	45.3	8.2
	Netherlands	57	36	43.9	40.4	15.7
	Austria	55	14.5	74.5	21.8	3.7
	Finland	39	30.8	51.3	35.9	12.8
	Hungary	25	36	32	64	4
Australian Shepherd	France	223	33.6	41.7	49.3	9
	Czech Republic	195	35.9	39	50.2	10.8
	Poland	90	45	26.7	56.7	16.6
	Austria	90	25	57.8	34.4	7.8
	Germany	87	34.5	42.5	46	11.5
	Belgium	49	29.6	46.9	46.9	6.2
	Hungary	24	39.6	37.5	45.8	16.7
White Swiss Shepherd	Denmark	93	18.8	62.4	37.6	0
	Spain	37	9.5	81	19	0
	Poland	31	24.2	64.5	29	6.5
	Slovakia	16	18.75	62.5	37.5	0
	Hungary	12	12.5	75	25	0
	France	10	20	60	40	0

hereditary diseases and this limits breeders options in designing selective breeding strategies and maintaining a wide gene pool at the same time.

Conflict of interest statement

The authors report no conflicts of interest in this work.

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