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Letter to the editor

A dog carrying mutations in *AVP-NPII* exhibits key features of central diabetes insipidus

Central diabetes insipidus (CDI) is a rare disease characterized by the excretion of copious amounts of diluted urine (polyuria), excess water intake (polydipsia), and a rise in serum sodium concentration (hypernatremia) (Christ-Crain et al., 2019). The neuropeptide arginine-vasopressin (AVP) is synthesized as a preprohormone along with its carrier protein neurophysin II (NPII) in hypothalamic supraoptic (SON) and paraventricular (PVN) magnocellular neurons, stored in the posterior pituitary, and secreted into the circulation. It binds to AVP receptor 2 in the kidney to promote the insertion of aquaporin channels (AQP2) that mediate antidiuretic effects. Deficits of AVP production and secretion cause CDI, while renal insensitivity to the antidiuretic effect of AVP causes nephrogenic diabetes insipidus (NDI) (Mahia and Bernal, 2021). Mice and rats carrying spontaneous or engineered mutations in the *AVP-NPII* gene present phenotypes similar to those of CDI patients (Valtin and Schroeder, 1964; Russell et al., 2003). However, the pathogenesis and complex clinical manifestations of CDI remain poorly understood.

Domestic dogs (*Canis familiaris*) are an attractive model organism for studying human diseases because their organ size, anatomy, physiology, pathological trajectory, and spatiotemporal brain proteomes are more similar to humans than rodents (Tsai et al., 2007; Zhao et al., 2021; Hong et al., 2022). In the present study, we sought to generate a dog model for CDI by mutating *AVP-NPII* via CRISPR/Cas9-mediated gene editing. Our findings from molecular, biochemical, and pharmacological assays, together with magnetic resonance imaging (MRI), demonstrate that the resulting mutant dog shows clinically typical CDI.

We used the Cas9/single guide RNA (sgRNA) method to disrupt the *AVP-NPII* gene in Beagle dogs following previous protocols (Feng et al., 2018). We targeted two sites (exons 1 and 2) of *AVP-NPII* with two independent sgRNAs to generate small indel mutations (Fig. 1A). We obtained five successful pregnancies containing 28 embryos after transferring 63 injected embryos into 10 surrogate mothers. Of three *AVP-NPII* mutant offsprings (all females), Mu220217 died on day 25 after birth without any discernible abnormalities; Mu220219 died on day 68 after birth (she appeared drinking more water, but died before we took any actions); Mu220216 is still alive. While the off-targets of CRISPR/Cas9-mediated editing remained to be systematically analyzed, genotyping analysis by PCR followed by DNA sequencing revealed that all three mutants contained mutations and lacked the wild-type (WT) sequence in the target regions. Two mutants contain mutations at site 1 (−3 bp in Mu220216 and +136/−36 in Mu220219; Fig. 1B). All three female offspring were positive for mutations at site 2 of exon 2 (−3 bp in Mu220216 and Mu220217, and −45 bp in Mu220219; Fig. 1B). These mutations resulted in single amino acid (aa) deletions in Mu220216 and Mu220217, and a frameshift at aa 21 and premature termination at aa 28 in Mu220219 (Fig. 1B). Then, we focused on the systematic phenotypic analysis of the viable mutant Mu220216.

The mutant dog Mu220216 appeared normal before weaning at 2 months old. During the weaning period (breastfeeding alternated with puppy food), we observed that the mutant dog was apparently thirsty and drank more water than the controls. To determine if the mutant dog displayed CDI, we carried out water deprivation and desmopressin (1-deamino-8-D-arginine vasopressin, a synthetic analogue of AVP commonly used to treat CDI patients) response tests (Fig. 1C) adopted from recommended protocols used for diagnosis of human patients (Christ-Crain et al., 2019). After water deprivation for 6 h and 7 h, the plasma osmolality and sodium concentration of the mutant dog at 4 months of age exceeded that of normal controls, but the urine osmolality remained greatly reduced compared with the WT dog (Fig. 1D; Table S1). At 1 h after desmopressin injection in the mutant muscle, however, there was a rapid increase in urine osmolality (from 129 mOsm/kg H_2O to 702 mOsm/kg H_2O) and a decrease in urine volume (from 10.5 mL to 3 mL) (Fig. 1C and 1D), demonstrating that the mutant was responsive to exogenous desmopressin. Thus, the mutant fully recapitulated the clinical manifestations of CDI patients.

