Cornification defect in the Golden retriever: clinical, histopathological, ultrastructural and genetic characterisation

Marie-Christine Cadiergues*, Anita Patel†, David H. Shearer‡, Ruth Fermor*, Suhel Miah§ and Anke Hendricks*

*Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK
†Dermatology Referrals, 23 Searchwood Road, Warlingham, Surrey CR6 9BB, UK
‡Vetcutis Limited, 104 Victoria Road, Diss, Norfolk IP22 4JG, UK
§The Institute of Orthopaedics and Musculoskeletal Science, University College London, Brockley Hill, Stanmore, Middlesex HA7 4LP, UK
Correspondence: Anke Hendricks, Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK. E-mail: ahendricks@rvc.ac.uk

Abstract

Veterinarians have recognised a non-pruritic skin disease in the Golden retriever breed characterised by excessive scaling of large, variably pigmented flakes of skin in otherwise healthy dogs. This prospective case series describes clinical, histopathological, ultrastructural and genetic features of this cornification defect in 17 affected dogs. The condition affects young dogs of either sex and is characterised by symmetrical, predominantly ventro-lateral scaling and hyperpigmentation of the trunk. Histopathological and ultrastructural changes of the stratum corneum are suggestive of delayed degradation of corneodesmosomes. A genetic aetiology is proposed and a single-trait autosomal recessive mode of inheritance discussed.

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Introduction

Over the years, veterinarians have recognised a non-pruritic skin disease in the Golden retriever breed characterised by excessive scaling of large, variably pigmented flakes of skin in otherwise healthy dogs. Although many dermatologists and dermatopathologists feel that they are seeing increasing numbers of affected dogs, this condition is as yet poorly documented in the literature.1,2 The diverse group of human diseases characterised by such a visible abnormality in epidermal function are grouped under the term ichthyosis. Only few dogs with ichthyosis-like skin disease have been reported in the veterinary literature. The best described syndrome is the heritable epidermolytic hyperkeratosis in one family of Norfolk terriers,3,4 but all the canine cases reported differ in their presentation from that seen in the Golden retriever breed, where this appears to be a more widespread problem. The aim of this prospective study is to (i) characterise the scaling disorder clinically, (ii) describe the structural changes associated with this disorder, and (iii) investigate its heritability.

Materials and methods

All procedures relating to this study were approved by the Ethics and Animal Welfare Committee of the Royal Veterinary College, London. Written informed consent was obtained from all owners.

Animals

Recruitment

With the help of a Golden retriever breeder and dog groomer, contact was established with a number of owners of potentially affected dogs. Furthermore, recruitment for the study was promoted to the referring veterinary surgeons of the Queen Mother Hospital for Animals of the Royal Veterinary College, London. A requirement of a minimum of 12 affected Golden retrievers matching the inclusion and exclusion criteria was calculated for estimating a mean age at onset (in weeks, based on an expected standard deviation (SD) of 3.5, an absolute error of 2 and a 95% level of confidence), and mean scaling and pigmentation scores (expressed per dog as sum of scaling scores and sum of pigmentation scores for all body regions, based on an expected SD of 30, absolute error of 17 and a 95% level of confidence). The aim was to recruit at least 15 dogs to allow for expected exclusions form the study. Finally, 20 Golden retriever dogs with a scaly appearance and a five-generation pedigree were recruited. The dogs were examined at veterinary practices in Surrey, at which one of...
the investigators (AP) worked or at the Queen Mother Hospital for Animals of the Royal Veterinary College.

Inclusion and exclusion criteria

Medical history: Dogs with a known underlying metabolic disease, a known history of other concurrent skin disease or on long-term pharmacotherapy for their skin condition were excluded. Non-steroidal anti-inflammatory drugs used to control pain and inflammation associated with osteoarthritis and antibiotics administered for wound management were permitted. Topical and systemic parasiticidal products and bathing with shampoos were also permitted; however, the owners were asked not to bath their dog within the week preceding the clinical examination.

