UNIVERSITY OF GEORGIA
College of Agricultural & Environmental Sciences

# SNP effects from ssGBLUP using the BLUPF90 family (postGSf90)

**Daniela Lourenco**
BLUPF90 Team – 11/2022

# Equivalence between GBLUP and SNP-BLUP

GBLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W}+\mathbf{G}^{-1}\boldsymbol{\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

$$\mathrm{Var}(\mathbf{u}) = ?$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{G}\sigma_u^2$$

SNP-BLUP (Ridge Regression)

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\mathbf{I}\boldsymbol{\lambda_2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix}$$

↓

**SNP effects**

$$\mathbf{u} = \mathbf{Za}$$

# Are GBLUP and SNP-BLUP equivalent?

- Assumption of GBLUP: $\mathrm{Var}(\mathbf{u}) = \mathbf{G}\sigma_u^2$
- In SNP-BLUP: $\mathbf{u} = \mathbf{Za}$

$$\mathbf{u} = \mathbf{Za}$$

$$\mathrm{Var}(\mathbf{u}) = \mathrm{Var}(\mathbf{Za})$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{Z}\,\mathrm{Var}(\mathbf{a})\,\mathbf{Z}'$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{ZZ}'\sigma_a^2$$

$$\sigma_a^2 = \frac{\sigma_u^2}{2\sum_{i=1}^{SNP} p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{ZZ}'\frac{\sigma_u^2}{2\sum_{i=1}^{SNP} p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \frac{\mathbf{ZZ}'}{2\sum_{i=1}^{SNP} p_i(1-p_i)}\sigma_u^2$$

Genomic relationship matrix VanRaden (2008)

$$\mathbf{G} = \frac{\mathbf{ZZ}'}{2\sum_{i=1}^{SNP} p_i(1-p_i)}$$

$$\mathrm{Var}(\mathbf{u}) = \mathbf{G}\sigma_u^2$$

GBLUP assumption!!!

# GBLUP and SNP-BLUP are equivalent!

If we can get **u** ($\mathbf{u} = \mathbf{Za}$) from SNP-BLUP, we can get **a** from GBLUP!

genes

MDPI

*Review*

**Single-Step Genomic Evaluations from Theory to Practice: Using SNP Chips and Sequence Data in BLUPF90**

Daniela Lourenco [1,*], Andres Legarra [2], Shogo Tsuruta [1], Yutaka Masuda [1], Ignacio Aguilar [3] and Ignacy Misztal [1]

https://www.mdpi.com/2073-4425/11/7/790

Pages 11-12

# ssGBLUP and ssSNP-BLUP are also equivalent!

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W}+\mathbf{H}^{-1}\frac{\sigma_e^2}{\sigma_u^2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

**ssGBLUP**

Misztal et al. (2009)
Legarra et al. (2009)
Aguilar et al. (2010)
Christensen & Lund (2010)

$$\begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z}\mathbf{M} & \mathbf{X}_n'\mathbf{Z}_n \\ \mathbf{M}'\mathbf{Z}'\mathbf{X} & \mathbf{M}'\mathbf{Z}'\mathbf{Z}\mathbf{M}+\mathbf{I}\frac{\sigma_e^2}{\sigma_\alpha^2} & \mathbf{M}_n'\mathbf{Z}_n'\mathbf{Z}_n \\ \mathbf{Z}_n'\mathbf{X}_n & \mathbf{Z}_n'\mathbf{Z}_n\mathbf{M}_n & \mathbf{Z}_n'\mathbf{Z}_n+\mathbf{A}^{nn}\frac{\sigma_e^2}{\sigma_g^2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\boldsymbol{\alpha}} \\ \hat{\boldsymbol{\epsilon}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{M}'\mathbf{Z}'\mathbf{y} \\ \mathbf{Z}_n'\mathbf{y}_n \end{bmatrix}$$

**ssSNPBLUP** or **ssBR**

Fernando et al. (2014)
Liu et al. (2014)
Mantysaari & Stranden (2016)

J. Dairy Sci. 101:10082–10088
https://doi.org/10.3168/jds.2018-14913


***Short communication:*** **Genomic prediction using different single-step methods in the Finnish red dairy cattle population**

H. Gao,*†[1] M. Koivula,‡ J. Jensen,* I. Strandén,‡ P. Madsen,* T. Pitkänen,‡ G. P. Aamand,† and E. A. Mäntysaari‡
*Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, DK-8830 Tjele, Denmark
†Nordic Cattle Genetic Evaluation, DK-8200 Aarhus, Denmark
‡Natural Resources Institute Finland (Luke), FIN-31600 Jokioinen, Finland

We confirmed that regular ssGBLUP and ssBR with an extra polygenic effect led to the same predictions.

# SNP effects in ssGBLUP

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W+H^{-1}\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix}$$

$$\hat{\mathbf{a}} = \alpha b \frac{1}{2\sum p_i(1-p_i)} \mathbf{Z'G^{-1}}\hat{\mathbf{u}}$$

Matrix of SNP content

Genomic relationship matrix

$\alpha =$ blending parameter for $\mathbf{G}$

$$\lambda = \frac{1}{n^2}\left(\sum_i \sum_j \mathbf{A22}_{ij} - \sum_i \sum_j \mathbf{G}_{ij}\right)$$

$$b = 1 - \frac{\lambda}{2}$$

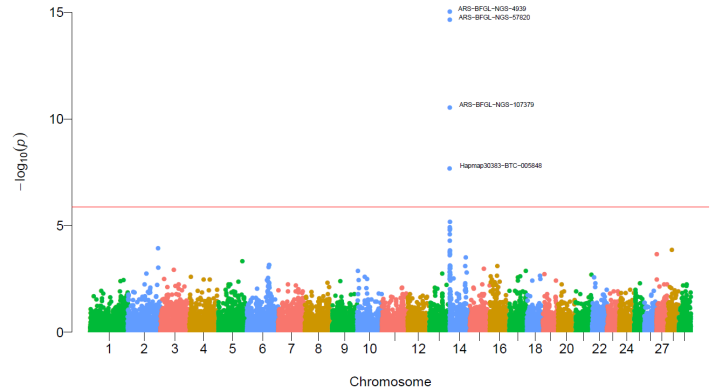
# What can we do with SNP effects?

