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Received: 21 December 2020 | Accepted: 14 July 2023

DOI: 10.1111/vde.13195

Veterinary Dermatology

ORIGINAL ARTICLE

p63 immunoexpression in hair follicles of normal and alopecia X-affected skin of Pomeranian dogs

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Abstract

Background: Alopecia X in Pomeranians is caused by a hair cycle deregulation, associated with downregulation of key regulatory genes of the Wnt and Shh pathways, and stem-cell markers. However, the pathogenesis remains unclear. p63 is an important transcription factor correlated with the aforementioned hair cycle modulating genes.

Hypothesis/Objectives: The aim of this study was to highlight possible changes of p63 immunohistochemical expression within the hair follicles in canine alopecia X compared with normal skin.

Animals: Skin biopsies from 19 alopecia X-affected and six control Pomeranians were analysed.

Materials and Methods: Serial histological sections of skin biopsies harbouring anagen, telogen and kenogen hair follicles were immunohistochemically evaluated for differences in p63 expression in the affected and control samples.

Results: Dogs with alopecia X had a significantly decreased immunoexpression of p63 in telogen and kenogen hair follicles.

Conclusions and Clinical Relevance: The decrease of p63 immunoexpression observed in canine alopecia X suggests an involvement of p63 in hair cycle.

KEYWORDS
alopecia X, dog, follicles, p63 immunoexpression, skin development, transcriptional factor defects

INTRODUCTION

Among domestic animals, noninflammatory alopecia is seen mainly in dogs and develops because of an impaired hair cycle. Although some of these alopecic conditions are acquired (e.g. endocrine alopecia), other types most likely have a genetic predisposition, because alopecia is associated with a clear breed predisposition and the onset of hair loss occurs at young age. Most alopecic conditions are associated with an impaired hair cycle (HC).[1]

During the normal HC, the hair follicle (HF) undergoes recurrent phases of controlled growth (anagen), regression of the lower follicle (catagen) and relative quiescence (telogen). After a variable time in telogen, initiation of a new anagen phase occurs through activation of stem cells.[2] The HC is tightly regulated and depends on a complex crosstalk of mesenchymal, epithelial and neuroendocrine skin components with the HF stem cells, their progenitors and the follicular keratinocytes. If one or more of these regulatory molecules are missing, the HF does not cycle properly, and noninflammatory alopecia develops. Histologically, noninflammatory alopecia is usually associated with a lack or reduced numbers of anagen follicles and a predominance of kenogen follicles, previously referred to as hairless telogen follicles. Kenogen follicles often become atrophic with time.[3] Alopecia X affects Pomeranians and is an example of noninflammatory alopecia with suggested hereditary background. The pathogenesis of alopecia X has been studied extensively, yet it remains unclear.[4,5] A recent transcriptome profiling study of skin biopsies from Pomeranians affected by alopecia X revealed downregulation of key regulatory genes of the Wnt and Shh pathways, both crucial for the HC, a significant downregulation of the HF stem cell markers *SOX9, LHX2, LGR5, TCF7L1* and *GLI1*, and a slight downregulation of the *TP63* gene.[6]

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The p63 transcription factor plays a fundamental role in skin development and homeostasis. It is a key regulator of the proliferative potential of basal keratinocytes in mature epidermis, keratinocyte differentiation[7] and survival,[8] as well as other aspects of keratinocyte biology, including cell–cell adhesion, basement membrane formation and skin barrier integrity.[9] p63 also is involved in HF growth and differentiation, as well as in the maintenance of the structure and function of other epithelia in the body. The *TP63* gene encodes at least six different isoforms of p63 with structures similar to those encoded by the *TP53* gene. Most of these isoforms contain an N-terminal transactivation (TA) domain, a DNA-binding domain and a carboxy-oligomerisation domain. The two main isoforms, TAp63 and ΔNp63, regulate a wide range of target genes that are essential for embryonal development and epithelial integrity.[10,11] The predominant p63 isoform, ΔNp63, can act both as transcriptional activator or repressor and is preferentially expressed in the post-developmental epithelial basal cells of the skin. It has been suggested that its expression blocks the growth-inhibitory and apoptosis-inducing activities of p53 or that it signals acting through p53. Thus, ΔNp63 could help to maintain the proliferative capacity of basal/progenitor cells.[7,12] However, p53 knockout mice exhibit no developmental epithelial disorders, which suggests a regulatory function distinct from that of p63.[13]

In scalp biopsies from human patients with androgenetic alopecia (AGA), the immunohistochemical evaluation of p63 expression (normally seen in basal/suprabasal cells of the epidermis, the outer root sheath and the hair matrix cells of the HF) of affected (frontal) and unaffected (occipital) areas showed that outer root sheaths from affected skin had significantly lower expression of p63 in comparison to unaffected areas.[14] No extensive studies on the immunohistochemical expression of p63 have been performed on canine non-inflammatory alopecic disorders, including alopecia X. Both human AGA and canine alopecia X are associated with an inhibition of genes related to the Wnt/β-catenin, Shh and *SOX9* signalling pathway, and p63 is a regulator of some of these pathways.[15,16]

Because p63 targeted deletion under the *FOXN1* promoter has been shown to induce severe hair loss in mice[17] and in light of the molecular evidence of p63 involvement to regulate different HC-related genes, some of which resulted to be downregulated in alopecia X,[6] we wanted to investigate the potential changes in p63 protein expression and distribution in the hair follicles of canine alopecia X-affected skin. To this aim, we analysed the immunohistochemical expression of p63 in anagen, telogen and kenogen HFs of Pomeranians affected by alopecia X, both in 'alopecic/hypotrichotic areas' and in 'clinically unaffected areas', as well as in the skin of control Pomeranians. The results of our study may give insights into the possible involvement of p63 in HC deregulation occurring in alopecia X.

