

SUPPLEMENTARY MATERIAL

A homozygous missense variant in the alkaline phosphatase gene *ALPL* is associated with a severe form of canine hypophosphatasia

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CONTENTS

Supplementary Figure 1. Radiographic findings in 2-week-old affected dogs.

Supplementary Figure 2. Conservation of the Val⁴³⁴ position in 136 vertebrate species.

Supplementary Table 1. Serum biochemistry in an affected and healthy 2-week-old littermate.

Supplementary Table 2. Samples used in NGS variant filtering.

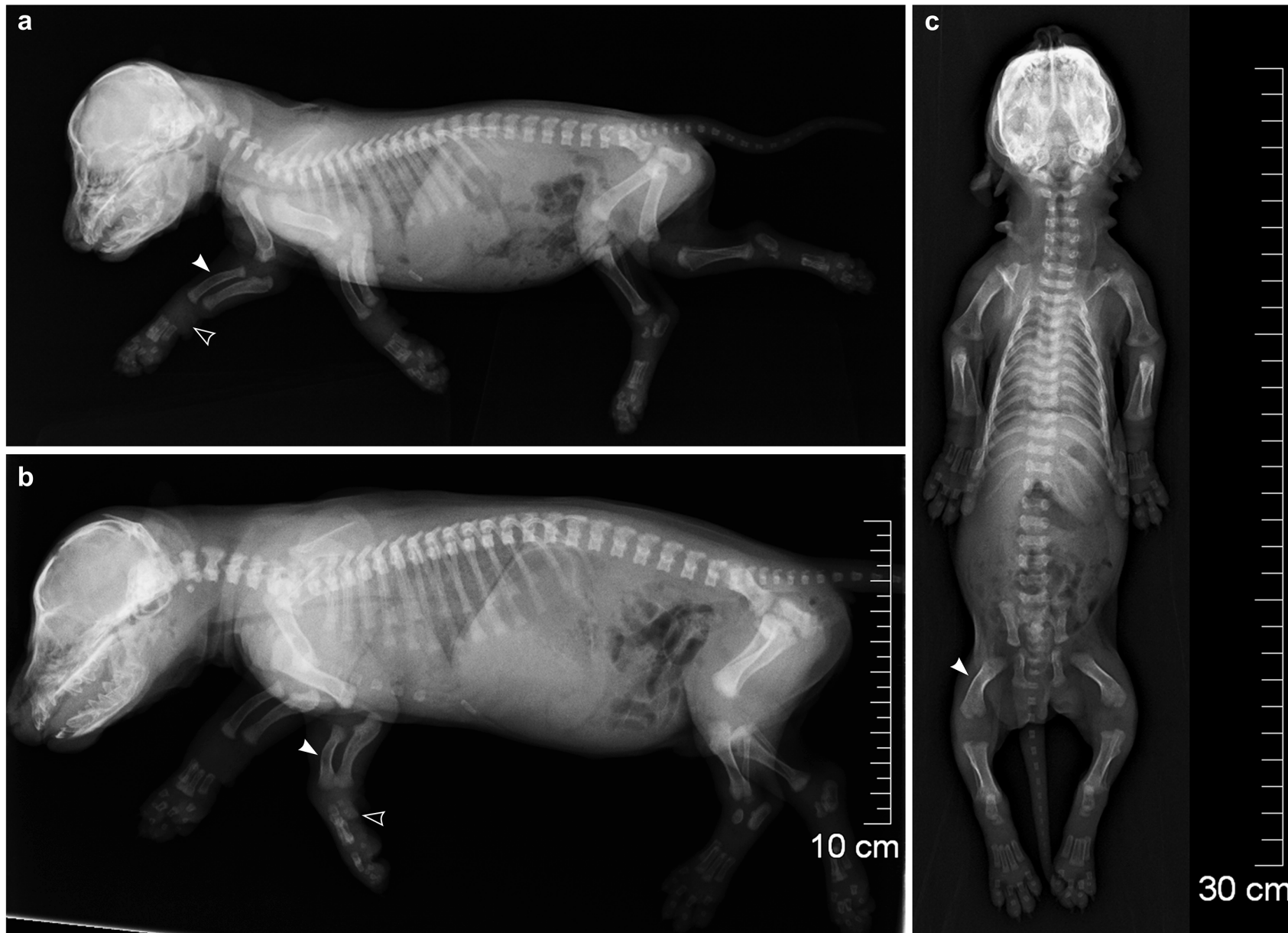
Supplementary Table 3. Prioritized homozygous variants in the affected dog.

