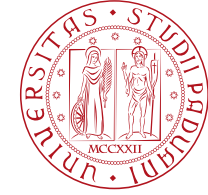




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University of Natural Resources
and Life Sciences, Vienna



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DI PADOVA

On the genomic regions affecting milk lactose content in dairy cattle

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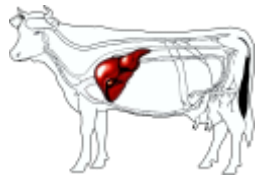
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70th Annual Meeting of the
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City of Ghent (Belgium), 26 - 30 Aug 2019

Lactose is the most abundant **solid** in bovine milk \approx 4.80-4.90%



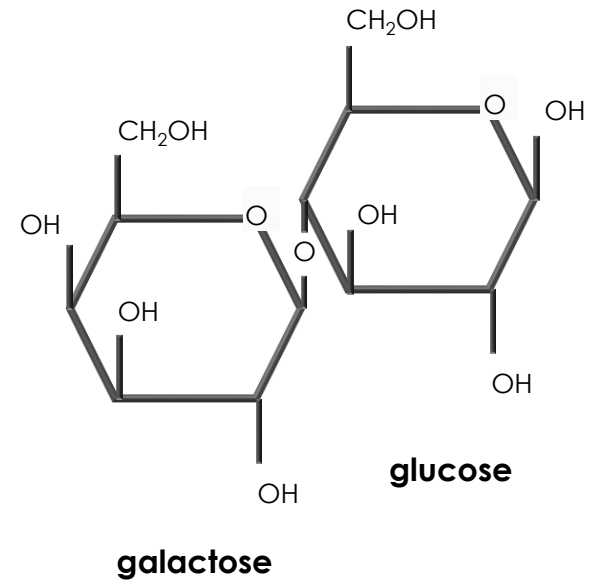
proprionate \rightarrow glucose



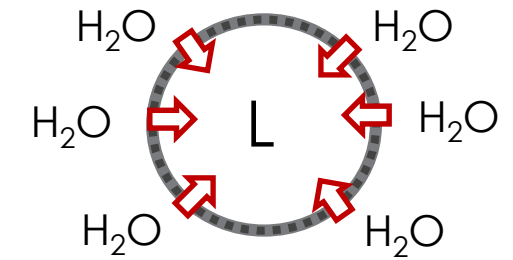
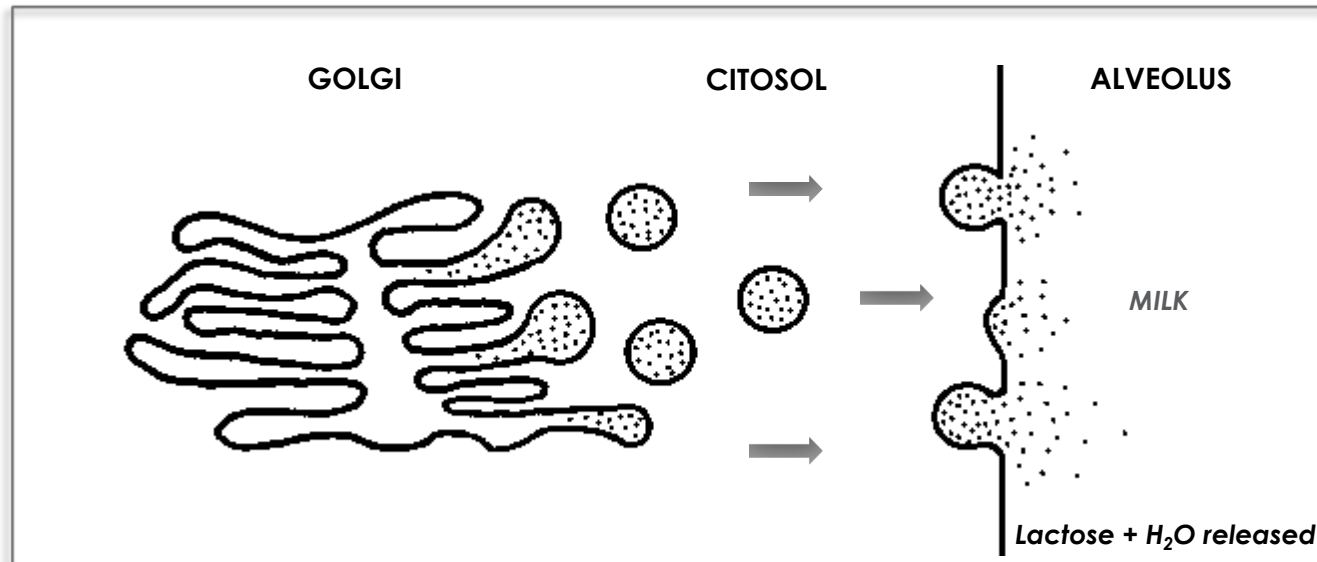
glucose \rightarrow galactose

galactose + glucose = **lactose**

LACTOSE SYNTHASE



Lactose (disaccharide) is packed in secretory vesicles for transport → CAN NOT pass the membrane



- ↑ **[L]** in secretory vesicles
- ↑ **osmotic pressure**
- ↑ **call of H₂O**

Lactose acts as osmole and thus directly determines milk volume.

