

1 **Genome-wide association study and heritability estimate for ectopic ureters in**

2 **Entlebucher mountain dogs**

3

4 Milena Gallana¹, Yuri Tani Utsunomiya², Gaudenz Dolf³, Rafaela Beatriz Pintor

5 Torrecilha², Ann-Kristin Falbo¹, Vidhya Jagannathan³, Tosso Leeb³, Iris Reichler¹, Johann

6 Sölkner⁴, Claude Schelling⁵

7

8 ¹Clinic for Reproductive Medicine, Vetsuisse-Faculty, University of Zurich,

9 Winterthurerstrasse 260, 8057 Zurich, Switzerland

10 ²São Paulo State University (Unesp). School of Agricultural and Veterinarian Sciences,

11 Jaboticabal, Department of Preventive Veterinary Medicine and Animal Reproduction, São

12 Paulo, Brazil

13 ³Institute of Genetics, Vetsuisse-Faculty, University of Bern, Bremgartenstrasse 109a,

14 3012 Bern, Switzerland

15 ⁴Department of Sustainable Agricultural Systems, Division of Livestock Sciences,

16 University of Natural Resources and Life Sciences, Gregor Mendel Straße 33, 1180

17 Vienna, Austria

18 ⁵Clinic for Reproductive Medicine and Center of Clinical Studies, Vetsuisse-Faculty,

19 University of Zurich, Eschikon 27, EHB F 22.1, 8315 Lindau, Switzerland

20 **Abstract**

21

22 An ectopic ureter is a congenital anomaly which may lead to urinary incontinence and
23 without a surgical intervention even to end-stage kidney disease. A genetic component
24 contributes to the development of this anomaly in Entlebucher mountain dogs (EMD).
25 However, its nature remains unclear. Using the Illumina CanineHD bead chip, a case-control
26 genome-wide association study was performed to identify SNPs associated with the trait.
27 Six loci on canine chromosomes 3, 17, 27 and 30 were identified with 16 significantly
28 associated SNPs. There was no single outstanding SNP associated with the phenotype and
29 the association signals were not close to known genes involved in human congenital
30 anomalies of the kidney or lower urinary tract. Additional research will be necessary to
31 elucidate the potential role of the associated genes in the development of ectopic ureters in
32 the EMD breed.

33

34

35

36 **Keywords:** GWAS, urinary tract development, *Canis lupus familiaris*, dog, CAKUT,
37 CALUT, Entlebucher mountain dog

38

39

40

41

42

43

44

45

46 In dogs, an ectopic ureter (EU) is a rare congenital anomaly in which one or both ureteral
47 orifices are not located at the anatomically correct position at the vesicular trigone of the
48 bladder (Osborne *et al.* 1995). Instead, the ureteral openings can be found in the bladder
49 neck, the urethra or even in the genital system (Dean 1988; Owen 1973). The most common
50 clinical sign of affected dogs is urinary incontinence (Fossum 1997). However, many dogs,
51 especially males, which show a higher prevalence for EU than females in the Entlebucher
52 mountain dog (EMD) (Fritsche *et al.* 2014), are continent for years and may show
53 incontinence only at an advanced age (Holt & Moore 1995; Reichler *et al.* 2012). Affected
54 dogs are predisposed for ascending infections of the urinary tract including pyelonephritis;
55 they may have or develop hydroureter and hydronephrosis, as well as changes of kidney
56 size or distorted internal kidney architecture (Holt & Moore 1995; Lamb & Gregory 1998;
57 Niesterok 2016). This may result in fatal end-stage kidney disease if no surgery is performed.
58 The EMD is one of the dog breeds that have a higher risk for EU (Fritsche *et al.* 2014). Out
59 of 552 classified EMD nearly half showed intravesicular ectopia, i.e. 130 females and 132
60 males. One fifth, i.e. 25 females and 84 males, had at least one extravesicular ectopic
61 termination. Urinary incontinence was a complaint in 3% and 27% of them. Hydronephrosis
62 and/or hydroureter was noticed by ultrasonography in 1% and 14% of the intravesicular and
63 extravesicular cases, respectively. In one third of them this was an incidental finding
64 (Fritsche *et al.* 2012). The breed predisposition indicates a genetic background, however
65 exaggerated breeding restrictions to reduce the risk of clinically affected dogs in the offspring
66 are of concern, as the EMD has already a high average inbreeding coefficient around 40%
67 (Schrack *et al.* 2017). Therefore, selection based on genotypes would be desirable, but first
68 attempts to associate five suitable candidate genes, selected from mouse studies (Uetani &
69 Bouchard 2009), to EU in EMD were unsuccessful (North *et al.* 2009). In humans, congenital
70 anomalies of the kidney and urinary tract (CAKUT) are a genetically heterogeneous group
71 of developmental disorders (syndromic and non-syndromic) with highly varying phenotypes.