General physical and dermatological examination. Only dogs with unremarkable general physical examination and excessive scaling skin lesions were included. Dogs with inflammatory or alopecic skin lesions in association with scaling were excluded.

Diagnostic tests. Inclusion required absence of ectoparasite infestation or microbial skin disease as determined by microscopic examination of skin scrapes, hair plucks, coat brushings and acetate tape strips from the skin surface and fungal culture of hair and scales (incubation on Dermasel agar (Oxoid, Basingstoke, UK) at 26 °C for 4 weeks). Furthermore, inclusion in the study required absence from systemic disease as determined by complete blood count and serum biochemistry values (total protein, albumin, total globulin, urea, creatinine, total bilirubin, triglycerides, glucose, cholesterol, alkaline phosphatase, alanine aminotransferase, total thyroxine and thyroid-stimulating hormone) within reference ranges or of no clinical significance.

Control dogs for histopathological and electron microscopy studies. Skin samples to serve as control tissue for histopathological and electron microscopy studies. Control dogs for histopathological and electron microscopy studies.

Clinical evaluation

History
The medical history including age at onset, progression, seasonality, known or suspected triggers, presence of pruritus, clinical signs, previous investigations/treatments and their outcome was collected from the owners. The owners were also asked to grade the severity of the condition on a 0–10 scale, where 0 = no scale visible and 10 = several layers of scales visible in the hair coat. When the identity of the sample was revealed, this dog was found to show histopathological and ultrastructural abnormalities were confined to the hair follicles.

Lesion scoring
The extent and the severity of the condition were visually assessed in 36 different sites: face, pinna (left and right, convex and concave), neck (dorsal, ventral, left and right lateral), thorax (dorsal, left and right lateral), lumbar dorsal, left and right flanks, perineum, dorsal and ventral tail, all four limbs (medial and lateral), all four feet, left and right axillae, sternum, left and right inguinal and abdomen. For each body site, scaling and pigmentation were assessed and scored from 0 to 3:

(1) Scaling:
(a) Average size of scale – 0: none; 1: ≤ 2 mm; 2: between 2 and 5 mm; 3: > 5 mm
(b) Amount of scale in the hair coat – 0: none; 1: mild; 2: moderate; 3: several layers of scales
(c) Amount of scale on the skin surface – 0: none; 1: mild; 2: moderate; 3: thick layer of scales adherent to the skin.

A maximum score of 9 per body site was possible.

(2) Pigmentation:
(a) Scale pigmentation – 0: white; 1: light grey/brown; 2: mottled dark grey/brown; 3: mottled black/dark brown
(b) Skin pigmentation – 0: white; 1: light grey/brown; 2: dark grey/brown; 3: black

A maximum score of 6 per body site was possible.

Analysis of lesion scores
Symmetry of lesions was tested by comparing the scores recorded on the left- and the right-hand sides, using a paired $t$ test. In the absence of significant differences, the scores of symmetrical regions (convex and concave pinna, lateral neck, lateral thorax, flanks, fore and hind limbs (medial and lateral), fore and hind feet, axillary and inguinal regions) were averaged.

The correlations between the size of the scales, the amount of scale on the skin and in the hair coat were tested using a Pearson correlation test. Provided the Pearson coefficient was over 0.9, the scores for these three parameters were added to result in a single ‘scaling’ score, which ranged between 0 and 9 for each body region. The correlation between the pigmentation of the scales and the pigmentation recorded on the skin was similarly tested and their respective scores were merged in a single ‘pigmentation’ score, which ranged between 0 and 6 for each body region. The scaling and pigmentation scores were also expressed as the percentage of the maximum score, 207 and 138, respectively.