Since there were mutations in target sites 1 or 2 of *AVP-NPII* in all three mutants, and no WT alleles remained, we predicted greatly reduced AVP levels in the viable mutant blood. Indeed, AVP levels at 6 h after water deprivation, which stimulates AVP release from the pituitary into blood, were 0.19 pg/mL in Mu220216, while the levels of AVP in three WT controls were 5.97 pg/mL, 7.64 pg/mL, and 7.78 pg/mL, respectively (Fig. 1E).

AVP is stored in the posterior pituitary where there is a bright spot in the sagittal T1-weighted image of both humans and dogs by MRI (Teshima et al., 2008; Yang et al., 2019). As the mutant showed clinical features of CDI together with a greatly reduced blood AVP level, we suspected that the posterior pituitary gland where AVP is stored might display reduced signals in T1-weighted image, as previously shown for some CDI patients (Gudinchet et al., 1989; Turkkahraman et al., 2015; Yang et al., 2019; Garcia-Castano et al., 2020). This was indeed the case; Fig. 1F showed a T1-weighted image from a WT Beagle with a marked hyper-intensity in the posterior pituitary (highlighted in the enlarged image), while no hyper-intensity was observed in the posterior pituitary of the mutant Beagle. The size of the pituitary gland in the mutant was smaller than in WT controls (56.63 μL in control vs. 48.88 μL in the mutant; Fig. 1F), probably due to degeneration of AVP-positive neurons resulting from mutated AVP-NPII, as previously

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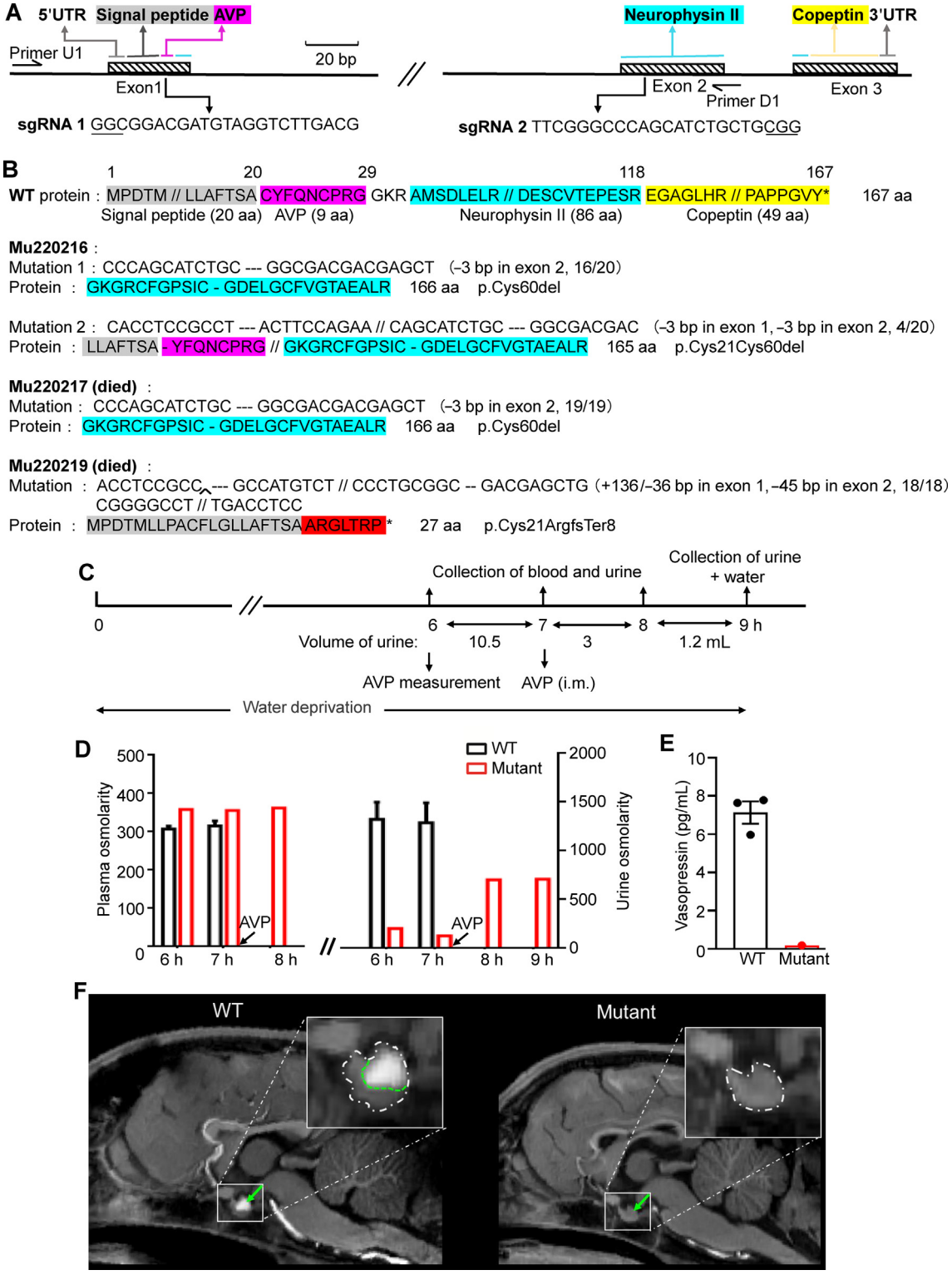