72 Even though single gene mutations were shown to cause renal anomalies in mice, the
73 elucidation of CAKUT cases in humans remains difficult (Nicolaou *et al.* 2016). Genetic
74 heterogeneity, modifier genes, allelic variation in gene expression, epigenetic effects,
75 complex modes of inheritance and environmental effects may hamper the clarification of the
76 genetic basis of sporadic and familial CAKUT (Yosypiv 2012).

77 Using complex segregation analysis, our group previously showed a genetic background for
78 EU in EMD and the possible involvement of a major gene for the EU-3 phenotype (Fritsche
79 *et al.* 2014), while the evaluation of an X-linked mode of inheritance, which could explain the
80 observed male predominance for extraventricular ectopia was not successful (Fritsche *et al.*
81 2014). The goal of the present study was to re-estimate the heritability for EU-3 and identify
82 genetic variants associated with EU and candidate genes within chromosomal regions of
83 such variants. Ureteric openings were determined for 1421 EMDs born between 1996 and
84 2016 and registered by official national kennel clubs. The majority of dogs were between six
85 and twelve months old when ultrasonographical screening was performed by authorized
86 private veterinarians and university clinics. This screening method was previously validated
87 by comparison with dissection (Balogh *et al.* 2015) and already used to elucidate the mode
88 of inheritance of EU by multivariate mixed logistic regression (Fritsche *et al.* 2014). All EU
89 diagnoses were finally assessed by one researcher in order to avoid a bias through the first
90 examiner. The dogs were classified based on the more caudally placed ureteral opening
91 into three phenotype classes, namely EU-1 (both ureters in the correct anatomical position,
92 i.e. a distance between the more caudal ureteral opening and the vesicourethral junction of
93 at least 1.8 cm (Balogh *et al.* 2015; Rozear & Tidwell 2003)), EU-2 (a distance of the more
94 caudal ureteral opening and the vesicoureteral junction between 1.1 and 0.1 cm) and EU-3
95 (at least one ureteral opening located extraventrically in the urethra or genital tract). Dogs
96 which had not been examined or for which no conclusive diagnosis was attained, were
97 classified as EU-0. Pedigree information for the dogs was available through an in-house

98 EMD database, merging all EMDs into one single family. We re-estimated the narrow sense
99 heritability (h^2) of EU-3 under a threshold-liability model (Lee *et al.* 2011) using phenotypes
100 of 98 cases (73 males and 25 females) and 151 EU-1 controls (32 males and 119 females)
101 and pedigree information of 4522 dogs. The analysis included sex as a fixed effect. EU-3
102 was found to be heritable, with estimates of 0.713 and 0.960 in the 0-1 risk and liability
103 scales, respectively. These findings led us to hypothesize that a major risk locus may
104 underlie EU-3, which could be presumably detected through a genome-wide association
105 study (GWAS).