Supplementary Table 4. Pathogenicity prediction results.

Supplementary Video Legends:

Supplementary Video 1. An affected puppy at 10 weeks of age (litter 1).

Supplementary Video 2. An affected puppy at 2 weeks of age (litter 2).



Supplementary Figure 1. Radiographic findings in 2-week-old affected dogs. (a) Carpal bones are non-mineralized (open arrowhead) but radius appears normal (arrowhead). **(b)** Mineralized carpal bones in one limb (open arrowhead). Radius is bowed cranially (arrowhead). **(c)** Right femur is abnormally curved (arrowhead).

Supplementary Table 1. Serum biochemistry in an affected and healthy 2-week-old littermate.

Metabolite	Healthy puppy	Affected puppy	Reference range (adult dogs)	Unit
Albumin	23*	29	25-44	G/L
Alkaline phosphatase	98	10*	20-150	U/L
Alanine aminotransferase	18	14	10-118	U/L
Amylase	338	332	200-1200	U/L
Total bilirubin	8	4	2-10	UMOL/L
Blood urea nitrogen	5.7	10.0*	2.5-8.9	MMOL/L
Calcium	2.86	3.09*	2.15-2.95	MMOL/L
Phosphorus	3.12*	3.01*	0.94-2.13	MMOL/L
Creatinine	35	49	27-124	UMOL/L
Glucose	7.6*	6.9*	3.3-6.1	MMOL/L
Sodium	132*	138	138-160	MMOL/L
Potassium	5.4	5.0	3.7-5.8	MMOL/L
Total protein	40*	54*	54-82	G/L
Globulin	17*	24	23-52	G/L

*Out of reference range (only adult dog control ranges were available).

Supplementary Table 2. Samples used in NGS variant filtering.

Breed	WGS	WES	WGS + WES	Animals
Karelian Bear Dog, affected		1	1	1
Karelian Bear Dog, unaffected*	5	9	14	13
Affenpinscher	1		1	1
Afghan Hound		1	1	1
Airedale Terrier	2	6	8	8
Akita		3	3	3
Alaskan Husky mix	1		1	1
Alaskan Malamute	2	5	7	7
Alpine Dachsbracke	1		1	1
American Bulldog	2		2	2
American Hairless Terrier		5	5	5
American Staffordshire Terrier	1		1	1
Australian Cattle Dog	5		5	5
Australian Kelpie	5	12	17	13
Australian Shepherd	1		1	1
Australian Terrier	1	13	14	14
Basenji	6		6	6
Basset Griffon Vendéen	4		4	4
Basset Hound	3		3	3
Bavarian Hound	1		1	1
Beagle	6		6	6
Bearded Collie	11		11	11
Belgian Shepherd, gronendael		6	6	6
Belgian Shepherd, malinois	6	3	9	9
Belgian Shepherd, tervueren	2	7	9	9
Bichon Frisé	6	7	13	13
Black Russian Terrier	1		1	1
Border Collie	64	4	68	68
Border Terrier	1		1	1
Boston Terrier		1	1	1
Boxer	1	4	5	5
Bull Terrier	3		3	3
Bulldog		1	1	1
Bullmastiff	2		2	2
Cairn Terrier	2		2	2
Cane Corso	5		5	5
Cavalier King Charles Spaniel	3		3	3
Central Asian Shepherd Dog	1		1	1
Chihuahua	3	2	5	5
Chinese Crested Dog	1		1	1