MATERIALS AND METHODS

We evaluated 26 formalin-fixed paraffin-embedded (FFPE) skin biopsies from 19 alopecia X-affected Pomeranians. Biopsies from six control dogs were taken during postmortem (≤24/48 h after death) examination, from animals that died of causes unrelated to skin diseases. Alopecia X was diagnosed based on a detailed signalment, physical examination, laboratory tests—including thyroid profile (t4, TSH, Ft4), urinary cortisol: creatinine ratio (UCCR), complete blood count and general biochemical profile—and the histopathological findings. Anamnestic data, time of onset, time of the year when the dogs were biopsied, and sites of biopsies are presented in Table S1 in the Supporting information. For the alopecia X group, biopsies were taken in fully alopecic areas or areas with the most severe hypotrichosis in case no completely alopecic areas were present. From nine alopecia X dogs, clinically unaffected skin also was biopsied (Table S1). Biopsies from canine patients were taken with informed owner consent after sedation and/or local anaesthesia. Ethical approval was not required. All biopsies were routinely processed for histological investigation, sectioned at 4 μm and stained with haematoxylin and eosin. Immunohistochemical evaluation (IHC) was performed using a primary antibody (Ab) specific for p63 (1:500, clone 4A4, DAKO), whose cross-reactivity with canine tissues has been demonstrated previously[18–20] and recognising all p63 isoforms.[21] Deparaffinised and rehydrated sections were incubated with 3% H_2O_2 in deionised water for 30 min at room temperature (RT) to inhibit endogenous peroxidase activity and then rinsed in 0.05 M Tris-buffered saline (TBS) pH 7.6 for 5 min. Antigen retrieval was performed by heat treatment in citrate buffer, pH 6.0 in a water bath for 2 h at 80°C. To reduce nonspecific binding, slides were incubated with bovine serum albumine 5% in PBS for 20 min, then incubated with normal goat serum (NGS; S-1000; Vector laboratories) for 20 min at RT. Thereafter, slides were incubated overnight with the primary Ab. Immune complexes were treated with ImmPRESS HRP Universal Antibody (horse Anti-mouse/rabbit IgG) Polymer Detection Kit, peroxidase (MP-700; Vector laboratories) for 30 min at RT. Peroxidase activity was detected by a 5 min application of 3–3′-diaminobenzidine 0.8% solution (D5905; Sigma–Aldrich) with 0.1% H_2O_2, followed by counterstaining with Mayer's haematoxylin, before final dehydration and mounting. A negative control was performed in all instances by omitting the primary Ab and incubating tissue sections with TBS and/or replacing it with an antibody of irrelevant specificity (mouse anti-human α-smooth muscle actin, clone 1A4; Dako).

p63 nuclei quantification and staining intensity

In order to count the total number of nuclei of the selected HFs, an automated count, using ImageJ analysis system (National Institutes of Health) was applied in most cases. In particular, a set of commands (macro) was developed by the authors to count all nuclei; when the automatic analysis was not able to count each nucleus individually, a manual count was performed. The number of negative nuclei was counted manually in all cases, and the number of positive nuclei was calculated

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as the difference between the total number of nuclei and the negative nuclei. Counting of the negative/positive nuclei were done blindly by two operators (LB, MM). Finally, the percentage of positive nuclei was calculated in anagen, telogen and kenogen HFs for each case.

The staining intensity was scored for positive p63 nuclei as strong, moderate and weak according to Nasiri et al.[22] and blindly by two operators (LDS, MR). The basal epidermal layer served as strong internal intensity reference control for p63.

All analyses were performed in the same predefined ROI (region of interest), that was delineated manually in the isthmus of anagen, telogen and kenogen HFs. The HF stage was determined based on typical morphological features associated with each stage. In some biopsies derived from dogs with alopecia X, few or no anagen HFs were present. Furthermore, as shown in Table S1, in four biopsies from alopecic skin, only dilated, hyperkeratotic HF infundibula were present and thus these biopsies were excluded. As a consequence, HFs from six normal skin biopsies and 26 skin biopsies (including 17 alopecic/hypotrichotic areas and nine clinically unaffected skin areas) from Alopecia X-affected dog were included in the final statistical analysis.

Statistical analysis

Preliminary assessment of data distribution was performed using the Shapiro–Wilk test.

Statistical differences among pathological and control cases were evaluated via the Wilcoxon–Mann–Whitney *U*-test for nonparametric distributions. Statistical differences between control cases and 'alopecic/hypotrichotic areas' (Area A) or 'clinically unaffected skin area' (Area B) of pathological cases were evaluated through Kruskal–Wallis test followed by Dunn's post hoc multiple comparison test. Differences in p63 staining intensity were evaluated using Fisher's test; p-values <0.05 were considered significant.

