Review

Thyroid Dysplasia in Wistar Hannover GALAS Rats

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Abstract: Thyroid dysplasia was recognized in WistarHan GALAS rats and confirmed as a heritable congenital disorder. The gene or genes involved were not identified, but homozygous animals with thyroid dysplasia also exhibited stunted growth, had reduced pituitary gland growth hormone (GH) and were hypothyroid. Heterozygous animals exhibited thyroid dysplasia with normal thyroid hormonal homeostasis and no difference in the incidence of preneoplastic or neoplastic lesions in oncogenicity studies. (J Toxicol Pathol 2009; 22: 247–254)

Key words: thyroid gland dysplasia, pituitary dwarfism, GALAS rats

Introduction

In 1997, it was noted that approximately 25% of the offspring of the Hannover Wistar GALAS rats derived from BRL Füllinsdorf were affected by thyroid lesions, had growth retardation, were hypothyroid however the pituitary growth hormone could not be demonstrated by immunohistochemistry. Similarly, studies in other companies, including the breeders of the GALAS program, were affected by this same lesion. The probable genetic origin as well as the histological details, led to the term ‘thyroid gland dysplasia’. The lesion was initially presented at the GTP Seminar at Zürich in 1998. In spring 1999 it was decided between RCC Ltd and the members of the GALAS program to institute a selective breeding and culling program for the colony in order to completely eradicate the thyroid alteration from the RCC breeding colony by 2000. In 2001, a vacuolar change in the thyroid follicular cells rats representing the same lesion as well as dwarfism in GALAS were reported1,2. Furthermore, bone characteristics were described in dwarfs derived from GALAS rats3.

Material and Methods

Procedures performed at Schering AG, Berlin

Selected litter mates, dwarfs and normal stature animals of the same litters were euthanatized at approximately 8–9 weeks of age. Blood samples were collected at necropsy and serum levels of T3, T4 and TSH were measured by the following method. Serum levels of T3, T4 and TSH were using commercially available EIA kits (for TSH: Amersham Pharmacia Biotech code RPN 2564; for T3: Biomerica code No. 7013; for T4: Biomerica code No. 7012).

The thyroid glands were collected, weighed, fixed in 4% neutral phosphate-buffered formaldehyde solution, trimmed, processed, embedded in paraffin wax, cut at an approximate thickness of 4 μm, stained by haematoxylin and eosin, and examined by light microscopy.

Selected paraffin sections of enlarged thyroid glands of dwarfs and normal stature litter mates as well as litter mates without any gross lesion were stained with antibodies against growth hormone (hGH) and thyroglobulin (TGH). Briefly, polyclonal antibodies Anti-Growth hormone (Catalog No. AR028-5R, Clone No. PU028-UP, BioGenex, dilution of 1:100) and Rabbit Anti-Human Thyroglobulin antibodies (Code No. A 0251, Lot 096, DAKO, dilution 1:8000 over night) were used on phosphate-buffered formaldehyde fixed material, embedded in paraffin wax, and cut at a nominal thickness of 4 μm. For detection, DakoCytomation EnVision+ System-HRP (DAB) was used according to the test kit description.

Enlarged thyroid glands of dwarfs and normal stature
litter mates as well as litter mates without any gross lesion were also sampled and fixed in 5% phosphate buffered glutaraldehyde for examination by transmission electron microscopy.

Selective breeding and culling procedures

At RCC Itingen, Switzerland, all pups from parental WistarHan GALAS rat breeder pairs 141 and 46 and three randomly selected pups of 250 parental WistarHan GALAS rat pairs, respectively, underwent necropsy at approximately 5 to 6 weeks of age to determine the presence of the ‘thyroid gland dysplasia’. The thyroid glands were collected, fixed in 4% neutral phosphate-buffered formaldehyde solution, trimmed, processed, embedded in paraffin wax, cut at an approximate thickness of 4 μm, stained by haematoxylin and eosin, and examined by light microscopy. This procedure was performed during three sequential screenings of animals on 119, 44 and 36 parental WistarHan GALAS rat breeder pairs, respectively.

Hormone study at RCC

Twenty five offspring per sex of selected breeding pairs, known to transmit the disease, were selected at 6–8 weeks of age and maintained for 13 weeks under optimal hygienic standard conditions. Blood samples were collected on days 1, 28 and 90. Serum levels of T3, T4 and TSH were using commercially available EIA kits (for TSH: Amersham Pharmacia Biotech code RPN 2564; for T3: Biomerica code No. 7013; for T4: Biomerica code No. 7012). Histologic examination was performed on the thyroid glands of all animals.

