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From genetic correlation to causation between milk yield, mastitis and and health traits in German Holstein cattle

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Background

Genetic correlations can **indicate causality**
☹ Detailed **causal inference** is **not feasible**

Gold standard to assess causal effects: **Randomized Control Trials**
☹ Can be **challenging** and sometimes even **unethical** to perform

Alternative: **Mendelian randomization**, analysis to investigate causality using a set of genetic variants (randomized at conception)

(Pickrell et al. 2015)

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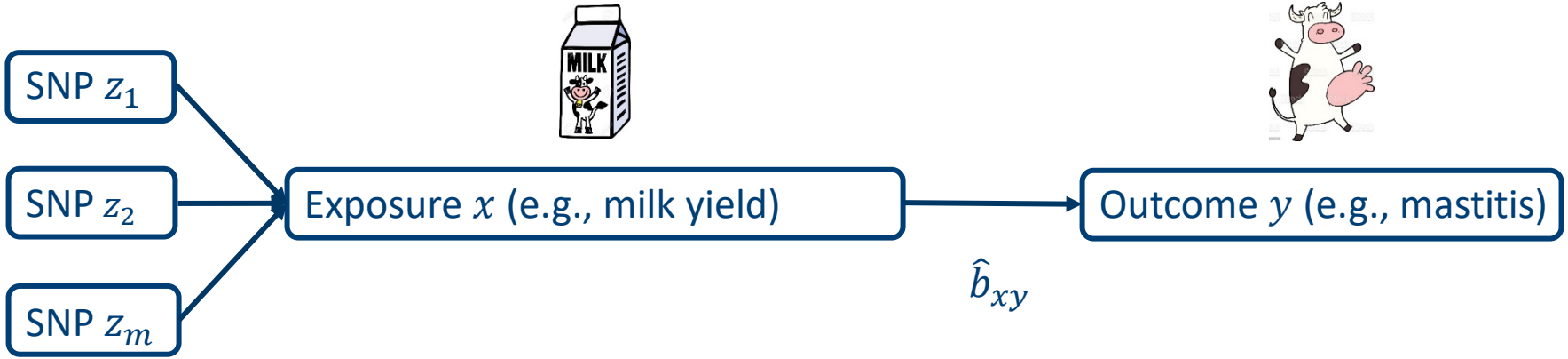
Mendelian randomization - Concept



If x has a causal effect on y ($\hat{b}_{xy}$)

- Every instrumental variable (IV) z having an effect on x ($\hat{b}_{zx}$) will also have an effect on y ($\hat{b}_{zy}$) via x

- $\hat{b}_{xy}$ will be identical at each variant ($\hat{b}_{zy}$ is linearly proportional to $\hat{b}_{zx}$)

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Aim of this Study

Mendelian randomization analysis to investigate the **causal effect of milk yield** on **mastitis** and **six claw health traits**

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Material

26,437 - 34,497 **German Holstein** cows (first lactation 2015 - 2020)

50K chip & imputed **WGS** data

De-regressed proofs (DRP) for

- Milk yield

Seven health traits:

- Mastitis
- Six claw health traits (Digital phlegmon, Digital dermatitis, Claw ulcers, Interdigital hyperplasia, Laminitis, White line disease)

vit
Service & Daten aus einer Quelle

→ **DRP** and **50K** genotypes provided by the **vit** (Vereinigte Informationssysteme Tierhaltung w.V., Verden)

→ Imputed **WGS** data provided by workgroup of Prof. Dr. **Tetens** (University of Göttingen)

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Methods (all analyses were carried out using GCTA (Yang et al. 2011))

Step 1: Bivariate GREML analysis using the 50K chip data

- r_g between milk yield and each health trait

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Step 2: GWAS for each trait using the imputed WGS data

- **Summary statistics** are **required** for the **next step**

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Methods (all analyses were carried out using GCTA (Yang et al. 2011))