RESULTS

In control cases, nuclear p63 immunoexpression was detected in the basal/suprabasal epidermal keratinocyes and in cells of all layers of the outer root sheath of the isthmus of telogen and anagen HFs. In anagen HFs, staining also was present in the outer root sheath of the inferior portion of the follicle and in the matrix cells of the bulbs. The dermal papilla and the noncornified

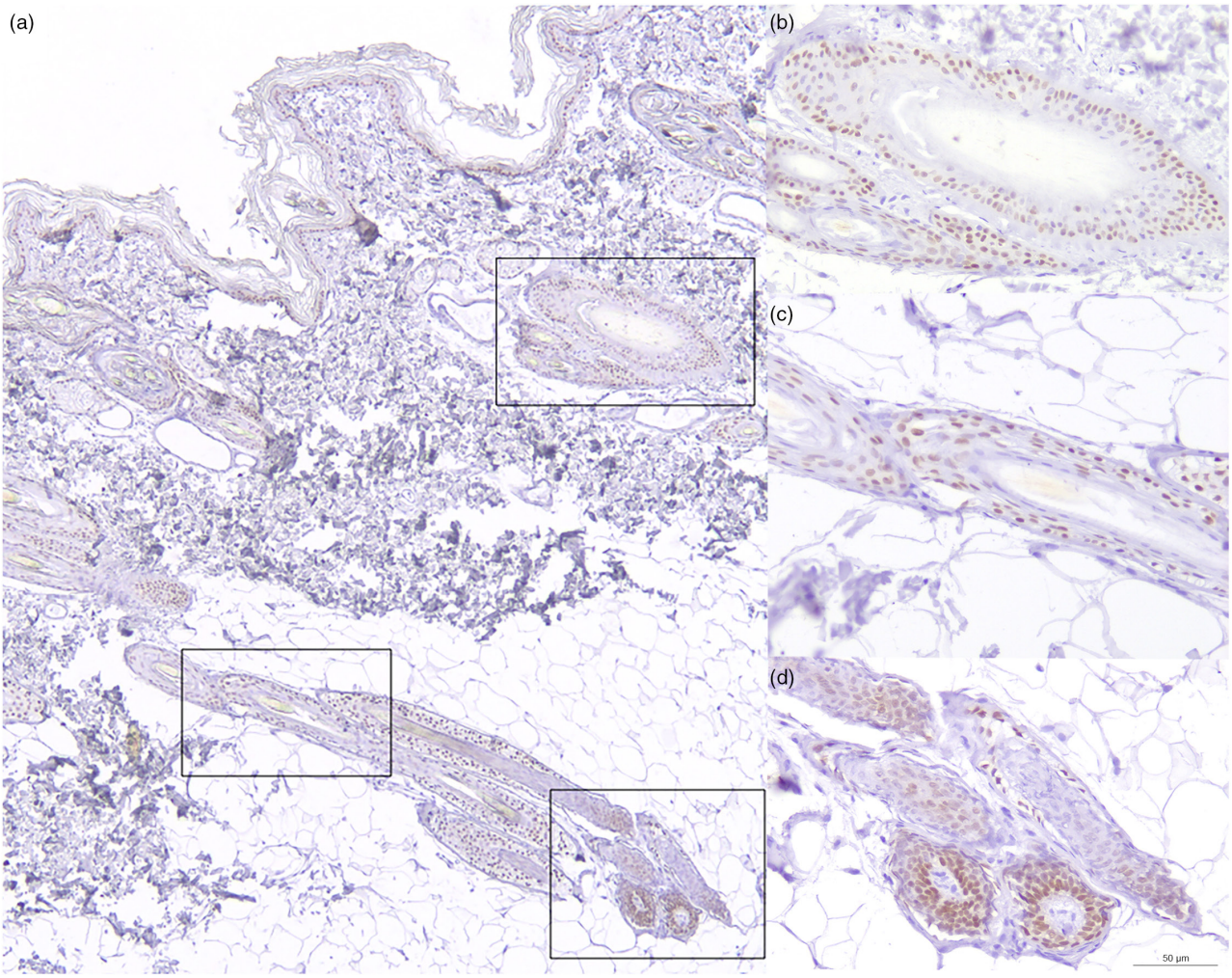

FIGURE 1 Normal skin from a Pomeranian dog. (a) Overview of the p63 immunostaining pattern in the different epithelial skin structures: the epidermis shows p63 nuclear staining in the basal/suprabasal keratinocyes. Positive nuclei are evident in the outermost layer of sebaceous glands; the hair follicles (HFs) show positive nuclei in all layers of the outer root sheath of the isthmus of telogen HFs (b), in the outer root sheath of the inferior portion (c) and in the matrix cells of the bulbs (d) of the anagen HFs. However, the dermal papilla (d) and the noncornified inner root sheath of the inferior portion of HFs (c) are negative.

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inner root sheath of the inferior portion of the HFs were negative (Figure 1).

Data analysis showed a significantly lower percentage of p63 positive nuclei in the isthmus of telogen and kenogen HFs in skin biopsies from dogs with alopecia X cases (including both clinically unaffected and alopecic/hypothricotic areas) in comparison to control cases [76.69% (67.84%–86.52%) (median with interquartile range) of alopecia X biopsies (mean rank=14.88) *versus* 94.24% (70.31%–96.04%) of control (mean rank=23.50), $p=0.0430$, Wilcoxon–Mann–Whitney $U=36$], while no significant difference was identified in anagen HFs among the same groups [86.85% (82.97%–93.12%) of alopecia X biopsies (mean rank=9.333) *versus* 90.61% (72.76%–92.75%) of control (mean rank=9.833), $p=0.8916$, $U=34$], as shown in Figures 2a,c and 3. The percentage of p63-positive nuclei in telogen and anagen HFs from Area A and Area B, determined by the Kruskal–Wallis test followed by Dunn's post hoc multiple comparison test, did not differ significantly between the two groups (Figure 2b,d) [telogen: 94.24% (70.31%–96.04%) in the control (mean rank=23.50) *versus* 81.71% (68.72%–86.73%) in Area A (mean rank=15.76) *versus* 75.11% (66.13%–83.11%) in Area B (mean rank=13.22), $p=0.1030$, Kruskal–Wallis statistic: 4.544; anagen: 90.61% (72.76%–92.75%) in the control (mean rank=9.833) *versus* 86.68% (82.75%–93.01%) in Area A (mean rank=9.167) *versus* 87.86% (78.48%–93.52%) in Area B (mean rank=9.500), $p=0.9809$, Kruskal–Wallis statistic: 0.04678].