Hormone study at participating Swiss company

One hundred and fifty offspring per sex were randomly selected at approximately 4 weeks of age and maintained for 14 weeks under specified pathogen free standard laboratory conditions. Blood samples were collected at weeks 5 and 14. Plasma levels of T3, T4 and TSH were measured using commercially available EIA kits (for TSH: Amersham Pharmacia Biotech code RPN 2564; for T3: Biomerica code No. 7013; for T4: Biomerica code No. 7012). The thyroid glands of all animals were examined histologically using standard light microscopy.

Results

Mechanistic study at Schering AG, Berlin

The thyroid glands were enlarged and absolute and relative organ weights were increased. At light microscopy
examination of enlarged thyroid glands, there was a diffuse, rarely focal or multifocal change consisting of enlarged follicle cells which typically had apically placed nuclei located on a subcellular structure (colloid-containing vesicle) (Fig. 1). The incidence of affected animals in 3 litters was: 5 dwarfs (22%), 12 normal growing animals with thyroid dysplasia (52%) and 6 unaffected littersmates (26%) (Fig. 2). By electron microscopy, in dwarfs as well as...
normal growing animals with thyroid dysplasia, the basally located rough endoplasmic reticulum was dilated and displaced the normally basally located nucleus to an apical position. The apically located endoplasmic reticulum was not affected (Figs. 3–7).

Dwarfs with reduced body weights (less than one third of littermates) showed features of a dysplastic thyroid gland, a 6–8 fold increase in serum TSH levels along with a severe decrease of T4 leading to abnormal TSH/T4 ratios (hypothyroid). In contrast, animals showing normal growth with dysplastic thyroid glands appeared to be normal for both TSH and T4 (euthyroid) (Table 1). There was no difference in TSH-levels in the pituitary glands of dwarfs compared to the animals with normal growth using immunohistochemistry. GH-levels in the pituitary glands of dwarfs were remarkably reduced when compared to animals with normal growth and no difference was seen between normal growing animals with thyroid dysplasia and unaffected animals (Figs. 8–9).

Regarding TGH, in follicle epithelia of dwarfs, there was a clumpy immunohistochemical reaction product. In generally smaller follicles, the reactivity was irregular or missing. In contrast, in the follicle cell cytoplasm of control animals, the reaction product took the form of fine droplets, and within the follicular colloid it was fine disperse. In heterozygotes, both aforementioned reactivity patterns were found (Figs. 10–12).

### Table 1. Summarized Results of Mechanistic Study Performed at Schering AG, Berlin

| Feature | BW (g) | Sex | Thyroid feature | GH stain | TSH (μg/l) | T4 (nmol/l) | TSH/T4 |
|---------|--------|-----|----------------|----------|------------|-------------|--------|
| dwarf   | 36.0   | F   | dysplastic     | normal   | 6777       | 1           | 6777   |
| dwarf   | 32.7   | F   | dysplastic     | normal   | 5371       | 2           | 2685   |
| normal  | 151.8  | F   | dysplastic     | normal   | 731        | 34          | 21.5   |
| normal  | 148.8  | F   | dysplastic     | normal   | 225        | 35          | 6.4    |
| normal  | 147.3  | F   | dysplastic     | normal   | 664        | 47          | 14.1   |
| normal  | 149.7  | F   | dysplastic     | normal   | 568        | 44          | 12.9   |
| normal  | 216.7  | M   | dysplastic     | normal   | 473        | 75          | 6.3    |
| normal  | 229.3  | M   | dysplastic     | normal   | 2594       | 45          | 57.6   |
| normal  | 150.7  | M   | normal         | normal   | 87         | 31          | 2.8    |
| normal  | 135.2  | F   | normal         | normal   | 403        | 46          | 8.8    |
| normal  | 147.3  | M   | normal         | normal   | 705        | 31          | 22.7   |

All pups of the same litter.

Fig. 8. Immunohistochemical localization of Growth Hormone (hGH) in a control rat pituitary gland. Many cells reacting positively. hGH antibody was obtained from BioGenex (USA). Secondary HRP-conjugated anti-mouse antibody was obtained from Dako (Germany).