Milk lactose content (**L%**):

- Routinely **recorded** worldwide in milk (predicted by IR spectroscopy)
- Medium to high **heritability** in dairy cattle (often > 0.40)
- Negative genetic/phenotypic **correlation with SCS** and **mastitis** (related to epithelial integrity)
- Quite constant (low variability in physiological conditions = healthy cows)

- $CV_p < 5\%$
- $CV_g < 3\%$

Published so far...

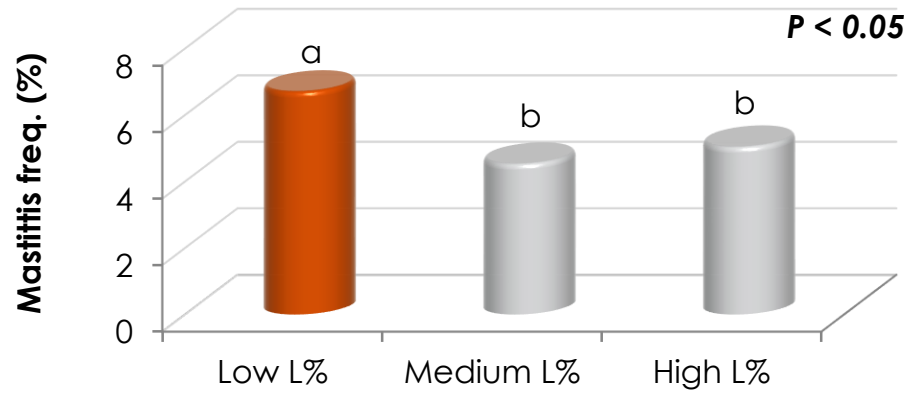
Reference	Test days, no.	Breed	Model	Heritability	Repeatability
Miglior et al. (2007)	60,645	Holstein	Random regression	0.50	—
Stoop et al. (2007) ¹	5,581	Holstein	Linear	0.64 (0.10)	0.72 (0.01)
Loker et al. (2012)	86,331	Holstein	Random regression	0.52 (0.03)	—
Ptak et al. (2012)	48,859	Holstein	Random regression	0.24 (0.03)	—
Tiezzi et al. (2013)	63,470	Holstein	Linear	0.33	0.56
Ederer et al. (2014)	97,146 ²	Fleckvieh	Linear	0.32 (0.01)	—
Sneddon et al. (2015)	15,366	Mix	Linear	0.25 (0.04)	0.60 (0.01)
Belay et al. (2017)	717,915	Norwegian Red	Linear	0.43 (0.06)	—
Haile-Mariam and Pryce (2017)	724,325	Mix	Random regression	0.34	—
Satola et al. (2017)	104,875	Holstein	Random regression	0.30	—
Visentin et al. (2017)	128,510	Mix	Random regression	0.36 (0.02)	0.49 (0.01)
Costa et al. (2018)	59,811	Holstein	Linear	0.43 (0.03)	0.63 (0.01)
Costa et al. (2019)	142,285 ³	Fleckvieh	Linear	0.57 (0.01)	0.62 (0.01)

Reference	Test days, no.	Cows, no.	Breed	SCC/SCS	
				Phenotypic correlation	Genetic correlation
Stoop et al. (2007)	5,581	1,953	Holstein	-0.24 (0.02)	-0.44 (0.21)
Miglior et al. (2007)	60,645	5,022	Holstein	-0.23	-0.20
Gillon et al. (2010)	590,083	113,905	Mix	-0.38	-0.35
Hosseini-Zadeh and Ardalan (2011)	458,408	57,301	Holstein	-0.15	-0.19
Sneddon et al. (2015)	15,366	4,378	Mix	-0.19 (0.01)	-0.07 (0.14)
Vilas Boas et al. (2017)	680	268	Gyr	-0.66	—
Visentin et al. (2017)	128,510	9,824	Mix	—	-0.28
Costa et al. (2018)	59,811	4,355	Holstein	-0.25 (0.01)	-0.22 (0.08)

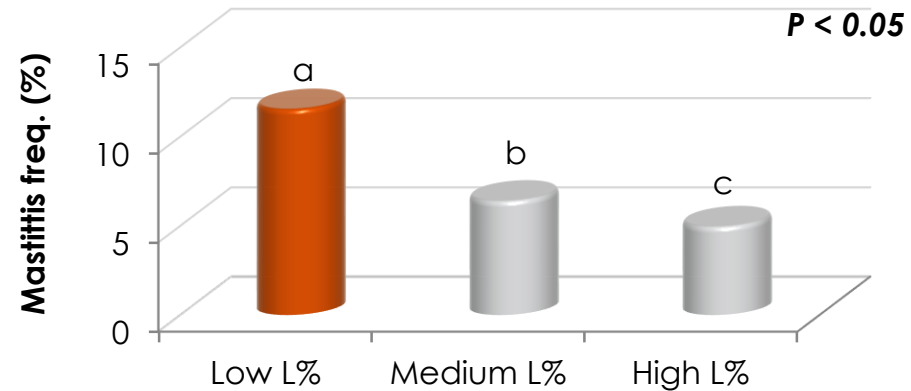
L% - relationships with mastitis

r_g	DIM	Source
-0.18	-10 to 150	Costa et al., 2019 (https://doi.org/10.3168/jds.2018-15883)
-0.24	5 to 65	Bastin et al., 2016 (http://dx.doi.org/10.3168/jds.2)
-0.10	5 to 305	

