Central Hypothyroidism in Miniature Schnauzers

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Background: Primary hypothyroidism is a common endocrinopathy in dogs. In contrast, central hypothyroidism is rare in this species.

Objectives: The objective of this article is to describe the occurrence and clinical presentation of central hypothyroidism in Miniature Schnauzers. Additionally, the possible role of the thyroid-stimulating hormone (TSH)-releasing hormone receptor (TRHR) gene and the TSHβ (TSHB) gene was investigated.

Animals: Miniature Schnauzers with proven central hypothyroidism, based on scintigraphy, and the results of a 3-day-TSH-stimulation test, or a TSH-releasing hormone (TRH)-stimulation test or both, presented to the Department of Clinical Sciences of Companion Animals at Utrecht University or the Department of Medicine and Clinical Biology of Small Animals at Ghent University from 2008 to 2012.

Methods: Retrospective study. Pituitary function tests, thyroid scintigraphy, and computed tomography (CT) of the pituitary area were performed. Gene fragments of affected dogs and controls were amplified by polymerase chain reaction (PCR). Subsequently, the deoxyribonucleic acid (DNA) sequences of the products were analyzed.

Results: Central hypothyroidism was diagnosed in 7 Miniature Schnauzers. Three dogs had disproportionate dwarfism and at least one of them had a combined deficiency of TSH and prolactin. No disease-causing mutations were found in the *TSHB* gene and the exons of the *TRHR* gene of these Schnauzers.

Conclusions and clinical importance: Central hypothyroidism could be underdiagnosed in Miniature Schnauzers with hypothyroidism, especially in those of normal stature. The fact that this rare disorder occurred in 7 dogs from the same breed suggests that central hypothyroidism could have a genetic background in Miniature Schnauzers.

Key words: Disproportionate dwarfism; TSHB gene; TRHR gene; Pituitary; Canine.

Hypothyroidism is one of the most common endocrinopathies in dogs. 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). The condition is rare compared with primary hypothyroidism.[1]

Adult-onset central hypothyroidism may be because of a tumor of the pituitary or adjacent regions,[2] lymphocytic adenohypophysitis,[3] head trauma,[4] nontraumatic intracranial hemorrhage,[5] or hypophysectomy.[6]

Abbreviations:

99mTcO$_4^-$	radioactive pertechnetate
CT	computed tomography
cDNA	complementary DNA
CRH	corticotropin-releasing hormone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
gDNA	genomic DNA
GH	growth hormone
GHRH	GH-releasing hormone
IRMA	immunoradiometric assay
IGF-1	insulin-like growth factor-1
P/B	pituitary height/brain area
PCR	polymerase chain reaction
rh	recombinant human
RIA	radioimmunoassay
SNP	single nucleotide polymorphism
TRH	TSH-releasing hormone
TRHR	TRH receptor
TSH	thyroid-stimulating hormone
TT$_4$	total thyroxine

Juvenile central hypothyroidism may be because of either isolated TSH deficiency or combined pituitary hormone deficiency (CPHD). In dogs, the best-known example of congenital central hypothyroidism is that of pituitary dwarfism in German Shepherd Dogs, characterized by a combined deficiency of all adenohypophyseal hormones except ACTH.[7] Combined pituitary hormone deficiency, including TSH deficiency, has also been described in a 12-week-old dog after head injury.[8] Isolated TSH deficiency is very uncommon in dogs being reported in a family of Giant Schnauzers,[9] in a

young Boxer,[10] and in a 2-week-old Portuguese Water Dog.[11]

This retrospective study describes the occurrence of central hypothyroidism in Miniature Schnauzers and suggests that this rare disorder may have a genetic background in this breed.

Materials and Methods

Dogs

Medical records of Miniature Schnauzers, presented to the Department of Clinical Sciences of Companion Animals at Utrecht University or the Department of Medicine and Clinical Biology of Small Animals at Ghent University from 2008 to 2012, were evaluated for inclusion in the study. All Miniature Schnauzers with proven central hypothyroidism, based on the combination of clinical signs, low plasma total thyroxine (TT_4) concentration, absent or insufficient thyroid uptake of radioactive pertechnetate ($99mTcO_4^-$) and either a plasma TSH concentration in the lower part of the reference range that did not increase after TRH administration or increased thyroid uptake of $99mTcO_4^-$ after 3 days of TSH stimulation combined with a rise in plasma TT_4 concentration after repeated TSH administration, were included. Data from signalment, clinical history, physical examination, complete blood count, biochemical blood testing, hormone determinations, diagnostic imaging, and the effect of treatment with levothyroxine were recorded.