Fig. 9. Immunohistochemical localization of Growth Hormone (hGH) in a dwarf rat pituitary gland. Only a few cells reacting positively. hGH antibody was obtained from BioGenex (USA). Secondary HRP-conjugated anti-mouse antibody was obtained from Dako (Germany).
Results obtained by selective breeding and culling procedures in GALAS colonies

At RCC Itingen, three sequential screenings with subsequent culling and selective breeding were performed to eliminate the undesirable traits of thyroid dysplasia and stunted growth from the colony. In the first screening, all pups from 141 litters were evaluated, whereby 28 (19.6%) of the litters were affected. The incidence of this lesion ranged within littersmates between 12.5 and 64.0%. After rotation breeding, in the second screening, three randomly selected pups of 250 litters were evaluated. Three (1.2%) of the litters were affected. Therefore, after a next rotation breeding, all pups of 46 litters were examined and no case of thyroid gland dysplasia was recorded.

The following incidences were recorded at histology for other GALAS breeders: Breeder 1: 9 (7.6%) of 119 litters were affected with an incidence within the litters of 25.0%–75.0%; Breeder 2: 10 (22.7%) of 44 litters were affected with an incidence within the litters of 6.7%–100.0%; Breeder 3: 6 (16.7%) of 36 litters were affected with an incidence within the litters of 36.5.0%–67.0%.

Hormone studies

In thyroid hormone studies of 13 weeks duration performed both at RCC Ltd and at an agrochemical company showed that there were no relevant differences in TSH, T3 and T4 levels between normal control animals (not exhibiting thyroid dysplasia) and normally growing, dysplastic animals (Tables 2 and 3). More generally, the study of the agrochemical company showed that there were no in-life parameters that could indicate the presence or absence of the condition of thyroid dysplasia.

Discussion

In 1997, a spontaneous lesion was detected in the thyroid glands of approximately 25% of the offspring of a
Thyroid Dysplasia in GALAS Rats

WistarHan: Brl (GALAS) rat colony at Schering AG, Berlin. The thyroids were enlarged and absolute and relative organ weights were increased. There was no clinical or biochemical alteration. However, well proportioned, hypothyroid dwarfs with GH-cell hypoplasia in the pituitary gland were also encountered in isolated litters. A similar lesion had been detected in 1996 in a satellite group of 50 young Wistar rats (12–13 weeks of age) from an oncogenicity study that were histologically examined at RCC Itingen in Switzerland. In the high dose group, treatment-related hepatocellular hypertrophy accompanied by an uncommon type of diffuse follicular hypertrophy in the thyroid gland was noted. Since there is a known relationship between both findings, i.e. increased turnover of

| Table 2. Serum Levels for T3, T4 and TSH in 25 Offsprings Per Sex of Selected Parental Animals |
|-----------------------------------------------|
| Males                          | Animals without dysplasia | Animals with dysplasia |
|                               | T3 (nmol/l) | T4 (nmol/l) | TSH (μg/ l) | T3 (nmol/l) | T4 (nmol/l) | TSH (μg/ l) |
|---------------------------------|--------------|--------------|-------------|--------------|--------------|--------------|
| Day 1                           | n            | 12           | 12          | 12           | 13           | 13           |
| Mean                            | 0.84         | 37.4         | 7.21        | 0.75         | 38.44        | 6.32         |
| Stand.Dev.                      | 0.18         | 11.07        | 1.92        | 0.17         | 5.49         | 2.48         |
| Day 28                          | n            | 12           | 12          | 12           | 13           | 13           |
| Mean                            | 0.83         | 34.33        | 7.65        | 0.76         | 41.26        | 5.91         |
| Stand.Dev.                      | 0.12         | 14.91        | 3.15        | 0.10         | 5.31         | 1.43         |
| Week 13                         | n            | 12           | 12          | 12           | 13           | 13           |
| Mean                            | 0.86         | 30.42        | 6.91        | 0.83         | 42.31        | 5.53         |
| Stand.Dev.                      | 0.14         | 13.42        | 1.73        | 0.12         | 15.40        | 1.92         |
| Females                         | Animals without dysplasia | Animals with dysplasia |
|---------------------------------|--------------|--------------|-------------|--------------|--------------|--------------|
| Day 1                           | n            | 13           | 13          | 11           | 12           | 12           |
| Mean                            | 0.97         | 24.27        | 9.28        | 0.98         | 30.42        | 6.36         |
| Stand.Dev.                      | 0.22         | 7.41         | 2.64        | 0.15         | 13.38        | 1.76         |
| Day 28                          | n            | 13           | 13          | 12           | 12           | 12           |
| Mean                            | 0.85         | 28.05        | 11.77       | 0.88         | 32.65        | 7.82         |
| Stand.Dev.                      | 0.09         | 6.66         | 3.67        | 0.17         | 12.28        | 1.37         |
| Week 13                         | n            | 13           | 13          | 12           | 12           | 12           |
| Mean                            | 0.94         | 29.33        | 8.69        | 1.01         | 32.44        | 6.85         |
| Stand.Dev.                      | 0.19         | 9.58         | 2.31        | 0.26         | 8.91         | 1.29         |