Lactation 1



Lactation ≥ 2



Class¹ of L%

LS-means of SCS

Lactation 1

Low L%

2.454^a (1.564)

Medium L%

1.512^b (1.261)

High L%

1.014^c (1.130)

Lactation ≥ 2

Low L%

3.538^a (1.764)

Medium L%

1.848^b (1.510)

High L%

1.084^c (1.269)

¹Classes of L% defined according to mean ± 2 SD

(Costa et al., 2019, <https://doi.org/10.3168/jds.2018-15883>)

Background → genetic relationship L% - mastitis

Idea → L% may be potential indicator of udder health



AIMS

- to identify significant genes for L%
- to detect overlapping regions with udder health traits

in Fleckvieh (Austrian dual-purpose Simmental) cattle breed

1. Input (> 7,000 bulls)

 Pseudo-phenotypes → de-regressed EBV of Fleckvieh bulls for L% (lactation 1) (MiX99; Lidauer et al., 2015)

 Genotypes → 54K Illumina BovineSNP50 BeadChip

2. Quality control (PLINK 1.9; Purcell et al., 2007, Chang et al., 2015)

SNP criteria:

- call rate ≥ 0.90
- MAF ≥ 0.01
- H-W equilibrium

40,486 SNP

Bulls criteria:

- EDC ≥ 10
- call rate ≥ 0.90

2,854 bulls

3. GWAS with linear approach (*GEMMA; Zhou and Stephens, 2012*)

$$\mathbf{y} = \mu + \mathbf{x}\beta + \mathbf{u} + \boldsymbol{\varepsilon}$$

\mathbf{y} = vector of phenotypes (de-regressed EBV of LP or LY)

μ = intercept

\mathbf{x} = vector of marker genotypes

β = effect size of the markers

\mathbf{u} = vector of random individual effects

$\boldsymbol{\varepsilon}$ = vector of errors

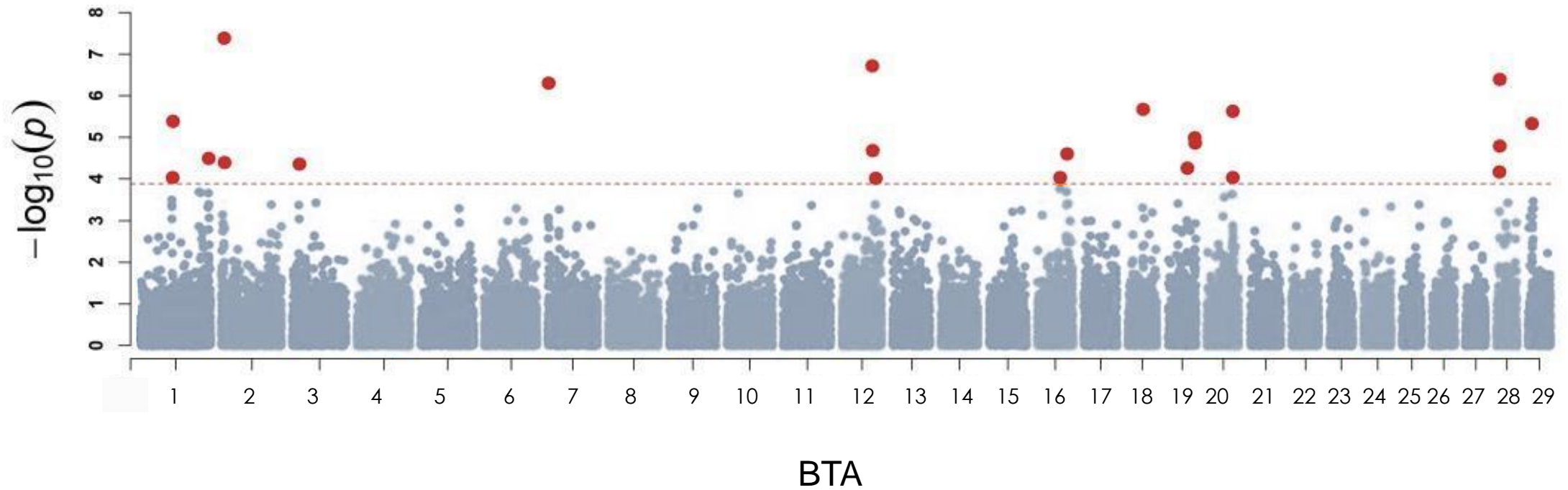
G-matrix added to correct for population structure

FDR cut-off at 0.20 (*Efron, 2007*) $\rightarrow P < 0.00013$

4. % genetic variance explained by SNP (*GCTA; Yang et al., 2011*)

