Project background

Multi-omics of Celiac in Down syndrome

- Celiac disease (CD) is highly influenced by genetics.
- Most (but not all!) genetic risk for CD is conferred by variation in the HLA-DQ region (chr6, genes HLA-DQA1 and HLA-DQB1).
- Sharp et al., 2019 developed a genetic risk score (GRS) for Celiac disease that accurately predicts CD using a combination of *both* HLA-DQ genotypes *and* non-HLA-DQ SNPs.

Received: 10 December 2019 | First decision: 5 January 2020 | Accepted: 10 May 2020
DOI: 10.1111/apt.15826
APT Alimentary Pharmacology & Therapeutics WILEY

A single nucleotide polymorphism genetic risk score to aid diagnosis of coeliac disease: a pilot study in clinical care

Seth A. Sharp[1] | Samuel E. Jones[1] | Robert A. Kimmitt[2] | Michael N. Weedon[1] | Anne M. Halpin[3] | Andrew R. Wood[1] | Robin N. Beaumont[1] | Seema King[4] | David A. van Heel[5] | Patricia M. Campbell[3] | William A. Hagopian[6] | Justine M. Turner[4] | Richard A Oram[1,2]

[1]Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK
[2]Royal Devon & Exeter, NHS Foundation Trust, Exeter, UK
[3]Division of Nephrology and Transplant Immunology, University of Alberta, Edmonton, AB, Canada
[4]Faculty of Medicine and Dentistry, Pediatrics, University of Alberta, Edmonton, AB, Canada
[5]Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK
[6]Pacific Northwest Research Institute, Seattle, WA, USA

Correspondence
Richard A. Oram, RILD Level 3, University of Exeter Medical School, Royal Devon and Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW UK.
Email: r.oram@exeter.ac.uk

Funding information
This research was supported by funding from the Women and Children's Health Research Institute, University of Alberta. R.A.O. is supported by a Diabetes UK Harry Keen Fellowship (16/0005529). S.S. is supported by a Diabetes UK PhD studentship (17/0005757). W.A.H. is supported by Grant U01DK063829. M.N.W. is supported by the Wellcome Trust Institutional Support Fund (WT097835MF). The funding was provided by the Wellcome Trust (084743 to DAvH), by grants from the Coeliac Disease Consortium and an Innovative Cluster approved by the Netherlands Genomics Initiative. This research utilised resources supported by National Institutes of Health grant U01-DK062418.

Summary

Background: Single nucleotide polymorphism–based genetic risk scores (GRS) model genetic risk as a continuum and can discriminate coeliac disease but have not been validated in clinic. Human leukocyte antigen (HLA) DQ gene testing is available in clinic but does not include non-HLA attributed risk and is limited by discrete risk stratification.

Aims: To accurately characterise both HLA and non-HLA coeliac disease genetic risk as a single nucleotide polymorphism–based GRS and evaluate diagnostic utility.

Methods: We developed a 42 single nucleotide polymorphism coeliac disease GRS from a European case-control study (12 041 cases vs 12 228 controls) using HLA-DQ imputation and published genome-wide association studies. We validated the GRS in UK Biobank (1237 cases) and developed direct genotyping assays. We tested the coeliac disease GRS in a pilot clinical cohort of 128 children presenting with suspected coeliac disease.

Results: The GRS was more discriminative of coeliac disease than HLA-DQ stratification in UK Biobank (receiver operating characteristic area under the curve [ROC-AUC] = 0.88 [95% CIs: 0.87-0.89] vs 0.82 [95% CIs: 0.80-0.83]). We demonstrated similar discrimination in the pilot clinical cohort (114 cases vs 40 controls, ROC-AUC = 0.84 [95% CIs: 0.76-0.91]). As a rule-out test, no children with coeliac disease in the clinical cohort had a GRS below 38th population centile.

Conclusions: A single nucleotide polymorphism–based GRS may offer more effective and cost-efficient testing of coeliac disease genetic risk in comparison to HLA-DQ stratification. As a comparatively inexpensive test it could facilitate non-invasive coeliac disease diagnosis but needs detailed assessment in the context of other diagnostic tests and against current diagnostic algorithms.

