Validation of QTL affecting mastitis in dairy cattle

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Mastitis

- The most costly disease in dairy cattle worldwide
  - more than 6 million cows within the EU affected annually
  - Average cost €600 per cow (including premature culling, Heikkila et al. 2012)

- Major animal welfare and environmental problem (antibiotic use)

- Resistance to mastitis is difficult to be included in breeding programs, complex biological background, low heritability
  - MSCC often used as an indicator trait

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Quantomics

GWAS in the Finnish Ayrshire and Brown Swiss

- 50K genotypes for 1786 FAY and 1030 BSW sires
- 238 FAY and 192 BSW regenotyped with Illumina HD chip, and remaining bulls imputed to HD
- GWAS e.g. with GenABEL (using genomic kinship matrix)

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Best QTLRs (Mb) based on QTL, 50K/HD GWAS results

- **BTA1: 78-79, 85, 89-90, 95;**
- **BTA3: 82, 111;**
- **BTA6: 89-90;**
- **BTA8: 104;**
- **BTA18: 4.8-6.6;**
- **BTA19: 22.5-22.8;**
- **BTA21: 66;**
- **BTA27: 18-19.8**

- These genomic regions (+/- 2.5 Mb) were analyzed for polymorphisms from 20X whole-genome sequences of 38 ancestral bulls of the two populations

Variants within the regions

- For details on SNP calling see poster/abstract no. 17515 by Holm et al.

- Quality filtering (SVS7, Golden Helix)
  - 327,037 SNPs in QTLR in Finnish Ayrshire -> 240,051
  - 299,733 SNPs in QTLR in Italian Brown Swiss -> 166,933

- Prioritizing to choose SNPs for genotyping in new samples

SNP prioritization

The SNPs called within the regions were ranked according to their estimated effect:

- Stop gain/loss
- Affecting splice site
- coding -> Variant Effect Predictor / SIFT -> deleterious
- miRNA or miRNA target site
- SNP in UTR or ncSNP overlapping with GERP element
- Effect on RNA structure (RNAsnp)

- Both unique and shared (FAY/BSW) SNPs chosen for validation

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BTA	QTLR (Mb)	SNPs	stopgain	splicing	deleterious	miRNA	GERP	missense
1	75.5-81.5	28		1	6	1	5	15
1	82.5-97.5	46		2	11	1	2	30
2	119.5-124.5	30		1	16		8	5
3	79.5-84.5	17	1	1	8		1	6
3	108.5-113.5	51		3	12	3	3	30
5	0.1-3.0	10			1			9
6	86.5-92.5	54	2	5	13	1	11	22
8	101.5-106.5	44			15	5	8	16
18	2.3-9.1	37			11	2	16	8
19	20.0-25.3	35	1	2	9			
21	63.5-68.5	7			2	2		3
27	15.5-22.3	25		1	8		6	10

The set of 384 prioritized SNPs for genotyping on Illumina BeadXpress

Validation phenotypes

- Finnish Ayrshire: daughter yield deviations (M.Lidauer) for:
  - mastitis incidence 1: -15 – 150 days of lactation, 2: 151 - 300 days of first lactation; 530 sires
  - Milk somatic cell score MSCC 1: across 1st lactation, 2: across 2nd lactation, 3: across 3rd lactation, 386 sires
  - Udder conformation UA: attachment, UD: depth; 386 sires
- Valdostana:
  - EBVs (reliability as covariate) for MSCC for 220 bulls
  - Bacteriological data (not yet analysed)
- Danish Red:
  - de-regressed EBVs (weighted with n of daughters) for clinical mastitis index (CMI) and somatic cell score index (SCSI)