5. Mapping with ARS-UCD1.2 assembly

22 significant signals



BTA	Mb	SNP	P-value
1	71.263	ARS-BFGL-NGS-5124	9.29 E-05
1	72.737	Hapmap42521-BTA-35582	4.12 E-06
1	154.087	ARS-BFGL-NGS-95240	3.26 E-05
2	5.757	ARS-BFGL-NGS-39978	4.13 E-08
2	6.701	Hapmap49624-BTA-47893	4.09 E-05
3	15.526	ARS-BFGL-NGS-64215	4.37 E-05
7	1.009	ARS-BFGL-NGS-110962	5.05 E-07
12	69.320	BTA-123122-no-rs	1.92 E-07
12	70.141	Hapmap50646-BTA-29027	2.09 E-05
12	77.316	ARS-BFGL-NGS-57541	9.68 E-05
16	51.811	ARS-BFGL-NGS-74373	9.34 E-05
16	67.704	BTA-26576-no-rs	2.50 E-05
18	34.127	ARS-BFGL-NGS-119782	2.14 E-06
19	44.547	ARS-BFGL-NGS-19774	5.58 E-05
19	61.017	Hapmap25852-BTA-148919	1.03 E-05
19	61.441	ARS-BFGL-NGS-55564	1.37 E-05
20	58.241	BTB-01648514	2.37 E-06
20	58.265	BTB-01648552	9.26 E-05
28	6.547	BTB-00874839	6.83 E-05
28	6.575	BTB-00874898	4.08 E-07
28	6.888	ARS-BFGL-NGS-40170	1.62 E-05
29	9.320	Hapmap32898-BTA-66437	4.73 E-06

**Genetic variance explained =
4.90%**

BTA	Mb	SNP	P-value
1	71.263	ARS-BFGL-NGS-5124	9.29 E-05
1	72.737	Hapmap42521-BTA-35582	4.12 E-06
1	154.087	ARS-BFGL-NGS-95240	3.26 E-05
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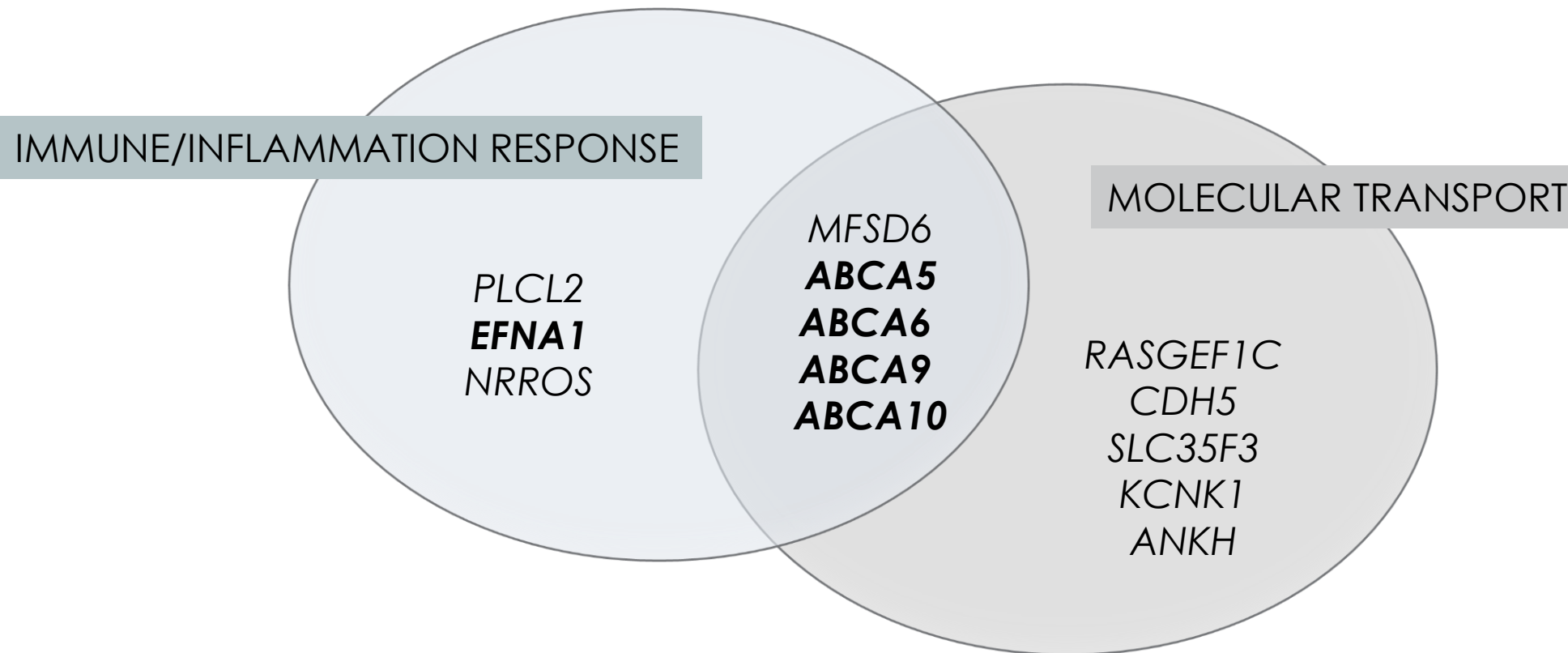
Regions detected in other GWAS for L%

- Holstein + Jersey in NZ (*Lopdell et al., 2017*)
- Holstein in NL (*Wang and Bovenhuis, 2018*)