Step 1: Bivariate GREML analysis using the 50K chip data

- r_g between milk yield and each health trait

Step 2: GWAS for each trait using the imputed WGS data

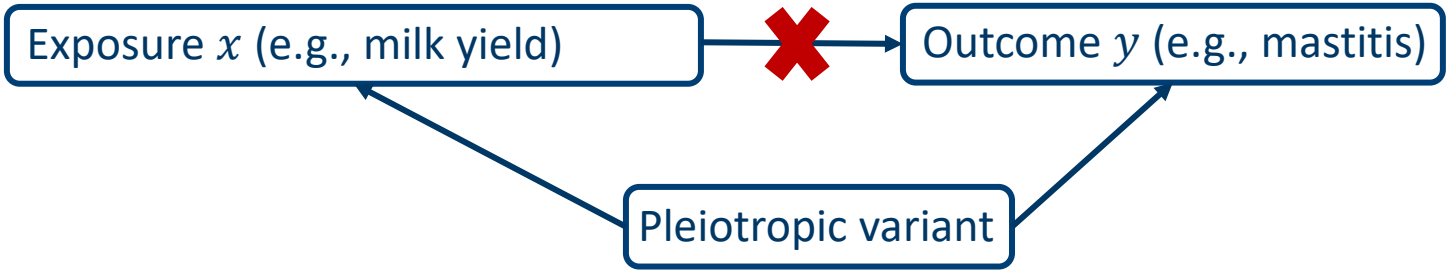
- **Summary statistics** are **required** for the **next step**

Step 3: Generalized summary-data based MR (**GSMR**) (Zhu et al. 2018)

- Variants that are **highly associated** ($p_{GWAS} < 5 * 10^{-8}$) with milk yield and **quasi independent** ($r^2 < 0.05$) are **selected as IVs**
- **GSMR** contains a filterstep to **remove pleiotropic variants**, the **HE**terogeneity **I**n **D**ependent **I**nstruments (**HEIDI**) outlier filter

→ One analysis with (HEIDI) and one without (noHEIDI) removing the pleiotropic variants was performed

Methods



In case of **pleiotropy**: **effect** of IV´s **on y not entirely mediated via x**

→ Concept of MR not fulfilled

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Results

Negative $\hat{b}_{xy}$ = milk yield ↑, health ↓

Health trait	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.041 (0.021)	0.049	105	-0.041 (0.021)	0.049	105
Digital dermatitis	-0.054 (0.021)	0.009	103	-0.048 (0.021)	0.020	105
Digital phlegmon	**-0.098 (0.020)**	**<0.001**	104	**-0.098 (0.020)**	**<0.001**	105
Interdigital hyperplasia	-0.039 (0.020)	0.058	105	-0.039 (0.020)	0.058	105
Laminitis	-0.034 (0.021)	0.058	105	-0.034 (0.021)	0.058	105
Mastitis	**-0.131 (0.020)**	**<0.001**	102	**-0.133 (0.020)**	**<0.001**	105
White line disease	-0.042 (0.020)	0.038	105	-0.042 (0.020)	0.038	105

N = Number of instrumental variables

→ **Negative experiment-wide significant** causal effect (p value < 0.0071) of **milk yield** on **mastitis** and **digital phlegmon**

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Results

Negative $\hat{b}_{xy}$ = milk yield ↑, health ↓

Health trait	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	**-0.041 (0.021)**	**0.049**	105	**-0.041 (0.021)**	**0.049**	105
Digital dermatitis	**-0.054 (0.021)**	**0.009**	103	**-0.048 (0.021)**	**0.020**	105
Digital phlegmon	**-0.098 (0.020)**	**<0.001**	104	**-0.098 (0.020)**	**<0.001**	105
Interdigital hyperplasia	-0.039 (0.020)	0.058	105	-0.039 (0.020)	0.058	105
Laminitis	-0.034 (0.021)	0.058	105	-0.034 (0.021)	0.058	105
Mastitis	**-0.131 (0.020)**	**<0.001**	102	**-0.133 (0.020)**	**<0.001**	105
White line disease	**-0.042 (0.020)**	**0.038**	105	**-0.042 (0.020)**	**0.038**	105

