Pituitary dwarfism, associated with growth hormone (GH) deficiency, is an autosomal, recessively inherited disorder in shepherd dogs. As outlined in reference [49], pituitary dwarfism is a serious illness and clinical signs are not limited to physical appearance. Instead, the dwarfs suffer from a whole range of clinical manifestations. Without proper treatment, the long-term prognosis is poor. Many dwarfs will not live more than 4 to 5 years, and although the prognosis improves significantly when dwarfs are properly treated with porcine GH and synthetic levo-thyroxine, their prognosis still remains guarded. The good news is that pituitary dwarfism is easily preventable. As long as mating between 2 carriers of the mutation that leads to pituitary dwarfism is prevented, no dwarfs will be born. Unfortunately, carriers of the mutation associated with pituitary dwarfism cannot be distinguished easily from dogs free of the mutation. The availability of a diagnostic DNA test, however, would enable breeders to prevent dwarfs form being born by testing the carrier status of potential breeding animals and applying a correct breeding policy. But before such a test could be developed, the mutated gene had to be identified first.

The results of the study described in reference [50] show that mutations in the LHX3 gene are associated with pituitary dwarfism in German shepherd dogs. LHX3 is a member of the LIM homeodomain protein family of DNA-binding transcription factors. These factors regulate the expression of genes that pattern the body and are critical for cell specialization during embryonic development [1]. Molecular defects in the LHX3 gene are associated with the combined pituitary hormone deficiency (CPHD) syndrome in humans [2][3][4][5][6][7]. Most human patients display a complete deficit of all pituitary anterior lobe hormones, except for adrenocorticotropic hormone. Also in mice, LHX3 is essential for differentiation and proliferation of pituitary cell lineages [8]. Homozygous LHX3-knockout mice display a complete absence of the differentiated hormone secreting cells, except for some
corticotropes [8][9]. Because the endocrinological phenotype of humans with \textit{LHX3} mutations and \textit{LHX3}-knockout mice is in accordance with the phenotype of the German shepherd dog dwarfs, \textit{LHX3} was considered an excellent candidate gene for involvement in pituitary dwarfism associated with GH deficiency in this dog breed. Analysis of intron 5 revealed that dwarfs have a deletion of 1 of 6 imperfect 7-base pair (bp) repeats. This deletion reduces the intron size to 68 bp and is associated with defective splicing of a proportion of the transcripts \textit{in vivo} and \textit{in vitro}. The aberrant splicing products result from skipping of exon 5 or retention of intron 5. Skipping of exon 5 results in a frame shift; the translation product will lack the homeodomain and will therefore probably not be functional [10]. Retention of the mutant intron also leads to a frame shift in the part of the mRNA that codes for the homeodomain. Splicing of the mutant intron 5 is expected to be hampered by its reduced size. Natural deletion mutants and \textit{in vitro} experiments indicate that there is a minimum size of 65–78 nucleotides (nt) of introns in higher eukaryotes [11][12][13][14][15][16][17][18]. The deletion in canine \textit{LHX3} shortened the distance between the splice donor and branch site to 48 nt. Therefore, splicing of the mutant intron 5 is expected to be hampered by its reduced size.

Congenital dwarfism associated with GH deficiency is also known in Saarloos wolfdogs and Czechoslovakian wolfdogs. Both are German shepherd dog-wolf cross-breeds. The study in reference [52] evaluated if pituitary dwarfism in these breeds is associated with the same mutations found in German shepherd dogs, by subjecting the Saarloos and Czechoslovakian wolfdog dwarfs to genetic testing. All dwarfs were found to be homozygous for the same 7-bp deletion in intron 5 of \textit{LHX3}. This mutation is identical to the one described in reference reference [50].

Although pituitary dwarfism associated with GH deficiency is a serious illness, the prevalence of the disease seems very low. The need for screening potential breeding animals could therefore be questioned. In the study described in reference [52], a large group of clinically healthy Saarloos and Czechoslovakian wolfdogs were screened for the mutation associated with pituitary dwarfism. The percentage of carriers of the mutated allele was 31% and 21%, respectively. These results clearly demonstrate that pituitary dwarfism is a relevant disorder and emphasize the need for screening. Pituitary dwarfism and the associated DNA defects most likely render the individuals so weak that they either die in the uterus or shortly after birth, which would explain why dwarfs are seen only occasionally.