1) Predictions for animals not included in the evaluation

   Indirect predictions

   Indirect Genomic Predictions

2) Genome-Wide Association Studies (GWAS)



# 1) Indirect Predictions

- Interim evaluations
  - Between official runs
- Not all genotyped animals are in the evaluations
  - Animals with incomplete pedigree increase bias and lower $R^2$
- Commercial products
  - e.g. GeneMax for non-registered animals

# 1) Indirect Predictions

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'W} \\ \mathbf{W'X} & \mathbf{W'W+H^{-1}\lambda_1} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{W'y} \end{bmatrix} \quad \Longrightarrow \quad \hat{\mathbf{a}} = \alpha b \frac{1}{2\sum p_i(1-p_i)} \mathbf{Z'G^{-1}} \hat{\mathbf{u}}$$

Indirect Prediction: $\mathbf{IP} = \boldsymbol{u}_{\boldsymbol{m}}^{*} = \mathbf{Z}\hat{\boldsymbol{a}}$

# 1) Indirect Predictions

Indirect Prediction: $\boldsymbol{u}_{\boldsymbol{m}}^{*} = \mathbf{Z}\hat{\boldsymbol{a}}$

↳ Fine if comparing among animals with IP

- Not fine if compare it with GEBV from the main evaluation
  - Put it in the pedigree scale

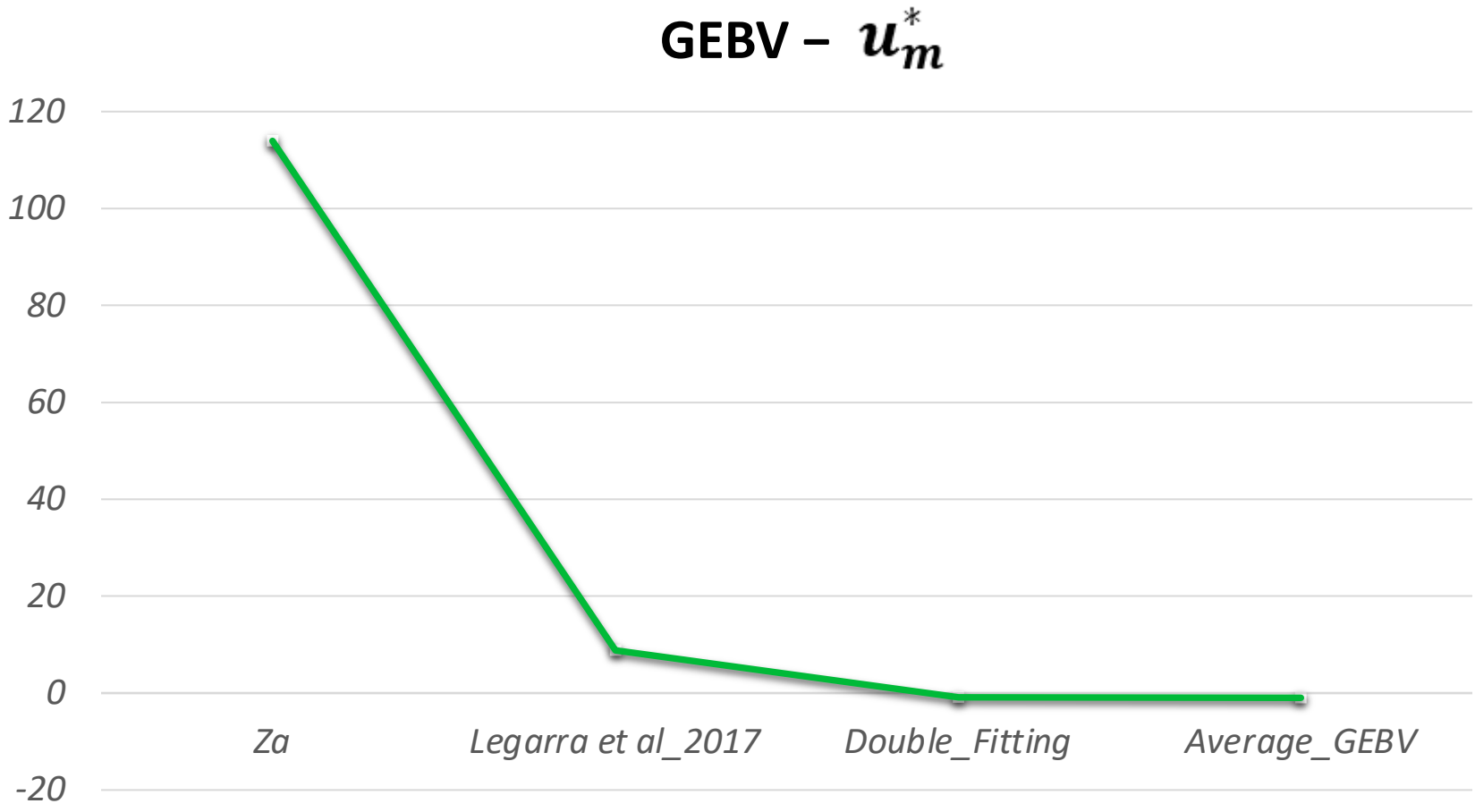
$$\boldsymbol{u}_{\boldsymbol{m}} = \hat{\boldsymbol{\mu}} + \boldsymbol{u}_{\boldsymbol{m}}^{*}$$

↳ $\hat{\boldsymbol{\mu}} = \alpha\lambda\mathbf{1}'\mathbf{G}^{-1}\hat{\mathbf{u}}$

$\alpha =$ blending parameter for $\mathbf{G}$

$$\lambda = \frac{1}{n^2}\left(\sum_i\sum_j \mathbf{A22}_{ij} - \sum_i\sum_j \mathbf{G}_{ij}\right)$$

# 1) Indirect Predictions



# How to compute Indirect predictions

1) Pedigree + phenotypes + genotypes

**2) `renumf90`**

3) preGSf90 to save clean files

**4) `blupf90+`** (save the clean files)

- Good practice to save time: `OPTION saveGInverse` + `OPTION saveA22Inverse`

**5) `postGSf90`** (with clean files)

- BLUPF90 family software to compute SNP effects (+more)
- Same parameter file as `blupf90+`
- Good practice to save time: `OPTION readGInverse` + `OPTION readA22Inverse`