Histological and ultrastructural investigations

Skin biopsy
Biopsy punches were used to obtain skin samples for histological (all affected and control dogs – three sites: dorsal, lateral and ventral thorax – Fig. 1, one 6-mm biopsy per site) and ultrastructural examination (three affected and two control dogs – lateral thorax, two 4-mm biopsy samples, removal of subcutis and most of the dermis in order to obtain samples of approximately 1 mm²) after local anaesthesia with lidocaine hydrochloride 2% plus adrenaline 0.001%. The biopsy sites were prepared only by carefully clipping hair with scissors prior to sampling, and care was taken to preserve the skin surface. If necessary, dogs were mildly sedated with medetomidine chlorhydrate 0.01 mg kg⁻¹ intravenously; sedation was reversed with atipamezole 0.05 mg kg⁻¹, intramuscularly.

Figure 1. Lesion maps showing the mean severity of scaling and pigmentation on the various sites, expressed as the percentage of the maximum score on an axial grey shading gradation from 0 (white) to 100% (black). Stars indicate the biopsy sites.
Histological evaluation
Punch biopsies were fixed in 10% neutralized buffered formalin. The specimens were then embedded in paraffin wax, and sections (3–4 μm) were stained with haematoxylin and eosin using an automatic staining system.

All specimens were examined in a blinded fashion by three of the investigators (DS, AH and MCC). Control dog specimens were examined first and used to define normality for each site (dorsum, lateral and ventral thorax). Biopsies from the affected dogs were then examined and the findings recorded in a standardised way for the following structures:

(i) Stratum corneum – presence of parakeratosis (none, patchy or complete), scale, hyperkeratosis, compact keratin (none, less than 50% of the surface, 50% of the surface or more) and pigmentation (none, patchy, complete).
(ii) Rest of the epidermis – presence of thickening (none, patchy, complete), pigmentation (none, patchy, complete), inflammation (none, patchy, complete) and nature of the infiltrate.
(iii) Dermis – presence of inflammation (none, mild, moderate or severe), distribution (superficial, periadnexal or deep) and nature of the infiltrate.
(iv) Adnexae – presence of follicular keratosis and any other abnormalities of the hair follicles, presence of sebaceous and sweat glands
(v) Subcutis – any abnormalities.

Ultrastructural examination on transmission electron microscopy (TEM)
Immediately after sampling, punch biopsies were fixed in freshly prepared 4% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 48 h at 4 °C. After rinsing in buffer, each biopsy was bisected and one piece of each was post-fixed in 0.5% RuO4 and 1% OsO4 in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C. The samples were then rinsed, dehydrated in a series of graded alcohol and then embedded in agar low viscosity resin. Ultrathin sections (70 nm thick) were collected on carbon-coated copper grids and stained with 2% uranyl acetate and 1% lead citrate. Preparations were viewed and photographed using a transmission electron microscope (Hitachi H-7650, Tokyo, Japan) operating at 80 kV.

Genetic investigations
Five-generation pedigrees were obtained from the owners or from a website which holds pedigree information on over 460 000 Golden retrievers bred in the UK.6 A group of age- and sex-matched control dogs was selected from this website. For each study case, a search of all dogs with the same birth date and the same sex was carried out which holds pedigree information on over 460 000 Golden retrievers bred in the UK. A random number generator (Microsoft Office Excel® 2003, Microsoft Corporation, Redmond, WA, USA) was used to define normality for each site (dorsum, lateral and ventral thorax). Biopsies from the affected dogs were then examined and the findings recorded in a standardised way for the following structures:

(i) Stratum corneum – presence of parakeratosis (none, patchy or complete), scale, hyperkeratosis, compact keratin (none, less than 50% of the surface, 50% of the surface or more) and pigmentation (none, patchy, complete).
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(iv) Adnexae – presence of follicular keratosis and any other abnormalities of the hair follicles, presence of sebaceous and sweat glands
(v) Subcutis – any abnormalities.

Statistical analysis
The statistical tests mentioned in the aforementioned sections were carried out using SPSS® 14.0 for Windows® (SPSS Inc., Chicago, IL, USA). A level of P < 0.05 was used to indicate a statistically significant difference. Sample size calculation was carried out using Win Episcope freeware version 2.0 (de Blas N, Ortega C, Frankena K, Noordhuizen K, Thrushfield M, CLIVE, Edinburgh, UK).

