Genomic analysis of hoof and leg conformation in Swiss dairy cattle identified a *HYAL1*-related recessive form of polysynovitis

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Background

- Traditional dairy breeding focused mainly on production traits, negatively impacting functional traits.
- Body conformation traits reflect important functional characteristics.
  - Poor hoof and leg conformation increases the risk of hoof lesions and lameness, a major welfare and economic issue in dairy cattle.
- Lameness is common in CH:
  - 14.8% of Swiss cows show clinical lameness
  - >80% of farms have at least one affected animal.

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Aim

1. Estimate the additive heritability (h^2) of hoof and leg conformation traits in Swiss Holstein (HO) and Brown Swiss (BS) cattle populations
2. Identify associated genomic regions using additive and non-additive GWAS based on whole genome sequenced level imputed SNV
3. Detect functional candidate genes and candidate variants in associated genome regions
4. Clinically characterize animals carrying recessive candidate variants

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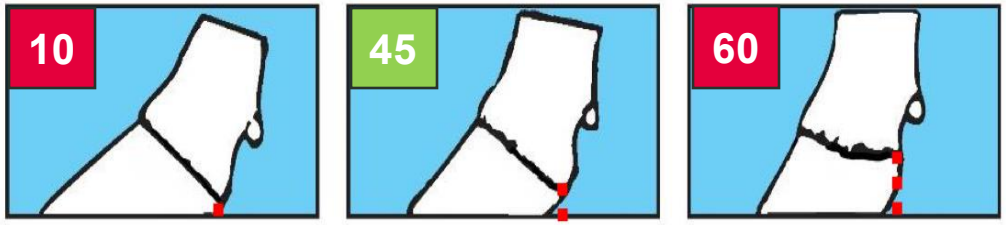
Hoof and leg conformation traits in HO

Heel depth (HDE)

millimeter: 10 to 60

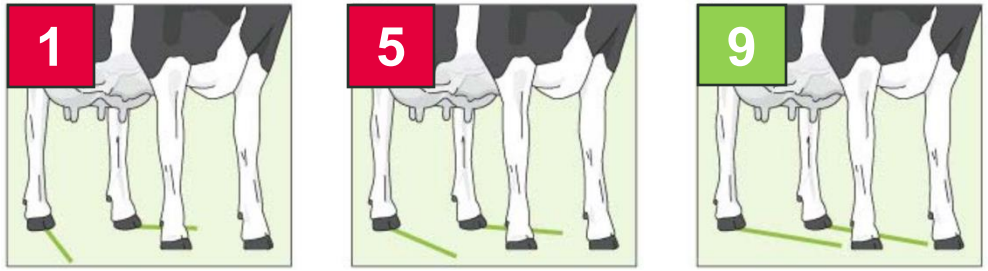


Foot angle (FAN)

score: 1 to 9

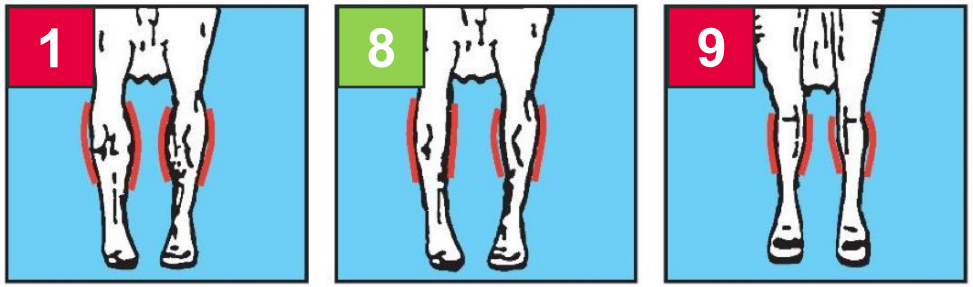
Locomotion (LOC)

score: 1 to 9



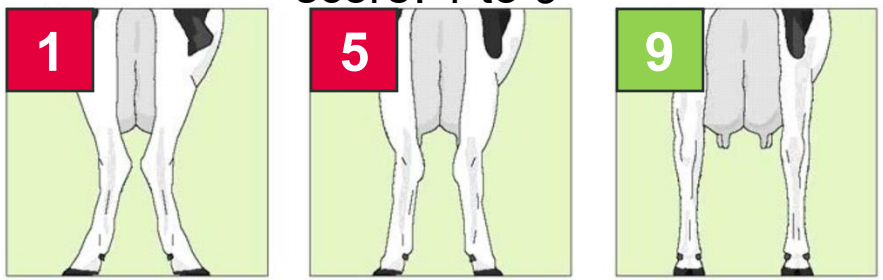
Bone structure (BST)

