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Genetic parameters, genome-wide associations and single-gene effects for intramuscular fat content in a local dual-purpose cattle breed

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Rotes Höhenvieh (RHV)

- RHV is a German dual-purpose cattle breed
- 2,389 females and 157 males registered in the German herd book (2021)
- RHV is included in the list of German endangered livestock populations for more than 20 years
- Today, RHV cattle are primarily used in suckler cow production systems, for landscape maintenance and quality beef production in low-input grazing systems
- Contribution to overall economy and preservation of the RHV breed by
 - focusing on niche markets, e.g., markets offering special meat products and aiming on meat quality (MQ) improvements
 - applying all available modern technologies such as genomic selection to achieve at least moderate genetic gain per year



Intramuscular fat content (IMF)

- IMF = the proportion of fat stored between the muscle fiber bundles, visible as marbling
- One of the most important MQ traits in beef cattle
- IMF is a quantitative trait affected by multiple genes, combined with environmental factors including sex, age, and feeding
- However, quite large h^2 for IMF is described (0.30 - 0.57) → enormous potential for selection and indication of major gene effects
- Significantly associated SNPs are identified in commercial beef cattle breeds
 - e.g. within *CAPN1*, *CAST*, *DGAT1*, *FABP4*, *RORC* and *TG*

Aims of the study

- To phenotype IMF in a broad number of German RHV cattle
- To associate genotypes of 6 different already known MQ gene SNPs in the RHV breed with IMF
- To estimate direct (h^2_d) and maternal genetic (h^2_m) heritability of IMF and genetic correlations with weight traits using different genomic models in RHV
- To perform a GWAS in order to identify genomic regions that are associated with direct and maternal genetic effects on IMF in RHV



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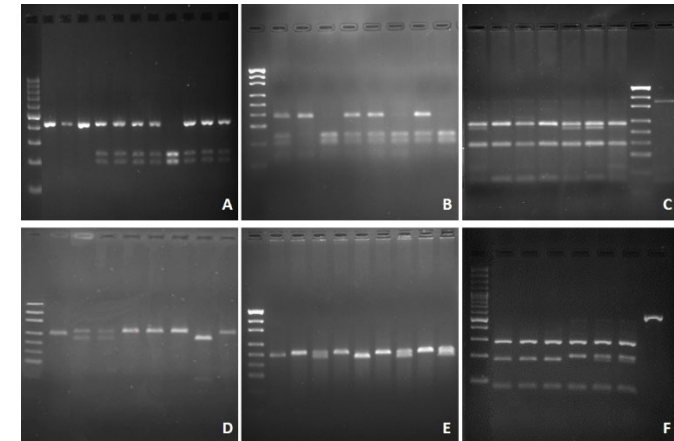
Animal material and phenotyping

- 528 RHV originating from 14 German herds, reflecting extensive pasture-based production systems
- Collection of meat samples
 - of the *musculus longissimus dorsi* between the 12th and 13th rib
 - with a thickness of 3.5 cm
- Phenotyping for IMF by near-infrared spectroscopy (NIRS)
 - mean of 2.50 % IMF (SD 2.80)
- Additionally, RHV were phenotyped for
 - birth weight (BTW)
 - 200-day weight (200dW)
 - 365-day weight (365dW)



DNA-based tests of MQ SNPs

- Typing of 6 MQ gene SNPs by DNA-based tests for all 528 RHV
 - *CAPN1* g.5709C>G (GenBank Acc. No. AH009246)
 - *CAST* g.2959A>G (GenBank Acc. No. AF159246)
 - *DGAT1* g.6829A>G (GenBank Acc. No. AY065621)
 - *FABP4* g.131C>G (GenBank Acc. No. KC660106)
 - *RORC* g.3290T>G (GenBank Acc. No. DQ667048)
 - *TG* g.422C>T (GenBank Acc. No. X05380)



Genotype, allele substitution and dominance effects of MQ gene SNPs

- Genotype effects:

$$y_{ijklmnopqr} = \mu + cp_i + cs_j + dg_k + fa_l + ro_m + tg_n + s_o + a_p + f_q + e_{ijklmnopqr} \quad [1]$$