Results
Among the 20 dogs initially recruited, three were excluded due to concurrent medication (long-term glucocorticoids, vitamin A), or absence of clinical signs. One dog had been on treatment for hypothyroidism for 3 years. However, medical records established that the scaling was present 3 years prior to the diagnosis of hypothyroidism and had not changed since, whereas the other clinical signs had resolved; therefore the two conditions were considered unrelated and the dog was included in the study (dog 3). Three dogs were receiving prescribed medication at the time of the examination: dog 3 received 1.2 mg of L-thyroxine tablets once daily (Soloxine®, Arnold, Shropshire, UK), dog 8 was receiving meloxicam (Metacam®, Boehringer Ingelheim Ltd, UK) for chronic arthritis and dog 12 was on antibiotics for a recent trauma to a dew claw. All three dogs were considered as affected if (i) owners confirmed that their dog had a history of visible scaling of no apparent cause and if the owners reported to be pruritic. Various treatments had been attempted on seven dogs (keratolytic, keratoplastic or anti-inflammatory) which was 0.25 in this type of mode of inheritance). The recessive mode of inheritance can be assumed if Z² < 3.84 and the estimated segregation frequency p ± SD includes the value 0.25.

Clinical features
History
The age at examination, sex and age at onset of scaling of the 17 dogs are summarized in Table 1. Seven dogs were male and 10 female. When examined, dogs were between 8 months, and 9 years and 9 months of age.

The median age at onset of scaling reported by the owner was 13 weeks (range: 0–4 years). Among the 17 dogs, nine (53%) showed clinical signs before they were 3 months of age, 11 (65%) before 6 months of age and 13 (76%) before 1 year of age. The duration of the disease at the time of examination ranged between 17 weeks (dog 13) and 8.7 years (dog 5).

In three cases (dogs 1, 3 and 12), the owners reported no change in the severity of the condition. Of the 14 cases for which variability was reported, eight dogs were examined at their average severity (dogs 2, 6, 9, 11 and 14 to 17); three dogs were examined when their condition was near to or at its best (dogs 4, 5 and 7) and three dogs were examined when their condition was close to or at its worst (dogs 8, 10 and 13). There were six dogs for which a specific factor was reported by the owner to worsen the condition (dogs 4, 5 and 14: moulting; dogs 8 and 10: seasonal change; dog 13: whelping). Dog 8 was the only dog reported to be pruritic. Various treatments had been attempted on seven dogs (keratolytic, keratoplastic or anti-inflammatory) which was 0.25 in this type of mode of inheritance). The recessive mode of inheritance can be assumed if Z² < 3.84 and the estimated segregation frequency p ± SD includes the value 0.25.
antimicrobial shampoos, moisturizers, systemic antibiotics, supplementation with evening primrose oil, cod liver oil, various vitamins including vitamin A and Chinese herbal remedies) with no persistent improvement.

**Skin lesions**

Skin lesions only consisted of scales and hyperpigmentation except in dog 7 who had also mild interdigital erythema. Skin lesions were widely distributed on the body surface, but in all cases the nasal planum and the footpads were spared. Ear canals were not affected with the exception of dog 4 that was diagnosed with aural *Malassezia* overgrowth; however, the owner was not aware of any aural clinical signs and the dog had never received any specific treatment. The symmetry of the lesions was confirmed by the absence of significant differences between the scores recorded on the left and the right sides independent of the body region and the criterion tested (paired t-test, \( P \geq 0.05 \) in all dogs).

**Variability of the clinical signs between sites**

Table 2 summarizes the total scores for scaling and pigmentation for the different body sites; Fig. 1 illustrates their variability.

**Scaling.** Large and flat scales were present on the surface of the skin (Figs 2 and 3) but also in the hair coat (Fig. 4). Their size varied between 1 and 2 mm to 13 mm (Fig. 5) and correlated strongly and positively with the amount of scale (Pearson coefficient 0.97, correlation significant at the 0.01 level). Similarly, the amount of scale observed in the hair coat correlated very well with the amount observed on the skin (Pearson coefficient 0.98, correlation significant at the 0.01 level).