p63 staining intensity ('strong', 'moderate' and 'weak'), as well as its distribution in telogen and anagen HFs among groups are shown in Figures 4 and 5, respectively. Fisher's exact test revealed a significant difference in the p63 nuclear intensity distribution among groups both in telogen and anagen HFs (telogen: $p=0.027$, χ^2 value=11.364; anagen: $p=0.006$, χ^2 value=12.455). In fact, plots show that all control cases exhibited strong p63 nuclear intensity and that weak intensity was present only in Area A of pathological cases.

Both percentage of p63 positive nuclei and staining intensity for individual cases are shown in Table S2.

DISCUSSION

In the present research study, differences in both percentage and intensity of p63 positive cells were observed in the isthmus region of HFs of skin biopsies from Pomeranian dogs affected by alopecia X in comparison with control dogs of the same breed. Previous immunohistochemical studies showed p63 protein localisation and expression in the basal/progenitor cells of several epithelial tissues, including cervix, tongue, oesophagus, mammary glands, prostate and urogenital tract.[13] As far as normal human and canine skin is

(a)

Groups	Telogen/kenogen HFs	Anagen HFs
Control	94.24 (70.31-96.04)	90.61 (72.76-92.75)
Alopecia X	76.69 (67.84-86.52)	86.85 (82.97-93.12)

(b)

Groups		Telogen/kenogen HFs	Anagen HFs
Control		94.24 (70.31-96.04)	90.61 (72.76-92.75)
Alopecia X	Area A	81.71 (68.72-86.73)	86.68 (82.75-93.01)
	Area B	75.11 (66.13-83.11)	87.86 (78.48-93.52)

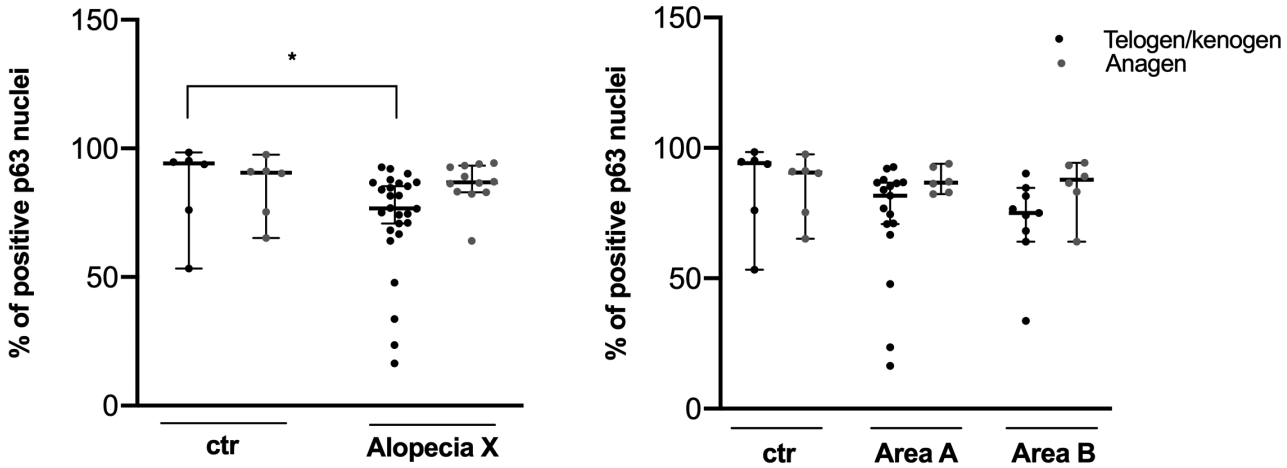

FIGURE 2 Percentage of positive nuclei in the isthmus of telogen/kenogen and anagen hair follicles (HFs). The median percentage of p63 positive nuclei with interquartile range in control and Alopecia X biopsies is shown in the tables (a,b). In the scatter dot plots (c,d), the lines represent the medians while the error bars represent their 95% confidence interval. A statistically significant difference (*$p=0.043$, Wilcoxon–Mann–Whitney $U=36$) is evident only for telogen/kenogen HFs of Alopecia X cases (26 biopsies from 19 dogs) when compared to control cases (six biopsies from six dogs) (c), while no significant differences were observed when they were grouped in 'alopecic/hypotrichotic areas' (Area A, 17 biopsies from 15 dogs) and 'clinically unaffected skin areas' (Area B, nine biopsies from nine dogs) (d). No statistical difference was observed for anagen HFs of alopecia X cases (12 biopsies from 9 dogs) when compared to control cases (six biopsies from six dogs), also when they were grouped in Area A (six biopsies from five dogs) and Area B (six biopsies from six dogs).