Twenty-five Miniature Schnauzers served as control dogs for the genetic studies. These dogs were presented with thyroid-unrelated illnesses, and therefore, thyroid function was not tested. Blood samples were collected with informed consent of the dog owners and genomic DNA (gDNA) was isolated from the samples by a salt extraction method.[12]

Hormone measurements

Plasma concentrations of TT_4 and TSH were evaluated by commercial homologous solid-phase chemiluminescent enzyme immunoassay activities (Immulite canine total T4, Immulite canine TSH).[a] Reference intervals for plasma TT_4 and TSH concentrations in euthyroid dogs are 1.48–3.57 μg/dL [19–46 nmol/L] and <0.60 ng/mL, respectively.

Plasma growth hormone (GH) concentration was measured by a commercially available radioimmunoassay (RIA) for porcine and canine GH, with a reference range of <5 μg/L.[b] Plasma insulin-like growth factor-1 (IGF-1) concentration was measured by a heterologous RIA, validated for the dog.[13] Plasma prolactin concentration was also measured by a heterologous RIA, validated for the dog.[14] Plasma ACTH concentration was measured by a commercially available immunoradiometric assay for humans,[c] validated for the dog,[15] with a reference interval of 5–85 pg/mL. Plasma cortisol concentration was measured by a RIA (Coat-A-Count Cortisol, DPC), validated for the dog,[15] with a reference interval of 0.40–4.93 μg/dL [11–136 nmoL/L].

Function tests

The thyroid glands were stimulated with 90 μg recombinant human (rh)TSH[d] administered IV on 3 consecutive days. Blood samples for the determination of plasma TT_4 concentration were collected in heparin-coated tubes immediately before and every 24 hours after rhTSH administration.[1] TRH-stimulation tests were performed by IV administration of 10 μg/kg TRH.[e] Blood samples for the determination of plasma TSH and prolactin concentrations were collected in chilled heparin-coated tubes immediately before and 10 and 20 minutes after TRH administration.

GH-releasing hormone (GHRH) stimulation tests were performed by IV administration of 1 μg/kg human GHRH.[f] Blood samples for determination of plasma GH concentration were collected in chilled EDTA-coated tubes immediately before and 20 minutes after GHRH administration.

The minimal interval between the stimulation tests was 1 day. After collection, blood samples were immediately centrifuged at 4°C for 10 minutes at 3000 × g. Plasma was stored at −25°C until assayed.

Diagnostic imaging

Scintigraphic imaging of the thyroid glands was performed with the Integrated ORBITER Gamma Camera System with Open Icon Workstation, equipped with a high-resolution parallel-hole collimator.[g] The dogs were injected IV with a dose of 80–90 MBq $99mTcO_4^-$. Forty-five minutes after administration of the radiopharmaceutical, planar images of the thyroid glands were made during 60 seconds (matrix, 256 × 256).[16]

Computed tomography images of the pituitary gland and hypothalamus were obtained, both before and after IV administration of 2 mL/kg of contrast medium,[h] with a single detector helical CT unit[i] by 2-mm-thick transverse slices with a pitch of 1 (120 kV, 180–260 mA). The pituitary height (in mm)/brain area (in mm^2) value was calculated as described previously.[17]

DNA sequence analysis of TSHB and TRHR

The localization of the exon-intron borders, PCR amplification reactions, PCR programs, DNA sequencing reactions, and methods of DNA sequence analysis are described in the supporting information file.

Results

Between 2008 and 2012, 7 Miniature Schnauzers (3 dwarfs, 4 nondwarfs) were diagnosed with central hypothyroidism. In five of these animals the secondary nature of the hypothyroidism was diagnosed by a 3-day TSH-stimulation test (2 dwarfs, 3 nondwarfs) and in 2 dogs by a TRH-stimulation test (1 dwarf, 1 nondwarf). In one of the dwarfs in which the 3-day-TSH stimulation test was performed, only thyroid scintigraphy (and not plasma TT_4 concentration measurement) was repeated after 3 days. Five dogs were intact males (4 nondwarfs, 1 dwarf) and 2 intact females (2 dwarfs). Ages ranged from 7 months to 2 years in the dwarfs and from 5 to 8 years in the nondwarfs. Pedigrees of 3 dwarfs and 3 nondwarfs were available for analyses (Fig 1). Two dwarfs were full siblings from different litters, and 1 dwarf and 3 nondwarfs had a common ancestor. The pedigrees display short inbreeding loops in the ancestry of 2 dwarfs and 4 nondwarfs, suggesting that a recessive gene variant plays a role in the etiology.