- y = observations for IMF
- μ = general mean
- cp_i = fixed effect for the genotype of the *CAPN1*-SNP (CC, CG or GG)
- cs_j = fixed effect for the genotype of the *CAST*-SNP (AA, AG or GG)
- dg_k = fixed effect for the genotype of the *DGAT1*-SNP (AA, AG or GG)
- fa_l = fixed effect for the genotype of the *FABP4*-SNP (CC, CG or GG)
- ro_m = fixed effect for the genotype of the *RORC*-SNP (GG, GT or TT)
- tg_n = fixed effect for the genotype of the *TG*-SNP (CC, CT or TT)
- s_o = fixed effect for sex (bull, oxen or female)
- a_p = fixed effect for slaughter age class (<2 yr, 2-2.5 yr, >2.5-3 yr, >3 yr)
- f_q = random farm effect
- $e_{ijklmnopqr}$ = random residual effect

- Allele substitution effects:

See [1], whereas genotypes were replaced by a linear regression on the number of the SNP-depending favorable alleles (0, 1, or 2)

- Dominance effects:

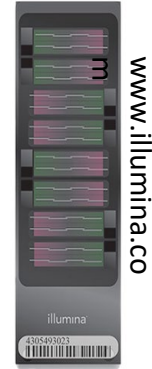
$$d = AB - \frac{1}{2}(AA + BB)$$

DNA-based tests of MQ gene SNPs

- Low minor allele frequencies were found for most MQ gene SNPs (e.g. *DGAT1**A = 2.1%, *FABP4**C = 9.7%)
 - No significant genotype effect of all 6 MQ gene SNPs ($P > 0.05$) on IMF
 - Allele substitution effects on IMF were small and did not differ from 0 after Bonferroni correction ($P > 0.05$) for all MQ gene SNPs, probably due to predominance of alleles
 - Dominance effects were small, but IMF values were highest in heterozygous animals for *CAST*- and *TG*-SNPs
- the 6 MQ gene SNPs with well-known effects in commercial beef cattle breeds, are of limited relevance in dual-purpose endangered RHV cattle with different breeding goals and strategies

777K SNP Bovine HD BeadChip

- Genotyping of $n = 343$ IMF-phenotyped RHV with the 777K SNP BovineHD BeadChip
- quality control by PLINK 1.9 package excluded SNPs
 - with a call rate < 0.90
 - with a minor allele frequency < 0.05
 - with a significant deviation from HWE
 - located on sex chromosomes



→ 581,304 SNPs were considered in the ongoing genomic analyses

Estimation of genetic parameters for IMF

- Application of single-trait models (only IMF) and of multiple-trait models (including IMF, BTW, 200dW and 365dW simultaneously)
- Consideration of different genetic relationship matrices in different runs:
 - pedigree-based relationship matrix (A)
 - genomic relationship matrix (G)
 - combined relationship matrix (H)
- Estimation of direct and maternal genetic effects and their correlations
- Fixed effects in all models: herd-year-season, sex, age at trait recording

Estimation of h^2 and variance components for IMF

Genetic parameters for IMF estimated applying the multiple-trait model with G-matrix, direct (d) and maternal (m) genetic effects, (SD)

trait	σ_p^2	σ_a^2	σ_m^2	σ_e^2	h_d^2	h_m^2
BWT	19.64 (0.45)	12.31 (1.11)	4.48 (0.56)	8.64 (0.59)	0.62 (0.05)	0.23 (0.03)
200dW	1343.10 (47.73)	740.18 (85.77)	369.45 (52.84)	594.89 (53.56)	0.55 (0.06)	0.27 (0.03)
365dW	2680.10 (69.36)	1192.61 (159.17)	605.22 (78.75)	1491.12 (87.23)	0.44 (0.05)	0.23 (0.03)
IMF	9.81 (1.45)	7.49 (1.44)	2.80 (0.49)	0.43 (0.12)	0.76 (0.09)	0.29 (0.05)