Score: 1 to 9



Rear leg rear view (RLR)

score: 1 to 9



Rear leg set (RLS)

score: 1 to 9

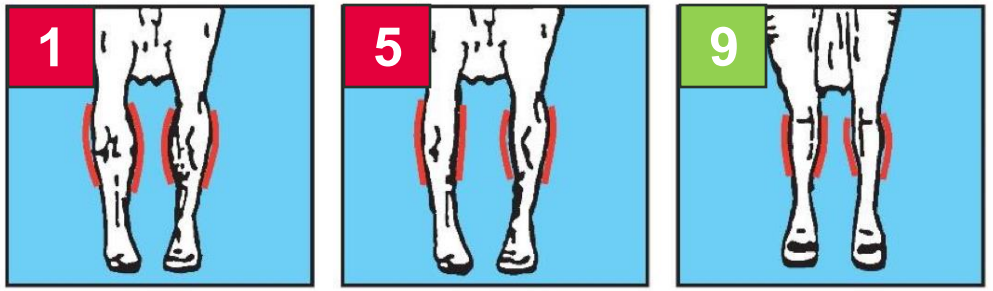
Hoof and leg conformation traits in BS

Rear leg side view (RLV)

Score: 1 to 9

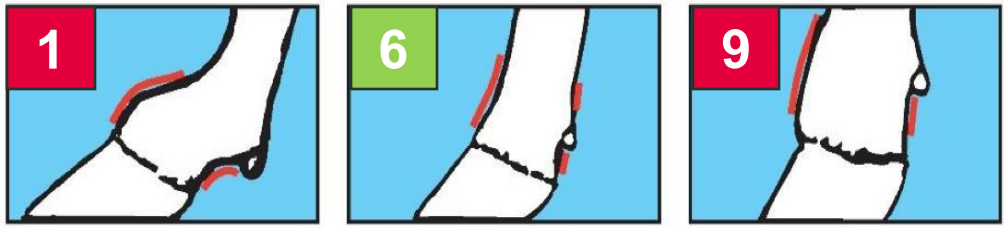
Hock quality (HQU)

Score: 1 to 9



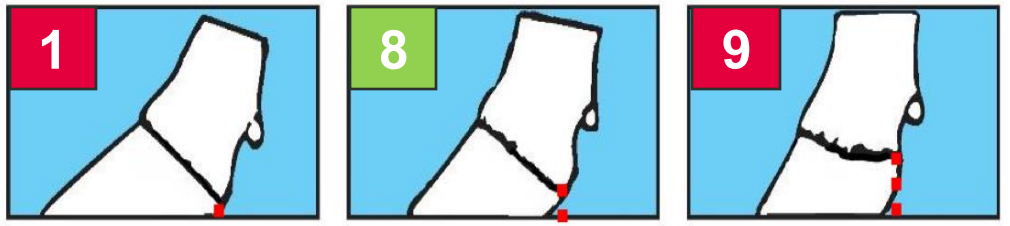
Foot angle (FAN)

Score: 1 to 9



Heel depth (HDE)

score: 1 to 9



Overview: Genotype and Phenotype

Number	Population	
	HO	BS
Animal genotyped	87,806	52,202
High density SNV panel	777,962	777,962
whole genome sequenced level imputed SNV panel	37,471,032	26,455,603
Records	1,141,329	1,132,160
Animal with genotype and phenotype	21,353	15,724

Estimated h^2 of hoof and leg conformation traits

Breed	Trait	Raw phenotype		Corrected phenotype		h^2 (SE)
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	
HO	BST	5.71 (1.26)	1–9	0.00 (1.14)	-4.82–3.84	**0.28** (0.01)
	HDE	35.58 (5.20)	10–60*	0.00 (4.76)	-25.06–25.57	**0.13** (0.008)
	FAN	5.46 (1.18)	1–9	0.00 (1.10)	-4.98–4.38	**0.14** (0.008)
	LOC	5.62 (1.26)	1–9	0.00 (1.14)	-4.88–3.95	**0.1** (0.007)
	RLR	5.73 (1.39)	1–9	0.00 (1.25)	-5.05–3.93	**0.21** (0.009)
	RLS	5.12 (1.12)	1–9	0.00 (1.06)	-4.50–3.82	**0.24** (0.009)
BS	HDE	5.40 (1.08)	1–9	0.00 (0.95)	-4.18–3.97	**0.1** (0.009)
	FAN	5.34 (1.20)	1–9	0.00 (1.10)	-5.29–4.11	**0.14** (0.01)
	HQU	5.40 (1.87)	1–9	0.00 (1.71)	-5.31–5.57	**0.26** (0.01)
	RLV	4.86 (1.12)	1–9	0.00 (1.04)	-4.15–4.61	**0.21** (0.01)

SD = standard deviation, Min = minimum, Max = maximum, h^2 = additive heritability, SE = standard error
BST = bone structure, DHE = heel depth, FAN = foot angle, LOC = locomotion, RLR = rear leg rear view, RLS = rear leg set, HQU = hock quality, RLV = rear leg side view
HO = Holstein, BS = Brown Swiss
*measured in mm