The dogs were referred for various clinical signs that were already present at a very young age (all dwarfs) or had been observed for the first time 4–8 weeks before admittance (all nondwarfs). History taking revealed mental dullness and lethargy in all dogs, superficial pyoderma in 2 nondwarfs, mild to moderate obesity in 1
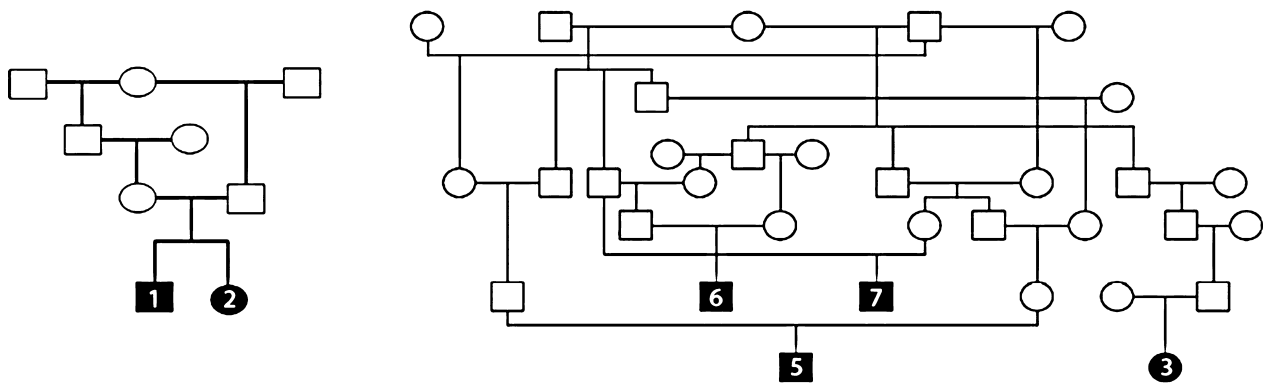
Fig 1. Pedigrees of 6 Miniature Schnauzers with central hypothyroidism. Males are depicted by squares and females by circles. Dogs with central hypothyroidism are indicated by the black symbols. Dogs 1–3 are dwarfs, and dogs 4–7 are of normal stature. Both pedigrees contain inbreeding loops.

dwarf and all nondwarfs, and impaired dentition in 2 dwarfs. The dwarf Miniature Schnauzers weighed 4.2–6 Kg (median 4.8 Kg) and the body weight of the nondwarfs ranged from 11.4 to 13.8 Kg (median 12.5 Kg). Physical examination of the dwarfs revealed disproportionate dwarfism (Fig 2) including a short neck, small head and short legs (n = 3), a scaly skin (n = 3), weak pulses (n = 1), mandibular prognathism (n = 2), kyphosis (n = 1), and lordosis (n = 1). Two dwarfs had hind leg weakness. In the nondwarfs, physical examination revealed a scaly skin (n = 3), alopecia between the shoulder blades, on the caudal back and tail (n = 2), weak pulses (n = 1) and hind leg weakness (n = 2). The neurological examination was otherwise unremarkable in all dogs and thyroid palpation was also unremarkable in all cases.

Complete blood count revealed mild anemia in 1 dwarf and 2 nondwarfs. Complete blood biochemistry profile was unremarkable in the dwarfs. In the nondwarfs, it revealed mild hypoalbuminemia (n = 1), mildly severely increased plasma alkaline phosphatase activity (n = 3), mildly increased plasma alanine

transaminase activity (n = 2), moderate to severe hypercholesterolemia, and mild to severe hyperlipidemia (n = 3).

Hormone measurements and function tests

Two dwarfs and 4 nondwarfs had a plasma TT_4 concentration <0.16 µg/dL (<2 nmol/L). The remaining dwarf had a plasma TT_4 concentration of 0.93 µg/dL (12 nmol/L). The plasma TSH concentration was below the detection limit of the assay in the dwarfs and ranged from 0.04 to 0.17 ng/mL (median, 0.06 ng/mL) in the nondwarfs.