# Output from postGSf90

```
snp_sol
```

http://nce.ads.uga.edu/wiki/doku.php?id=readme.pregsf90

contains solutions of SNP and weights

- 1: trait
- 2: effect
- 3: SNP
- 4: Chromosome
- 5: Position
- 6: SNP solution
- 7: weight

```
snp_pred
```

- 1st line: model, tuning, blending information
- 2nd line: Trait/effect info
- AF in 10 columns
- mu_hat, var_mu_hat
- SNP effects

# How to compute Indirect Predictions

**6)predf90**

- Have to provide a SNP file for the new genotyped animals to receive IP
  - same SNP as in the clean file

```
predf90 --snpfile newgen.txt --use_mu_hat
```

- The last statement adds the base, so that we have: $\boldsymbol{u_m = \hat{\mu} + u_m^*}$



http://nce.ads.uga.edu/wiki/doku.php?id=readme.predf90

# Output from predf90

SNP_predictions

| Animal ID | SNP call rate | Indirect Predictions |
|---|---|---|
| UGA50014 | 1.00 | 0.17414457 |
| UGA50016 | 1.00 | 0.72332874E-01 |
| UGA50042 | 1.00 | 1.0016705 |
| UGA50058 | 1.00 | 0.17190497 |
| UGA50060 | 1.00 | 0.98674759E-01 |
| UGA50065 | 1.00 | -0.60623702E-01 |
| UGA50073 | 1.00 | -0.17860851 |
| UGA50077 | 1.00 | -0.21597147 |
| UGA50079 | 1.00 | -0.69586390 |
| UGA50084 | 1.00 | 1.0600574 |
| UGA50085 | 1.00 | -0.28602412 |
| UGA50088 | 1.00 | -0.12758011 |

`predf90` can also compute accuracy of indirect predictions

OPTION snp_p_value #in `blupf90+`
OPTION snp_var #in `postGSf90`
--acc #in `predf90`

Garcia et al. Genetics Selection Evolution (2022) 54:66
https://doi.org/10.1186/s12711-022-00752-4


RESEARCH ARTICLE Open Access

Theoretical accuracy for indirect predictions based on SNP effects from single-step GBLUP

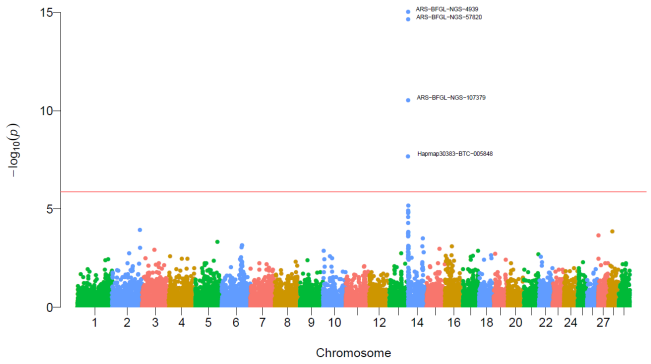
Andre Garcia[1*], Ignacio Aguilar[2], Andres Legarra[3], Shogo Tsuruta[1], Ignacy Misztal[1] and Daniela Lourenco[1]

# 2) Genome-wide Association Studies

# Current standard for GWAS

- Single marker regression with **G** to compensate for relationships
  - $\mathbf{y} = \mathbf{Xb} + \mathbf{z}_i\mathbf{a}_i + \mathbf{u} + \mathbf{e}$
    - **z:** gene content {0,1,2}
    - **a:** SNP effect

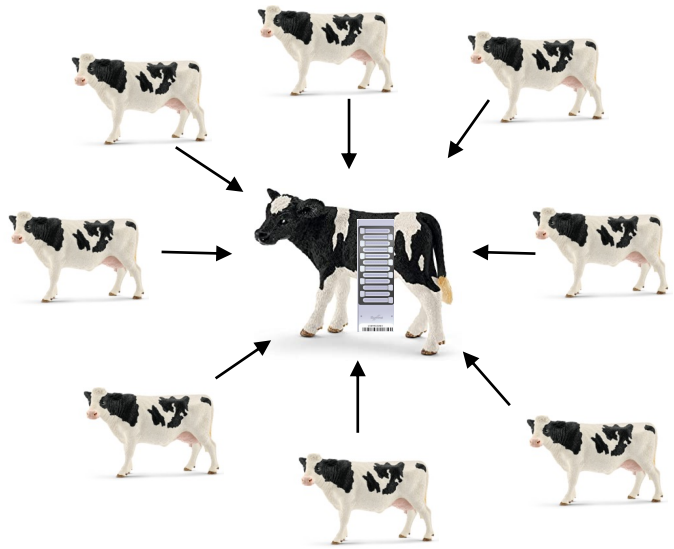
- Estimate SNP effects
- Get p-values as $pval_i = 2\left(1 - \Phi\left(\left|\frac{\hat{a}_i}{sd(\hat{a}_i)}\right|\right)\right)$
- Apply Bonferroni to correct for multiple testing




- **Assumption: Genotyped individuals have phenotypes**

# GWAS in livestock populations

- Most animals are non-genotyped
- Animals may not have phenotypes
- Some traits are sex-limited
  - milk, fat, protein



- Single marker regression
  - Only genotyped animals with phenotypes
  - Deregressed EBV

- Need a method that fits the livestock data
  - ssGWAS

# Single-step GWAS (historical)

SNP effects

GEBVs

$$\hat{\mathbf{a}} = \alpha b \frac{1}{2\sum p_i(1-p_i)} \mathbf{Z}'\mathbf{G}^{-1}\hat{\mathbf{u}}$$