Significant genes for mastitis/SCS in *Bos Taurus*

BTA	SNP location (Mb)	Gene	Nearby genes (± 0.1 Mb from SNP)
1	71.263	PAK2	NRROS, CEP19, LOC112447342, PIGX, SENP5, LOC112447342, LOC104970886
1	72.737	PLCL2	TBC1D5
1	154.087	-	LOC104970891
2	5.757	-	NEMP2, NAB1, MFSD6, LOC104971101
2	6.701	-	ANKAR, OSGELP1, ASNSD1, SLC40A1
3	15.526	-	EFNA1, EFNA3, EFNA4 , ADAM15, DCST1, DCST2, ZBTB7B, LENEPI, FLAD1, CKS1B, SHC1, LOC107132270, MIR92B, MUC1, TRIM46, KRTCAP2, DPM3, SLC50A1
7	1.009	RASGEF1C	MAPK9, GFPT2, LOC107132588, LOC100848388
12	69.320	LOC112449084	TMTC4, GGACT, MIR2892
12	70.141	-	-
12	77.316	LOC784305	-
16	51.811	LOC101904639	SPEN, LOC507787, LOC789035, FBLIM1, TMEM82, SLC25A34, LOC112441770, PLEKHM2, LOC515551, LOC112441934
16	67.704	-	PTGS2, LOC107133257, LOC112441859
18	34.127	CDH5	BEAN1
19	44.547	GJC1	DBF4B, HIGD1B, EFTUD2, MIR2343, CCDC103, FAM187A, GFAP, KIF18B, LOC104975097
19	61.017	ABCA6	ABCA5, ABCA9, ABCA10
19	61.441	LOC112442737	-
20	58.241	-	ANKH , LOC107131578, LOC112443072
20	58.265	-	ANKH , LOC107131578, LOC112443072
28	6.547	SLC35F3	-
28	6.575	-	KCNK1 , TRNAC-ACA
28	6.888	-	KCNK1 , TRNAC-ACA
29	9.320	-	ACTN2, HEATR1, LOC112444786, LOC101908221

(Meredith et al., 2013; Chen et al., 2015; Tiezzi et al., 2015; Kang et al., 2018; Wathes et al., 2019)



EFNA1 → maintenance of epithelial cells integrity in mammary gland

ABCA family → transmembrane transport of small molecules, lipid and glucose homeostasis, insulin-like growth factor-1 regulation, affecting neutrophils

Take home messages

L% is affected by **several regions** spread across the genome, with functions related to:

- immune/inflammation response
- molecular transport regulation

Some signals were within or nearby genes significant for mastitis/SCS, others were in regions with unclear/unknown functions.

→ Thus, an association with udder health at genomic level exists.



Perspectives

Define and investigate L% derived traits (potentially informative, able to catch L% variability)

Exploit new indicators of mastitis resistance/udder health/longevity

Thank you for the attention,
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On the genomic regions associated with milk lactose in Fleckvieh cattle