- h_d^2 over all models are high and lying within the range of estimates for different commercial beef cattle breeds
- Quite high h_m^2 due to effects of dam milk quality on MQ and weight traits of the offspring in suckler cow husbandry of RHV

σ_p^2 = phenotypic variance, σ_a^2 = additive genetic variance, σ_m^2 = maternal genetic variance, σ_e^2 = residual variance, h_d^2 = direct heritability, h_m^2 = maternal heritability

Genetic correlations

Genetic correlations between weight traits and IMF and direct-maternal genetic correlations for the four traits applying the multiple-trait model with G-matrix

Effect		Direct genetic				Maternal genetic			
	Trait	BWT	200dW	365dW	IMF	BWT	200dW	365dW	IMF
Direct genetic	BWT	...	...	...	...	-0.77 (0.04)	...	...	...
	200dW	...				...	-0.68 (0.04)		
	365dW	...				...		-0.71 (0.05)	
	IMF	-0.13 (0.09)	-0.15 (0.12)	0.01 (0.09)		...			-0.18 (0.09)

GWAS and gene annotations for IMF

- GWAS was carried out using the linear mixed model association method as implemented in the GCTA software:

$$y = 1\mu + x_i s_i + Zu + e$$

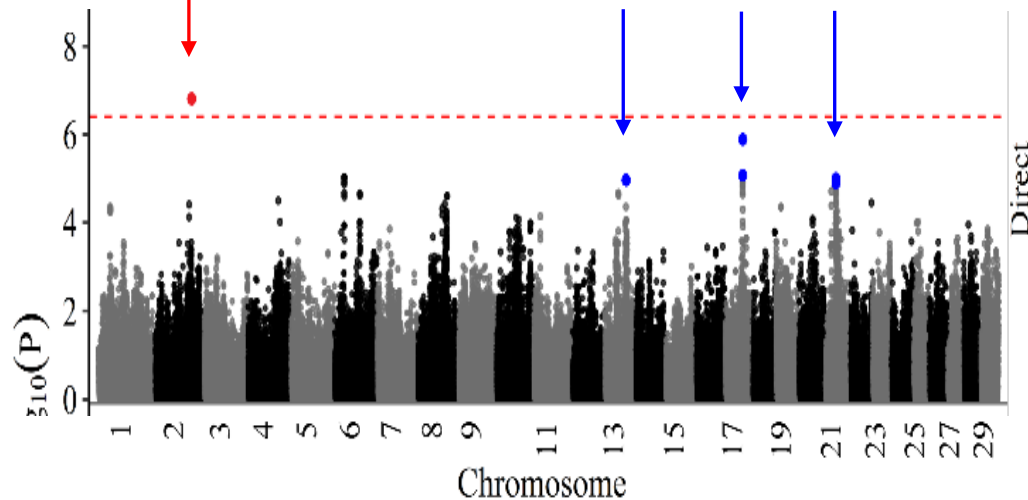
where y is the vector of direct or maternal de-regressed proofs for IMF; 1 is the vector of ones; μ is the overall mean effect; x_i is the vector of genotypes coded as 0, 1, or 2; s_i was the effect of the i th SNP; $u \sim N(0, G\sigma_u^2)$ is the vector of random polygenic effects, with G representing the genomic relationship matrix among animals, and $G\sigma_u^2$ is the polygenic variance; Z is an incidence matrix for u , and e is the residual random effect

- For the annotations of the candidate genes, a window frame of ± 250 kb around each significant SNP was used for mapping to the Bos taurus reference genome ARS-UCD1.2 with the biomaRt package in R

Manhattan plots for direct genetic effects on IMF

BTA2: rs137226468 next to
ENSBTAG00000052399 +
VWC2L

BTA13: rs108949054 next to *BMP7*
BTA17: e.g. rs109093435 in *TMEM132D*
BTA21: rs42815709 in *UBE2Q2*
BTA21: rs43136852 next to *CHRNA4*