VanRaden 2008
Stranden and Garrick 2009
Wang et al. 2012

a) Quadratic SNP variance (Falconer & Mackay, 1996)

$$d_i = \hat{a}_i^2 2p_i(1-p_i)$$

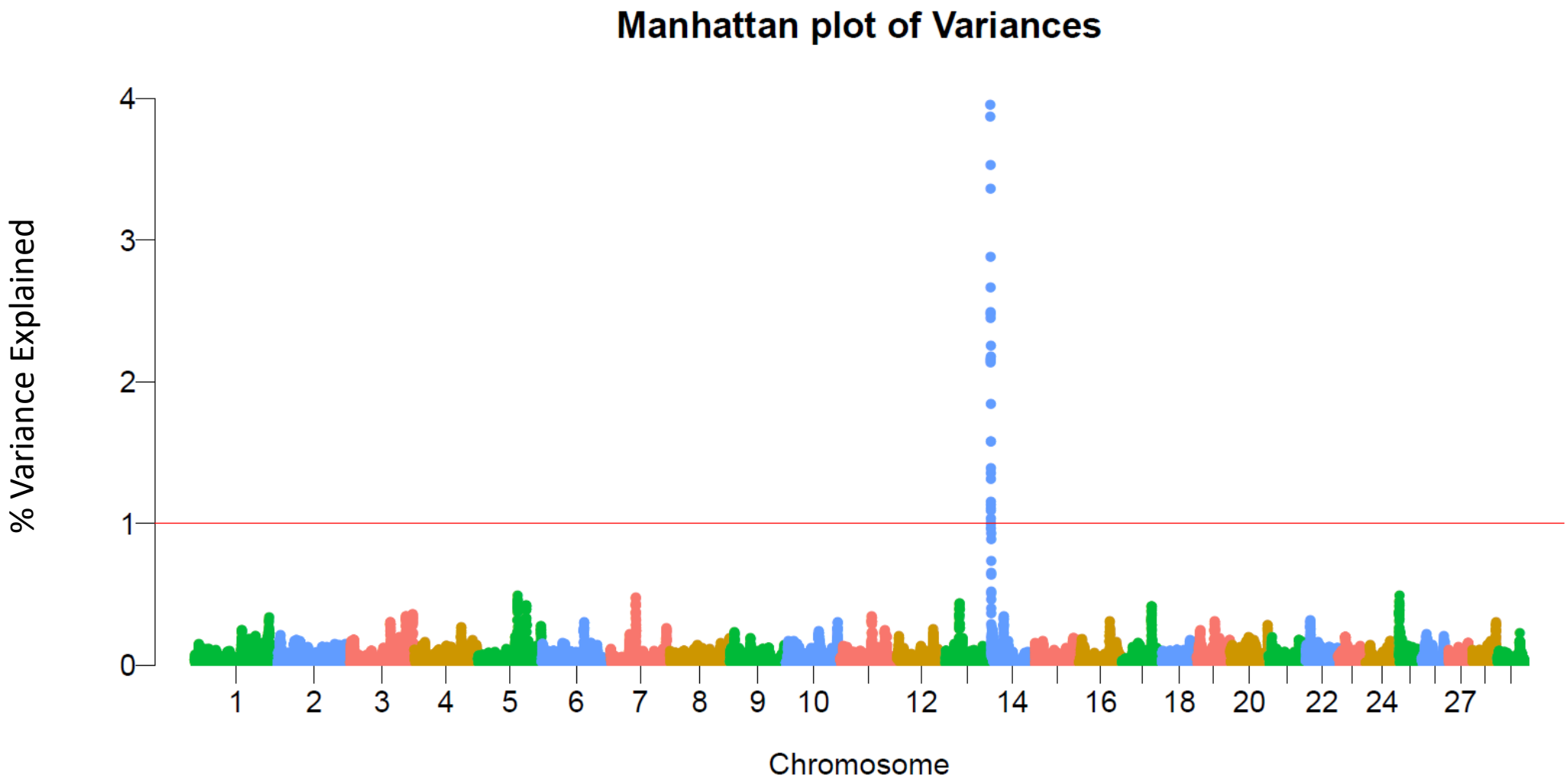
b) NonlinearA SNP variance (VanRaden, 2008)