The scaling was most pronounced on the lateral thorax, flanks, sternum and abdomen (56–66% of the maximum score). It was less pronounced but easily detectable on the lateral and ventral neck, lumbar region, perineum and hind limbs (44–50%). The dorsal neck, dorsal thorax, fore limbs and groin were only mildly scaly (31–39%), whereas the face, both aspects of the ears, the tail and the feet were hardly scaly or normal (≤ 16%).

**Pigmentation.** The pigmentation of the scales correlated positively with the pigmentation of the skin (Pearson coefficient 0.9, correlation significant at the 0.01 level). The pigmentation varied according to the site of examination (Fig. 5): it was particularly marked on the axillae, sternum, abdomen, lateral thorax, groin, hind limbs and ventral neck (52–73% of maximum score); it was less marked but easily detectable on the lateral neck, flanks and fore limbs (41–48%) or mild on the dorsal neck, dorsal thorax and feet (16–28%); on the remaining areas there was only mild (face, lumbar dorsal region and dorsal tail; 10–13%) or no pigmentation (pinnae, ventral tail; ≤ 3%).

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The scores for scaling and pigmentation generally correlated considering all body regions (Pearson coefficient 0.76, correlation significant at the 0.01 level). However, there were discrepancies for the axillary and inguinal regions, which were markedly hyperpigmented (percentages of the maximum score of 73 and 57%, respectively) but only mildly scaly (31 and 39%, respectively).

**Variability of scaling and pigmentation between individuals**

The average degree of scaling and pigmentation for the individual dogs are summarized in Table 3. It is obvious that within the population of dogs included in the study, the severity of scaling and pigmentation varied markedly between individuals. However, overall the two types of lesions correlated well and positively (Pearson coefficient 0.85, correlation significant at the 0.01 level).

**Histological examination**

The histopathological findings compared to control dogs are summarized in Table 4 and illustrated in Fig. 6.

**Stratum corneum**

Parakeratosis was never observed. Compact orthokeratotic keratin was observed in 46 samples (Fig. 6b,d,e,g), affecting more than 50% of the surface with one exception. This was observed in at least two of the three sites in all dogs but one (dog 7). The stratum corneum was generally hypereosinophilic (Fig. 6b,d,e,g); this was generally observed in specimens from all three sites and also in at least two of the three sites sampled of each dog.

The presence of scale(s) (Fig. 6b,d) was recorded in 34 samples, more commonly on the ventral samples. No scales were detected in any of the three sites for one dog (dog 7); scales were detected only in one site in four cases (lateral: dogs 4 and 14; ventral: dogs 1 and 13). Hyperpigmentation of the stratum corneum was present in 38 samples (Fig. 6g); it was patchy in all samples but one and more frequently observed in lateral and ventral samples. This lesion was observed only on the ventral site in two cases (dogs 4 and 13).

**Rest of the epidermis**

It was mildly thickened in 30 samples (Fig. 6b,g); the thickening was patchy in 20 samples and complete in the other 10. The acanthosis did not necessarily correspond with the presence and the degree of hypereosinophilia of the
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overlying stratum corneum and was observed in specimens from all three sites. All three sites were acanthotic in only one dog (dog 17). Hyperpigmentation was noted in 38 biopsies (Fig. 6g), mainly from the lateral and ventral sites and was principally patchy and interfollicular; however, it appeared more prominent ventrally than in the two other sites. Both the epidermis of the control dogs (Fig. 6f) and the epidermis of the affected Golden retrievers (Fig. 6g,h) featured unidentified variably vacuolated cells with shrunken basophilic nuclei within the upper stratum spinosum where this was present. However, more than five vacuolated cells per biopsy specimen (Fig. 6g,h) were observed in 42 samples (79%), and at all three sites in most dogs, whereas more than five vacuolated cells were only observed in 22% of control biopsies. This difference of proportions was significant (57.5%, 95% confidence interval (CI) 31–74%). Inflammation was rarely present in the epidermis (six samples) and if so, it was mild and patchy in all samples.