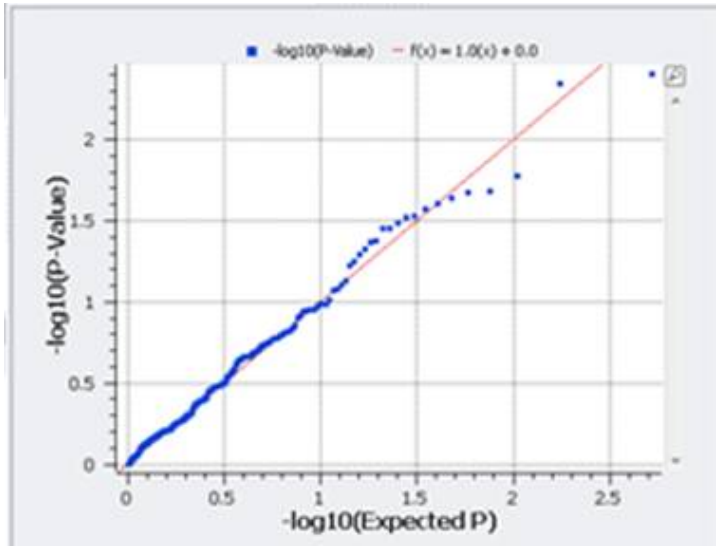
Validation by genotyping selected SNPs

- Finnish Ayrshire
  - 550 new, younger bulls
  - 261 SNPs with MAF >2%, call rate >0.9
- Valdostana:
  - 363 bulls
  - 219 SNPs with MAF >2%, call rate >0.69

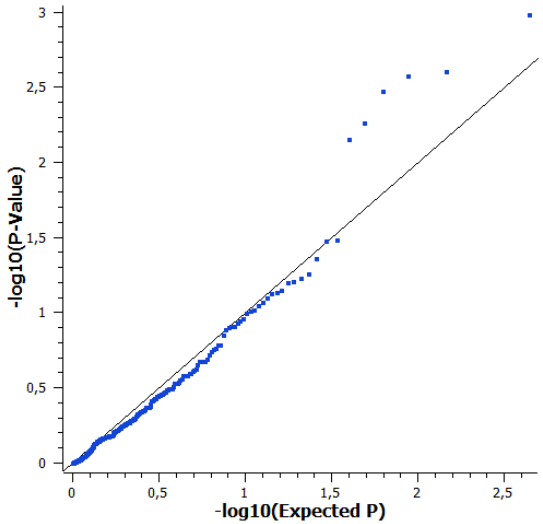
http://www.arev.it/allevatori/index.cfm/le-razze-bovine-valdostane

Association analyses in FAY and BSW

- Mixed model analysis by EMMAX (Kang et al. 2010) in SVS7
- Kinship matrix based on independent SNP genotypes (50K data) were used for correction of the population stratification



QQ-plot, FAY, CM1



QQ-plot, Valdostana, MSCC

Validation by QTLR *in silico*

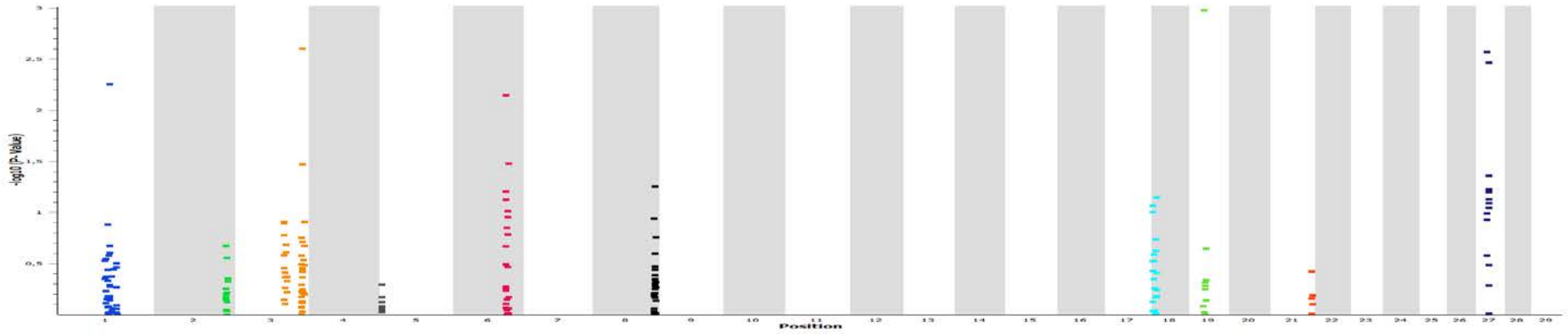
- Danish Red
  - 845 sires from Denmark
  - 50K genotypes imputed to HD
    (ref. population of 2036 Nordic bulls)
  - HD imputed to full sequence
    (WGS of 253 dairy bulls as reference)