$$d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a})}-2}$$

# Single-step GWAS

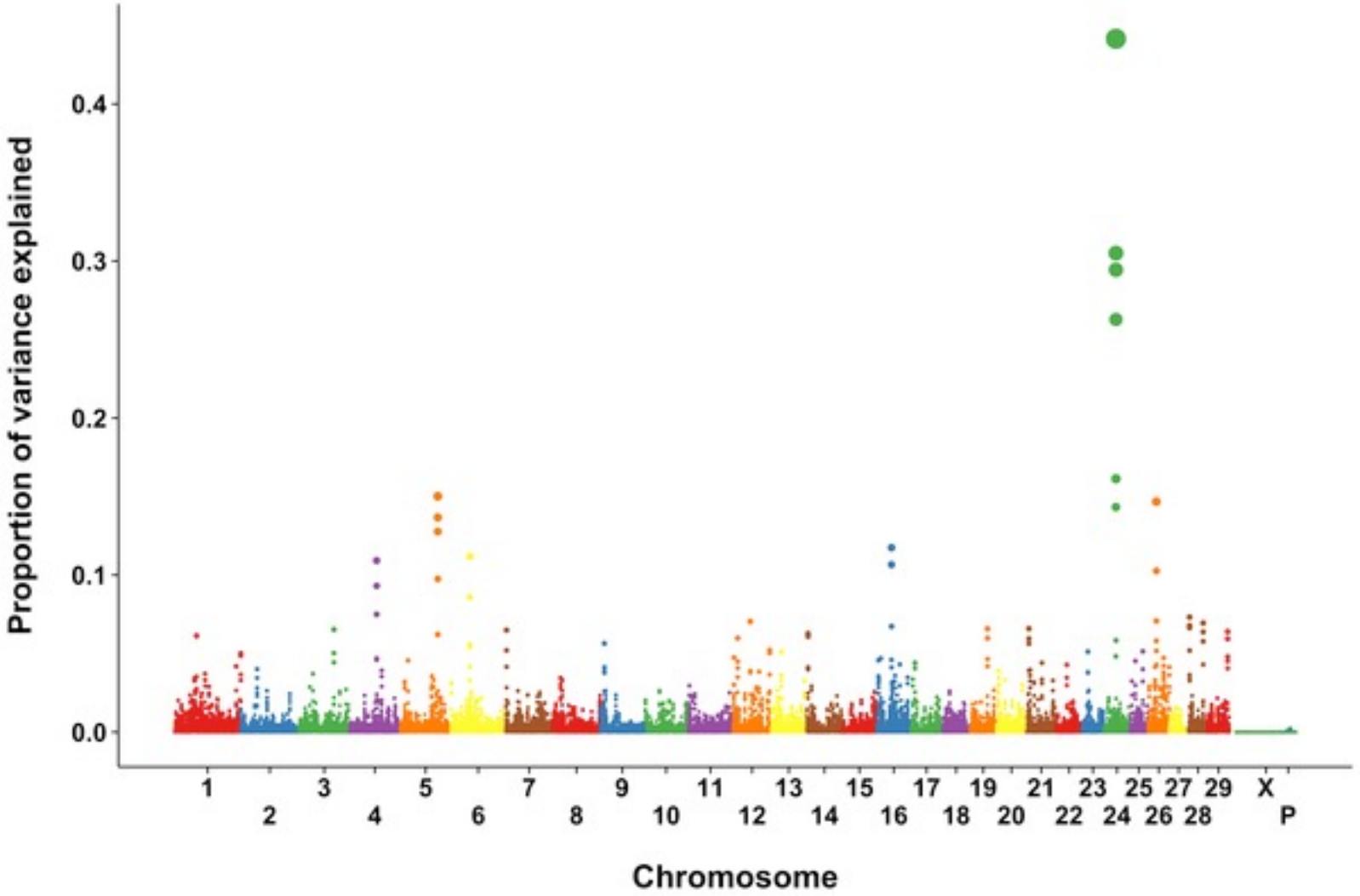
Fat – US Holsteins

No P-value!!!



# Single-step GWAS

**Figure 2. Proportion of SNP variance explained by 5-SNP moving windows for rectal temperature from a single-step GBLUP analysis**




No P-value!!!

PLOS ONE

Dikmen S, Cole JB, Null DJ, Hansen PJ (2013) Genome-Wide Association Mapping for Identification of Quantitative Trait Loci for Rectal Temperature during Heat Stress in Holstein Cattle. PLOS ONE 8(7): e69202. https://doi.org/10.1371/journal.pone.0069202
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069202

# Can we have p-values in ssGWAS?

Gualdrón Duarte *et al. BMC Bioinformatics* 2014, **15**:246
http://www.biomedcentral.com/1471-2105/15/246

BMC Bioinformatics

METHODOLOGY ARTICLE Open Access

## Rapid screening for phenotype-genotype associations by linear transformations of genomic evaluations

Jose L Gualdrón Duarte[1], Rodolfo JC Cantet[1], Ronald O Bates[2], Catherine W Ernst[2], Nancy E Raney[2] and Juan P Steibel[2,3*]

## Genome-Wide Association Analyses Based on Broadly Different Specifications for Prior Distributions, Genomic Windows, and Estimation Methods

**Chunyu Chen,[1] Juan P. Steibel, and Robert J. Tempelman**
Department of Animal Science, Michigan State University, East Lansing, Michigan 48824
ORCID ID: 0000-0002-7833-6730 (R.J.T.)

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics
doi: 10.1111/age.12378

## Meta-analysis of genome-wide association from genomic prediction models

Y. L. Bernal Rubio*†, J. L. Gualdrón Duarte*, R. O. Bates*, C. W. Ernst*, D. Nonneman‡, G. A. Rohrer‡, A. King‡, S. D. Shackelford‡, T. L. Wheeler‡, R. J. C. Cantet†§ and J. P. Steibel*¶

J. Dairy Sci. 101:3140–3154
https://doi.org/10.3168/jds.2017-13364


## Genome-wide association analyses based on a multiple-trait approach for modeling feed efficiency

Y. Lu,* M. J. Vandehaar,* D. M. Spurlock,† K. A. Weigel,‡ L. E. Armentano,‡ E. E. Connor,§ M. Coffey,# R. F. Veerkamp,‖ Y. de Haas,‖ C. R. Staples,¶ Z. Wang,** M. D. Hanigan,†† and R. J. Tempelman*[1]

Aguilar *et al. Genet Sel Evol* (2019) 51:28
https://doi.org/10.1186/s12711-019-0469-3

GSE Genetics Selection Evolution

SHORT COMMUNICATION Open Access

## Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle

Ignacio Aguilar[1], Andres Legarra[2*], Fernando Cardoso[3,4], Yutaka Masuda[5], Daniela Lourenco[5] and Ignacy Misztal[5]

# P-values in ssGWAS

1) Factorize and Invert LHS of ssGBLUP with YAMS (Masuda et al., 2014)

2) Solve the MME for $\begin{bmatrix}\hat{\boldsymbol{\beta}}\\ \hat{\mathbf{u}}\end{bmatrix}$ using the sparse Cholesky factor

**blupf90+**

Ignacio Aguilar

3) Extract coefficients for genotyped animals ($\mathbf{C}^{u_2u_2}$) from LHS$^{-1}$

4) Obtain individual prediction error variance of SNP effects:

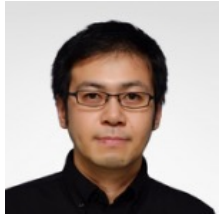
$$Var(\hat{a}_i) = \alpha b \frac{1}{2\sum p_i(1-p_i)} \mathbf{z}_i' \mathbf{G}^{-1}(\mathbf{G}\sigma_u^2 - \mathbf{C}^{u_2u_2})\mathbf{G}^{-1}\mathbf{z}_i \frac{1}{2\sum p_i(1-p_i)} \alpha b$$

(Gualdron-Duarte et al., 2014)

**postGSf90**

Andres Legarra

5) Backsolve GEBV to SNP effects ($\hat{a}$): $\hat{a} = \alpha b \frac{1}{2\sum p_i q_i} \mathbf{Z}'\mathbf{G}^{-1}\hat{u}$



Yutaka Masuda

6) p-value$_i$ $= 2\left(1 - \Phi\left(\left|\frac{\hat{a}_i}{sd(\hat{a}_i)}\right|\right)\right)$