45 associated suggestive genomic regions

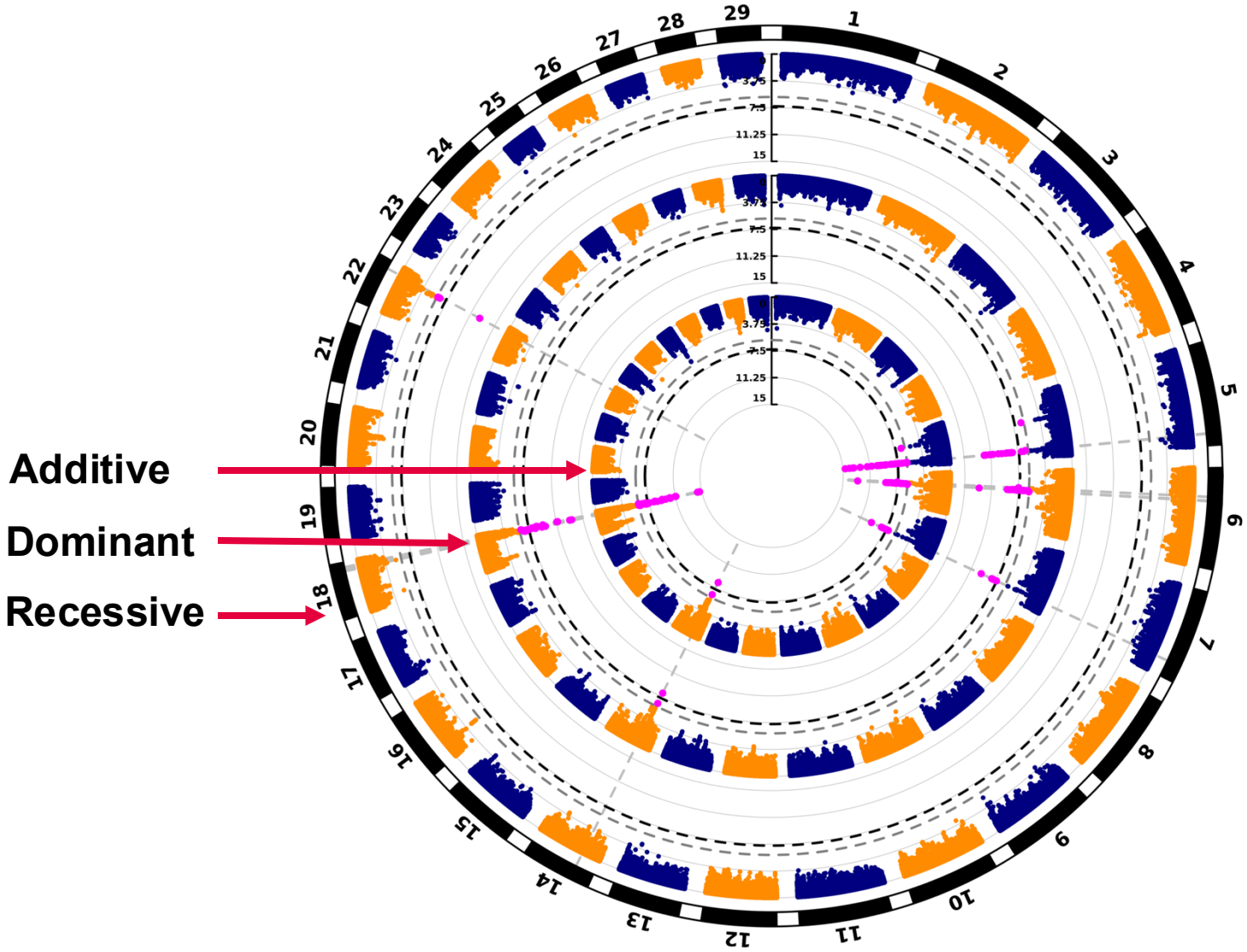
- GWAS analyses with a **subset of ~700k markers using GCTA**

Threshold:
$P \leq 1x10^{-6}$

Breed	Trait	Additive	Non-additive	
			Dominant	Recessive
HO	BST	5	5	1
	FAN	1	2	1
	HDE	1	1	0
	LOC	3	1	1
	RLR	0	0	1
	RLS	2	1	1
BS	HDE	3	2	1
	FAN	0	0	0
	HQU	5	3	0
	RLV	2	1	1
Total		**22**	**16**	**7**

