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Ancestral T-Box Mutation Is Present in Many, but Not All, Short-Tailed Dog Breeds

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Dogs differ greatly in their morphological characteristics including various tail phenotypes. Congenitally short-tailed dogs are present in many breeds; however, the causative mutation located in the T-box transcription factor *T* gene (C189G) had only been described in the bobtailed Pembroke Welsh Corgis. We investigated here the presence of the *T* gene mutation in 23 other breeds (360 dogs, including 156 natural short tailed) in which natural bobtailed dogs exist. In the 17 breeds in which the C189G mutation was observed, there was a perfect correlation between this mutation and the short-tail phenotype. However, 6 breeds did not carry the known substitution or any other mutations in the *T* gene coding regions. No dogs were found to be homozygous for the C189G mutation, suggesting that the homozygous condition is lethal. In order to study the effect of the *T* gene mutation on litter size, we compared the number of puppies born from short-tailed parents to that born from long-tailed parents. In the Swedish Vallhund breed, we observed a 29% decrease in the litter size when both parents were short tailed. Given that the *T* gene mutation is not present in all breeds of short-tailed dog, there must be yet other genetic factors affecting tail phenotypes to be discovered.

Key words: *dog, mutation, short-tail phenotype, T-box transcription factor T*

Dogs have become important models for genetic studies due to their extensive phenotypic variation, unique breed structure, as well as available genome sequence and genetic tools (Sutter and Ostrander 2004; Lindblad-Toh et al. 2005; Starkey et al. 2005; Tsai et al. 2007). Selective breeding has enriched many breed-specific morphological characteristics including tail length. Tail length depends on the number of the caudal vertebrae, which can vary significantly between individuals. Several dog breeds show very short tails (brachyury) or even complete absence of the tail vertebrae (anury) as illustrated in Figure 1. A genetic cause of short-tail phenotype has been identified in Pembroke Welsh Corgis (Haworth et al. 2001). The C189G mutation in exon 1 of the T-box transcription factor *T* gene affects the DNA-binding property of the T protein resulting in the bobtail phenotype. Pembroke Welsh Corgis heterozygous for the C189G mutation have short tails, and this mutation is thought to cause embryonic lethality in homozygotes (Haworth et al. 2001; Indrebø et al. 2007). *T* gene mutations in mouse cause early embryonic lethality and abnormalities in the development of mesodermal tissues, including the tail and spine, thus suggesting an essential role for the *T* gene during mammalian development (Wilson et al. 1995). In addition to the *T* gene, mutations in other genes like *Pax1* and *Wnt-3a* have been associated with tail development in mouse (Greco et al. 1996; Wilm et al. 1998).

In Pembroke Welsh Corgis, the length of the natural bobtail varies from a complete tailless to a short tail with half the length of a normal tail and occasional kinks. For

Figure 1. Illustration of Bourbonnais Pointer dogs showing a tailless (anury) phenotype on the left, a short-tail (brachyury) phenotype in the middle, and a long-tail phenotype on the right (photo: Michaël Comte).

comparison, another likely recessively inherited type of bobtail exists in Bulldogs where all dogs in the breed have short tails with multiple kinks (Whitney 1947). There are also occasional reports of short-tailed dogs born from long-tailed parents in some breeds, revealing multiple patterns of inheritance or variations in penetrance.

We studied here the presence of the *T* gene mutation in a large number of breeds to investigate its possible ancestral origin and to identify whether other genetic causes exist in association with short tails. We tested 23 different breeds and showed that the C189G mutation is present in all short-tailed dogs of 17 breeds, supporting a correlation of mutation with the phenotype. We also showed that breeding of 2 bobtailed Swedish Vallhund dogs with the *T* gene mutation decreases litter size, which confirms a major role of *T* gene during embryogenesis.

Materials and Methods

Animals and Definition of the Tail Phenotype

Samples were collected from 23 breeds including 360 dogs (156 short-tailed and 204 long-tailed dogs; Table 1). In addition, samples were collected from 80 dogs including 9 breeds presenting only the long-tail phenotype (American Cocker Spaniel, Bichon Frisé, English Setter, English Springer Spaniel, Golden Retriever, Long Haired Dachshund, Shih-Tzu, Smooth Dachshund, and Yorkshire Terriers).