--- adjusted Bonferroni-corrected genome-wide significance threshold

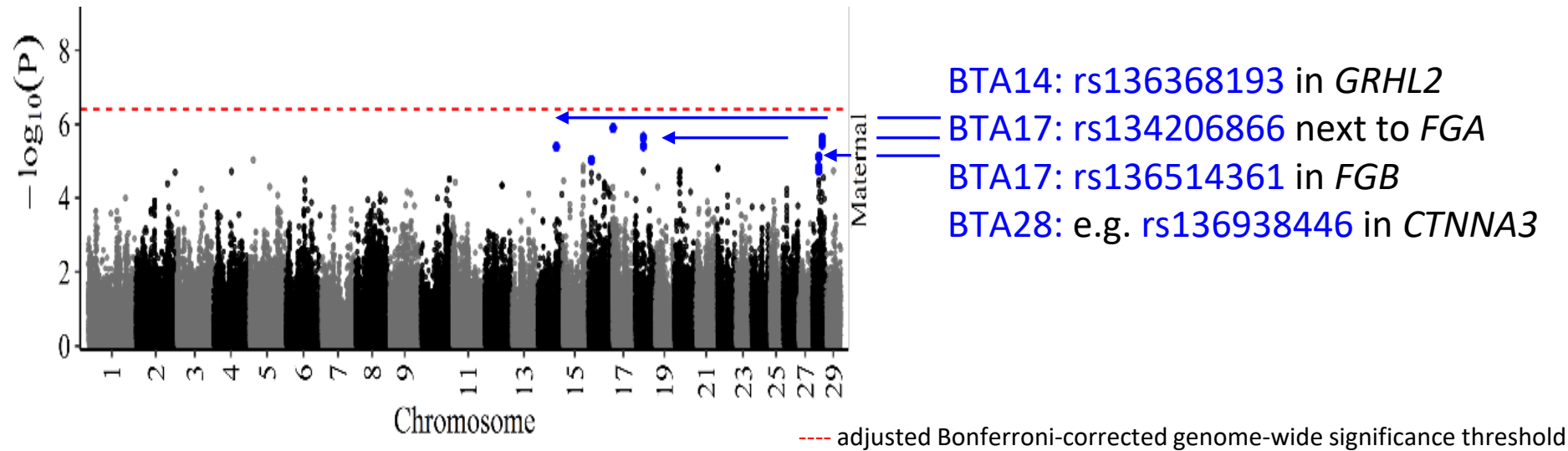
rs137226468 surpassed the genome-wide significance threshold for direct effects on IMF

- *ENSBTAG00000052399* = protein-coding gene with until now unknown function
- *VWC2L* = candidate gene for feed efficiency in pigs

- 7 SNPs surpassed chromosome-wide significance threshold for direct effects on IMF
 - *BMP7* = candidate gene for feed efficiency and associated with average daily gain and carcass traits (e.g. carcass marbling score) in beef cattle

- 14 — *TMEM132D* = candidate gene for body weight in broilers

Manhattan plots for maternal genetic effects on IMF



- 13 SNPs surpassed chromosome-wide significance threshold for maternal effects on IMF
 - *GRHL2* = already known maternal-related effect on IMF in cattle

- Identified candidate genes for direct and maternal genetic effects on IMF
- partly differing from association signals in other cattle breeds and
 - differing from the 6 MQ candidate genes, as no significant SNP was identified within or next to these genes

Conclusion

- High IMF genetic variance and h^2 underline RHVs' potential for quality meat production
- non-significant effects of the 6 MQ gene SNPs on IMF might be due to the breeding history of RHV:
 - focus on adaptation and resilience in harsh outdoor systems
 - loss of heterozygosity due to the very small initial population size during the early days of structured breeding
- However, further gene annotations based on GWAS suggest possibilities for MQ improvement
- Fine mapping of the annotated genes is useful for a RHV breed-specific selection strategy including IMF
- This gives the RHV breed the opportunity to produce high-quality beef, to achieve economic competitiveness and to be utilized sustainably as an animal genetic resource

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And thank you for your attention