Example: GWAS results for BST in HO

- Analyses with a **subset of ~700k markers using GCTA**



25 significant associated genomic regions

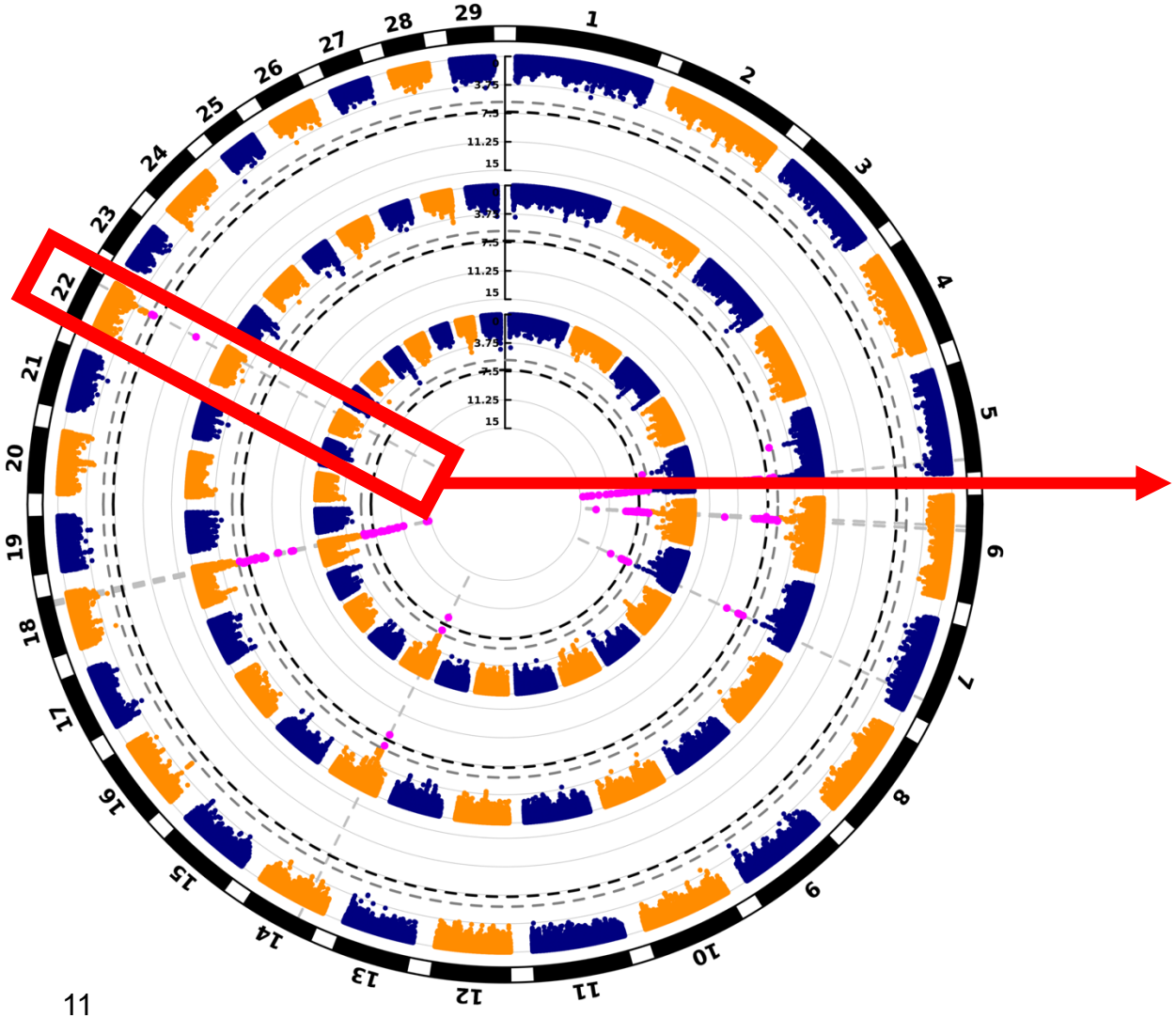
- Whole genome sequencing SNV imputed panel **using GCTA**

Threshold:
$P \leq 5x10^{-8}$

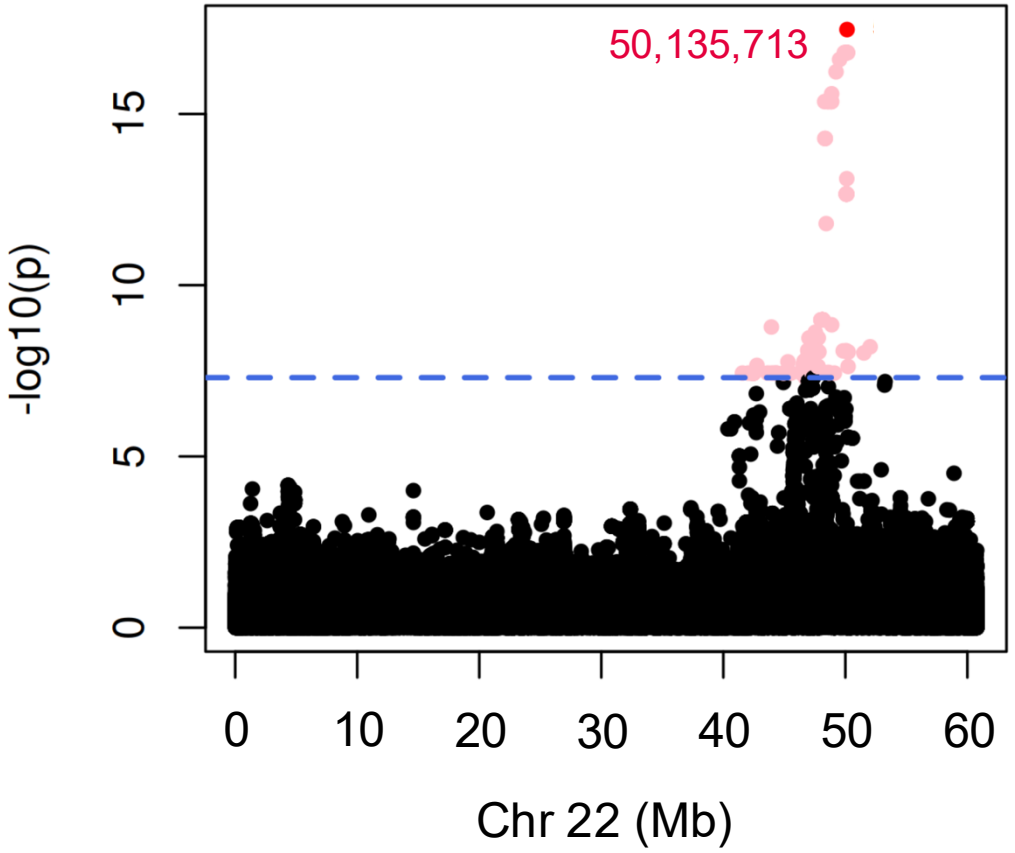
Breed	Trait	Additive	Non-additive	
			Dominant	Recessive
HO	BST	5	5	1
	FAN	0	0	0
	HDE	0	0	0
	LOC	2	1	1
	RLR	0	0	0
	RLS	0	0	0
BS	HDE	2	2	0
	FAN	0	0	0
	HQU	4	2	0
	RLV	0	0	0
Total		**13**	**10**	**2**