Dermis

Inflammation of the dermis was recorded in 18 samples (10 dogs), essentially from ventral and lateral sites. It affected the superficial dermis in 14 sections; the other four sections showed inflammation of the superficial and periadnexal dermis. Inflammation was considered as mild in 14 biopsies and moderate in the other four. The infiltrate was mononuclear except in three lateral sections in which it was mixed with neutrophils. The presence of a dermal inflammatory infiltrate was not always accompanied by acanthosis.

Adnexae and subcutis

The adnexae and the subcutis were considered as normal in all samples.

Ultrastructural examination (TEM)

In the stratum corneum of normal skin, distal to the most proximal three to four layers, corneocytes were loose and corneodesmosomes were scarcely observed (Fig. 7a). In affected retrievers, corneocytes remained cohesive and corneodesmosomes more numerous (Fig. 7b).

Genetic analysis

The inbreeding coefficients of the 17 affected dogs varied between 0.9 and 9.1% (mean 5.5, 95% CI: 4.2–6.8); those of the 17 control dogs ranged between 2.3 and 14.5% (mean 7.2, 95% CI: 5.5–9.1). The mean values of the two groups did not differ significantly (Mann–Whitney test, P = 0.18).

A complex family tree spanning four generations was constructed from the pedigrees of nine of the dogs examined for this study (Fig. 8). This included a total of 95 dogs, of which 23 were affected and 19 had an unknown phenotype. A clustering of affected dogs among close relatives, most of them not sharing the same environment, suggests a strong hereditary component to this scaling disorder.

Table 4. Histopathological lesions observed in 51 samples obtained from the dorsal, lateral and ventral thorax of 17 dogs and numbers of sites affected per dog

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dorsal</th>
<th>Lateral</th>
<th>Ventral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum corneum Compact keratin ≥50% surface</td>
<td>13</td>
<td>16</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>&lt;50% surface</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hypereosinophilia</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>46</td>
</tr>
<tr>
<td>Scales</td>
<td>9</td>
<td>11</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Pigmentation Patchy</td>
<td>6</td>
<td>14</td>
<td>17</td>
<td>37</td>
</tr>
<tr>
<td>Complete</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rest of epidermis Thickening</td>
<td>9</td>
<td>4</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Complete</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Pigmentation Patchy</td>
<td>6</td>
<td>14</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>Complete</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Superficial vacuolation† &gt;5 cells</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>Inflammation Patchy</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Complete</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dermis Inflammation Zone 1</td>
<td>11</td>
<td>5</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Zones 1 and 2</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

†number of unidentified variably vacuolated cells with shrunken basophilic nuclei within the upper stratum spinosum.
presence of several affected siblings is not supportive of a new mutation. Assuming monogenic Mendelian inheritance, the pattern of affected individuals is not compatible with simple autosomal dominant or X-linked modes of inheritance, neither it is compatible with mitochondrial inheritance. Due to the high number of affected offspring from an affected and an unaffected parent, digenic (or oligogenic) inheritance appears unlikely. The most compatible modes of inheritance are autosomal recessive, or possibly autosomal dominant with variable penetrance. The hypothesis of an autosomal recessive mode of inheritance was tested using segregation analysis. A total of 63 offspring from 10 litters that had at least one affected animal were included. At $Z^2 = 1.23$ ($P > 0.2$) and with an estimated segregation frequency of $0.32 \pm 0.07$, this was consistent with a simple recessive mode of inheritance.