N = Number of instrumental variables

→ **Negative nominal significant** causal effect (p value < 0.05) of **milk yield** on **almost each health trait**

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Results

Negative $\hat{b}_{xy}$ = milk yield ↑, health ↓

Health trait	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.041 (0.021)	0.049	105	-0.041 (0.021)	0.049	105
Digital dermatitis	**-0.054 (0.021)**	**0.009**	**103**	**-0.048 (0.021)**	**0.020**	**105**
Digital phlegmon	**-0.098 (0.020)**	**<0.001**	**104**	**-0.098 (0.020)**	**<0.001**	**105**
Interdigital hyperplasia	-0.039 (0.020)	0.058	105	-0.039 (0.020)	0.058	105
Laminitis	-0.034 (0.021)	0.058	105	-0.034 (0.021)	0.058	105
Mastitis	**-0.131 (0.020)**	**<0.001**	**102**	**-0.133 (0.020)**	**<0.001**	**105**
White line disease	-0.042 (0.020)	0.038	105	-0.042 (0.020)	0.038	105

N = Number of instrumental variables

→ **No substantial effect** of **pleiotropic** variants, **HEIDI** removed **six variants** with a pleiotropic effect

Results

Negative $\hat{b}_{xy}$, r_g = milk yield ↑, health ↓

Health trait - MY	HEIDI $\hat{b}_{xy}$ (SE)	r_g (SE)
Claw ulcers	-0.041 (0.021)	-0.069 (0.028)
Digital dermatitis	-0.054 (0.021)	-0.018 (0.026)
Digital phlegmon	-0.098 (0.020)	-0.182 (0.032)
Interdigital hyperplasia	-0.039 (0.020)	-0.106 (0.027)
Laminitis	-0.034 (0.021)	-0.069 (0.020)
Mastitis	-0.131 (0.020)	-0.303 (0.026)
White line disease	-0.042 (0.020)	-0.088 (0.035)

- **All estimates were negative**, pointing to the **unfavorable relationship** between **milk yield** and the **health traits**

→ BUT: **quantitative differences** between r_g & $\hat{b}_{xy}$

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Discussion

Only few pleiotropic variants removed, whereas **previous studies** found **extensive pleiotropy** in humans and farm animals? (Visscher and Yang 2016, Xiang et al. 2021)

- IVs were selected based on a very stringent threshold to avoid bias of $\hat{b}_{xy}$ (Zhu et al. 2018)
- Pleiotropic variants might be less significantly associated with milk production

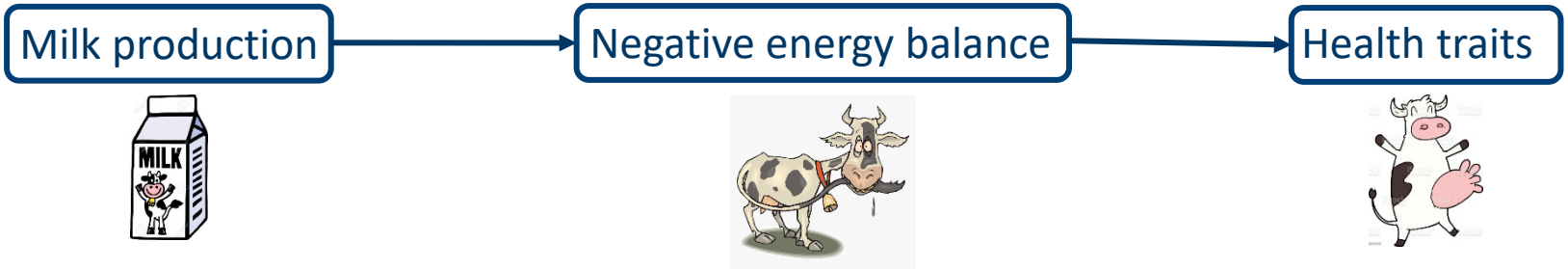
Discussion

Only few pleiotropic variants removed, whereas **previous studies** found **extensive pleiotropy** in humans and farm animals? (Visscher and Yang 2016, Xiang et al. 2021)