106 For the GWAS, genomic DNA was extracted from EDTA blood samples of 381 EMDs with
107 phenotype EU-1 (n=218), EU-2 (n=28) or EU-3 (n=135). Genotyping was performed using
108 the Illumina® CanineHD assay (Illumina Inc., San Diego CA, USA) at GeneSeek (part of
109 Neogen Corporation in Lincoln, USA). This chip contains approximately 173000 single
110 nucleotide polymorphism (SNP) markers distributed throughout the genome with an average
111 density of 70 markers per million base pairs, allowing for a robust within-breed association
112 analysis.

113 Due to the high selective pressure and inbreeding levels in EMD, our GWAS analysis
114 included dominance and autozygosity effects, apart from additive marker effects (see
115 supplementary material). The family-wise error rate was controlled by adopting a LD-
116 corrected Bonferroni significance level of $0.05/K$, where K is the effective number of
117 independent markers. The approach was similar to the simple method (Gao *et al.*, 2008),
118 except that K was estimated from the ratio between the total number of markers and the
119 average number of tag-partners per marker ($r^2 > 0.3$), instead of the eigenvalues required
120 to explain 99.5% of the variance in the genotype matrix. After scanning all autosomes (Fig.
121 1), we found six genome-wide significant loci (p -value $< 9.65 \times 10^{-5}$), which are presented in
122 Table 1.

123 Significant markers were observed in five regions on canine chromosomes (CFA) 3, 17, 27

124 and 30. On CFA 3, SNP BICF2P957732 is located near *MCTP2*, which encodes a
125 transmembrane protein with Ca⁺⁺ binding domains involved in signal transduction or
126 membrane trafficking (Shin *et al.* 2005). Lalani and coworkers (2013) found gross heart
127 anomalies in *Xenopus* embryos associated with morpholino knockdown of *MCTP2*. The
128 SNP BICF2P527992 is in an intron of the IQ motif containing GTPase activating protein 1
129 gene (*IQGAP1*) and presented a significant dominance effect on the investigated trait.
130 *IQGAP1* is an interesting functional candidate gene because it is involved in the regulation
131 of the beta-catenin/GATA3 pathway in *Xenopus* embryos (Goto *et al.* 2013). The beta-
132 catenin/GATA3 pathway is involved in the formation of the ureteric bud, and loss of function
133 of *GATA3* leads to ectopic ureteric bud formation and severe urogenital abnormalities (Grote
134 *et al.* 2008). However, *Iqgap1*-null mice did not show any observable phenotype (Li *et al.*
135 2000). There is no clear functional candidate gene for the association signal on CFA 17,
136 however, the underlying effects were due to autozygosity in this locus. The most intriguing
137 association signals are on CFA 27. There is one signal at ~1 Mb overlapping with the *HOXC*
138 gene cluster. *HOX* genes are obvious functional candidate genes for phenotypes that
139 involve potential defects in development (Mallo & Alonso 2013). The other association signal
140 at ~23 Mb on CFA 27 is located in an extremely gene poor region upstream of the *SOX5*
141 gene, which encodes another transcription factor involved in development. Coding variants
142 may lead to Lamb-Shaffer syndrome in humans, a neurodevelopmental disorder, sometimes
143 seen in combination with variable skeletal abnormalities (Nesbitt *et al.* 2015) but extremely
144 rarely with urogenital malformations (Lee *et al.* 2013). Therefore, it remains unclear whether
145 *SOX5* is the causative gene underlying this association signal. As the region of this
146 association signal is extremely gene poor, it might have important regulatory functions that
147 are not necessarily restricted to *SOX5* (Ovcharenko *et al.* 2005). The association signal on
148 CFA 30 is located between the *SMAD6* and *SMAD3* genes, encoding again important
149 transcription factors of the TGF-beta signaling pathway that also have a role in development

150 (Macias *et al.* 2015). While our GWAS failed to pick up any candidate genes known from
151 human studies, it is remarkable that 3 of the 5 putative association signals fall near the genes
152 for developmental transcription factors. As the p-values only just exceed the significance
153 threshold, the findings need to be regarded with caution and the associations should be
154 validated in a larger set of animals. In addition, nine genes which were previously shown to
155 be involved in congenital anomalies of the lower urinary tract (CALUT) in humans (reviewed
156 in Rasouly & Lu 2013) were analysed by comparing the genomes of 296 dogs of different
157 breeds and eight EMD genomes with either EU-1 or EU-3 phenotypes. We did not find any
158 protein-changing variants in these candidate genes (not shown).