$\Phi$ is the cumulative standard normal function

# How to run ssGWAS with p-values in BLUPF90

- After renumf90 and preGSf90 to save clean files:
  - blupf90+ to estimate GEBV
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION saveGInverse`
    - `OPTION saveA22Inverse`
    - `OPTION snp_p_value`
  - postGSf90 to backsolve GEBV to SNP effect
    - `OPTION SNP_file snp.dat_clean`
    - `OPTION map_file mrkmap.txt_clean`
    - `OPTION readGInverse`
    - `OPTION readA22Inverse`
    - `OPTION snp_p_value`
    - `OPTION windows_variance X #if need variance explained by X SNP`

# Output from postGSf90

`chrsnp_pval`

contains data to create plot by GNUPLOT

- 1: trait
- 2: effect
- 3: -log10(p-value)
- 4: SNP
- 5: Chromosome
- 6: Position in bp

Pft1e2.gnuplot

Pft1e2.R

`chrsnp`

contains data to create plot by GNUPLOT

- 1: trait
- 2: effect
- 3: values of SNP effects to use in Manhattan plots → [abs(SNP_i)/var(SNP)]
- 4: SNP
- 5: Chromosome
- 6: Position

Sft1e2.gnuplot

Sft1e2.R

# Output from postGSf90

```
snp_sol
```

contains solutions of SNP and weights

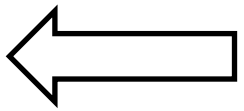
- 1: trait
- 2: effect
- 3: SNP
- 4: Chromosome
- 5: Position
- 6: SNP solution
- 7: weight

if `OPTION windows_variance` is used

- 8: variance explained by *n* adjacents SNP.

if `OPTION snp_p_value` is used

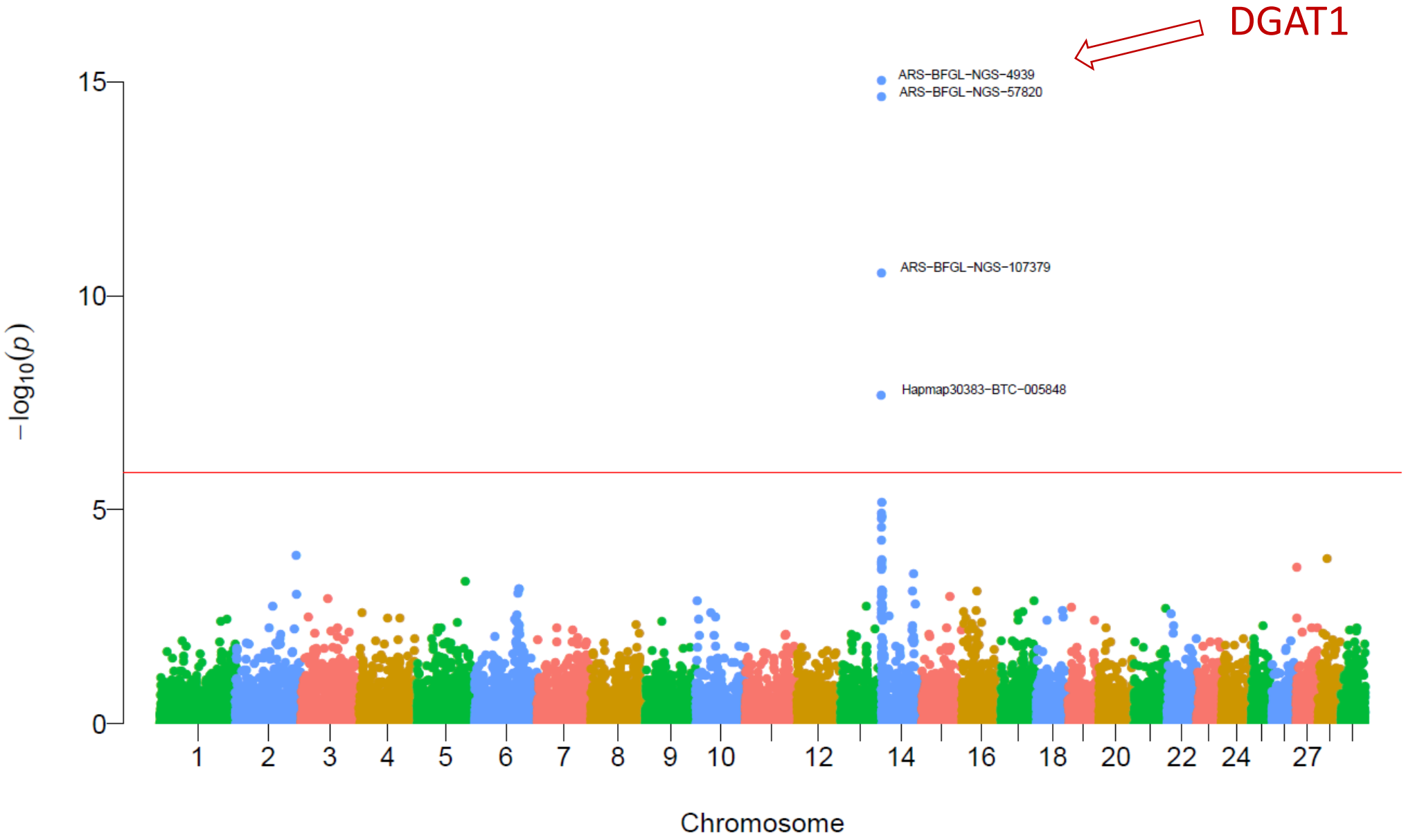
- 9: variance of the SNP solution (used to compute the p-value)



