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Short Communication

The mRNA expression of *PTTG1* is a strong prognostic indicator for recurrence after hypophysectomy in dogs with corticotroph pituitary adenomas

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ABSTRACT

Pituitary-dependent hypercortisolism (PDH) is a common endocrinopathy in dogs, but the promotors and initiators of the tumourigenesis of corticotroph pituitary adenomas remain unknown. Based on human data, we investigated mRNA expression of pituitary tumour transforming gene 1 (*PTTG1*) with quantitative RT-PCR in canine corticotroph pituitary adenomas. *PTTG1* was overexpressed in adenomas approximately 3-fold. A strong association was observed between *PTTG1* expression and disease-free interval; dogs with high *PTTG1* expression had a significantly (4 times; $P = 0.02$) shorter disease-free interval than dogs with low *PTTG1* expression. This paper shows that *PTTG1* expression is a negative prognosticator in relation to disease-free interval and recurrence in dogs undergoing transsphenoidal hypophysectomy as treatment for PDH.

Introduction

Pituitary-dependent hypercortisolism (PDH) is a frequently encountered endocrinopathy in canine practice and it is estimated that 1–2/1000 dogs per year are diagnosed with this disease (Willeberg and Priester, 1982). Although several factors have been reported to affect survival and disease-free interval (DFI) after transsphenoidal hypophysectomy, mutations in proto-oncogenes and tumour suppressor genes, which are often involved in other neoplasms, have received little attention in pituitary tumours (Dworakowska and Grossman, 2009). Pei and Melmed identified and characterized pituitary tumour transforming gene 1 (*PTTG1*) as highly expressed in rat pituitary tumour cells (Pei and Melmed, 1997). Since then, mutations in the *PTTG1* gene have also been reported in human pituitary adenomas (Asa and Ezzat, 2009; Melmed, 2011). This prompted us to investigate the differential *PTTG1* mRNA expression in pituitary adenomas of dogs with PDH that underwent pituitary surgery. In order to explore the prognostic value of *PTTG1* expression, its expression was related to survival and DFI in surgically treated dogs.

Pituitary adenoma tissue was collected during transsphenoidal hypophysectomy in 20 dogs in which the diagnosis of PDH was confirmed, as previously described (van Rijn et al., 2014). Pituitary adenoma tissue was snap frozen in liquid nitrogen and stored at −70 °C until analysis. Healthy pituitary tissue was obtained as surplus material from 20 healthy, adult, crossbreed dogs euthanased in other non-related experiments and approved by the Ethics Committee on Animal Experimentation at Utrecht University and in accordance with the 3R-policy of the university (DEC#: 2007.III.02.029). Animal care and handling was performed in accordance with the 'European Directive for the Protection of Vertebrate animals used for Experimental and Scientific Purpose, European Community Directive 86/609/CEE'. Pituitary gland processing was as described by van Rijn et al. (2014), and experiments were conducted with anterior lobe tissue only.

RT-qPCR conditions, primer design, validation, relative gene expression (dCq), and data analysis was performed as described previously (van Steenbeek et al., 2013). Expression normalization was performed using three reference genes; Tata box binding protein (*TBP*), hydroxymethylbilane synthase (*HMBS*), and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide (*YWHAZ*); expression stability was evaluated as required under MIQE-precise (Bustin et al., 2010). Details of primers, including sequence and annealing temperature, are listed in the Appendix: Supplementary Table 1. Statistical analysis was performed using commercially available statistical software (IBM SPSS Statistics for Windows, Version 24.0, IBM). Normality of data was assessed using Shapiro–Wilk testing and parametric or non-parametric tests were used accordingly. To compare gene expression levels between groups, Student's *t* test or Mann

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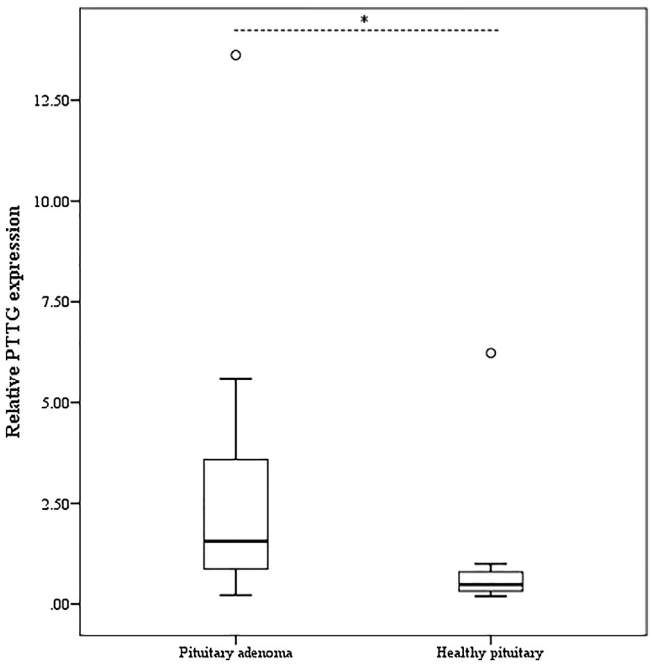

Fig. 1. Boxplot displaying the relative mRNA expression of *PTTG1*, normalized by expression of reference genes in healthy pituitary and pituitary adenoma ($P < 0.001$). ◦ represent outliers.

Whitney *U* test were used. Correlation coefficients were calculated using a Spearman ρ test. Furthermore, survival analysis was performed using Kaplan Meier curves; the Log Rank test was used to assess statistical significance. Dogs without initial remission were excluded from this analysis. Bonferroni corrections for multiple comparisons were applied when appropriate. Statistical significance was set at $P < 0.05$.