Study performed at RCC Ltd Itingen, Switzerland.

| Table 3. Serum Levels for T3, T4 and TSH in 140 Offsprings Per Sex of Randomly Selected Parental Animals |
|-----------------------------------------------|
| Males                          | Animals without dysplasia | Animals with dysplasia |
|                               | T3 (nmol/l) | T4 (nmol/l) | TSH (μg/ l) | T3 (nmol/l) | T4 (nmol/l) | TSH (μg/ l) |
|---------------------------------|--------------|--------------|-------------|--------------|--------------|--------------|
| Week 5                          | n            | 128          | 128         | 128          | 21           | 21           | 21           |
| Mean                            | 1.46         | 101.5        | 13.16       | 1.33         | 96.4         | 13.66        |
| Stand.Dev.                      | 0.31         | 12.1         | 2.46        | 0.26         | 10.1         | 2.45         |
| Week 14                         | n            | 128          | 128         | 128          | 21           | 21           | 21           |
| Mean                            | 1.65         | 102.7        | 12.70       | 1.77         | 101.5        | 12.98        |
| Stand.Dev.                      | 0.49         | 15.5         | 2.74        | 0.51         | 15.9         | 2.42         |
| Females                         | Animals without dysplasia | Animals with dysplasia |
|---------------------------------|--------------|--------------|-------------|--------------|--------------|--------------|
| Week 5                          | n            | 132          | 132         | 132          | 18           | 18           | 18           |
| Mean                            | 1.68         | 86.2         | 12.27       | 1.54         | 78.30        | 12.71        |
| Stand.Dev.                      | 0.57         | 16.7         | 3.33        | 0.38         | 16.80        | 3.96         |
| Week 14                         | n            | 132          | 132         | 132          | 18           | 18           | 18           |
| Mean                            | 1.46         | 75.7         | 13.94       | 1.49         | 76.10        | 14.39        |
| Stand.Dev.                      | 0.42         | 15.1         | 2.62        | 0.57         | 15.40        | 3.08         |

Study performed at participating Swiss company.
hormones by higher metabolic activity of the liver leading to hepatocellular hypertrophy and following adaptive response of the thyroids, i.e. follicular hypertrophy, the lesion was considered to be treatment-related. However, the thyroid gland findings were also seen in some control animals. After this 1996 study, follicular hypertrophy of the thyroid gland was noted in most studies, randomly distributed over all groups including the controls. From a retrospective evaluation, the first case appeared in RCC studies in 1995.

As mentioned above, approximately 25% of the offspring were affected by thyroid lesions along with growth retardation in single litter mates. The latter were hypothyroid and the pituitary growth hormone could not be demonstrated by immunohistochemistry. Within one litter, there were dwarfs, animals with normal body size along with thyroid dysplasia and unaffected animals (selected litters: 5 dwarfs, 12 normal growing animals with thyroid dysplasia, 6 normal animals, i.e. approximately 22%, 52% and 26%, respectively). Animals with normal growth patterns had no clinical or biochemical abnormalities including those with thyroid lesions. In contrast, dwarfs with growth retardation showed an almost complete lack of pituitary GH reactivity, reached half the size of the normal litter mates, and a high proportion of these animals displayed continuous abnormal hopping movements.

TGH in thyroid glands showed abnormalities when examined by immunohistochemistry consisting of a clumpy immunohistochemical reaction product in follicle epithelia and an irregular or missing reactivity in follicular colloid of dwarfs, whereas different reaction patterns were recorded in normal growing rats with thyroid dysplasia.