Although the screening test is available for all breeders, it is not yet commonly used by German shepherd dog breeders. By the beginning of 2015, only 44 German shepherd dogs have been tested by the genetic lab of the Department of Clinical Sciences of Companion Animals. In this group, the percentage of carriers of the mutated allele is no less than 30%. The impact of inappropriate breeding on the health status and general well-being of dogs receives more and more media attention. Therefore, breeders have come under the attention and scrutiny of the public eye. More importantly, from June 1\textsuperscript{st} 2014, Dutch law (“Besluit houders van dieren”, dictates breeders must do everything possible to prevent severe congenital diseases from being passed onto or occur in the offspring of their breeding
The availability of our genetic test enables breeders to prevent pituitary dwarfism. Therefore, Dutch law now obligates German shepherd dog breeders to use the genetic test. Hopefully German shepherd dog breeders will be persuaded by all this to start testing their breeding animals for the presence of the LHX3 mutations associated with pituitary dwarfism. If all breeding animals were genetically tested and a correct breeding policy would be implemented, pituitary dwarfism due to an LHX3 mutation could be eradicated completely.

In humans with pituitary dwarfism due to an LHX3 mutation, anatomical abnormalities in the occipito-atlantoaxial joints in combination with a basilar impression of the dens axis have been reported [6][7]. The study described in reference [53] is the first report of similar anatomical malformations of the atlanto-axial joint, leading to instability and dynamic compression of the cervical spinal cord, in Czechoslovakian wolfdogs and a German shepherd dog with pituitary dwarfism due to an LHX3 mutation. All dogs displayed neurological signs indicating a cervical spinal cord disorder. Magnetic resonance imaging (MRI) and computed tomography (CT) images revealed incomplete ossification of the atlas with 1 or more of the 3 suture lines between the 3 ossification centers of C1 still open in all dogs. The incomplete ossification of the bony elements of C1 is expected to have resulted in instability of C1. This instability and resulting movements between the bony elements of C1 probably caused the excessive soft tissue formation located in the open sutures in C1. Additionally, flexion of the C1 - C2 junction resulted in an increased dorsal displacement of the dens axis, causing compression of the cervical spinal cord.

Pituitary dwarfism associated with GH deficiency in German shepherd dogs has been seen for decades and dwarfs are born in purebred populations all over the world. The phenotype has extensively been described in numerous case reports [20][21][22][23][24][25][26][27][28][29][30][31][32]. So far, there have been no reports of atlanto-axial abnormalities in canine dwarfs. This raises the question whether these vertebral malformations are not just coincidental findings that are unrelated to the 7-bp deletion in LHX3. However, the atlanto-axial malformations occur in dwarfs of 2 different breeds, and similar findings are described in human pituitary dwarfism. Additionally, the phenotype of pituitary dwarfism is highly variable in other aspects as well, which could be due to possible variations in the level of residual activity of the LHX3 protein between dwarfs, as described in reference [50]. It is therefore concluded that the anatomical abnormalities of the atlanto-axial joint are associated with canine pituitary dwarfism due to an LHX3 mutation. Consequently, pituitary dwarfs should be monitored closely for neurological signs.

The human LHX3 gene consists of 7 exons. Alternate promoters lead to 2 transcript variants: variant 1, containing exon 1, and variant 2, containing exon 2 instead of exon 1. The first exon of both variants is spliced to exon 3 [33][34]. Protein isoforms LHX3a and LHX3b are translated from an ATG start codon in exon 1 and exon 2. In addition, a third protein isoform, M2-LHX3, has been described, which is translated from a start codon in exon 4 of transcript variant 1[35]. Earlier in vitro studies concluded that isoforms LHX3a and M2-LHX3 are potent gene activators in humans and that LHX3b is not [33][35]. The protein isoforms
LHX3a and LHX3b, to our knowledge, have not been demonstrated in vivo. By analysis of genomic DNA and cDNA sequences, the study described in reference [51] shows that in dogs the predicted start codon of LHX3a is followed shortly by a stop codon. LHX3a seems to be redundant in dogs and the function of exon 1 may be to circumvent exon 2 in order to direct production of isoform M2-LHX3. These results highlight the significance of isoform M2-LHX3 and the canine situation opens the possibility that also in other species the LHX3a isoform is redundant.