Table 1. Genotyping results of the *T* gene mutation (C189G) for 23 different breeds harboring the short-tail phenotype

	Total number of dogs	Number of long-tail dogs	Genotype at C189	Number of short-tail dogs	Genotype at C189
17 breeds with C189G mutation					
Australian Shepherd	70	42	C/C	28	C/G
Austrian Pinscher	2	1	C/C	1	C/G
Australian Stumpy Tail Cattle Dog	2	0		2	C/G
Bourbonnais Pointer	25	16	C/C	9	C/G
Brazilian Terrier	17	7	C/C	10	C/G
Brittany Spaniel	18	4	C/C	14	C/G
Croatian Sheepdog	3	1	C/C	2	C/G
Danish/Swedish Farmdog	2	1	C/C	1	C/G
Jack Russel Terrier	10	7	C/C	3	C/G
Karelian Bear Dog	6	3	C/C	3	C/G
Mudi	10	5	C/C	5	C/G
Polish Lowland Sheepdog	28	10	C/C	18	C/G
Pyrenean Shepherd	64	57	C/C	7	C/G
Savoy Sheepdog	17	15	C/C	2	C/G
Schipperke	12	4	C/C	8	C/G
Spanish Waterdog	7	3	C/C	4	C/G
Swedish Vallhund	22	6	C/C	16	C/G
6 breeds without C189G mutation					
Boston Terrier	4	0	C/C	4	C/C
English Bulldog	5	0	C/C	5	C/C
King Charles Spaniel	22	13	C/C	9	C/C
Miniature Schnauzer	6	4	C/C	2	C/C
Parson Russel Terrier	3	2	C/C	1	C/C
Rottweiler	5	3	C/C	2	C/C

Pedigrees and tail phenotype information (anury, brachyury, and long tail) were collected from sampled dogs. Anury corresponds to a complete lack of vertebrae and brachyury to a short tail with variable lengths. Tail phenotypes were recorded by the sample collector, taken from the breeding database (Finnish Kennel Club 2008) or directly reported by the owners.

Genomic DNA Extraction

Samples were either ethylenediaminetetraacetic acid-blood or buccal cell samples. Genomic DNA was extracted from blood and buccal cells using either the NucleoSpin Kit (Macherey-Nagel, Hoerdt, France) or the BuccalAmp DNA Extraction Kit (Epicentre Biotechnologies, Madison, WI). Some samples with low DNA yields were amplified using the V2 Genomiphi Kit (GE Healthcare, Buckinghamshire, UK).

Polymerase Chain Reaction and Sequencing

The C189G mutation in exon 1 of the *T* gene was tested by polymerase chain reaction (PCR) from genomic DNA as previously described by Haworth et al. (2001) or with the following primer pair: 5′-AGAGCCTGCAGTACC-GAGTG-3′ designed in exon 1 of the *T* gene and 5′-CCGAGACTTCTCCCAGAAAA-3′ designed in intron 1. The presence of the mutation in the amplified PCR product was detected by 1) restriction enzyme assay or 2) sequencing. 1) Restriction enzyme assay was performed with *BstE*II enzyme (New England Biolabs, Ipswich, MA) followed by visualization on agarose gel. All coding exons and surrounding splicing sites were sequenced for short-tailed dogs that did not have the mutation using primers and conditions as previously described by Haworth et al. (2001), except exon 8 in which we used the following primer pair: 5′-GCGGAGAAGGTGCCTTAGTA-3′ and 5′-CCTGG-GAGGTCAATCAAATC-3′. 2) PCR products were cleaned by ExoSAP-IT (GE Healthcare) and sequenced with the BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with ABI PRISM 3130XL DNA analyzer (Applied Biosystems). The resulting sequencing data were analyzed with the DNA Sequencing Analysis v5.2 software (Applied Biosystems). The nucleotide numbers indicated throughout this study have been defined from the *T* gene translation initiation codon (GenBank AJ245513).