Multi-omics of Celiac in Down syndrome

- At the Linda Crnic Institute for Down Syndrome, we applied the Sharp et al. GRS to a cohort of individuals with **Trisomy 21 +/- Celiac disease.**
- We evaluated…
  - How does the GRS perform in individuals with Down syndrome (DS), as compared to the typical population?
  - How do the individual components in the GRS relate to CD in DS?
  - Can we improve the performance of this CD GRS in DS with the addition of protein biomarkers?

Title: Multi-Omics Assessment of Genetic Risk for Celiac Disease in Down Syndrome

Authors: Marisa Gallant **Stahl**[1,2] MD MSCS, Jessica R **Shaw**[2], Neetha Paul **Eduthan**[2], Angela L **Rachubinski**[2], Keith P **Smith**[2], Belinda Enriquez **Estrada**[2], Matthew D **Galbraith**[2,3], Ronald J **Sokol**[1] MD, Sameer **Chavan**[4], Laura Ann **Leaton**[4,5], Katherine M **Kichula**[4,5], Paul J **Norman**[4,5] PhD, Jill M **Norris**[6] PhD MPH, Edwin **Liu**[1] MD & Joaquin M **Espinosa**[2,3] PhD

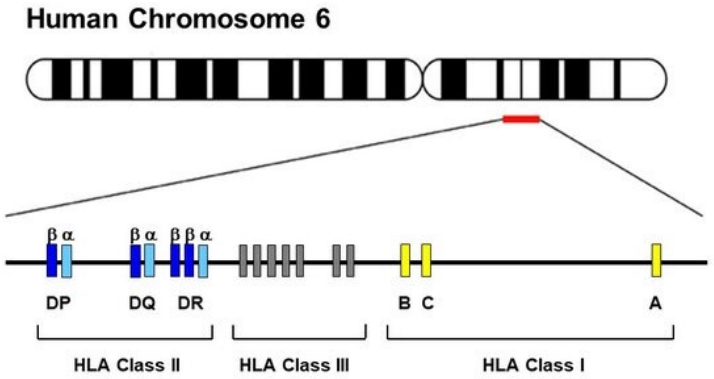
Affiliations:

1. Digestive Health Institute, Children's Hospital Colorado, Department of Pediatrics, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States.
2. Linda Crnic Institute for Down Syndrome, University of Colorado, Aurora, CO
3. Department of Pharmacology, University of Colorado Anschutz Medical Campus.
4. Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
5. Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA
6. Department of Epidemiology, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

JPGN
Journal of Pediatric Gastroenterology and Nutrition

Primer on HLA genetics of Celiac disease

Pairs of HLA-DQ alleles form HLA-DQ heterodimers



Alleles encoding CD-associated heterodimers

Heterodimer	DQA1* allele	DQB1* allele
DQ2.5	*05*	*0201*
DQ2.2	*0201*	*0202*
DQ8	*03*	*0302*
DQ7.5	*05*	*03*