Repeated administration of rhTSH during 3 consecutive days in 1 dwarf and 3 nondwarfs resulted in a clear increase in plasma TT_4 concentration (Fig 3). TRH was administered in 3 dwarfs and 3 nondwarfs and did not result in an increase in plasma TSH concentration (Fig 4).
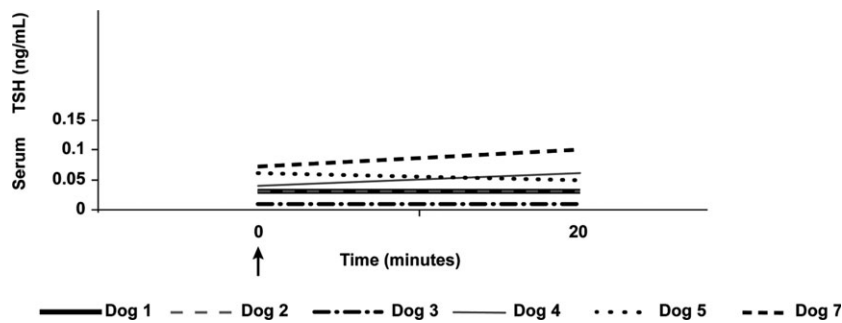
Plasma prolactin concentration after TRH administration was measured in 1 dwarf and 2 nondwarfs. In the dwarf, TRH administration failed to result in an

Fig 2. A 4-year-old female intact Miniature Schnauzer dwarf with central hypothyroidism standing in front of an unaffected dog of the same breed, age, and sex. Note the relatively short neck and limbs and the relatively small head of the dog.
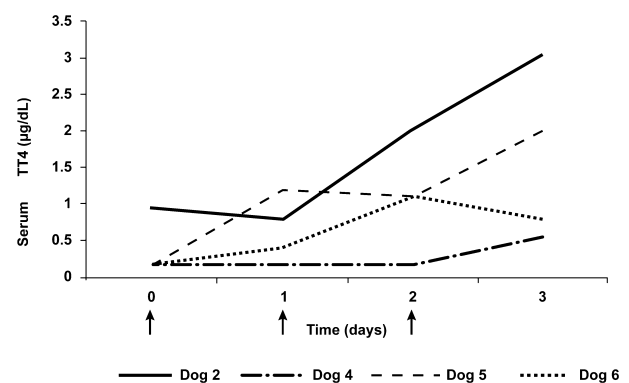
Fig 3. Effect of IV administration of 90 µg recombinant human thyroid-stimulating hormone (rhTSH) on 3 consecutive days on plasma total thyroxine (TT_4) concentration. In all dogs, repeated rhTSH administration resulted in a clear increase in plasma TT_4 concentration.

Fig 4. Thyrotropin-releasing hormone (TRH)-stimulation test. IV administration of TRH did not lead to an increase in plasma thyroid-stimulating hormone (TSH) concentration in any of the dogs tested.

increase in plasma prolactin concentration (maximal increase of 0.1 μg/L). In contrast, a clear rise was observed in the 2 nondwarfs (maximal increase of 10.2 and 13.2 μg/L).[18] Prolactin was not measured in the other dogs.

Basal plasma GH concentration was measured in 2 dwarfs and 2 nondwarfs. It was within reference interval in 1 dwarf (0.8 μg/L) and high (8.9 μg/L) in the other dwarf. In the 2 nondwarfs, plasma GH concentration was within the reference range (1.3 and 3.0 μg/L). Basal plasma IGF-I was measured in 3 dwarfs and 2 nondwarfs and ranged from 19 to 72 μg/L in the dwarfs and 100–118 μg/L in the nondwarfs.

Administration of GHRH to the 3 dogs (1 dwarf, 2 nondwarfs) with a basal plasma GH concentration ≤3.0 μg/L resulted in a clear rise of this parameter in the dwarf and in one of the nondwarfs (maximal increase of 3.4 and 5.6 μg/L). In the other nondwarf, there was no increase in plasma GH concentration after stimulation with GHRH. However, the plasma IGF-I concentration was 100 μg/L in this dog.