159 Despite the high heritability found for ectopic ureters the association study failed to come up
160 with a single strong association signal. There are at least two possible explanations for such
161 a finding: (1) Despite the high heritability it is possible that many genetic risk loci with small
162 effects are involved in the formation of EU. (2) It also cannot be excluded that a genetic
163 variant, which predisposes the dogs for the formation of ectopic ureters, is fixed in the EMD
164 population and therefore not detectable by the genome-wide association study.

165 In the latter scenario, modifier genes may still modulate the phenotype despite a unique
166 underlying ectopic ureter genotype, similar to humans (Yosypiv 2012). This assumption is
167 supported by the high average inbreeding coefficient of extant EMDs (Schrack *et al.* 2017),
168 as well as by the much lower prevalence of ectopic ureters in the related Appenzeller
169 mountain dog breed (Bitterli 2011). However, the high heritability seems to contradict this
170 hypothesis. Even if we failed to identify strong candidates known from human studies, the
171 signals seem to be quite clear for the relatively small number of animals, supporting an
172 oligogenic inheritance. However, before using some of those SNPs for marker-assisted
173 selection of breeding EMDs, further research is needed to support this data.

174

175 **Competing interests**

176 The authors declare that they do not have any competing interests.

177

178 **Acknowledgements**

179 The authors would like to thank Prof. M. Stoffel and Dr. A. von Rotz (Division of Veterinary
180 Anatomy, Vetsuisse Faculty, University of Bern) for their supporting information about ureter
181 development. The laboratory work was partly performed using the logistics of the Center for
182 Clinical Studies at the Vetsuisse Faculty Zurich, University of Zurich. This study was kindly
183 funded by the Albert Heim Stiftung, the Stiftung für das Wohl des Hundes, as well as the
184 Swiss Federal Food Safety and Veterinary Office. We thank the breeder associations and
185 especially Margret Epple, Herma Cornelese and Max and Gertrud Heller for their support
186 and the private veterinarians for their assistance in acquiring the clinical data.

187

188

189

190

191

192

193

194

195 **References**

196 Bitterli F. (2011) Prevalence and clinical relevance of ectopic ureters of the Entlebucher
197 Mountain dog and Appenzeller Mountain dog. Vetsuisse-Faculty Zurich, University of
198 Zurich.

199

200 Dean P.W. (1988) Canine Ectopic Ureter. The compendium on continuing education for
201 the practicing veterinarian 10, 146-157.
202

203 Fossum T.W. (1997) Surgery of the kidney and ureter - Ectopic Ureter. In: Small Animal
204 Surgery (ed 2), pp. 558-565. St. Louis, Mosby.
205

206 Fritsche R., Dolf G., Schelling C., Hagen R., Hungerbühler S.O., Dorsch R., Hittmair K.,
207 Vink-Nooteboom M.V., Zaal M., Reichler I.M. (2012) Inheritance of ectopic ureters in
208 Entlebucher Mountain Dogs BSAVA World Congress 2012, Birmingham, ISBN 978 1
209 905319 46 6.
210

211 Fritsche R., Dolf G., Schelling C., Hungerbuehler S.O., Hagen R. & Reichler I.M. (2014)
212 Inheritance of ectopic ureters in Entlebucher Mountain Dogs. Journal of Animal Breeding
213 and Genetics 131, 146-152.
214

215 Gao X., Starmer J. & Martin E.R. (2008) A multiple testing correction method for genetic
216 association studies using correlated single nucleotide polymorphisms. Genetic
217 Epidemiology 32, 361-369.
218

219 Goto T., Sato A., Adachi S., Iemura S., Natsume T. & Shibuya H. (2013) IQGAP1 protein
220 regulates nuclear localization of β -catenin via importin- β 5 protein in Wnt signalling. The
221 Journal of Biological Chemistry 288, 36351-36360.
222

223 Grote D., Boualia S.K., Souabni A., Merkel C., Chi X., Costantini F., Carroll T. & Bouchard
224 M. (2008) Gata3 acts downstream of b-catenin signaling to prevent ectopic metanephric
225 kidney induction. PLoS Genetics 4 (12), e1000316.