Example: GWAS results for BST in HO

GWAS with HD panel



GWAS with all imputed SNVs for the Chromosome 22



15 unique significant QTL

- Whole genome sequencing SNV imputed panel **using GCTA**

Breed	Trait	Additive	Non-additive	
			Dominant	Recessive
HO	BST	5	0	1
	FAN	0	0	0
	HDE	0	0	0
	LOC	2	0	0
	RLR	0	0	0
	RLS	0	0	0
BS	HDE	2	0	0
	FAN	0	0	0
	HQU	4	1	0
	RLV	0	0	0
Total		**13**	**1**	**1**

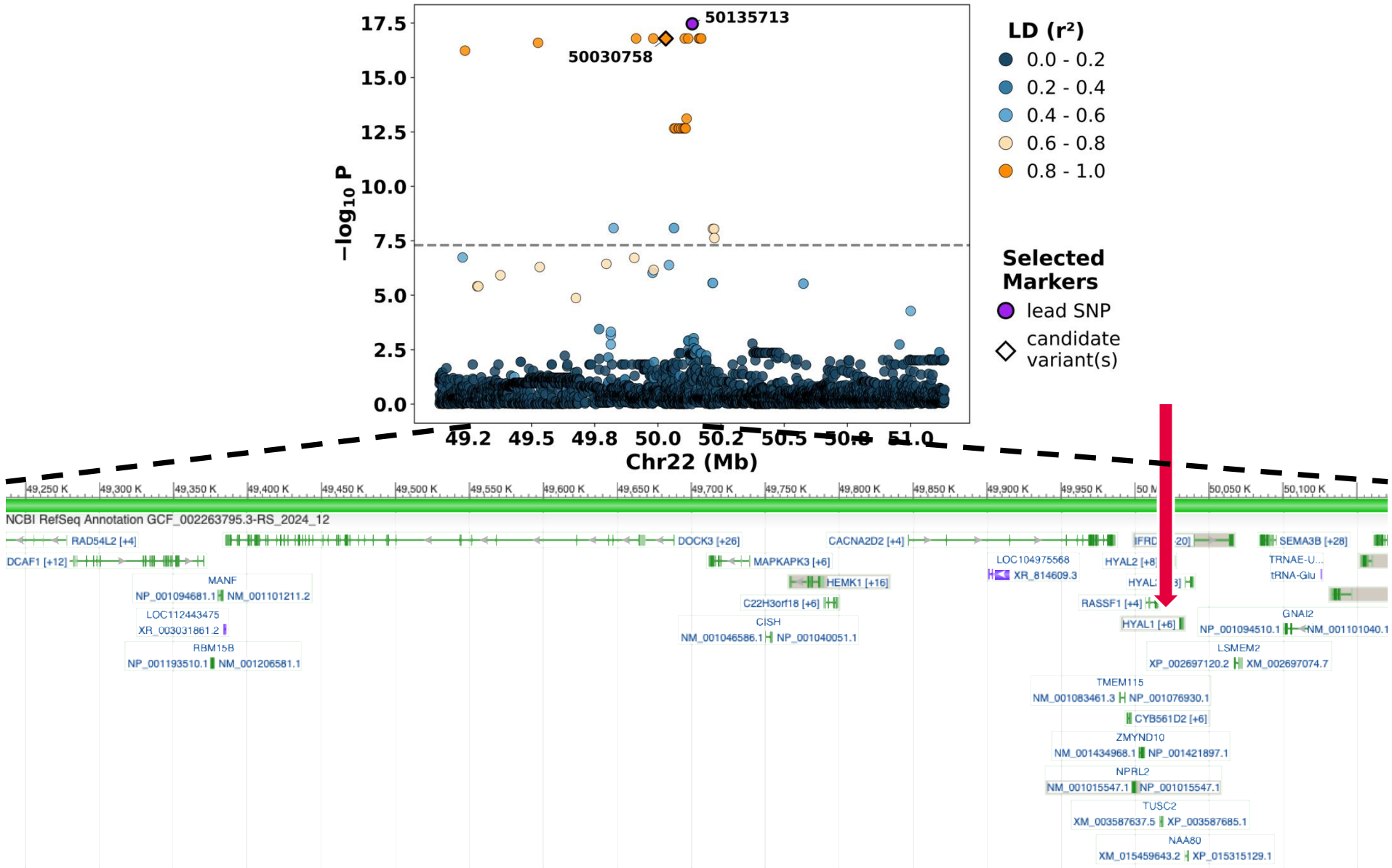
Positional candidate genes for the 15 QTL