Basal plasma ACTH (1 dwarf, 3 nondwarfs) and cortisol (1 dwarf, 1 nondwarf) concentrations were within reference range in all dogs (median, 27 pg/mL; range, 20–52 pg/mL; 1.1 and 2.1 μg/dL, respectively).

Diagnostic imaging

In all 7 dogs, little to no uptake of $99mTcO_4^-$ by the thyroid glands was seen (Fig 5a). Repeated administration of rhTSH during 3 consecutive days (2 dwarfs, 3 nondwarfs) resulted in increased thyroid uptake of $99mTcO_4^-$ on day 3 (Fig 5b). In 5 dogs (2 dwarfs, 3 nondwarfs), including the 2 dogs in which the secondary nature of the hypothyroidism was not proven by a 3-day-TSH-stimulation test, the thyroid glands could still be identified on the scintiscan and had a normal shape (Fig 5a), indicating central hypothyroidism.

A CT scan of the pituitary area including the hypothalamus (1 dwarf and 2 nondwarfs) revealed no
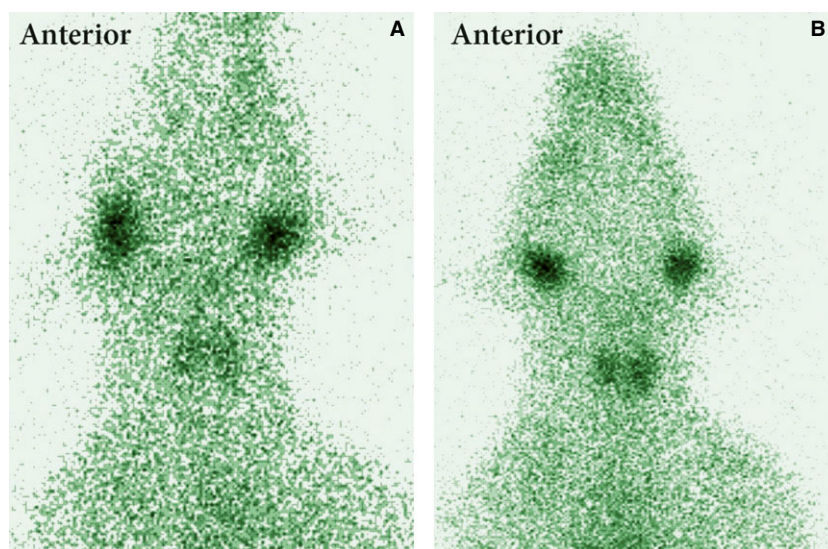
Fig 5. Scintigraphic image of the thyroids of a 1-year-and-8-month-old Miniature Schnauzer with central hypothyroidism. (A) Before stimulation with recombinant human thyroid-stimulating hormone (rhTSH). Note the severely decreased thyroid uptake of radioactive pertechnetate. The thyroids are of normal shape. (B) After 3 days of IV administration of 90 μg rhTSH. Note the clearly improved uptake of radioactive pertechnetate by the thyroids.

abnormalities. The P/B values were within the lower quarter of the reference range (0.14–0.16 [0.14–0.31[17]]).

Follow-up

The animals were treated with synthetic levothyroxine,[j] with a starting dose of 10–20 μg/kg q 12 hour. After 1–3 months (median 6 weeks), the dogs came for checkup. All owners reported that their animals became more active. In the 2 nondwarfs with alopecia, the hair coat was restored. In all dogs of normal stature, obesity improved (total weight loss ranged from 300 g to 3.7 Kg [median 2.8 Kg]). The weakness of the hind limbs also resolved in the 2 dwarfs and 2 nondwarfs. The plasma TT_4 concentration ranged from 2.18 to 5 μg/dL (28–64 nmol/L) in the dwarfs and 1.3–3.5 μg/dL (14–45 nmol/L) in the nondwarfs.

DNA sequence analysis of TSHB

DNA was collected from 3 dwarfs, 3 nondwarfs, and 25 control Miniature Schnauzers. The DNA sequence of the 3 exons and the introns of *TSHB* were analyzed in these animals. Compared to the reference dog genome, all cases were homozygous for T instead of A at position c.-63, which is part of the putative TATA-box. However, 6 of the 25 control dogs were also homozygous for T at this position and 13 dogs were heterozygous for A/T. In intron 1 at position c.-2 + 371, all cases were homozygous for C, whereas the reference dog genome was homozygous for T. Of 5 control dogs, 3 were also homozygous for C and 2 were heterozygous for C/T. Finally, in intron 1 at position c.-1−639, all cases were homozygous for A, whereas the reference dog genome was homozygous for G. Of 5 control dogs, 2 were homozygous for A and 3 were heterozygous for A/G at this position. Otherwise, the sequence of *TSHB* of the cases was in agreement with the reference dog genome.