Neonatal hypothyroidism is known to be associated with a significant decrease in pituitary GH content. In rats, GH cells first appear on day 18 of gestation. The synergism of glucocorticoid and thyroid hormones in the GH expression is described. Thyroid hormone exert a stimulatory effect on GH expression in the fetal pituitary gland. The fetal thyroid hormone level is low until the onset of fetal thyroid function, which occurs between days 17 and 18 of gestation. The action on GH expression is evident as early as day 17 of gestation in rats. Therefore, it is concluded that also in the present alteration thyroid hormone levels directly influenced pituitary GH leading to the appearance of dwarfs. The cause of dwarfism by primary hypothyroidism in GALAS rats was also considered by others. It may be assumed that protein folding and secreting disorders underlie the hypothyroidism similar to the mechanism described for rdw rats.

The morphological alteration noted by light microscopy included diffuse, rarely focal or multifocal change, consisting of enlarged follicle cells which typically have apically placed nuclei located on a subcellular structure (colloid-containing vesicle). The subcellular structure was identified by electron microscopy as dilated endoplasmic reticulum that has displaced the normally basally located nucleus. Only the basally located endoplasmic reticulum structures became dilated, in contrast to the apical portion leading to the assumption of a polar affection of cell organelles. Lesions described elsewhere in GALAS rats were consisted with these alterations.

Despite the lack of growth hormone in the pituitary demonstrated by immunohistochemistry as well as a 3–5 fold increase in serum TSH levels along with a severe decrease of T4 leading to pathological TSH/T4 ratios (hypothyroid) in dwarfs, a morphological difference in the thyroid glands between dwarfs and normal sized animals with thyroid dysplasia could not be detected.

The proportion of dwarfs, normal size animals with thyroid dysplasia, and unaffected animals at a relation of approximately 1:2:1 led to the assumption of an autosomal recessive hereditary disorder.

These findings gave rise to the possibility that normal sized animals with thyroid dysplasia may react differently than normal animals in toxicity studies. Problems that could arise include increased tumor incidences, exaggerated reactions to weak goitrogens or secondary to hepatocellular hypertrophy. It was also unclear if the hormonal status changed with increasing age. Moreover, animals suffering from this hereditary disorder which produce a high number of dwarfs are not suitable for reprotoxicity studies.

Therefore, it was proposed to all GALAS members that a histological examination of the thyroid glands from all pups of numbered breeding pairs be performed at RCC Itingen, Switzerland. All littermates would receive the number of the breeder parents. All parental breeding pairs that had affected pups, would be eliminated from further breeding. The remaining parental animals would be bred crossover for a further search for thyroid-dysplasia gene bearers. RCC Ltd performed this procedure in three steps. In RCC litters, the incidence of this lesion ranged within littermates between 12.5 and 64.0%. From 2000 onwards, no new case of this disorder has been detected. Only a selected number of litters were examined from all other GALAS breeders showing a similar incidence of lesions within their colonies (7.6%–22.7%) and litters (6.7%–100%), respectively. Hence, it is concluded that at the stage when these investigations were performed, approximately 8.9% of all breeding animals of the GALAS colonies were affected by the involved gene(s) assuming an incidence of approximately 20% of affected litter mates (Hardy-Weinberg: p2+2pq+q2=1 leading to a relation of 79.2% normal animals : 19.6% normal sized but dysplastic animals: 1.2% dwarfs. A total of approximately 98.8% of the litter mates would appear to be normal.

In addition, hormone studies were performed to establish the validity of studies already performed with such animals. A number of companies took part in this test, either in cooperation with RCC or they performed in-house studies by themselves. In 13-week studies performed at RCC Itingen, Switzerland, and independently also in a agrochemical company, no changes could be detected in levels of T3, T4 and TSH in normal growing animals with thyroid dysplasia. This result was confirmed by other companies that performed similar in-house studies.
Published data on T3, T4 and TSH in normal growing animals were comparable\(^1\). In addition, there was no indication of increased preneoplastic and neoplastic lesions in oncogenicity studies performed with normal sized but thyroid dysplastic animals.

Summarized, thyroid dysplasia was recognized and confirmed as congenital disorder. It is hypothesized that homozygotes are dwarfs in which a reduction of pituitary gland GH, thyroid dysplasia and hypothyroidism is present. Animals with normal growth but dysplastic thyroids were not expressing higher incidences of pre-neoplastic or neoplastic changes in the thyroid gland but are not suitable for reprotoxicity studies because of the possibility of giving birth to dwarfs.

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