Discussion

This cornification defect of Golden retrievers appears to affect dogs from a young age, with no sex predilection. The condition may be stable or run a waxing and waning course. Clinically, the disorder spares the nasal planum, footpads and ear canals, and the lesions consist of large, flat and variably pigmented scales present in the hair coat and on the skin, itself variably hyperpigmented. The distribution is symmetrical and mainly ventral and lateral. The overall severity of the condition varies markedly between individuals, but there is a consistent pattern of lesion distribution. The severity of the scaling correlates very well with the intensity of the pigmentation with the exception of the axillary and inguinal regions which are more pigmented than scaly; this discrepancy may be explained by the fact that on these two regions the scales, although in

![Figure 6. Representative photomicrographs of normal canine epidermis (a, c and f) and epidermis from an affected Golden retriever (b, d, e, g and h). Haematoxylin and eosin.](image-url)

Compared to control skin compact, eosinophilic orthokeratosis is obvious (b, d, e and g). Scale is visible in images b and d. g illustrates stratum corneum and malphigian hyperpigmentation. Some biopsies showed mild focal acanthosis (b and g). Vacuolated cells can be found in control (f) and, more numerously, affected skin (g and h).
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excessive amount, are mechanically removed due to friction in these areas.

For the purposes of the study, it would have been preferable to compare lesional histopathology to that of normal skin from healthy Golden retrievers only, rather than dogs of a variety of breeds. However for legal reasons, biopsy of healthy privately owned dogs was not possible, and specimens of normal skin had to be sourced from fresh cadavers of dogs euthanized for reasons other than poor health potentially affecting skin structure and function. More than one such cadaver of the Golden retriever breed was not available during the study period of several months. Despite the variability of lesion scores between dogs and between sites, all cases showed the characteristic compact structure and hypereosinophilic staining of the stratum corneum in the lateral and ventral biopsies. While this appears to represent a major abnormality in haematoxylin and eosin-stained formalin-fixed skin affected by this disorder, the presence of scale is less consistently observed. It is possible that scale present at the time of sampling may have detached during transport or processing.

The patchy distribution of the pigment granules, which appears to be interfollicular both in the stratum corneum and in the deeper epidermis, matches the mottled appearance of the scales and the skin observed grossly.

While in most biopsies from normal dogs unidentified variably vacuolated cells with shrunken basophilic nuclei within the upper stratum spinosum can be found, they appear to be more prominent in most specimens from affected dogs. These findings are generally in agreement with those described by Mauldin et al.\textsuperscript{11} Epidermal thickening and occasional epidermal and dermal inflammation may accompany the main histopathological features. However when present, these changes are mild. It is conceivable that this cornification disorder is associated with functional impairment of the epidermal barrier and that these inflammatory changes reflect this.

The aetiology of the Golden retriever cornification defect remains to be determined. The age at onset of this cornification defect in Golden retrievers and the familial links between affected dogs are striking features of this condition and are highly suggestive of a genetic defect as the underlying cause. In our sample, the level of inbreeding was low and does not explain the familial clustering. While the lack of complete phenotypic information within litters injects uncertainty into the pedigree analysis, a Mendelian autosomal recessive mode of inheritance may be inferred from the pattern of affected dogs in the composite family tree. This hypothesis was supported by the simple segregation analysis. However, a dominant autosomal mode with incomplete penetrance or an oligogenic mode of inheritance cannot be ruled out. Although the marked scaling of affected dogs is obvious even to the untrained eye, for those dogs whose phenotypic information was obtained from owner reports rather than clinical examination, misclassification may have occurred in mildly affected cases and may have affected analysis. Ultimately, proof of a mode of inheritance requires breeding studies, identification of a linked marker through linkage analysis, or identification of the specific causative mutation.

A classification of hereditary canine ichthyotic skin diseases into epidermolytic and non-epidermolytic forms has been proposed.\textsuperscript{12,13} Based on clinical and histopathological features this cornification disorder in Golden retrievers falls into the non-epidermolytic category. Non-epidermolytic hyperkeratosis has also been reported in Cairn terriers,\textsuperscript{14} Jack Russell terriers\textsuperscript{15} and American bulldogs.\textsuperscript{14} Anecdotal reports of individual or few cases only are available for West Highland white terriers,\textsuperscript{1} soft-coated Wheaten terrier,\textsuperscript{16} pit bull terrier,\textsuperscript{17} a terrier and a Doberman pinscher,\textsuperscript{18} an Australian shepherd-type\textsuperscript{19} and Rottweiler.\textsuperscript{1} The disorders described are heterogeneous with regard to their clinical signs and reported structural features, and distinct from the condition observed in the Golden retriever breed\textsuperscript{11} and reported here.