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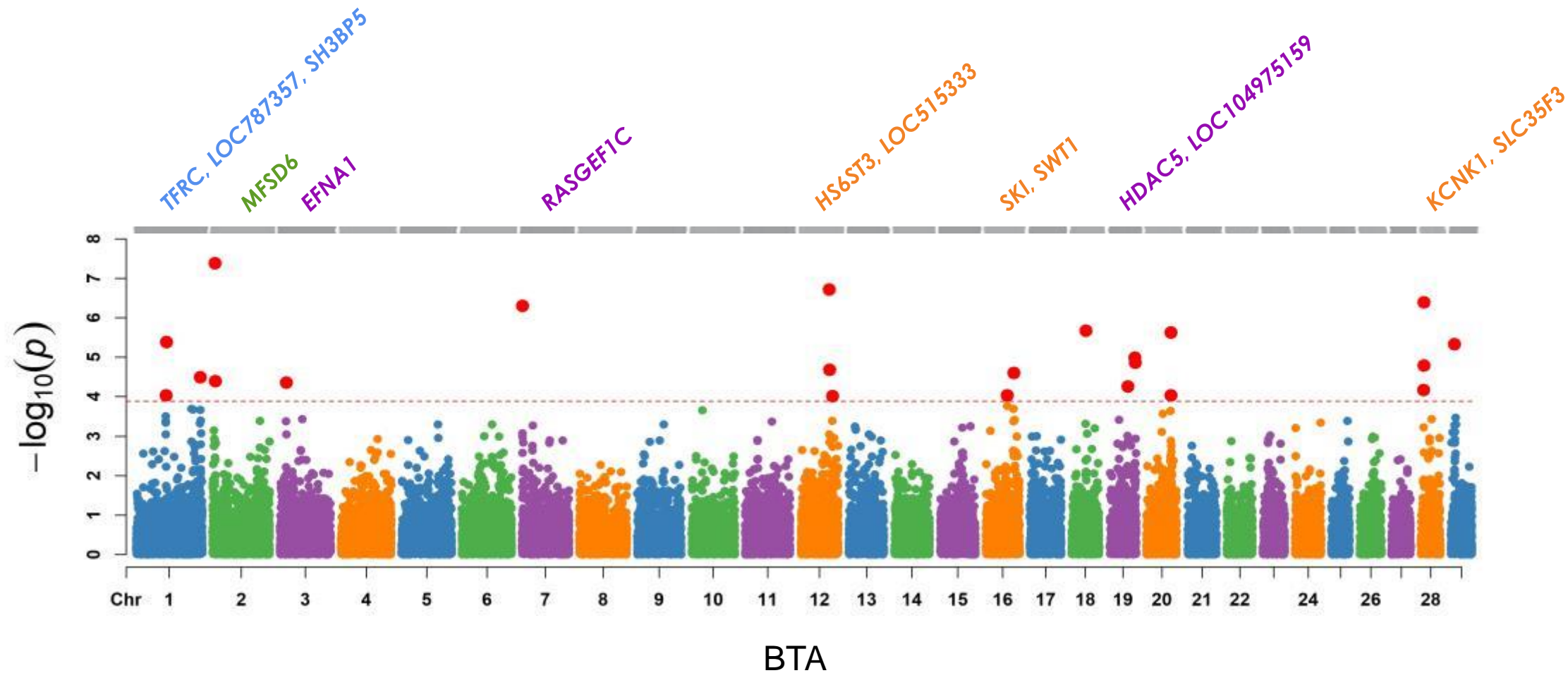
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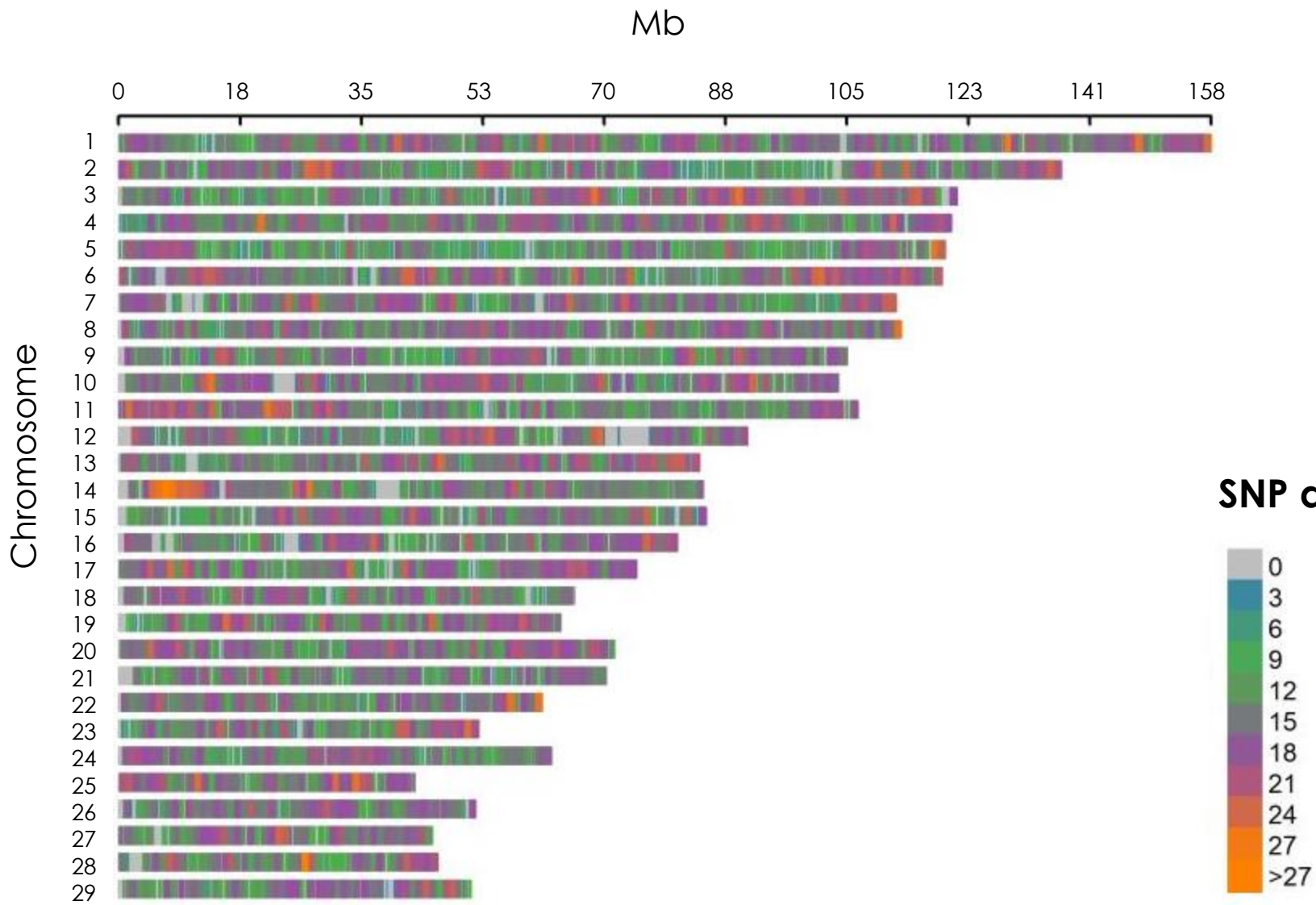
Founded by University of Padova, Italy (Ricerca Scientifica fondi DOR – 2017, project **DOR1721792/17**), in collaboration with **COMET-Project D4Dairy** supported by Austrian Ministry for Transport, Innovation and Technology (BMVIT), the Federal Ministry for Digital and Economic Affairs (BMDW) of Austria, and the provinces of Lower Austria and Vienna.

