A PUTATIVE GAIN-OF-FUNCTION MUTATION IN *CRHR1* GENE CAUSING ACTH-DEPENDENT HYPERCORTISOLISM IN A POODLE DOG. V. De Marco[1,2,3], LR Carvalho[1], AEC Billerbeck[1], PSL Oliveira[4] & BB Mendonca[1]. 1 Laboratório de Hormônios e Genética Molecular LIM/42, Faculdade de Medicina da Universidade de São Paulo, Brazil. 2 Universidade Guarulhos, SP, Brazil. 3 Pompéia Veterinary Hospital, São Paulo, Brazil. 4 Heart Institute (INCOR), Faculdade de Medicina da Universidade de São Paulo, Brazil.

Pituitary-dependent hypercortisolism (PDH) is a very common endocrinopathy in dogs, generally caused by an ACTH-secreting corticotroph adenoma although the underline pathogenesis is still unknown. There is a high incidence of PDH in Poodles and familial pituitary-dependent hypercortisolism have been identified suggesting a genetic involvement. An increased *CRHR1* expression was demonstrated in human and canine ACTH-secreting pituitary adenomas, despite the autonomous ACTH secretion and the low portal levels of CRH. In addition, prolonged exposure of human corticotroph adenoma cells to CRF does not result in receptor desensitization. The aim of the present study was to screen the *Crhr1* gene for mutations in genomic DNA from Poodles with PDH. Fifty Poodle dogs (33 female, median age of 8.71 years, range 1.5 to 14 years) with PDH were studied. The diagnosis was based on clinical signs (polyphagia, polyuria, polydipsia, abdomen enlargement, panting) and cortisol levels > 1,4 µg/dl after dexamethasone suppression test, plasmatic ACTH levels > 17 pg/ml, bilateral adrenal enlargement at abdominal ultrasound, hyperlipidemia, hyperphosphatasemia, low urinary density. Genomic DNA was isolated from peripheral blood, amplified by PCR using intronic primers to amplify 13 exons, and submitted to automatic sequence. The coding sequence was compared to the canine Boxer genomic DNA sequence available at NCBI site. We found the heterozygous allelic variation p.V97M (GTG > ATG) in exon 4 in one dog. To investigate if this variation is a mutation or a single nucleotide polymorphism, we studied fifty normal poodle dogs (32 female, median age of 9,38 years, range 6 - 16 years) and p.V97M was not found in one hundred normal alleles. The codon 97 is located in the extracellular aminoterminal domain of the *CRHR1* and is extremely important for high affinity ligand binding. The molecular study of the quaternary structure of normal and mutated proteins showed a structural rearrangement of the mutated protein by changing the contact surface between the CRH and its receptor, Crhr1, resulting in a 17% higher binding energy comparing to the wild type. In conclusion, this study identified a Crhr1 gain-of-function mutation, probably responsible for ACTH-dependent hypercortisolism in a poodle dog of our cohort.

Como citar esse resumo (ABNT/NBR 6023):
DE MARCO V.; CARVALHO L.R.; BILLERBECK, A.E.C.; OLIVEIRA, P.S.L.; MENDONCA, B.B. A putative gain-of-function mutation in *CRHR1* gene causing ACTH-dependent hypercortisolism in a poodle dog. In: **Proceedings of the 2010 American College of Veterinary Internal Medicine Forum**, Anaheim: ACVIM, 2010. p.87-88. 2010. CD ROM.