- IVs were selected based on a very stringent threshold to avoid bias of $\hat{b}_{xy}$ (Zhu et al. 2018)
- Pleiotropic variants might be less significantly associated with milk yield

Causal associations in a **low** range?

- Might be explained by a third variable that mediates the effect of x on y (e.g., the NEB)



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Conclusion

The results of this study

… indicate that **milk production** and **health** traits are **not only negatively correlated**

→ **unfavorable causal associations** have been found as well, potentially mediated by the negative energy balance

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Conclusion

The results of this study

… indicate that **milk production** and **health** traits are **not only negatively correlated**

→ **unfavorable causal associations** have been found as well, potentially mediated by the negative energy balance

… motivate the **application** of **mendelian randomization** analyses to gain **more insight** in the **genomic connection** between complex traits

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Mendelian randomization analysis of 34,497 German Holstein cows to infer causal associations between milk production and health traits

H. Schneider, V. Haas, A.-M. Krizanac, C. Falker-Gieske, J. Heise , J. Tetens, G. Thaller, J. Bennewitz

published in *Genetics Selection Evolution* (2024), 56(27)

GSE Genetics Selection Evolution

Thank you for your attention!

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Results

Negative $\hat{b}_{xy}$ = fat yield ↑, health ↓

Health trait / Fat yield	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.035 (0.028)	0.223	61	-0.035 (0.028)	0.223	61
Digital dermatitis	**0.061 (0.028)**	**0.028**	61	**0.061 (0.028)**	**0.028**	61
Digital phlegmon	0.047 (0.028)	0.089	61	0.047 (0.028)	0.089	61
Interdigital hyperplasia	0.039 (0.027)	0.154	61	0.039 (0.027)	0.154	61
Laminitis	-0.015 (0.028)	0.590	61	-0.015 (0.028)	0.590	61
Mastitis	**0.059 (0.026)**	**0.024**	61	**0.076 (0.026)**	**0.004**	60
White line disease	-0.026 (0.026)	0.342	61	-0.026 (0.026)	0.342	61

N = Number of instrumental variables

→ **Negative experiment-wide significant** causal effect of **fat yield** on **mastitis, nominal significant on mastitis** and **digital dermatitis**

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Results

Negative $\hat{b}_{xy}$ = fat yield ↑, health ↓

Health trait / Fat yield	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.035 (0.028)	0.223	61	-0.035 (0.028)	0.223	61
Digital dermatitis	0.061 (0.028)	0.028	61	0.061 (0.028)	0.028	61
Digital phlegmon	0.047 (0.028)	0.089	61	0.047 (0.028)	0.089	61
Interdigital hyperplasia	0.039 (0.027)	0.154	61	0.039 (0.027)	0.154	61
Laminitis	-0.015 (0.028)	0.590	61	-0.015 (0.028)	0.590	61
Mastitis	**0.059 (0.026)**	**0.024**	**61**	**0.076 (0.026)**	**0.004**	**60**
White line disease	-0.026 (0.026)	0.342	61	-0.026 (0.026)	0.342	61

N = Number of instrumental variables

→ **No substantial effect** of **pleiotropic** variants, **HEIDI** removed **one variant** with a pleiotropic effect