Breed	WGS	WES	WGS + WES	Animals
Chinese indigenous dog	38		38	38
Chow Chow	1		1	1
Cocker Spaniel	1		1	1
Curly Coated Retriever	1		1	1
Dachshund	6	2	8	8
Dalmatian	3	4	7	7
Dandie Dinmont Terrier	1		1	1
Deerhound	1		1	1
Dobermann	5	9	14	14
Dogue de Bordeaux	6		6	6
Dutch Shepherd Dog	2		2	2
Elo	1		1	1
English Cocker Spaniel	2		2	2
English Springer Spaniel		1	1	1
Entelbuch Cattle Dog	8		8	8
Eurasier	2		2	2
Finnish Hound		3	3	3
Finnish Lapphund	2	6	8	6
Finnish Reindeer Herder		4	4	4
Finnish Spitz		9	9	9
Flanders Cattle Dog		2	2	2
Flat Coated Retriever	1		1	1
Foxterrier		4	4	4
French Bulldog	4	1	5	5
German Hunting Terrier	2		2	2
German Pinscher		4	4	4
German Pointing Dog	1	3	4	4
German Shepherd Dog	17	1	18	18
German Shepherd mix	3		3	3
Golden Retriever	8	3	11	11
Golden Retriever mix	1		1	1
Great Dane	1	16	17	17
Great Pyrenees	1		1	1
Greater Swiss Mountain Dog	6		6	6
Greyhound	1		1	1
Griffon	3		3	3
Grosspitz	1		1	1
Havanese	3		3	3
Heideterrier	1		1	1
Hovawart	1		1	1
Irish Soft Coated Wheaten Terrier	1	7	8	8
Irish Terrier	2		2	2

Breed	WGS	WES	WGS + WES	Animals
Irish Wolfhound	2		2	2
Italian Greyhound	2	1	3	3
Jack Russell Terrier	3		3	3
Japanese Chin	1		1	1
Kerry Blue Terrier	1		1	1
King Charles Spaniel		2	2	2
Kromfohländer	1		1	1
Kuvasz		4	4	4
Labrador Retriever	42	10	52	52
Labrador Retriever mix	1		1	1
Lagotto Romagnolo	9	4	13	11
Lancashire Heeler		2	2	1
Landseer	2	2	4	4
Leonberger	54	4	58	57
Miniature Bullterrier	1		1	1
Miniature Schnauzer	25	2	27	27
Mixed Breed	1		1	1
Newfoundland	2	4	6	6
Norfolk Terrier		3	3	3
Norwegian Elkhound, grey		2	2	2
Norwegian Lundehund		1	1	1
Norwich Terrier	8	1	9	9
Nova Scotia Duck Tolling Retriever	1		1	1
Old English Sheepdog	1		1	1
Papillon	3		3	3
Parson Russell Terrier		4	4	4
Pomeranian	4		4	4
Poodle	22		22	22
Portuguese Water Dog	3	2	5	5
Pug	20		20	20
Pyrenean Sheepdog		1	1	1
Rhodesian Ridgeback	4	2	6	6
Rottweiler	2	3	5	5
Russian Black Terrier		4	4	4
Saint Bernard Dog	2		2	2
Saluki	5	2	7	7
Samoyed		1	1	1
Schnauzer	1	5	6	6
Shetland Sheepdog	2	3	5	5
Shih Tzu	4		4	4
Siberian Husky	3	2	5	5
Sloughi	3		3	3

Breed	WGS	WES	WGS + WES	Animals
Spanish Waterdog	1		1	1
Stabyhoun	5		5	5
Staffordshire Bull Terrier	1		1	1
Swedish Vallhund	3	2	5	5
Tibetan Mastiff	10	3	13	13
Tibetan Spaniel	1		1	1
Vizsla	2		2	2
Weimaraner	2		2	2
Welsh Corgi	2		2	2
Welsh Springer Spaniel	3		3	3
West Highland White Terrier	19	2	21	20
Whippet	4	3	7	7
White Swiss Shepherd Dog	3	2	5	5
Wolf	8		8	8
Yorkshire Terrier	67		67	67
Total samples in initial filtering	658	243	901	890
Grand total	663	252	915	903

NGS = Next generation sequencing, WGS = Whole genome sequence, WES = Whole exome sequence

*The unaffected KBDs were excluded from initial filtering

Supplementary Table 3. Prioritized homozygous variants in the affected dog.