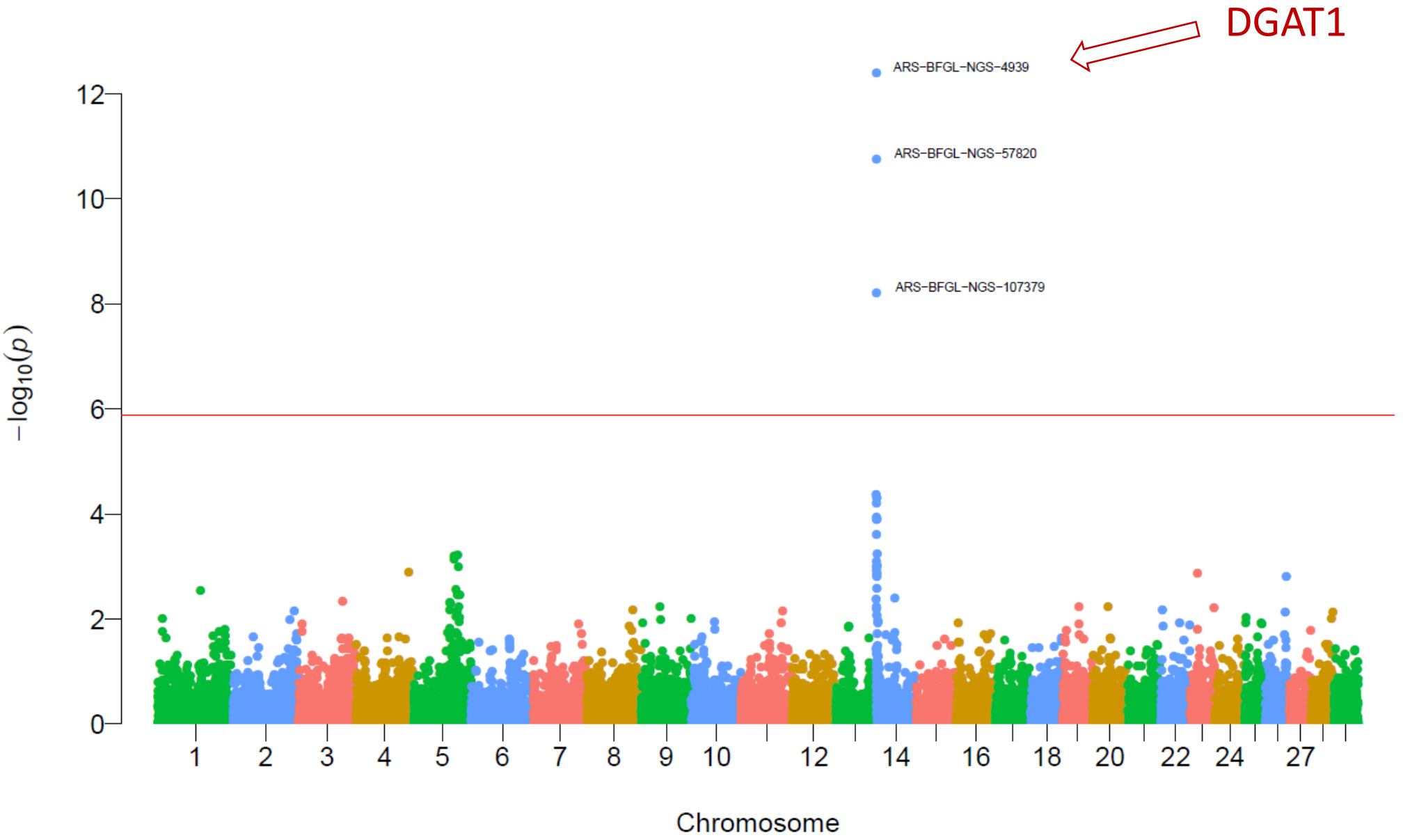
# P-values in ssGWAS for US Holsteins

- US HOL 2009 data: milk, fat, protein
- Single-trait models
  - 10k genotyped bulls
  - 752k records for 100k daughters
  - 303k animals in ped