Fig. 1. *AVP-NPII* mutant dogs display key features of central diabetes insipidus. **A**: Schematic diagram of the dog *AVP-NPII* gene (*AVP-NPII*; NM_001197149.1) and the sgRNA target sites in exons 1 and 2. **B**: Sequencing results for target regions of three F0 AVP-NPII mutants with a 100% mutation rate. The ratio (in brackets) represents the number of mutant clones vs. total clones sequenced. The red shaded 7-aa peptide in Mu2220219 is artificial due to a frameshift. Two of the three mutants died at 25 days and 68 days after birth for unknown reasons. aa, amino acid. **C**: Timeline for experimental manipulations and assays of dogs at four months old. i.m. : intramuscular injection **D**: Osmolality in the serum and urine of control and mutant dogs at four months old after water deprivation and desmopressin response tests. Data for WT are presented as mean ± SEM. Arrows indicate the time when AVP analogue was administered. **E**: AVP levels in the mutant were markedly lower than in controls. **F**: Sagittal T1-weighted images highlighting pituitary glands in WT and mutant Beagles. Green arrows indicate the pituitary gland and dashed lines depict the pituitary gland enlarged in white boxes. The high intensities in the posterior pituitary gland in WT were lost in the mutant (right panel).

suggested (Russell et al., 2003). The absence of a bright spot in the sagittal T1-weighted image of the posterior pituitary in the mutant dog may result from disrupted production, transport, or both of AVP. In summary, we successfully generated an *AVP-NPII* mutant dog displaying clinically typical CDI.

In addition to its antidiuretic effect in the periphery, AVP modulates complex social behaviour and social cognition in animal models (Donaldson and Young, 2008), which is supported partially by the fact that AVP receptors are expressed in specific brain regions of rodents (Christ-Crain et al., 2021). Consistently, CDI patients show a higher rate of psychiatric phenotypes such as depression and anxiety (Aulinas et al., 2019; Atila et al., 2022). However, the functions of AVP and its receptors in the brain are not completely understood (Donaldson and Young, 2008; Christ-Crain et al., 2019). Dogs show a higher similarity to humans in brain anatomy and behaviors than rodents. During the long-term domestication and co-evolution with humans, dogs have developed remarkable human-like social cognitive abilities. Dogs are thus particularly suited to uncover the functions of AVP in the brain and understand the psychiatric aspects of CDI patients. Meanwhile, several candidate drugs for CDI are under clinical trial (https://clinicaltrials.gov; http://www.chinadrugtrials.org.cn), as the currently used desmopressin therapy often leads to hyponatremia in CDI patients (Christ-Crain et al., 2021). Though the present study is limited by characterizing only one viable mutant, further studies on the mutant and its progeny which can be produced by regular breeding, somatic cloning, or both can be carried out to explore the pathogenesis, especially the neuropathologenesis of the psychiatric aspects of CDI, and develop better therapies for this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgg.2022.11.003.

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Huijuan Xu
State Key Laboratory for Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

Haiyang Hao
Beijing Sinogene Biotechnology Co. Ltd, Beijing 100005, China

Shirui Wang
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Xueru Liu
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Wen Lyu
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

State Key Laboratory for Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Zhentao Zuo
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Hefei Comprehensive National Science Center, Institute of Artificial Intelligence, Hefei, Anhui 230088, China

Yan Zhuo
State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Jidong Mi
Beijing Sinogene Biotechnology Co. Ltd, Beijing 100005, China

Yong Q. Zhang
College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

State Key Laboratory for Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Rui Tian*
State Key Laboratory for Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Huijuan Zhu**
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

* Corresponding author.

** Corresponding author.
E-mail addresses: rtian@genetics.ac.cn (R. Tian), shengxin2004@163.com (H. Zhu).

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