Breed	Trait	Model	Chr	QTL interval	Candidate gene
HO	BST	Additive	5	104,547,532-106,547,170	*NTF3, KCNA1, NDUFA9, FGF6, FGF23, CCND2*
			6	36,048,590-38,048,179	*PIGY, PKD2, SPP1, MEPE, IBSP*
			7	89,812,839-91,811,827	*ADGRV1*
			14	23,325,792-25,325,229	*PLAG1, BPNT2*
			18	56,574,893-58,574,848	*POLD1, ETFB, MIR99B, MIR125A, PPP2R1A*
		Recessive	22	49,135,960-51,134,158	*HYAL1, TRAIP, GMPPB, DAG1, AMT, RHOA, QARS1, MIR191, NDUFAF3, SLC25A20*
	LOC	Additive	5	107,761,169-109,760,788	*CACNA1C, IL17RA, ADA2, ATP6V1E1, PEX26, TUBA8, SOX10*
			11	43,806,510-45,806,350	*EDAR, RANBP2, SLC5A7*
BS	HDE	Additive	13	60,329,758-62,328,857	*CSNK2A1, RBCK1, ID1, BCL2L1, POFUT1, ASXL1, DNMT3B*
			25	484,002-2,483,588	*PIGQ, STUB1, GNPTG, CLCN7, TELO2, IFT140, MRPS34, IGFALS, GFER, TSC2, PKD1, ABCA3, CCNF, TBC1D24, KREMEN2*
	HQU	Additive	3	117,733,467-119,733,028	*TWIST2, HDAC4, NDUFA10*
			18	34,316,206-36,314,919	*CBFB, MIR328, CTCF, ACD, LCAT, PRMT7, CDH3, CDH1*
			25	226,786-2,226,229	*AXIN1, PIGQ, STUB1, GNPTG, CLCN7, TELO2, IFT140, MRPS34, IGFALS, GFER, TSC2, PKD1, ABCA3, CCNF, TBC1D24*
			26	22,249,367-24,248,244	*BTRC, POLL, DPCD, FBXW4, FGF8, GBF1, NFKB2, MIR146B, SUFU, TRIM8, CYP17A1, CNNM2, NT5C2*
		Dominant	6	87,208,448-89,208,308	*ADAMTS3, ALB, CXCL8, PPBP, PF4*