Statistical Analysis

The possible effect of the homozygous *T* gene mutation for embryonic viability was estimated by following the litter sizes of short-tailed parents compared with long-tailed parents in Swedish Vallhund breed. Statistical significance of the variation between the study groups was measured by Student's *t*-test.

Results

We analyzed the presence of the known T-box transcription factor *T* gene mutation, C189G (Ile63Met), in 23 breeds by genotyping 360 dogs including 156 short-tailed and 204 long-tailed dogs. We identified 17 breeds in which the short-tailed dogs carried the mutation and 6 breeds that did not (Table 1). Moreover, this mutation was not found in any dog from a set of 80 dogs belonging to 9 breeds that do not display naturally the short-tail phenotype (American Cocker Spaniel, Bichon Frisé, English Setter, English Springer Spaniel, Golden Retriever, Long Haired Dachshund, Shih-Tzu, Smooth Dachshund, and Yorkshire Terriers). These breeds increase the list of breeds analyzed by Haworth et al. (2001) from 19 to 28, thus confirming that this variant is not a nucleotide polymorphism. In the 6 breeds without the *T* gene mutation, in order to find any other variations in the *T* gene that could underlie the short-tail phenotype, we sequenced all the coding exons, exon/intron boundaries, and untranslated regions (UTR). The sequencing did not reveal any causative mutations in any of the 6 breeds. In addition to the several polymorphic sites described by Haworth et al. (2001), one novel nucleotide change present at the 5′ UTR (G-6A) was found in Miniature Schnauzers. However, this change did not segregate with the phenotype, indicating that it was a noncausative polymorphic site. This fact tends to exclude the entire T locus as causal for the phenotype in this Miniature Schnauzer pedigree, in which the short-tailed dogs are descendants of long-tailed parents. These results demonstrate that the *T* gene mutation is present in many but not all short-tailed dogs and that also other genetic factors are likely to regulate tail length.

Among the 315 dogs belonging to the 17 breeds identified here, all the 133 short tailed were heterozygous for the C189G *T* gene mutation and all the 182 long-tailed dogs did not carry the mutation. This result indicates the full penetrance and the lethality of the homozygous mutation. To further investigate the homozygous lethality, we calculated litter sizes for 2 parent combinations (long tailed × long tailed and short tailed × short tailed) for Swedish Vallhunds using the KoiraNet Breeding Database from the Finnish Kennel Club (2008) (Figure 2). Altogether, 253

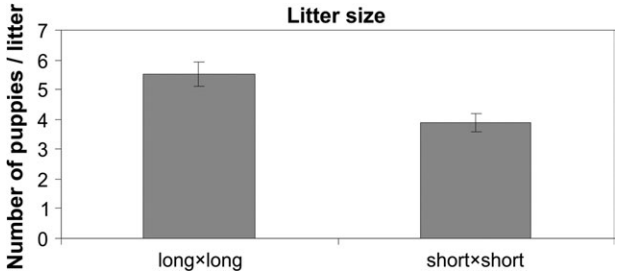

Figure 2. Comparison of litter sizes from different crosses in Swedish Vallhund breed. The average litter size is 5.5 puppies/litter with long-tail × long-tail crosses and 3.9 puppies/litter with short-tail × short-tail crosses. The litter size is reduced of 29% with short-tail × short-tail crosses (***$P < 0.001$) compared with the average litter size of long-tail × long-tail crosses. Results are expressed as mean (column value) ± standard error of the mean (bars on the top of the column).

puppies from 56 litters born between years 2000 and 2007 were included in the calculation. The average litter sizes were 5.5 puppies for long × long and 3.9 puppies for short × short crosses. The litter size reduction was 29% ($P = 0.0008$) for short × short crosses as compared with the long × long crosses. The observed 29% decrease, suggestive of an in utero lethality, is compatible with the expected 25% reduction in the litter size for short × short crosses because one-fourth of the puppies will inherit the mutation from both parents.