The most important endocrine differential diagnosis of pituitary dwarfism due to GH deficiency is juvenile hypothyroidism. Defects at any level of the hypothalamus-pituitary-thyroid axis can lead to deficient secretion of thyroid hormones. Hypothyroidism can be classified as primary or central, and both forms can be congenital or acquired. In central hypothyroidism the thyroids are not affected primarily but are deprived of stimulation by thyroid stimulating hormone (TSH). Primary hypothyroidism is a common endocrinopathy in dogs. In contrast, central hypothyroidism is rare in this species. Isolated TSH deficiency has only been reported in a family of Giant Schnauzers [36], in a young Boxer [37], and in a 2-week-old Portuguese water dog [38]. The study outlined in reference [54] describes the occurrence and clinical presentation of central hypothyroidism in Miniature Schnauzers.

Primary hypothyroidism is diagnosed regularly in Miniature Schnauzers, based either on thyroid scintigraphy or a TSH-stimulation test. However, due to secondary atrophy of thyroid tissue these tests produce the same results in dogs with primary hypothyroidism as in dogs with central hypothyroidism [39][40]. Central hypothyroidism might therefore be an underdiagnosed disorder that could be quite common in Miniature Schnauzers and should be considered in Miniature Schnauzers with symptoms indicative of deficient thyroid hormone secretion.

If, however, central hypothyroidism is as rare as it seems, the fact that 7 dogs of the same breed are affected by the same disorder strongly suggests that central hypothyroidism has a genetic background in Miniature Schnauzers. Two possible candidate genes were the TSHB (TSHB) gene and the thyrotropin-releasing hormone receptor (TRHR) gene. Thyroid-stimulating hormone is a heterodimeric glycoprotein that contains both an α- and a β-subunit. The α-subunit, α-GSU, is common to TSH, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The β-subunit, TSHβ, is unique to TSH [41]. In humans, several mutations of the TSHB gene are associated with secondary hypothyroidism [42][43][44][45][46]. Thyrotropin-releasing hormone stimulates the thyrotropes and lactotropes to secrete TSH and prolactin, respectively. In humans, spontaneous mutations in the TRHR gene are known to give rise to a combined deficiency of TSH and prolactin [47]. Genetic analysis of the TSHB gene and the exons of the TRHR gene revealed 3 single nucleotide polymorphisms (SNPs) in TSHB, and 2 SNPs in TRHR. However, no disease causing mutations were found in either genes.

Due to its retrospective nature, not all pituitary hormone stimulation tests were performed. Therefore, the Miniature Schnauzers may have different forms of central hypothyroidism. Further studies to determine the secretory capacity of all
adenohypophyseal hormones are needed to get insight in the underlying cause(s) of central hypothyroidism in this breed.

**Conclusions** [48]

- A contraction of a 7-bp DNA repeat in intron 5 of canine *LHX3* leads to deficient splicing and is associated with pituitary dwarfism in German shepherd dogs. Splicing of the mutant intron 5 is expected to be hampered by its reduced size.
- In dogs, the predicted start codon of LHX3a is followed shortly by a stop codon, which makes LHX3a seem to be redundant in this species. The function of exon 1 may be to circumvent exon 2 in order to direct production of isoform M2-LHX3, highlighting the significance of isoform M2-LHX3.
- Saarloos and Czechoslovakian wolfdog dwarfs have the same 7-bp deletion in intron 5 of *LHX3* as do German shepherd dog dwarfs.
- The frequency of carriers of this mutation among clinically healthy Saarloos and Czechoslovakian wolfdogs used for breeding was 31% and 21%, respectively, emphasizing the need for screening before breeding.
- If all breeding animals were genetically tested for the presence of the *LHX3* mutation and a correct breeding policy would be implemented, pituitary dwarfism due to an *LHX3* mutation could be eradicated completely.
- In canine pituitary dwarfs with neurological signs indicative of a cervical problem, atlantoaxial abnormalities that resemble those encountered in human CPHD patients with an *LHX3* mutation, may be identified. These findings suggest an association between the *LHX3* mutation in dogs with pituitary dwarfism and atlanto-axial malformations. Consequently, pituitary dwarfs should be monitored closely for neurological signs.
- Central hypothyroidism might be an underdiagnosed disorder that could be quite common in Miniature Schnauzers and should be considered in Miniature Schnauzers with symptoms indicative of deficient thyroid hormone secretion. The fact that this rare disorder occurred in 7 dogs from the same breed suggests that central hypothyroidism may have a genetic background in Miniature Schnauzers.
- No mutations were found in the *TSHB* gene and the exons of the *TRHR* gene that could explain the presence of central hypothyroidism in Miniature Schnauzers.
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