Genes identified with assembly **UMD_3.1.1**

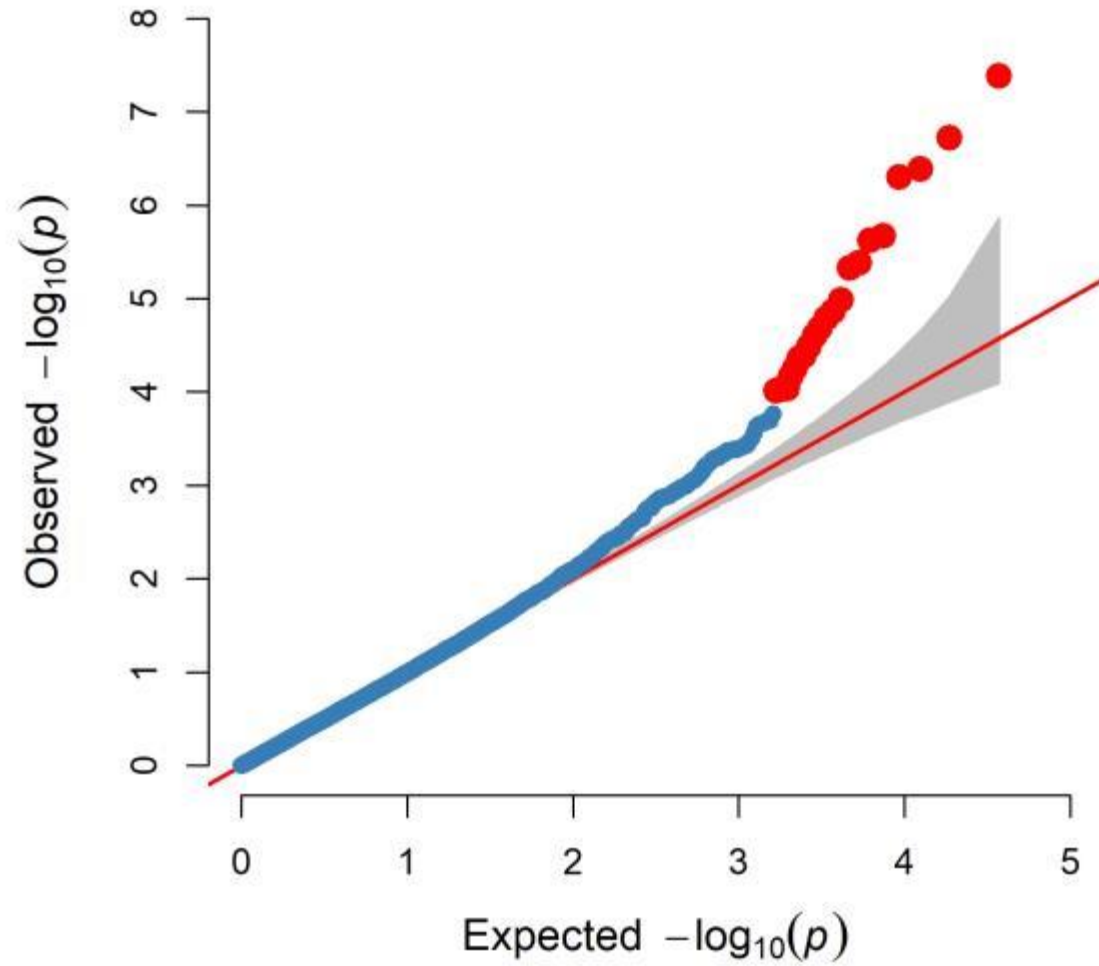


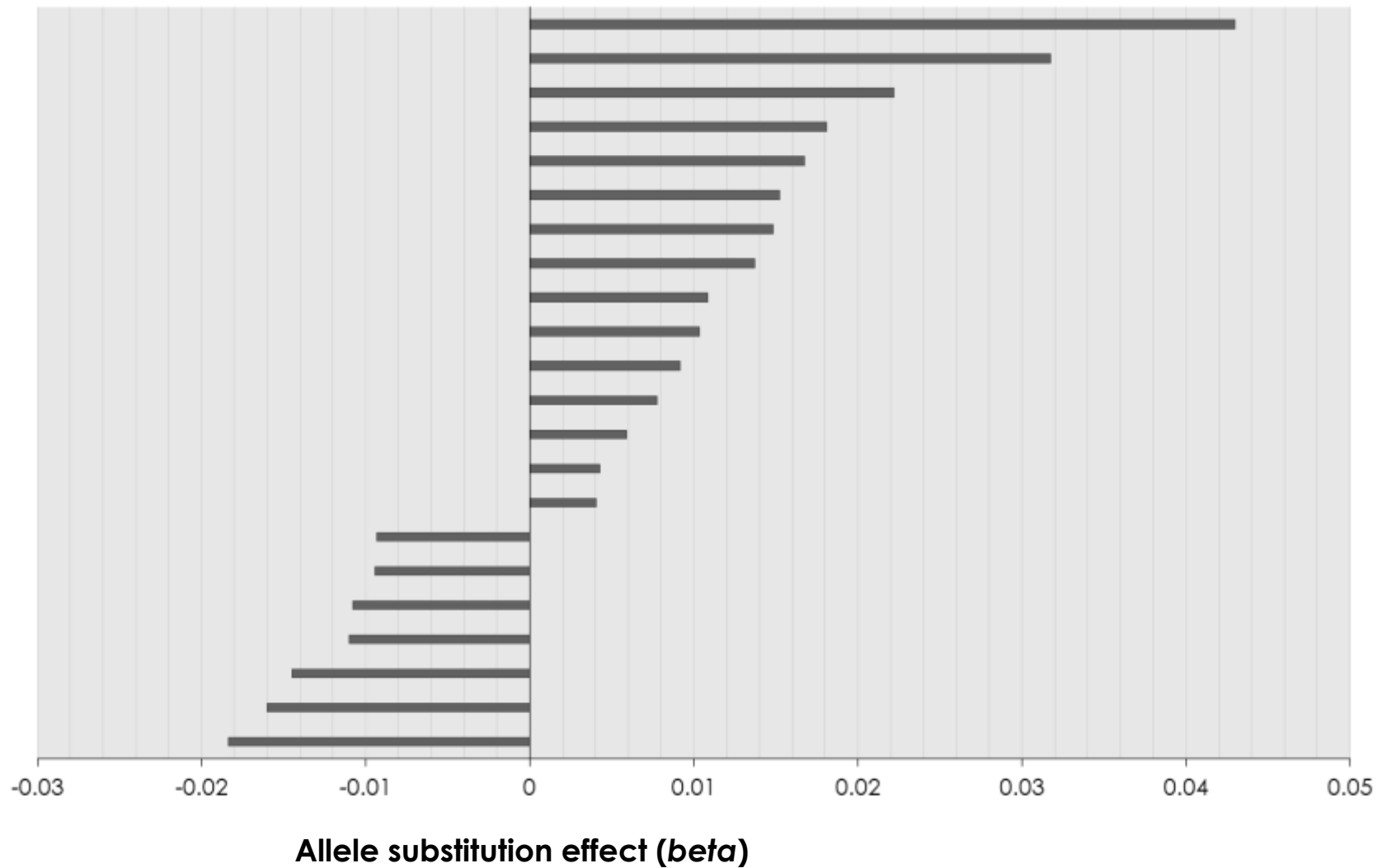
Functions of genes identified with assembly **UMD_3.1.1**

Chr	Gene	Functions
1	<i>TFRC</i>	Transmembrane transport of small molecules, vesicle-mediated transport , iron transport, endocytosis.
1	<i>SH3BP5</i>	Regulation of Bruton's tyrosine kinase (B-lymphocytes development); interaction with MAPK8 (mitogen-activated protein kinase 8, related to cell apoptosis).
1	<i>LOC787357</i>	Dorsalization regulation and axin interaction.
2	<i>MFSD6</i>	Macrophage reception and intra/extra cellular transport facilitation .
3	<i>EFNA1</i>	Regulation of ephrin and inflammatory response .
7	<i>RASGEF1C</i>	Regulation of membrane-associated molecular activity, intracellular signaling pathways, cell differentiation and proliferation, cytoskeletal organization, vesicle trafficking , and nuclear transport.
12	<i>HS6ST3</i>	Glycosaminoglycan (heparan sulfate) biosynthesis.
12	<i>LOC515333</i>	Transmembrane molecular transport; regulation of ATP-binding cassette transporter molecules; hemostasis regulation.