Chr:position	REF > ALT	Variant type	Gene	Gene information	Variant details (ref. sequence)
1:47568351	C > T	splicing SNV	SYNJ2	Synaptojanin 2: inositol polyphosphate 5-phosphatase, associated to hearing loss in mice.	c.3423+11C>T (XM_014115768.2)
1:115347272	A > G	nonsynonymous SNV	ZNF527	Zinc finger protein 527: unknown function.	c.430A>G; p.R144G (XM_014115577.2)
1:115788268	C > T	nonsynonymous SNV	LOC100687072	ZFP30 zinc finger protein: unknown function.	c.505G>A; p.G169R (XM_014115673.2, low quality ref.)
1:116699524	G > GTAGTAGGCT	nonframeshift insertion	LOC102155339	IgGFC-binding protein-like: unknown function.	c.4112_4113insAGCCTACTA; p.G1371insGAYY (XM_022406688.1)
1:117146140	G > T	splicing SNV	DMKN	Dermokine: epidermal differentiation, endosome trafficking, upregulated in inflammatory disease.	c.693+8G>T (XM_022403921.1)
1:117321334	G > A	nonsynonymous SNV	LSR	Lipolysis stimulated lipoprotein receptor: lipid metabolism, lipoprotein clearance.	c.1022C>T; p.P341L (XM_005616718.3, partial ref.)
2:77561953	A > C	nonsynonymous SNV	ALPL	Alkaline phosphatase, liver/bone/kidney: mutations cause hypophosphatasia in humans.	c.1301T>G; p.V434G (XM_005617214.3)
6:7824739	G > C	nonsynonymous SNV	CUX1	Cut like homeobox 1: transcription factor.	c.1602C>G; p.N534K (XM_005621000.3)
14:27216415	C > T	nonsynonymous SNV	VWDE	Von Willebrand factor D and EGF domains: unknown function.	c.4973G>A; p.G1658D (XM_022427414.1)
14:35556623	G > A	nonsynonymous SNV	DNAH11	Dynein axonemal heavy chain 11: mutations cause primary ciliary dyskinesia in humans.	c.5020G>A; p.G1674S (XM_022427467.1, low quality ref.)
15:63661871	G > A	nonsynonymous SNV	DDX60	DExD/H-box helicase 60: antiviral RNA helicase.	c.4840C>T; p.H1614Y (XM_532716.6)
18:49806934	T > G	nonsynonymous SNV	ALDH3B1	Aldehyde dehydrogenase 3 family member B1: aldehyde detoxification.	c.1094A>C; p.Y365S (XM_014121253.2)
18:55366074	G > T	nonsynonymous SNV	TMEM132A	Transmembrane protein 132A: unknown function.	c.1748C>A; p.P583H (XM_005631694.3)
21:32354052	C > A	nonsynonymous SNV	NRIP3	Nuclear receptor interacting protein 3: unknown function.	c.157G>T; p.G53C (XM_846198.4, low quality ref.)
25:50721076	C > T	nonsynonymous SNV	LOC102152569	Uncharacterised protein model: unknown function.	c.647C>T; p.P216L (XM_005635935.3)

REF = reference allele, ALT = alternative allele, SNV = single nucleotide variant

Supplementary Table 4. Pathogenicity prediction results.

Algorithm	Prediction	PredictSNP expected accuracy	Score	Patogenicity cutoff
PredictSNP consensus	Neutral	0,63	-	-
PredictSNP: MAPP	Neutral	0,65	-	-
PredictSNP: PhD-SNP	Deleterious	0,68	-	-
PredictSNP: PolyPhen-1	Neutral	0,67	-	-
PredictSNP: PolyPhen-2	Deleterious	0,40	-	-
PredictSNP: SIFT	Neutral	0,68	-	-
PredictSNP: SNAP	Neutral	0,58	-	-
PredictSNP: PANTHER	Deleterious	0,65	-	-
PROVEAN	Neutral	-	-2.446	<= -2.5
MutationTaster2*	Disease causing	-	0,999999992	1 is highest probability of accurate prediction

*The Mutation Taster2 algorithm was used with a human *ALPL* reference (NM_000478.5, NP_000469.3), and the other algorithms with the canine reference ((XM_005617214.3, XP_005617271.1)

Supplementary Video Legends

Supplementary Video 1. An affected puppy at 10 weeks of age (litter 1). The puppy is walking in a crouched position and showing both plantigrade and digitigrade gait (metatarsals and metacarpals on the ground).

Supplementary Video 2. An affected puppy at 2 weeks of age (litter 2). The affected puppy is having a seizure.