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Results

Negative $\hat{b}_{xy}$ = protein yield ↑, health ↓

Health trait / Protein Yield	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.015 (0.035)	0.676	45	-0.026 (0.036)	0.463	44
Digital dermatitis	-0.038 (0.035)	0.270	45	-0.038 (0.035)	0.270	45
Digital phlegmon	**-0.142 (0.034)**	**<0.001**	45	**-0.142 (0.034)**	**<0.001**	45
Interdigital hyperplasia	-0.033 (0.034)	0.339	45	-0.035 (0.035)	0.318	43
Laminitis	-0.034 (0.034)	0.321	45	-0.034 (0.034)	0.321	45
Mastitis	**-0.211 (0.033)**	**<0.001**	45	**-0.229 (0.033)**	**<0.001**	44
White line disease	-0.025 (0.033)	0.451	45	-0.029 (0.034)	0.389	43

N = Number of instrumental variables

→ **Negative experiment-wide significant** causal effect of **protein yield** on **mastitis and digital phlegmone**

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Results

Negative $\hat{b}_{xy}$ = protein yield ↑, health ↓

Health trait / Protein Yield	HEIDI			noHEIDI		
	$\hat{b}_{xy}$ (SE)	p value	N	$\hat{b}_{xy}$ (SE)	p value	N
Claw ulcers	-0.015 (0.035)	0.676	45	-0.026 (0.036)	0.463	44
Digital dermatitis	-0.038 (0.035)	0.270	45	-0.038 (0.035)	0.270	45
Digital phlegmon	-0.142 (0.034)	<0.001	45	-0.142 (0.034)	<0.001	45
Interdigital hyperplasia	**-0.033 (0.034)**	**0.339**	**45**	**-0.035 (0.035)**	**0.318**	**43**
Laminitis	-0.034 (0.034)	0.321	45	-0.034 (0.034)	0.321	45
Mastitis	**-0.211 (0.033)**	**<0.001**	**45**	**-0.229 (0.033)**	**<0.001**	**44**
White line disease	**-0.025 (0.033)**	**0.451**	**45**	**-0.029 (0.034)**	**0.389**	**43**

N = Number of instrumental variables

→ **No substantial effect** of **pleiotropic** variants, **HEIDI** removed **five variants** with a pleiotropic effect

Results

Negative $\hat{b}_{xy}$, r_g = fat yield ↑, health ↓

Health trait - FY	HEIDI $\hat{b}_{xy}$ (SE)	r_g (SE)
Claw ulcers	-0.035 (0.028)	0.032 (0.050)
Digital dermatitis	0.061 (0.028)	0.108 (0.073)
Digital phlegmon	0.047 (0.028)	-0.085 (0.027)
Interdigital hyperplasia	0.039 (0.027)	-0.028 (0.051)
Laminitis	-0.015 (0.028)	-0.094 (0.028)
Mastitis	0.059 (0.026)	0.178 (0.080)
White line disease	-0.026 (0.026)	-0.160 (0.089)

- **All estimates were negative**, pointing to the **unfavorable relationship** between **milk yield** and the **health traits**

→ BUT: **quantitative differences** between r_g & $\hat{b}_{xy}$

Results

Negative $\hat{b}_{xy}$, r_g = protein yield ↑, health ↓

Health trait - PY	HEIDI $\hat{b}_{xy}$ (SE)	r_g (SE)
Claw ulcers	-0.015 (0.035)	0.059 (0.050)
Digital dermatitis	-0.038 (0.035)	0.120 (0.074)
Digital phlegmon	-0.142 (0.034)	-0.160 (0.086)
Interdigital hyperplasia	-0.033 (0.034)	-0.167 (0.053)
Laminitis	-0.034 (0.034)	-0.038 (0.029)
Mastitis	-0.211 (0.033)	0.347 (0.092)
White line disease	-0.025 (0.033)	-0.048 (0.030)

- **All estimates were negative**, pointing to the **unfavorable relationship** between **milk yield** and the **health traits**

→ BUT: **quantitative differences** between r_g & $\hat{b}_{xy}$