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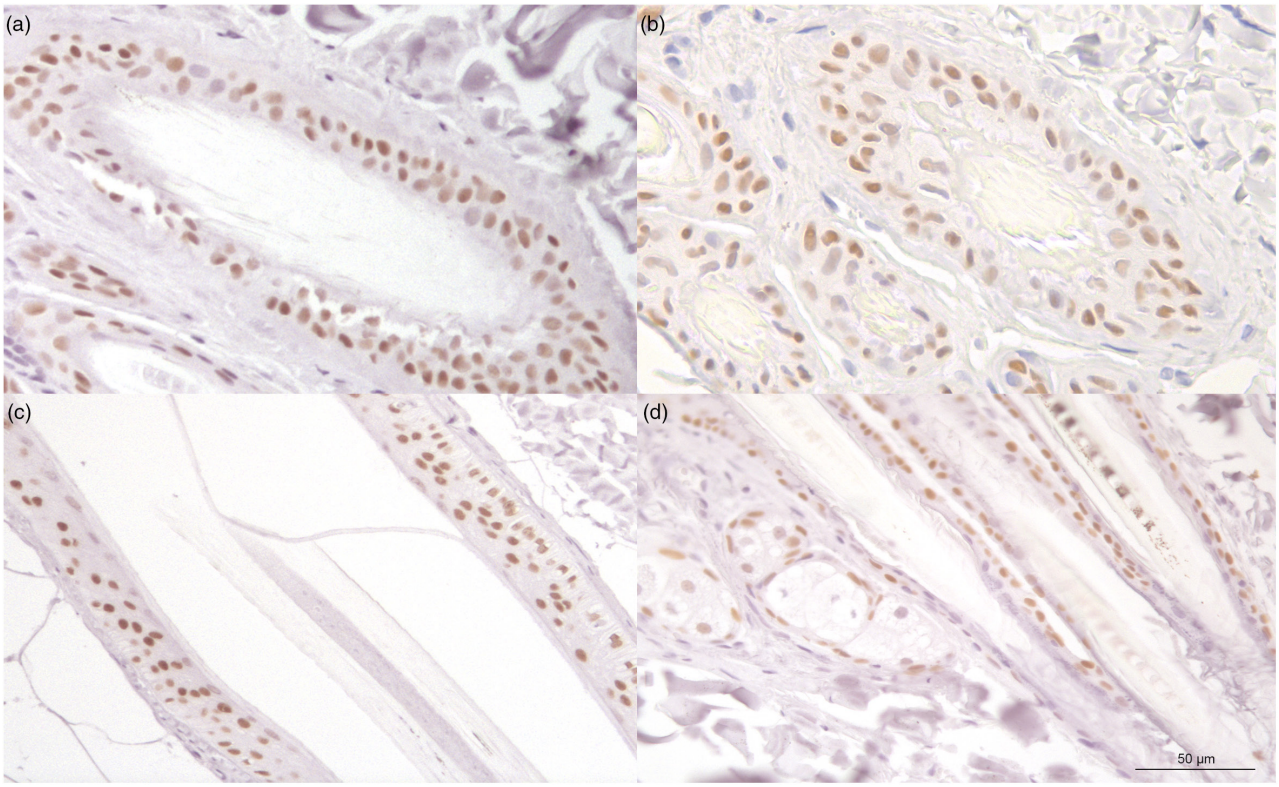

FIGURE 3 p63 positive nuclei in control and alopecic cases. (a) High percentage of cells with strong intensity of p63 immunostaining in the outer rooth sheath (ORS) of the isthmus of a telogen hair follicle (HF) from a control dog. (b) Lower percentage of cells with moderate-to-weak intensity of the p63 immunostaining in the ORS of the isthmus of a telogen HF from a clinically affected area. (c) High percentage of cells in the ORS of the isthmus of an anagen HF with strong intensity of p63 immunostaining from a normal skin biopsy of a control case. (d) Low percentage of positive cells in the ORS of the isthmus of an anagen HF with moderate intensity of p63 immunostaining from a clinically unaffected area of a pathological case.

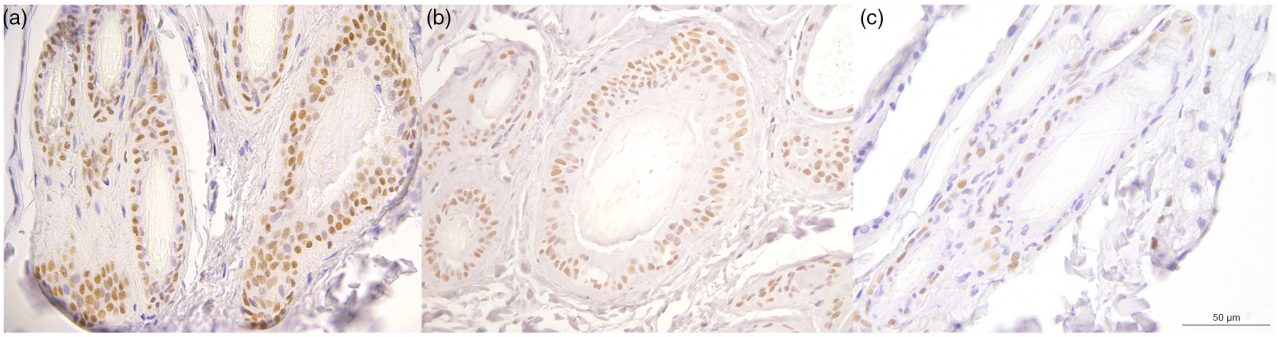

FIGURE 4 p63 nuclear immunostaining intensity in telogen HF. p63 strong nuclear intensity is evident in a telogen HF from normal skin (a), while moderate (b) and weak intensity (c), together with scarce p63 positive nuclei, are observed in affected cases.

concerned,[7,23] p63 expression has been found in basal/suprabasal cells of the epidermis, outer root sheath, hair matrix, seboblasts located in the outermost layer of sebaceous glands, outer layer cells of the ductal portion, and myoepithelial cells of the secretory portion of sweat glands, thus suggesting a similar role for p63 in both humans and dogs. Our results obtained in all normal and pathological samples confirmed that papilla and inner root sheath were negative for p63, as reported by Saraiva et al.[23]