226

227 Holt P.E. & Moore A.H. (1995) Canine ureteral ectopia: an analysis of 175 cases and
228 comparison of surgical treatments. *Veterinary Records* 136, 345-349.

229

230 Lalani S.R., Ware S.M., Wang X., Zapata G., Tian Q., Franco L.M., Jiang Z., Bucasas K.,
231 Scott D.A., Campeau P.M., Hanchard N., Umana L., Cast A., Patel A., Cheung S.W.,
232 McBride K.L., Bray M., Craig Chinault A., Boggs B.A., Huang M., Baker M.R., Hamilton S.,
233 Towbin J., Jefferies J.L., Fernbach S.D., Potocki L. & Belmont J.W. (2013) MCTP2 is a
234 dosage-sensitive gene required for cardiac outflow tract development. *Human Molecular*
235 *Genetics* 22, 4339-4348.

236

237 Lamb C.R. & Gregory S.P. (1998) Ultrasonographic findings in 14 dogs with ectopic ureter.
238 *Veterinary Radiology & Ultrasound* 39, 218-223.

239

240 Lee S.H., Wray N.R., Goddard M.E. & Visscher P.M. (2011) Estimating missing heritability
241 for disease from genome-wide association studies. *American Journal of Human Genetics*
242 88, 294–305.

243

244 Lee R.W.Y., Bodurtha J., Cohen J., Fatemi A., Batista D. (2013) Deletion 12p12 Involving
245 SOX5 in Two Children With Developmental Delay and Dysmorphic Features. *Pediatric*
246 *Neurology* 48, 317-320.

247

248 Li S., Wang Q., Chakladar A., Bronson R.T. & Bernards A. (2000) Gastric hyperplasia in
249 mice lacking the putative Cdc42 effector IQGAP1. *Molecular and Cellular Biology* 20, 697-
250 701.

251

252 Macias M.J., Martin-Malpartida P. & Massagué J. (2015) Structural determinants of SMAD
253 function in TGF- β signaling. Trends in Biochemical Sciences 40, 296-308.
254

255 Mallo M. & Alonso C.R. (2013) The regulation of Hox gene expression during animal
256 development. Development 140, 3951-3963.
257

258 Nesbitt A., Bhoj E.J., McDonald Gibson K., Yu Z., Denenberg E., Sarmady M., Tischler T.,
259 Cao K., Dubbs H., Zackai E.H. & Santani A. (2015) Exome sequencing expands the
260 mechanism of SOX5-associated intellectual disability: a case presentation with review of
261 SOX-related disorders. American Journal of Medical Genetics Part A 167, 2548-2554.
262

263 Nicolaou N., Puli S.L., Nijman I.J., Monroe G.R., Feitz W.F., Schreuder M.F., van Eerde
264 A.M., de Jong T.P., Giltay J.C., van der Zwaag B., Havenith M.R., Zwakenberg S., van der
265 Zanden L.F., Poelmans G., Cornelissen E.A., Lilien M.R., Franke B., Roeleveld N., van
266 Rooij I.A., Cuppen E., Bongers E.M., Giles R.H., Knoers N.V. & Renkema K.Y. (2016)
267 Prioritization and burden analysis of rare variants in 208 candidate genes suggest they do
268 not play a major role in CAKUT. Kidney International 89, 476-486.
269

270 Niesterok C., Koehler C., Alef M. & Kiefer I. (2016) Causes of hydronephrosis in dogs and
271 cats. Ultraschall in der Medizin 37, PS1_02.
272