16	<i>SKI</i>	Regulation of growth factor TGF- β pathway (involved in cell growth, differentiation, apoptosis, homeostasis).
16	<i>SWT1</i>	Transcriptional protein.
19	<i>HDAC5</i>	Crucial role in transcriptional regulation, cell cycle progression, and developmental events.
19	<i>LOC104975159</i>	-
28	<i>SLC35F3</i>	Solute carrier, thiamine transport.
28	<i>KCNK1</i>	Potassium and sodium channel activity; stabilization of membrane potential.



QQ plot





- BTA19 - ARS-BFGL-NGS-19774
- BTA28 - BTB-00874898
- BTA3 - ARS-BFGL-NGS-64215
- BTA28 - BTB-00874839
- BTA12 - BTA-123122-no-rs
- BTA12 - Hapmap50646-BTA-29027
- BTA19 - ARS-BFGL-NGS-55564
- BTA28 - ARS-BFGL-NGS-40170
- BTA7 - ARS-BFGL-NGS-110962
- BTA18 - ARS-BFGL-NGS-119782
- BTA2 - ARS-BFGL-NGS-39978
- BTA19 - Hapmap25852-BTA-148919
- BTA1 - ARS-BFGL-NGS-5124
- BTA1 - Hapmap42521-BTA-35582
- BTA29 - Hapmap32898-BTA-66437
- BTA16 - BTA-26576-no-rs
- BTA12 - ARS-BFGL-NGS-57541
- BTA1 - ARS-BFGL-NGS-95240
- BTA16 - ARS-BFGL-NGS-74373
- BTA2 - Hapmap49624-BTA-47893
- BTA20 - BTB-01648514
- BTA20 - BTB-01648552