A recent study investigated the transcriptome profile and differential p63 gene expression in telogen and late anagen micro-dissected HFs of healthy dogs by using RNAseq, without revealing significant differences.[24] However, a slight downregulation of p63 gene expression at the limit of significance (a ratio of −0.53, where upregulated and downregulated genes had ratios of 0.58 and −0.58, respectively) was demonstrated in a recent transcriptome profiling study on Pomeranians affected by alopecia X.[6] This study evaluated different genes by analysing 'in full' 6 mm biopsies, including the whole pilosebaceous unit and the epidermis. However, in our study the evaluation of distribution and intensity of p63 immunoexpression was focused exclusively on the isthmus parts of the HFs in both normal and pathological cases, revealing a low expression of this molecule at the protein level in pathological cases when compared to healthy controls. Interestingly, percentages of p63-positive nuclei were significantly different between normal and pathological cases only in

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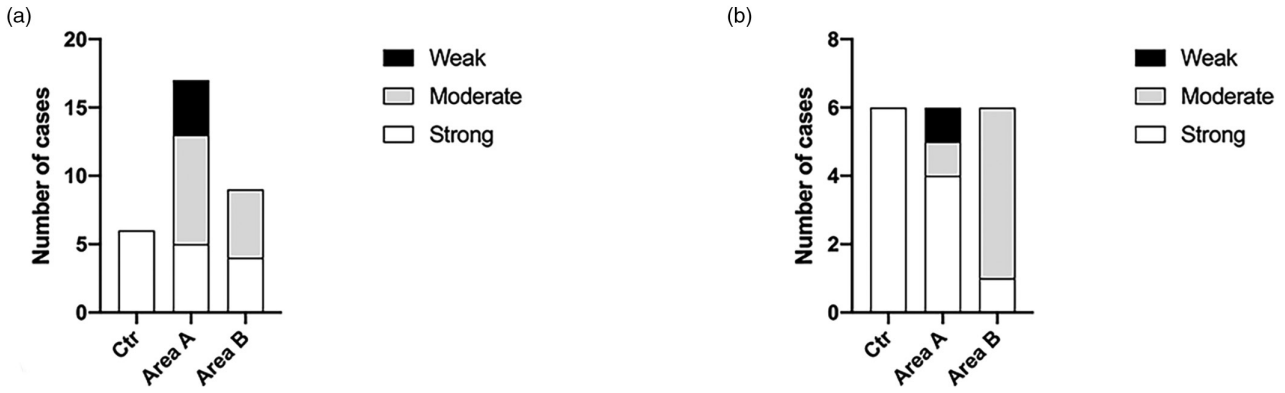

FIGURE 5 p63 nuclear intensity distribution in control and alopecia X cases. Figure shows the distribution of p63 nuclear staining intensity of telogen HFs (a) and anagen HFs (b) in control group (ctr) and pathological cases group (Area A and Area B). (Fisher's exact test for telogen $p=0.027$, $\chi^2=11.364$; Fisher's exact test for anagen $p<0.006$, $\chi^2=12.455$).

telogen and not in anagen HFs, while immunostaining intensity reduction was evident in both phases. Skin biopsies from Alopecia X-affected Pomeranian dogs showed rare anagen phase HFs, as expected in this disease. The scarce presence of anagen HFs in skin biopsies from affected dogs could, at least in part, explain the lack of statistical difference in p63 immunoexpression between normal dogs and alopecia X-affected Pomeranians in our study, as well as the only slight differences in p63 gene expression observed previously by bulk transcriptome analysis.[6] Furthermore, no significant differences in the percentage of p63-positive nuclei were present among controls and different areas of pathological cases, although assessment of clinically unaffected skin areas was possible for only nine of 19 pathological dogs.

It has been shown that mutations in the *Tp63* gene cause epidermal abnormalities characterised by ectodermal dysplasia.[11] ΔNp63, a p63 protein isoform predominantly expressed in epithelial cells, plays a crucial role in the regulation of cell cycle progression and cell growth. ΔNp63 transgenic animals exhibit a depleted HF stem-cell niche, which further contributes to the overall cycling defects observed in mutant animals.[8] In addition, studies of p63-mutant thymus in vivo and clonogenic assays in keratinocytes further support a key role for p63 in maintaining the proliferative potential of stem cells in stratified epithelia.[25] However, p63 does not seem to be a marker of epithelial stem cells in canine skin in vivo, because it is expressed in all basal cells, as well as in a significant number of suprabasal cells of epidermis and HF outer root sheath. Moreover, p63 is highly expressed in the HF matrix cells of the bulb, which is thought to contain only cells with limited growth capacity.[26] Taken together, literature data indicate that p63 may be required for the maintenance of certain progenitor cell populations that are necessary for epithelial turnover and development, while ΔNp63 may promote N-terminal transactivation cell conversion and keratinocyte terminal differentiation.[11]

As described above, while the difference in p63 nuclear positivity between healthy and alopecic Pomeranians was evident in telogen phase, the evaluation of staining intensity score of p63 immunoexpression revealed that the immunolabelling was generally weaker in HFs from alopecic dogs both in anagen and telogen phases when compared to HFs of normal skin samples, suggesting an involvement of p63 decrease during HC in canine alopecia X. It could be hypothesised that reduced p63 protein levels in alopecic skin of alopecia X-affected dogs may be associated with a lack or reduced return to cycle (anagen) of HFs, which showed a similar anatomical distribution of p63-positive nuclei when compared to control cases, although with reduced intensity of p63 immunostaining in anagen phase.