DNA sequence analysis of TRHR

The DNA sequence of the 2 exons and the intron boundaries of *TRHR* were analyzed in 3 dwarfs, 3 nondwarfs, and 6 control dogs. DNA sequence analysis revealed 2 variations. Three cases, 1 dwarf and 2 nondwarfs, were homozygous for A at position c.-73, whereas the reference dog genome was homozygous for G. These dogs were also homozygous for C at position c.-94, whereas the reference dog genome was homozygous for T. One case was heterozygous for both variations. No other mutations were found.

Discussion

This study demonstrates that central hypothyroidism could be an underdiagnosed disorder in Miniature Schnauzers with signs indicative of deficient thyroid hormone secretion. Whereas primary hypothyroidism is commonly diagnosed in dogs, central hypothyroidism is rarely reported.[7–11] The fact that this rare disorder occurred in 7 dogs from the same breed suggests that central hypothyroidism could have a genetic background in Miniature Schnauzers. However, no disease-causing mutations were found in the *TSHB* gene and exons of the *TRHR* gene.

Differentiating primary and central hypothyroidism is problematic because TSH deficiency in central hypothyroidism results in atrophy of the thyroid gland, with resultant inadequate thyroxine secretion or radionuclide uptake on TSH stimulation testing and nuclear scintigraphy, respectively.[19–21] The relatively low sensitivity of increased plasma TSH concentration in dogs with primary hypothyroidism contributes to the difficulty in the diagnosis of central hypothyroidism. The situation in Miniature Schnauzers opens the possibility that secondary hypothyroidism is more common than thought in other breeds as well and may in part explain the low sensitivity of the canine TSH assay.

Absence of an increase in plasma TSH concentration after TRH administration is not fully diagnostic for central hypothyroidism. Also in dogs with primary hypothyroidism, TRH administration does not result in a significant rise in plasma TSH concentration.[19–21] In 5 of the dogs, the central nature of the hypothyroidism was confirmed by administering rhTSH on 3 consecutive days.[1] Cases of long-standing central hypothyroidism with subsequent thyroid atrophy often fail to respond to a single dose of TSH.[22] Repeated administration of TSH will result in the reactivation of thyroid tissue, which will not occur in primary hypothyroidism.[9] The reactivation of thyroid tissue was shown by an increase in circulating TT_4 concentration (4 dogs) and thyroid uptake of $99mTcO_4^-$ (5 dogs). In the remaining 2 dogs, the diagnosis of central hypothyroidism was based on scintigraphy. In these dogs, the thyroid glands could still be identified and had a normal shape. In contrast, in dogs with primary hypothyroidism, immune-mediated destruction will result in no or a very irregular uptake of $99mTcO_4^-$.

Central hypothyroidism may be because of either isolated TSH deficiency or combined pituitary hormone deficiency. To determine whether a dog also has a deficiency of GH, ACTH, prolactin, or gonadotropins, the pituitary can be stimulated with GHRH, corticotropin-releasing hormone (CRH), TRH, and gonadotropin-releasing hormone (GnRH).

In the previous reports about congenital central hypothyroidism in dogs, GH response to provocative testing was blunted.[9,10] Because these dogs did not show signs of GH deficiency, the authors suggested that this suboptimal response was not because of a defect of the somatotropic cells. In the present report, 2 of the 3 Miniature Schnauzers in which a GHRH-stimulation test was performed, responded to GHRH administration with a normal increase in plasma GH concentration, indicating that the somatotropic cells were unaffected. Interestingly, one of the Miniature Schnauzers did not respond with a rise in plasma GH concentration after GHRH administration. In contrast to the other 2 Miniature Schnauzers, this dog was already treated with levothyroxine at the time of the

GHRH-stimulation test. The absence of a rise in plasma GH concentration after GHRH administration may be explained by the feedback effect of the exogenous thyroid hormones at the hypothalamic level, resulting in an increased somatostatin tone,[20] which will suppress both the basal GH secretion and the effect of provocative testing. This, combined with the fact that this Miniature Schnauzer did not display growth retardation and had a plasma IGF-I concentration of 100 μg/L, makes GH deficiency unlikely in this animal.