273 North C., Kruger J.M., Venta P.J., Miller J.M., Rosenstein D.S., Randall E.K., Fitzgerald
274 S.D., Estero F.L. (2009) Clinical and genetic characterization of a congenital Entlebucher
275 mountain dog urinary tract syndrome. In ACVIM 2009, Vancouver.
276

277 North C., Kruger J.M., Venta P.J., Miller J.M., Rosenstein D.S., Randall E.K., White B. &
278 Fitzgerald S.D. (2010) Congenital ureteral ectopia in continent and incontinent-related

279 Entlebucher mountain dogs: 13 cases (2006-2009). *Journal of Internal Medicine* 24, 1055-
280 1062.

281

282 Osborne C., Johnston G. & Kruger J. (1995) Ectopic ureters and ureteroceles. In: *Canine*
283 *and feline nephrology and urology*, Vol Williams & Wilkins, pp. 608-620.

284

285 Ovcharenko I., Loots G.G., Nobrega M.A., Hardison R.C., Miller W. & Stubbs L. (2005)
286 Evolution and functional classification of vertebrate gene deserts. *Genome Research* 15,
287 137-145.

288

289 Owen R.R. (1973) Canine ureteral ectopia – a review. 2. Incidence, diagnosis and
290 treatment. *Journal of Small Animal Practice* 14, 419-427.

291

292 Rasouly H.M. & Lu W. (2013) Lower urinary tract development and disease. *Wiley*
293 *Interdisciplinary Reviews: Systems Biology and Medicine* 5, 307-342.

294

295 Reichler I.M., Eckrich Specker C., Hubler M., Boos A., Haessig M. & Arnold S. (2012)
296 Ectopic ureters in dogs: clinical features, surgical techniques and outcome. *Veterinary*
297 *Surgery* 41, 515-522.

298

299 Rozear L., Tidwell A.S. (2003) Evaluation of the ureter and ureterovesicular junction using
300 helical computed tomographic excretory urography in healthy dogs. *Veterinary Radiology*
301 *& Ultrasound* 44(2), 155-164.

302

303 Schrack J., Dolf G., Reichler I.M. & Schelling C. (2017) Factors influencing litter size and
304 puppy losses in the Entlebucher Mountain dog. *Theriogenology* 95, 163-170.