Relative mRNA expression of *PTTG1* was approximately 3-fold higher in adenoma tissue compared to healthy canine pituitary tissue (3.2; 95% CI: 2.2–5.2; $P < 0.001$; Fig. 1). Differences were not found in *PTTG1* expression by sex ($P = 0.34$) or breed ($P = 0.85$). *PTTG1* expression was not significantly different between dogs with and without recurrence of hypercortisolism ($P = 0.08$). Within the adenoma samples two groups were formed; high *PTTG1* expression samples and low *PTTG1* expression samples. The average dCq value of all adenoma tissue used for qRT-PCR analysis served as the cut off value (1.95). Between the two groups there were no significant differences in age, sex, breed, UCCR and pituitary height to brain area (P/B) ratio (a measure of pituitary size). The data followed an accelerated failure time model and thus the differences between groups relied on time (DFI) instead of proportion of events (recurrence). Survival analysis demonstrated that DFI was significantly shorter ($P = 0.02$) for dogs with high *PTTG1* expression (<1.95 dCq) than for dogs with low *PTTG1* expression. This significant decrease demonstrated that dogs in the *PTTG1*-high group relapsed approximately four times faster than dogs in the *PTTG1*-low group (Fig. 2A). There was no significant difference in survival time between the groups (Fig. 2B; $P = 0.30$).

In agreement with earlier rat and human studies, we report higher *PTTG1* expression in canine pituitary adenomas compared to normal pituitary tissue. *PTTG1* is an oncogene known to play a role in pituitary cell transformation and tumour formation (Pei and Melmed, 1997; Zhang et al., 1999). *PTTG1* disruption could result in unrestrained cellular growth and tumorous processes, resulting in tumour regrowth and recurrence of hypercortisolism. This could explain the shorter DFI in dogs with a high *PTTG1* expression. However, the exact mechanism needs to be elucidated and would require additional studies.

Because analysis of mRNA expression levels in pituitary tissue can only be performed after surgery, *PTTG1* cannot be used as a pre-operative marker of prognostic factor. However, its expression could be used to guide the intensity and management of post-surgical follow-up.

In dogs undergoing transsphenoidal hypophysectomy as treatment for PDH, *PTTG1* mRNA expression is a negative prognosticator, specifically DFI. Further studies should aim to investigate the exact mechanism behind this. The known role of *PTTG1* in sister chromatid separation and cell cycle regulation provides a starting point for these investigations (Pei and Melmed, 1997; Sapochnik et al., 2016).

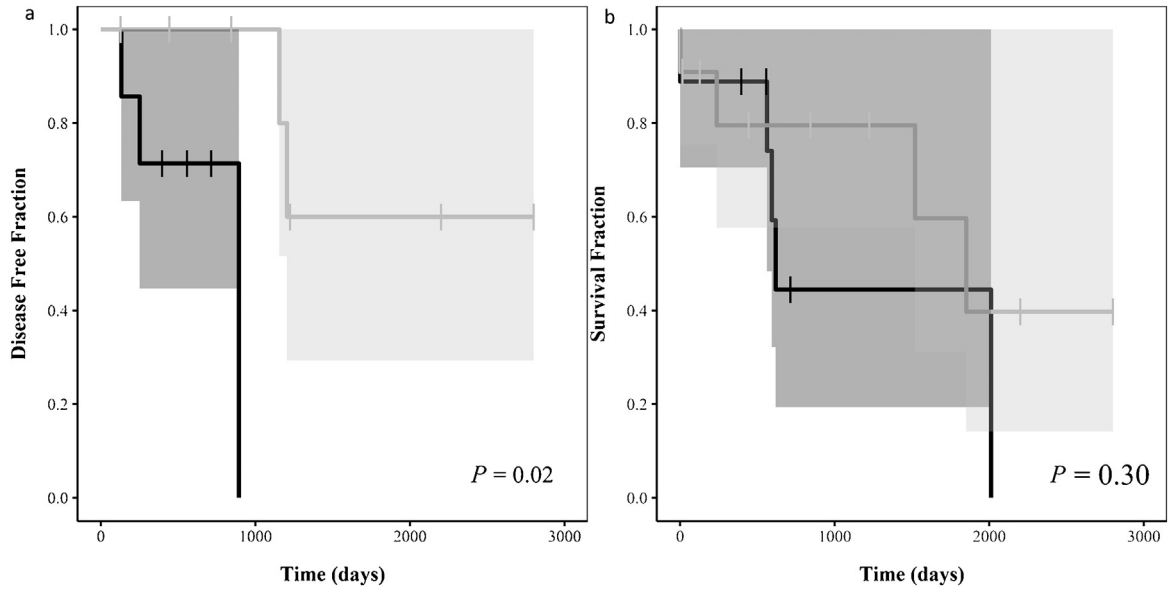

Fig. 2. Kaplan Meier survival curves. Disease free interval (A) and survival time (B) in days, comparing dogs with high expression of pituitary tumour transforming gene 1 (*PTTG1*, dCq < 1.95; continuous line) and low expression of *PTTG1* (dCq > 1.95; dotted line). Vertical bars indicate censored cases. Shaded areas represent 95% confidence intervals.
Dogs with a high expression of *PTTG1* have a 4× shorter disease-free interval than dogs with a low expression of *PTTG1* ($P = 0.02$).

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tvjl.2018.08.012.

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