Discussion

Natural bobtailed dogs are present in many breeds but the causative mutation has been identified only for the Pembroke Welsh Corgis (Haworth et al. 2001), in which a dominantly inherited C189G mutation of the T-box transcription factor *T* gene (Ile63Met) results in a short-tail phenotype. In this study, we identified 17 additional breeds with this mutation, suggesting an ancestral origin of the *T* gene mutation. Indeed, these 17 breeds mainly belong to 2 groups: sheepdog and hunting breeds. All analyzed short-tailed dogs were heterozygous for the C189G mutation as previously shown for Corgis (Haworth et al. 2001). For the Swedish Vallhunds, analysis of the litter sizes from short-tailed × short-tailed crosses revealed a 29% reduction in litter size, further supporting recessive embryonic lethality of the mutation. In the Brittany Spaniel and Bourbonnais Pointer short-tailed × short-tailed crosses, such a reduction was not reported by the breeders; however, this observation being based on only few crosses, the reduction percentage could not be significantly calculated. Homozygosity for the mutation appears to cause either embryonic or early postnatal lethality due to serious developmental defects (Indrebø et al. 2007). Similar observations have been previously seen in different mouse mutants for the *T* gene (Gluecksohn-Schoenheimer 1938; Wilson et al. 1995). Recently, Pembroke Welsh Corgi puppies with severe anatomical defects having the homozygous mutation have been characterized. These puppies lacked tails, manifested anorectal atresia with severe alterations in the posterior lumbar region and spine, and had a failure to thrive (Indrebø et al. 2007). In contrast with homozygous, heterozygous bobtailed dogs have not been reported to manifest any other abnormalities (Indrebø et al. 2007). In mouse mutants, additional spinal defects have been described in heterozygous *T* gene mutants. These phenotypic differences are most likely due to the different type of mutations in dogs and mice. Whereas the mutation in dogs affects only the T-box domain, mice carry large deletions that cover the whole *T* gene and may also affect the other genes in the region such as the *T2* gene (Herrmann et al. 1990; Rennebeck et al. 1998).

Although the *T* gene mutation is present in many breeds, it does not explain all short tail phenotypes. Boston Terrier, English Bulldog, King Charles Spaniel, Miniature Schnauzer, Parson Russell Terrier, and Rottweiler breeds all have natural short-tailed dogs but the analyzed bobtails carried neither the known C189G *T* gene mutation nor the other novel causative mutations within the same gene. The short-tail phenotype includes either a complete lack of vertebrae or a short tail with variable length. The *T* gene mutation can cause both anury and brachyury as illustrated in Figure 1. The 6 breeds above have both anury and brachyury, but the length and the number of kinks in these breeds are highly variable and could indicate heterogeneous genetic backgrounds. In mouse, mutations in genes such as *Pax1*, *Wnt-3a*, *Dll3*, and *Noto* have been associated with short and kinked tails and, thus, remain as potential candidates (Gruneberg 1961; Greco et al. 1996; Wilm et al. 1998; Abdelkhalek et al. 2004; Dunty et al. 2008). Short tail with multiple prominent kinks is a very common phenotype in King Charles Spaniel. Further analyses are underway to determine the mode of inheritance and the cause of the short-tailed phenotype in the breed. The rare short-tailed Miniature Schnauzers, Parson Russell Terrier, and Rottweilers that were part of the study were all born from long-tailed parents, suggesting a recessive model, spontaneous developmental abnormality (congenital and not hereditary), or a sporadic mutation. We are currently extending sample and pedigree collections in these breeds to address these questions. Boston Terriers and English Bulldogs all either lack or have very short and kinky tails, indicating that the phenotype is fixed and has become part of the breed characteristics. The mode of inheritance has been suggested to be recessive (Whitney 1947).

Tail docking is prohibited in many European countries, and the docked dogs cannot participate in official dog shows or trials unlike natural bobtails. The owner of the bobtailed dog needs a certificate from a veterinarian to prove the natural short tail to get access to shows. In the breeds harboring the mutation, veterinary inspections can now be replaced by the simple genetic test that confirms natural bobtails. Our study extends significantly the list of breeds that could benefit from the genetic testing.

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