305

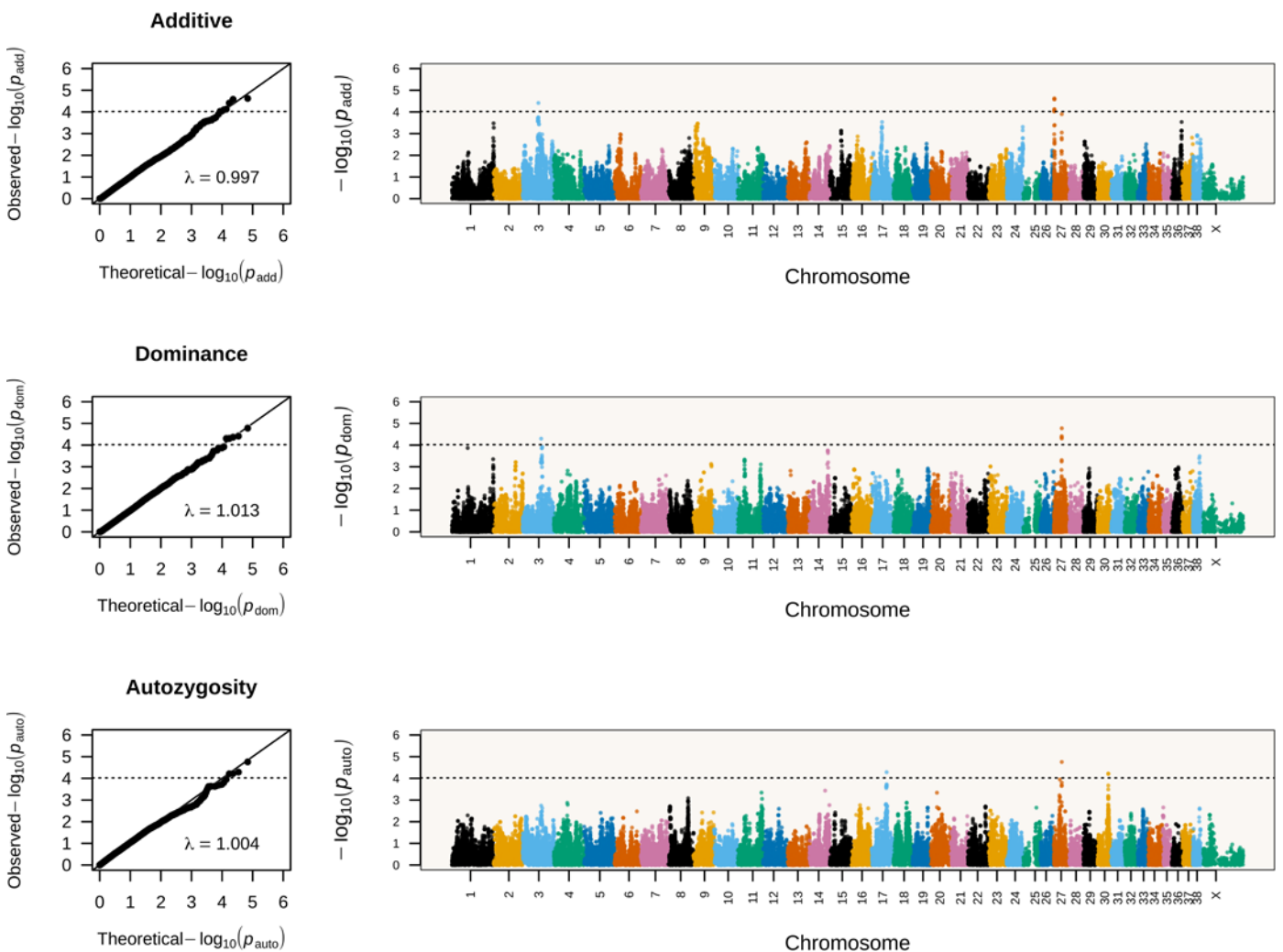
306 Shin O.H., Han W., Wang Y. & Sudhof T.C. (2005) Evolutionarily conserved multiple C2
307 domain proteins with two transmembrane regions (MCTPs) and unusual Ca(2+) binding
308 properties. The Journal of Biological Chemistry 280, 1641-1651.

309

310 Uetani N., Bouchard M. (2009) Plumbing in the embryo: developmental defects of the
311 urinary tracts. Clinical Genetics 75(4), 307-317.

312

313 Yosypiv I.V. (2012) Congenital anomalies of the kidney and urinary tract: A genetic
314 disorder? International Journal of Nephrology 2012, 909083.



315

316 **Figure 1** | Quantile-quantile and Manhattan plots for additive (add), dominance (dom) and autozy-
317 gosity (auto) effects. The dashed horizontal line corresponds to the LD-adjusted Bonferroni thresh-
318 old ($p = 9.65 \times 10^{-5}$).

319 **Table 1.** Summary statistics for significant markers associated with ectopic ureters in Entlebucher mountain dogs