However, besides p63-altered expression, downregulation of other genes implicated in HC and cellular maturation has been identified. In the study carried out by Brunner et al.,[6] *Tp63* downregulation was associated with a downregulation of other related genes, such as those of Wnt and Shh pathways. In fact, a number of signalling pathways and transcription factors have been identified to affect p63 expression in epithelial cells, such as Wnt, Shh, Notch and EGFR pathways.[27]

Chromatin immunoprecipitation and reporter genes suggested that murine *Tp63* is directly regulated by the WNT pathway through binding of Lef1/Tcf with β-catenin to a region between the TA and ΔNp63 promoters.[28,29] β-catenin/WNT signalling also is essential for the development of all skin appendages from the earliest stages, and onwards downregulation of these two genes also may be critical for the consequences of the p63 loss of function,[30] although in humans *SOX2* also may regulate *Tp63* to control corneal epithelial stem/progenitor cell function. Repression of *SOX2* has been found to reduce *Tp63* expression and, in this respect, *SOX2* can regulate C38–C40 enhancers and may control the transcription of *Tp63* mRNA.[31]

The immunohistochemical evaluation of p63 expression in our cases revealed a pattern similar to the results obtained in human skin and HFs by Fiuraskova et al.,[14] in which the outer root sheaths from normal occipital skin had significantly higher expression of p63 in comparison with frontal alopecic areas of AGA-affected human patients. A greater number of cases with biopsy samples in clinically unaffected/hypotrichotic areas would clarify whether p63 immunoexpression in these areas significantly differs from normal control cases and clinically alopecic areas.

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CONCLUSIONS

p63 plays a key role in regulating the self-renewal and long-term proliferative capacity of stem cells,[31] and its expression is required for basal and suprabasal epidermal cells maintenance and maturation, as well as of outer root sheath and matrix cells of the HFs.[7] Thus, the observed reduction of p63 immunohistochemical expression in HFs from alopecia X-affected dogs gives further insight into a possible involvement of p63 in HC deregulation occurring in canine alopecia X.

AUTHOR CONTRIBUTIONS

Leonardo Della Salda: Conceptualization; funding acquisition; investigation; resources; supervision; validation; writing – review and editing. **Laura Bongiovanni:** Investigation; methodology; writing – review and editing. **Marcella Massimini:** Formal analysis; investigation; methodology; writing – review and editing. **Mariarita Romanucci:** Supervision; writing – original draft; writing – review and editing. **Antonella Vercelli:** Data curation; investigation; supervision. **Alessia Colosimo:** Writing – review and editing. **Ramona Di Matteo:** Investigation. **Sabrina Vanessa Patrizia Defourny:** Formal analysis; investigation; methodology; writing – original draft.

ACKNOWLEDGEMENTS

In memory of Paolo Simoni, unforgotten teacher and friend. We thank Marina Baffoni for technical support, Romina Di Costanzo (Vet. Clin. L'Arca, Teramo) for submitting a control case, and Monika Welle (Vetsuisse Faculty, University of Bern, Bern, Switzerland) for submitting most cases and for critically reviewing the study proposal.

FUNDING INFORMATION

This study was self-funded.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Della Salda L, Bongiovanni L, Massimini M, Romanucci M, Vercelli A, Colosimo A, et al. p63 immunoexpression in hair follicles of normal and alopecia X-affected skin of Pomeranian dogs. Vet Dermatol. 2023;34:567–575. https://doi.org/10.1111/vde.13195

摘要

背景: 博美犬的X脱毛是由毛发周期失调引起的，与Wnt和Shh通路的关键调节基因以及干细胞标志物的下调有关。然而，其发病机制尚不清楚。P63是与上述毛发周期调节基因相关的重要转录因子。

假设/目的: 本研究的目的是强调与正常皮肤相比，X脱毛患犬的毛囊内p63免疫组织化学表达的可能变化。

动物: 对19例X脱毛患犬和6例对照博美犬的皮肤活检进行了分析。

方法和材料: 用免疫组织化学方法对含有生长期、休止期和成熟期毛囊的皮肤活检的一系列组织学切片进行评估，以确定p63在发病样本和对照样本中表达的差异。

结果: X脱毛患犬的静止期和无毛期毛囊中p63的免疫表达显著降低。

结论和临床相关性: 在犬X脱毛中观察到p63免疫表达的降低表明p63参与了毛发生长周期。

Résumé

Contexte: L'alopécie X chez les Poméraniens est causée par une dérégulation du cycle pilaire, associée à une régulation à la baisse de gènes régulateurs clés des voies Wnt et Shh, et de marqueurs de cellules souches. Cependant, la pathogénie demeure obscure. P63 est un important facteur de transcription en corrélation avec les gènes modulateurs du cycle pilaire mentionnés ci-dessus.

Hypothèse/Objectifs: L'objectif de cette est de mettre en évidence les modifications possibles de l'expression immunohistochimique de p63 dans les follicules pileux, dans l'alopécie X canine, par rapport à la peau normale.

Animaux: Des biopsies de peau provenant de 19 Poméraniens atteints d'alopécie X et de six Poméraniens témoins sont analysées.

Méthodes et matériel: Des coupes histologiques en série de biopsies cutanées contenant des follicules pileux anagènes, télogènes et kénogènes ont été évaluées par immunohistochimie pour déterminer les différences d'expression de p63 dans les échantillons atteints et les échantillons témoins.