http://www.vikinggenetics.com

  - single-locus regression analysis for each SNP separately,
    using linear mixed model (Yu et al., 2006) $y = 1\mu + mg + Zu + e$
    fitted by REML using DMU (Madsen and Jensen, 2011)
  - t-test against a null hypothesis of g = 0

Results /prioritized SNPs; FDR<30%

- Valdostana: MSCC
  - BTA1: 84.4 Mb
  - BTA3: 110.9 Mb
  - BTA6: 87.1 Mb
  - BTA19: 21.7 Mb
  - BTA27: 15.5 Mb; 19.0 Mb; no LD between these two



Results /prioritized SNPs; FDR<30%

- FAY
  - CM1, CM2: none
  - MSCC1, MSCC2, MSCC3:

  BTA6: 7 SNPs in **86.9-88.1 Mb**

  BTA8: 2 SNPs around 104 Mb

  BTA19: 2 SNPs within 25.0-25.2 Mb
  - UA, UD:

  BTA6: 3 SNPs around **86.8-87.0 Mb**

  BTA27: **15.5 Mb; 18.7 Mb**

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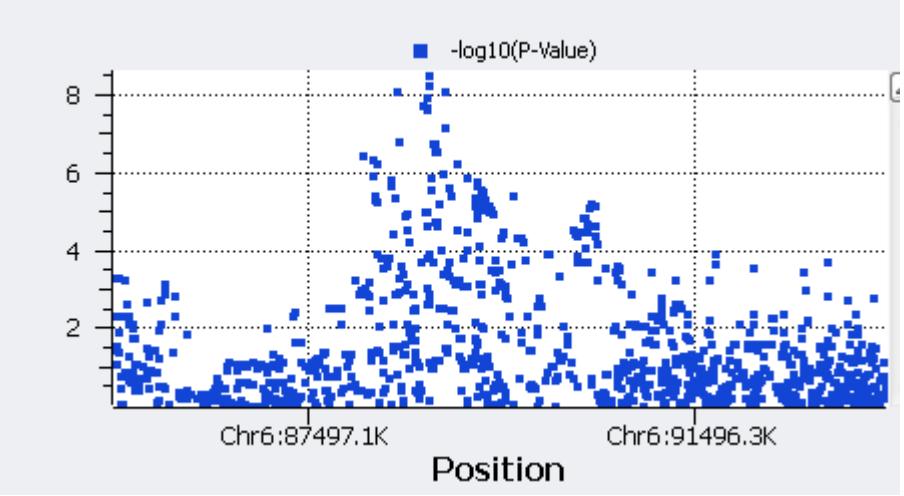
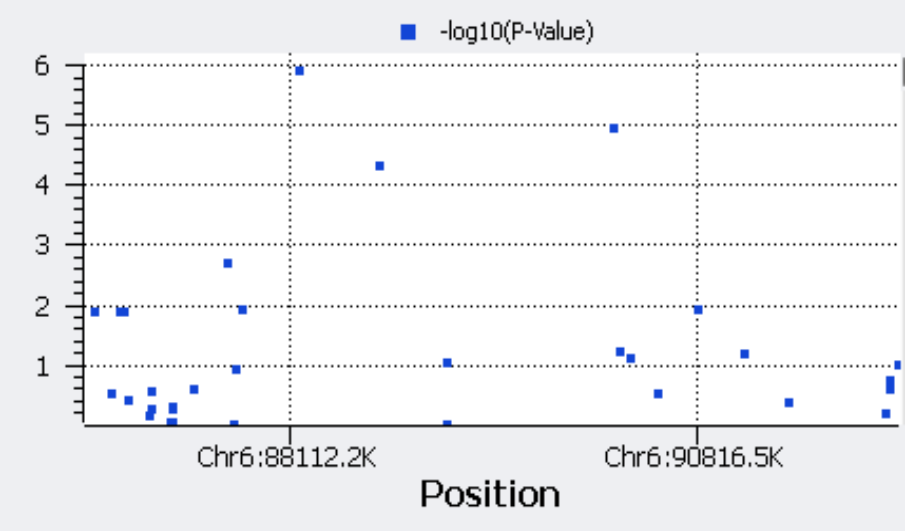
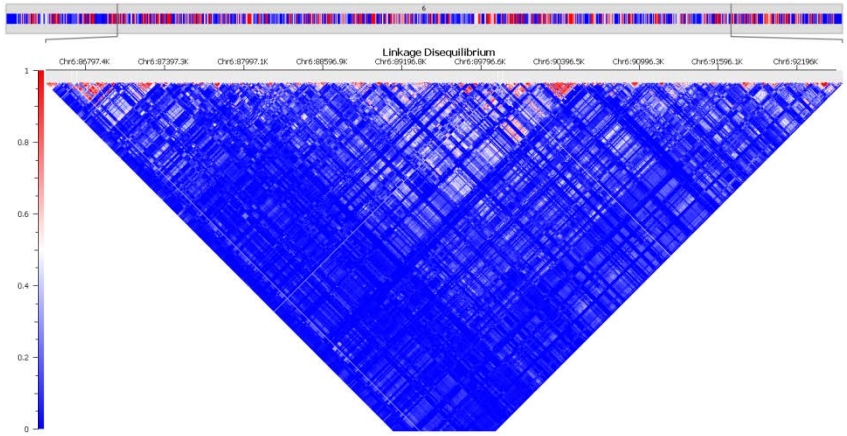
Danish Red results best SNP/ QTLR