320

| CFA ¹ | Position ² | SNP ³ | Position ³ | ⁴ Alleles | ⁵ ♂ control | ⁶ ♂ case | ⁷ ♀ control | ⁸ ♀ case | ⁹ Inheritance model | ¹⁰ P-value | ¹¹ nearest gene | ¹² Distance |
|------------------|-----------------------|-------------------------|-----------------------|----------------------|------------------------|---------------------|------------------------|---------------------|--------------------------------|-----------------------|----------------------------|------------------------|
| 3 | 45655143 | BICF2P957732 | 45655143 | A/C | 23/9/0 | 39/27/6 | 95/22/2 | 9/13/3 | Additive | 3.89E-005 | MCTP2 | 187,272 |
| 3 | 53778402 | BICF2P527992 | 53778402 | T/C | 13/16/3 | 43/23/7 | 57/56/6 | 18/5/2 | Dominance | 5.05E-005 | IQGAP1 | 0 |
| 17 | 43412582 | BICF2P1360326 | 43412582 | C/T | 29/3/0 | 57/15/0 | 89/29/1 | 21/3/0 | Autozygosity | 5.23E-005 | CTNNA2 | 59,764 |
| 27 | 1007730 | TIGRP2P347803_rs8943392 | 1007730 | T/C | 20/12/0 | 65/8/0 | 95/23/1 | 24/1/0 | Additive | 9.38E-005 | NEF2 | 0 |
| 27 | 1168218 | BICF2S22927985 | 1168218 | G/A | 21/11/0 | 67/6/0 | 97/21/1 | 24/1/0 | Additive | 9.21E-005 | HOXC4 | 41,292 |
| 27 | 1411816 | BICF2G630137589 | 1411816 | G/A | 13/17/2 | 54/19/0 | 73/42/4 | 19/6/0 | Additive | 2.63E-005 | HOXC13 | 88,346 |
| 27 | 1415865 | BICF2G630137593 | 1415865 | T/C | 13/17/2 | 54/19/0 | 73/42/4 | 19/6/0 | Additive | 2.63E-005 | HOXC13 | 109,704 |
| 27 | 1428628 | BICF2G630137612 | 1428628 | G/A | 14/16/2 | 54/19/0 | 74/41/4 | 19/6/0 | Additive | 7.91E-005 | HOXC13 | 12,763 |
| 27 | 1433130 | BICF2G630137624 | 1433130 | C/T | 13/16/3 | 52/21/0 | 71/44/4 | 19/6/0 | Additive | 2.38E-005 | HOXC13 | 17,265 |
| 27 | 1437735 | BICF2G630137629 | 1437735 | G/A | 14/16/2 | 53/19/0 | 74/41/4 | 19/6/0 | Additive | 7.47E-005 | HOXC13 | 21,870 |
| 27 | 22783765 | BICF2G630149192 | 22783765 | C/T | 19/12/1 | 45/25/2 | 70/46/3 | 16/5/4 | Dominance | 4.39E-005 | SOX5 | 73,896 |
| 27 | 22929398 | BICF2P1108722 | 22929398 | A/G | 18/13/1 | 43/28/2 | 70/46/3 | 16/5/4 | Dominance | 3.88E-005 | lncRNA | 0 |
| 27 | 22994047 | BICF2P830285 | 22994047 | T/G | 18/13/1 | 41/30/2 | 69/46/4 | 16/5/4 | Dominance | 5.00E-005 | lncRNA | 0 |
| 27 | 23114194 | BICF2P595351 | 23114194 | T/C | 18/13/1 | 40/31/2 | 69/44/6 | 16/5/4 | Dominance | 1.67E-005 | lncRNA | 0 |
| 30 | 31140902 | BICF2P906072 | 31140902 | C/T | 22/10/0 | 46/23/3 | 78/37/4 | 16/8/1 | Autozygosity | 6.06E-005 | SMAD3 | 105,411 |
| 30 | 31236521 | BICF2P664860 | 31236521 | A/G | 22/10/0 | 46/24/3 | 78/37/4 | 16/8/1 | Autozygosity | 6.23E-005 | SMAD3 | 9,792 |

321

322 ¹ Canine chromosome, ²SNP position in the corresponding chromosome (CanFam3.1 genome version) Annotation Release 103, ³SNPs in the
 323 Illumina® CanineHD bead chip, ⁴minor allele/major allele, ⁵⁻⁸SNP genotype distribution, ⁹gene effect, ¹⁰P-value, ¹¹gene symbol of the nearest gene
 324 of the reported SNP, ¹²Distance in bp between SNP and nearest gene (0=SNP within gene).