The histological observation of compact keratin, mostly in the absence of acanthosis, is matched by the ultrastructural evidence within the stratum corneum of more cohesive corneocytes and more numerous desmosomes, suggesting abnormal desquamation due to delayed degradation of corneodesmosomes. These features also

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure7.png}
\caption{Transmission electron micrograph showing the stratum corneum of normal skin (a) and affected dogs (b). The stars indicate keratinocytes of the last layer of the stratum granulosum. In normal skin, distal to the most proximal three to four layers, corneocytes appear loose and corneodesmosomes are scarcely observed. In affected skin, corneocytes remain cohesive and corneodesmosomes more numerous.}
\end{figure}
seen in mouse skin treated with cholesterol sulphate, a model used to investigate the role of cholesterol sulphate accumulation in the pathomechanism of human recessive X-linked ichthyosis (RXLI). In fact, normal epidermal thickness with compact orthokeratosis and variable suprabasal vacuolation and persistence of corneodesmosomes high into the stratum corneum are features of RXLI. Clinical similarities of this cornification disorder to human RXLI include scaling, which may not be obvious at birth but develops as the child ages, and large hyperpigmented scale sparing the face, palms and soles. In RXLI, steroidal sulphatase deficiency leads to accumulation of cholesterol sulphate in skin and other tissues. Accumulated cholesterol sulphate is thought to contribute to the delayed degradation of corneodesmosomes in RXLI in a number of ways including the inhibition of stratum corneum serine proteases. Since in dogs the steroid sulphatase gene is also located on the X chromosome and the disease in retrievers showed no evidence of X-linked inheritance, the two diseases are unlikely to be analogous. However, the mechanism of desquamation is complex and it is conceivable that deficiency in or inhibition of stratum corneum-specific proteases or glycosidases by some other mechanism results in delayed desquamation in affected Golden retrievers.

In conclusion, this study describes a clinically and histopathologically distinct cornification disorder in the Golden retriever, which affects young dogs of either sex and is characterised by predominantly ventro-lateral scaling and hyperpigmentation of the trunk and histopathological and ultrastructural changes of the stratum corneum. A genetic aetiology is proposed, and a single-trait autosomal recessive mode of inheritance discussed.

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References
Cornification defect in Golden retrievers


Résumé Une dermatose non prurigineuse du Golden retriever, caractérisée par des squames plus ou moins pigmenteées a été récemment rapportée. Cette étude de cas, prospective, décrit les caractéristiques cliniques, ultrastructurales et génétiques de ce trouble de la cornification chez 17 chiens. La maladie atteint des jeunes des deux sexes, et est caractérisée par un squamosis et une hyperpigmentation à prédominance tronculaire ventrolatérale. Les modifications histopathologiques et ultrastructurales sont en faveur d’une dégradation retardée des cornéodesmosomes. Une étiologie génétique est proposée et un mode de transmission autosomal récessif est discuté.

Resumen Clínicos veterinarios han reconocido la presencia de una enfermedad no pruriginosa en perros de raza Golden retriever que se caracteriza por excesiva descamación con escamas pigmentadas y de gran tamaño en la piel de perros que por lo demás aparecen sanos. Este estudio prospectivo describe las características clínicas, histopatológicas, ultraestructurales y genéticas de este defecto de cornificación detectado en 17 animales. La condición afecta a animales jóvenes de cualquier sexo y se presenta con descamación simétrica, predominantemente ventrolateral e hiperpigmentación del tronco. Los cambios histopatológicos y ultraestructurales del estrato corneal sugieren una degradación retardada de los desmosomas en corneocitos. Se propone una etiología genética heredada como defecto simple autosómico y recesivo.