BTA	Region Mb	N of SNPs	CMI/Mb	-log$_{10}$(P)	SCSI/Mb	-log$_{10}$(P)
1	75.5-81.5	16,669	76.37	3.16	*79.1*	*4.45*
1	82.5-97.5	31,451	88.06	2.75	*96.38*	*3.76*
2	119.5-124.5	16,218	*120.8*	*3.92*	123.8	2.83
3	79.5-84.5	13,217	na	na	81.4	2.85
3	108.5-113.5	17,567	**111.2**	**3.64**	112.9	2.51
5	0.1-3.0	8,756	0.92	2.00	1.91	3.37
6	86.5-92.5	24,124	**89.14**	**4.60**	90.72	3.19
8	101.5-106.5	24,486	na	na	**104.5**	**2.99**
18	2.3-9.1	28,376	7.52	2.86	7.71	3.27
19	20.0-25.3	15,675	**24.05**	**3.93**	**22.77**	**3.75**
21	63.5-68.5	16,464	na	na	63.58	3.12
27	15.5-22.3	23,044	na	na	**15.67**	**2.93**

FAY, CM1; new SNPs imputed for 1561 sires

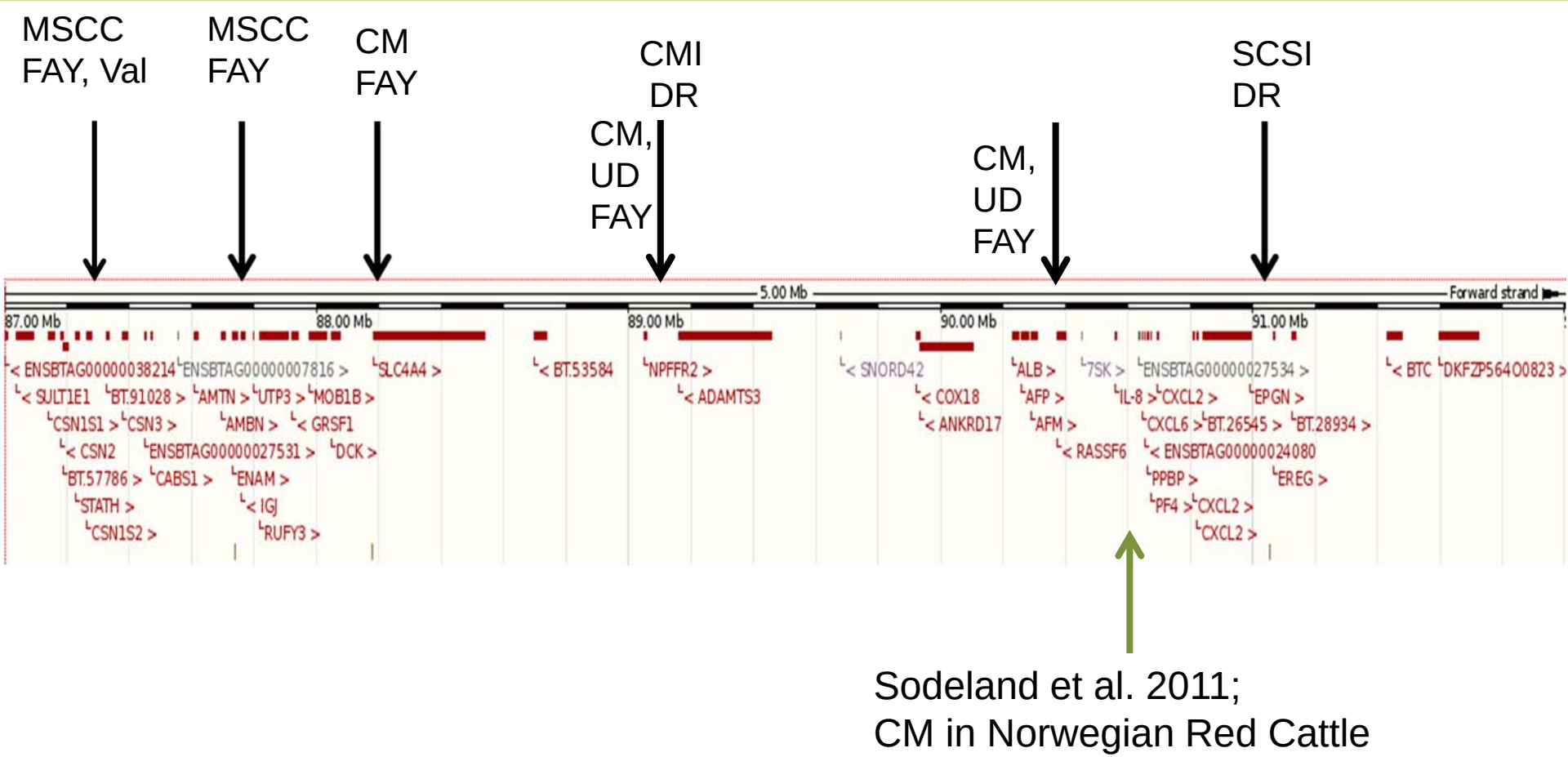






Best SNP: 5'-SLC4A4, GERP

BTA6 QTLR



Sodeland et al. 2011;
CM in Norwegian Red Cattle

Conclusions

- 5 regions (BTA3, BTA6, BTA8, BTA19, BTA27) agree within 1 Mb, but no identical associated SNPs across populations
  - High power needed to confirm results (imputation to full populations to be done)

- Most results on BTA6 within a 5 Mb region: MSCC detected in all three populations (CM and UD when phenotypes available)
  - Several QTL in BTA6?

- Background of mastitis resistance is complex (e.g. immunology, structure, energy balance)
  - Using several traits may help in defining candidate genes

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Thank you for your attention!

And everybody involved during the study!

MTT

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