In the dwarf Giant Schnauzers,[9] ACTH response to CRH was reported as normal. In these Miniature Schnauzers, no CRH-stimulation test was performed. However, basal plasma concentrations of ACTH and cortisol were determined in 4 (1 dwarf, 3 nondwarfs) and 2 (1 dwarfs, 1 nondwarf) dogs, respectively, and were within their respective reference ranges.[15] These findings suggest normal corticotropic function. Nevertheless, a CRH-stimulation test would be required in all cases for optimal evaluation of the hormone secreting capacity of the corticotropic cells.

Although all dogs had central hypothyroidism, there seemed to be at least 2 different subclasses. Three dogs displayed disproportionate dwarfism, a hallmark of congenital hypothyroidism, whereas the other dogs were of normal stature. Also the age of disease onset differed between these 2 groups. This, however, does not necessarily exclude congenital central hypothyroidism in the dogs of normal stature. It may be that the TSH deficiency in these dogs was mild compared with the dwarf group, which allowed normal growth. However, it is also possible that the dwarfs and nondwarfs suffer from a different form of hypothyroidism.

The TRH-stimulation test failed to result in an increase in plasma prolactin concentration in one of the dwarf Miniature Schnauzers, but resulted in a clear rise of this hormone in the 2 dogs of normal size. This could also suggest that these animals are affected by 2 different disorders and that dwarf Miniature Schnauzers could have a combined deficiency of TSH and prolactin. It is however not possible to draw firm conclusions on the results of TRH response testing in just 3 dogs.

The dogs had no history of head trauma that could explain low TSH secretion. To evaluate if there was any indication that the central hypothyroidism was acquired in another way, CT scanning of the pituitary area was performed. Instead of being increased, as can be expected in acquired forms of central hypothyroidism such as lymphocytic adenohypophysitis[3] or a pituitary tumor, the P/B value was in the low end of the reference range in both the dwarfs and the nondwarfs, making congenital maldevelopment of the pituitary a more feasible option in both groups. Also 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.

If central hypothyroidism in Miniature Schnauzers were to have a genetic background, *TSHB* would serve as a prime candidate gene.[23,24] All tested case dogs were homozygous for T instead of A at c.-64. This A to T mutation affects the putative TATA-box. The TATA-box is an important element in many gene promoters of vertebrates and the functionality of the homologous TATA-box in *tshb* of the mouse has been demonstrated.[25] However, 6 out of 25 control Miniature Schnauzers were homozygous for T at c.-64 as well. If this mutation would be responsible for central hypothyroidism, it would have a very low penetrance. We cannot rule out the possibility that the effect of this mutation on *TSHB* expression is enhanced by another genetic or environmental factor in the patients. The other 2 variations that were found in intron 1 of *TSHB* at position c.-2 + 371 and at position c.-1−639 were regarded as single nucleotide polymorphisms (SNPs), because the cases and a number of control dogs were homozygous for these variants.

A candidate gene that could explain both TSH and prolactin deficiency is *TRHR*,[26] because TRH stimulates both the thyrotropes and the lactotropes. The variations found in the noncoding region of exon one of *TRHR* could be related to the promoter region of this gene and therefore to the expression of the protein. However, the corresponding haplotype was only found in 3 of 6 cases, of which 2 belonged to the dwarf and 1 to the nondwarf group (without prolactin deficiency) and therefore regarded as insignificant.

Because of its retrospective nature, this study has some limitations. Because not all pituitary hormone stimulation tests were performed, it is not clear if an isolated or combined form of TSH deficiency is present in these Miniature Schnauzers and if the dogs have the same or different forms of central hypothyroidism. Further studies to determine the secretory capacity of all adenohypophyseal hormones are needed to determine the underlying cause(s) of the central hypothyroidism in this breed.

Footnotes

Acknowledgments

Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Appendix S1. DNA sequence analysis of *TSHB* and *TRHR*

Table S1. Oligonucleotides used for the amplification of canine *TSHB* DNA fragments.

Table S2. Oligonucleotides used for the amplification of canine *TRHR* DNA fragments.