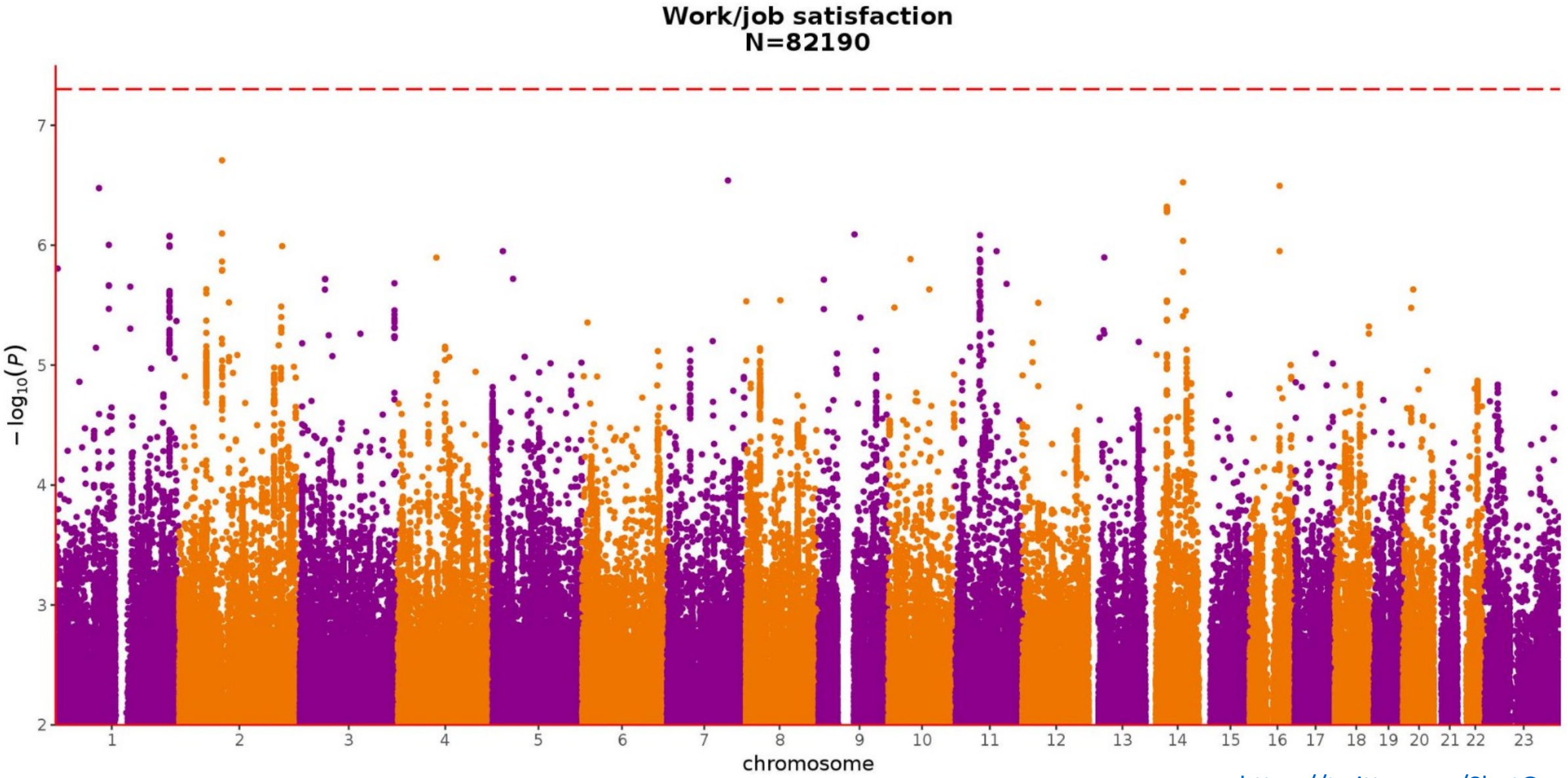
# P-values in ssGWAS - Milk



# P-values in ssGWAS - Fat



# Non-significant hits



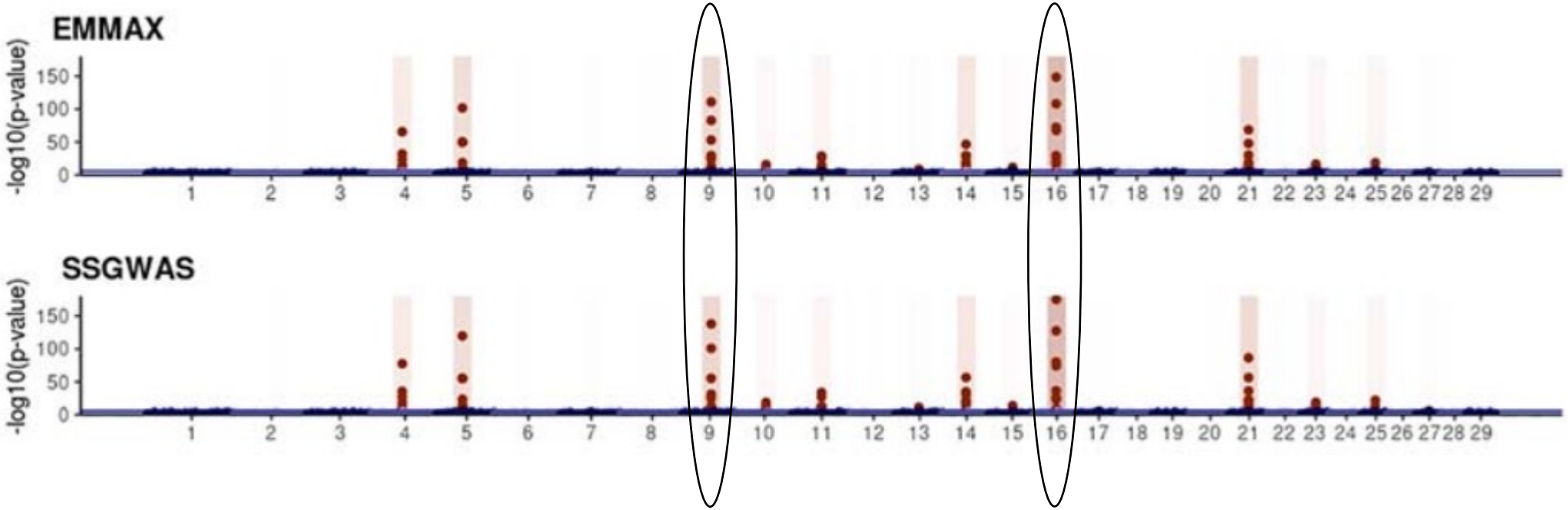

https://twitter.com/SbotGwa

# ssGWAS vs. EMMAX

- Simulated population (1 QTN per CHR)

14k genotyped sires
Deregressed EBV
(10 daughters)

14k genotyped sires
500k Pedigree
250k phenotypes




| Association | EMMAX (Khang et al., 2010) | ssGWAS (Aguilar et al., 2019) |
|---|---|---|
| True Positive | 55.2[a] (3.7) | 61.6[a] (8.7) |
| False Positive | 0.0 | 0.0 |

Mancin et al. (2021)

# postGSf90 options

http://nce.ads.uga.edu/wiki/doku.php?id=readme.pregsf90

```
OPTION Manhattan_plot
```

Uses GNUPLOT to plot the Manhattan plot (SNP effects) for each trait and correlated effect.

```
OPTION Manhattan_plot_R
```

Uses R to plot the Manhattan plot (SNP effects) for each trait and correlated effect.
`pdf` images are created: *manplot_St1e2.pdf*, but other formats can be specified.
Note: *t1e2* corresponds to trait 1, effect 2.

```
OPTION Manhattan_plot_R_format <format>
```

Control the format type to create images in R
`format` values accepted:

- pdf (default)
- png
- tif

```
OPTION plotsnp <n>
```

Control the values of SNP effects to use in Manhattan plots

- 1: plot regular SNP effects: abs(val)
- 2: plot standardized SNP effects: abs(val/sd) (default)

# nce.ads.uga.edu/wiki

start

## BLUPF90 Family of Programs

**Now with support for genomic selection**

*Ignacy Misztal and collaborators, University of Georgia*

BLUPF90 family of programs is a collection of software in Fortran 90/95 for mixed model computations in animal breeding. The goal of the software is to be as simple as with a matrix package and as efficient as in a programming language. For general description, see a paper from the CCB'99 workshop or see a paper on BGF90 at 7th WCGALP.

For variance component estimation, the family offers choices for simple and complicated models; see paper "Reliable computing in estimation of variance components" . From 2009 the programs are successively modified for genomic selection using a single-step approach (or ssGBLUP) by Ignacio Aguilar and Shogo Tsuruta.

For support, join blupf90 group at yahoo.com.

**Troubleshooting**

If the software crashes with segmentation fault, please change settings in your operating system. See FAQ:Segmentation fault for details. Also, The FAQ pages provide useful suggestions and solutions.

## Headline

- History
- Modules
- Condition of use
- Distribution / Download
- Documentation / Manual / Tutorial
- Application program details
- Support
- FAQ
- Tricks / Tips
- To Do
- Courses
- Sample data
- Undocumented options