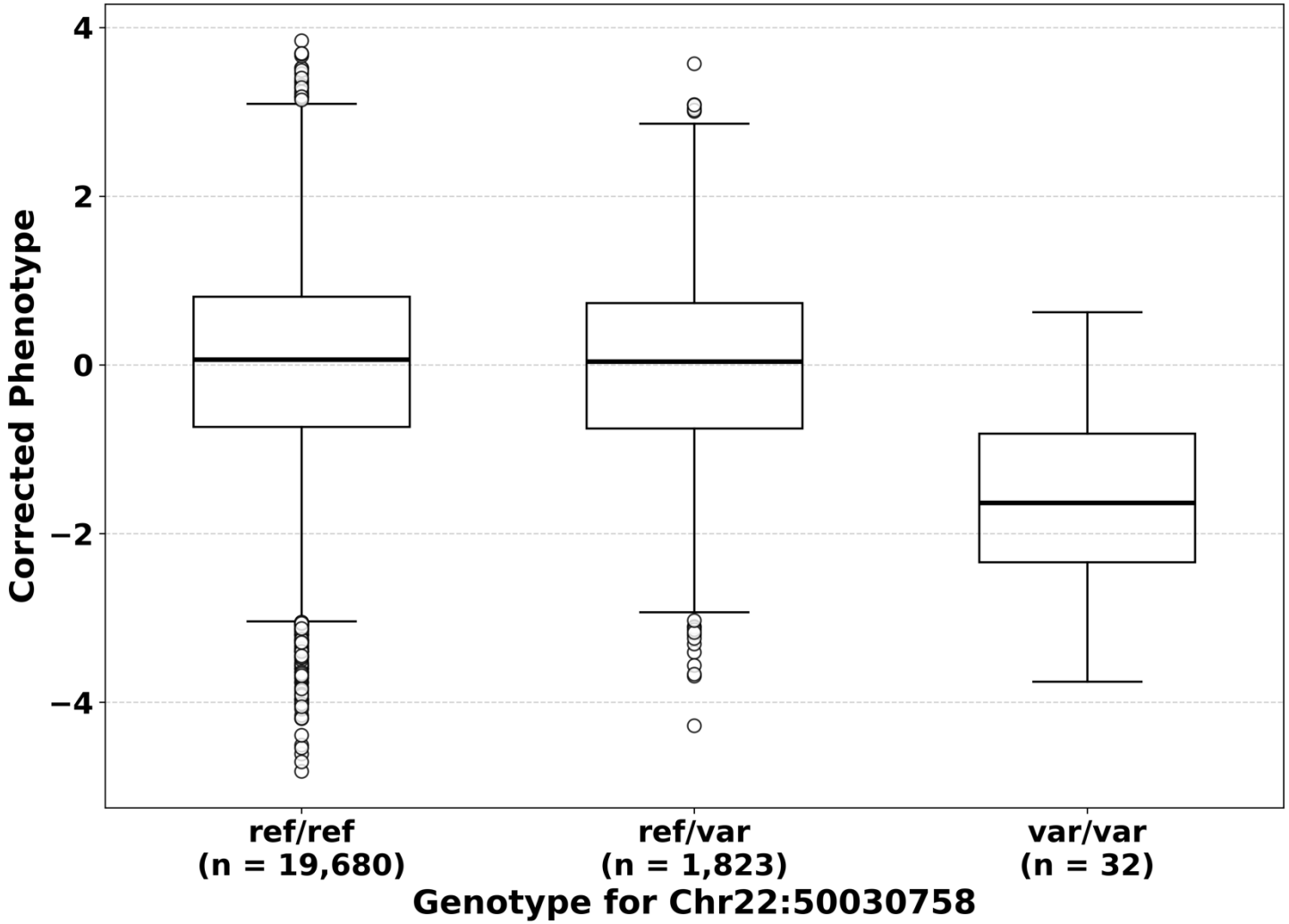
17 candidate variants

Breed	Trait	Model	Chr	Position (bp)	MAF	R^2	Effect size	SE	p-value	Gene	Variant type
HO	BST	Recessive	22	50,030,758	0.043	0.991	-1.594	0.187	1.62×10^{-17}	*HYAL1*	Stop gained
	LOC	Additive	5	108,760,988	0.31	1	0.089	0.015	5.42×10^{-9}	*CACNA1C*	Intronic
			11	44,828,627	0.343	0.944	0.096	0.012	7.31×10^{-15}	*GCC2*	Missense
BS	HDE	Additive	13	61,320,050	0.117	0.995	-0.115	0.020	1.99×10^{-8}	*TPX2*	Synonymous
			25	1,171,241	0.453	0.951	-0.091	0.015	1.55×10^{-9}	*TELO2*	Upstream
				1,340,769	0.45	0.908	-0.097	0.015	1.55×10^{-10}	*MAPK8IP3*	Intronic
				1,690,471	0.464	0.959	-0.098	0.015	1.15×10^{-10}	*TRAF7*	Upstream
				1,836,217	0.466	0.945	-0.096	0.015	2.04×10^{-10}	*ABCA3*	Upstream
	HQU	Additive	3	118,271,945	0.348	0.919	-0.171	0.033	3.12×10^{-7}	*HDAC4*	Intronic
				118,888,292	0.348	0.975	-0.181	0.033	5.38×10^{-8}	*NDUFA10*	Intronic
			18	35,124,569	0.456	0.794	0.175	0.032	6.7×10^{-8}	*RIPOR1*	Frameshift
			25	1,148,147	0.295	0.978	0.174	0.033	9.85×10^{-8}	*CLCN7*	Synonymous
			26	22,262,227	0.272	0.845	-0.191	0.035	5.76×10^{-8}	*BTRC*	Intronic
				22,433,281	0.273	0.851	-0.194	0.035	3.81×10^{-8}	*FBXW4*	Intronic
				22,503,920	0.273	0.852	-0.2	0.035	1.44×10^{-8}	*FGF8*	Missense
				23,248,278	0.303	1	-0.2	0.034	3.24×10^{-9}	*SUFU*	Downstream
		Dominant	6	88,208,422	0.154	1	-0.297	0.045	5.42×10^{-11}	*ANKRD17*	Intronic

Example: non-additive QTL for BST in HO



Recessive *HYAL1* variant for BST in HO



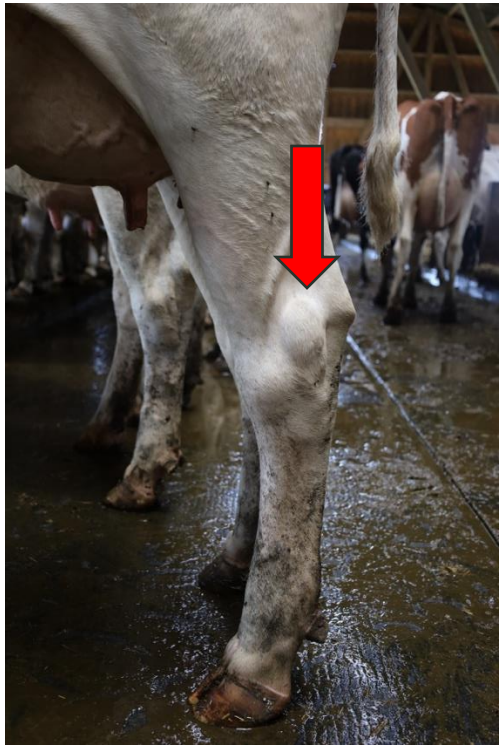
u^b *HYAL1* as functional candidate gene