Résultats: Les chiens atteints d'alopécie X présentent une immunoexpression significativement réduite de p63 dans les follicules pileux télogènes et kénogènes.

Conclusions et pertinence clinique: La diminution de l'immunoexpression de p63 observée dans l'alopécie X canine suggère une implication de p63 dans le cycle pilaire.

Zusammenfassung

Hintergrund: Die Alopecia X der Pomeranier wird durch eine Störung der Haarzyklusregulierung verursacht, die zusammen mit einer Herabsetzung von Schlüssel-regulatorischen Genen wie Wnt und Shh Zyklus, und Stammzellmarkern gesehen wird. Die Pathogenese bleibt jedoch unklar. P63 ist ein wichtiger Transkriptionsfaktor, der mit den zuvor erwähnten Haarzyklus-modulierenden Genen korreliert.

Hypothese/Objektive: Das Ziel dieser Studie war es, mögliche Veränderungen der p63 Exprimierung innerhalb der Haarfollikel bei der caninen Alopecia X im Vergleich zu normaler Haut zu unterstreichen.

Tiere: Es wurden Hautbiopsien von 19 Pomeraniern mit Alopecia X und von sechs Kontrollen analysiert.

Methoden und Materialien: Es wurden Serienschnitte von Hautbiopsien, die Haarfollikel im anagenen, telogenen und kenogenen Stadium beinhalteten, immunhistochemisch auf Unterschiede in der p63 Exprimierung in den betroffenen und in den Kontrollproben evaluiert.

Ergebnisse: Hunde mit Alopecia X zeigten eine signifikant niedrigere Immunexprimierung von p63 in telogenen und kenogenen Haarfollikeln.

Schlussfolgerungen und klinische Bedeutung: Die Abnahme der p63 Immunexprimierung, die bei der caninen Alopecia X beobachtet wird, weist darauf hin, dass p63 beim Haarzyklus eine Rolle spielt.

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要約
背景: ポメラニアンの脱毛症Xは、毛周期の調節不全によって引き起こされ、WntおよびShh経路の主要な調節遺伝子や幹細胞マーカーのダウンレギュレーションと関連している。しかし、その病態はまだ不明である。P63は、前述のヘアサイクル調節遺伝子と相関する重要な転写因子である。
仮説/目的: 本研究の目的は、犬の脱毛症Xにおける毛包内のp63免疫組織化学的発現が、正常皮膚と比較して変化している可能性を明らかにすることであった。
対象動物: 19頭の脱毛症X罹患犬および6頭の対照ポメラニアンの皮膚生検を解析した。
材料と方法: 成長期、休止期、kenogenの毛包を有する皮膚生検の連続組織切片を免疫組織化学的に評価し、罹患サンプルと対照サンプルにおけるp63発現の違いを調べた。
結果: 脱毛症Xの犬では、休止期およびkenogen毛包におけるp63の免疫発現が有意に低下していた。
結論と臨床的関連性: 犬の脱毛症Xで観察されたp63免疫発現の減少は、毛周期におけるp63の関与を示唆していた。

Resumo
Contexto: Alopecia X em lulus da Pomerânia é causada por desregulação do ciclo folicular, associada à inibição de genes chave reguladores das vias Wnt and Shh, e marcadorres de células tronco. Entretanto, a patogênese permanece não esclarecida. P63 é um fator de transcrição importante correlacionado com os genes moduladores do ciclo folicular mencionados anteriormente.
Hipótese/Objetivos: O objetivo deste estudo foi destacar possíveis alterações da expressão imuno-histoquímica do p63 dentro dos folículos pilosos na alopecia X canina em comparação com a pele normal.
Animais: Foram analisadas biópsias de pele de 19 lulus da Pomerânia afetados por alopecia X e seis controles.
Métodos e Materiais: Cortes histológicos seriados de biópsias de pele contendo folículos pilosos anágenos, telógenos e kenógenos foram avaliadas por imuno-histoquímica quanto a diferenças na expressão de p63 nas amostras afetadas e de controle.
Resultados: Cães com Alopecia X tiveram uma imunoexpressão significativamente diminuída de p63 em folículos pilosos telógenos e kenógenos.
Conclusões e Relevância Clínica: A diminuição da imunoexpressão do p63 observada na alopecia X canina sugere um envolvimento do p63 no ciclo do pelo.

Resumen
Introducción: la alopecia X en pomerania es causada por una deregulación del ciclo del pelo, asociada con la regulación a la baja de genes reguladores clave de las vías Wnt y Shh, y marcadores de células madre. Sin embargo, la patogenia sigue sin estar clara. P63 es un factor de transcripción importante correlacionado con los genes moduladores del ciclo del pelo antes mencionados.
Hipótesis/Objetivos: El objetivo de este estudio fue investigar los posibles cambios en la expresión inmunohistoquímica de p63 dentro de los folículos pilosos en la Alopecia X canina en comparación con la piel normal.
Animales: Se analizaron biopsias de piel de 19 perros Pomerania afectados por Alopecia X y seis de control.
Métodos y materiales: Se evaluaron inmunohistoquímicamente secciones histológicas seriadas de biopsias de piel que albergaban folículos pilosos anágenos, telógenos y kenógenos para determinar las diferencias en la expresión de p63 en las muestras afectadas y de control.
Resultados: Los perros con alopecia X tenían una inmunoexpresión significativamente menor de p63 en los folículos pilosos telógenos y kenógenos**.**
Conclusiones y relevancia clínica: La disminución de la inmunoexpresión de p63 observada en la Alopecia X canina sugiere una implicación de p63 en el ciclo del pelo.

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