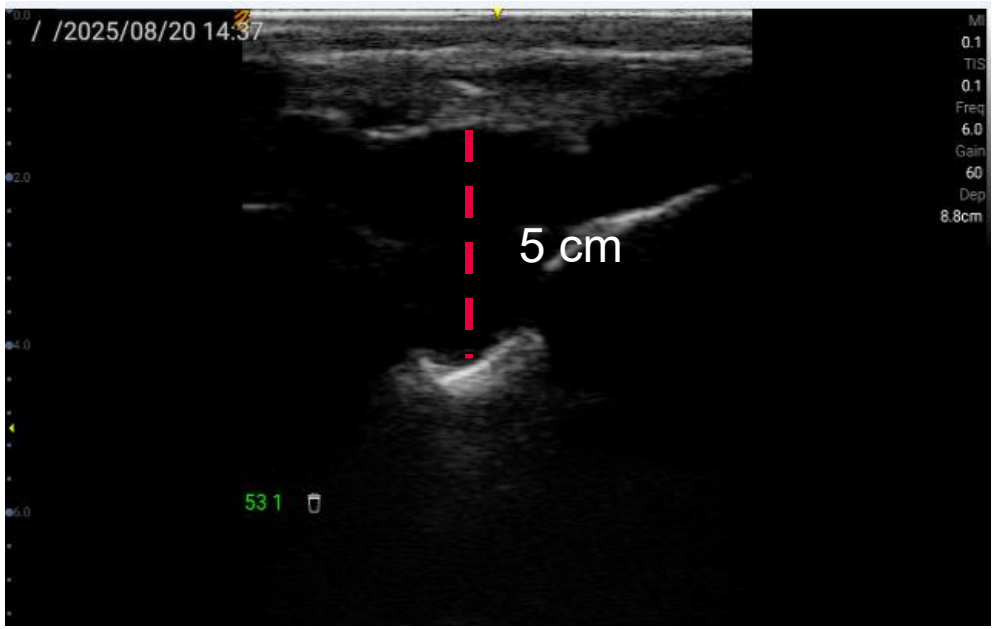
- Function
  - Produces hyaluronidase-1, an **enzyme that breaks down hyaluronic acid**
- Effect
  - Mucopolysaccharidosis Type IX in humans
    - soft tissue masses, joint swelling, cartilage abnormalities, and reduced bone quality
- Predict effect on cattle
  - A stop-gained variant may disrupt hyaluronic acid breakdown, leading to weaker cartilage, altered hoof/bone structure and accumulation of hyaluronic acid

Imundo et al. (2011)

Polysynovitis in *HYAL1* homozygous HO cattle

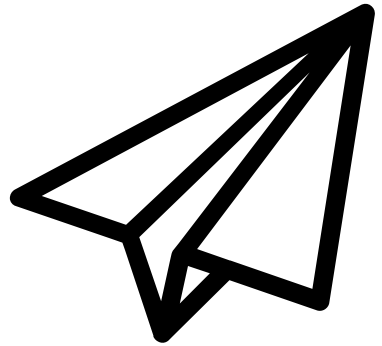
- **On-farm Examination** of 7 HO females
  - Three heifers 7 mo. old
  - Four cows 3.5 y. old
- **Clinical findings**
  - hock enlargement
  - Joints soft, non-warm, and non-painful
  - Lameness in one cow (score 3/5)
- **Ultrasonography**
  - abundant anechogenic content in affected joints
- **Clinical Diagnosis**
  - **Polysynovitis**
    (mostly in hindlimbs and 1 cow in the forelimbs)





Take home message

- First high-resolution study of the genetic architecture of hoof and leg conformation traits in Swiss dairy cattle.
- Traits show a polygenic background, driven by many small-effect loci.
- Non-additive GWAS models reveal important large-effect recessive variants (e.g., *HYAL1*).
  - Monitoring deleterious alleles in breeding populations.



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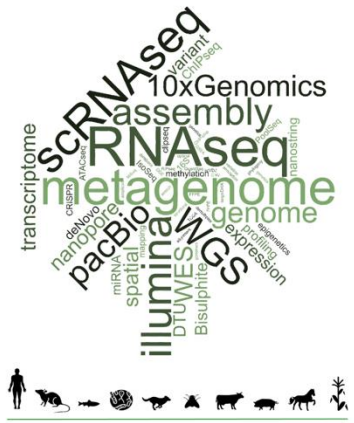
Thank you !



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