Subset of many possible HLA alleles at DQA1 and DQB1

DQ2.5
α-chain DQA1*05 Dosage>=1
β-chain DQB1*02:01 Dosage>=1

DQ2.2
α-chain DQA1*02:01
β-chain DQB1*02:02

DQ8
α-chain DQA1*03
β-chain DQB1*03:02

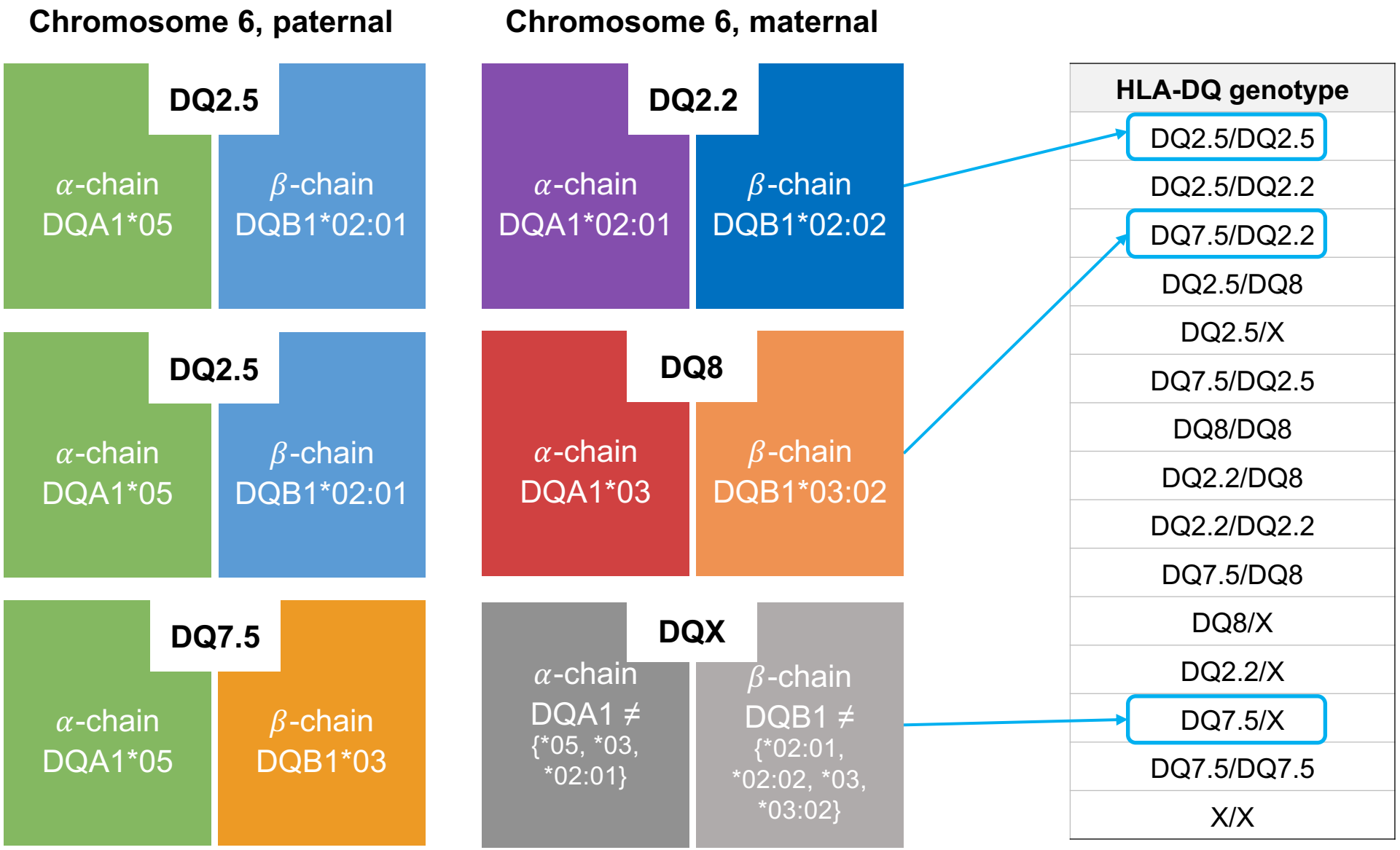
DQ7.5
α-chain DQA1*05
β-chain DQB1*03

DQX
α-chain DQA1 ≠ {*05, *03, *02:01}
β-chain DQB1 ≠ {*02:01, *02:02, *03, *03:02}

Figure adapted from:
Megiorni, Francesca, and Antonio Pizzuti. "HLA-DQA1 and HLA-DQB1 in Celiac Disease Predisposition: Practical Implications of the HLA Molecular Typing." *Journal of Biomedical Science*, vol. 19, no. 1, 2012, p. 88. *DOI.org (Crossref)*, https://doi.org/10.1186/1423-0127-19-88.

Pairs of HLA-DQ heterodimers form HLA-DQ genotypes

For example:



HLA-DQ genotype
DQ2.5/DQ2.5
DQ2.5/DQ2.2
DQ7.5/DQ2.2
DQ2.5/DQ8
DQ2.5/X
DQ7.5/DQ2.5
DQ8/DQ8
DQ2.2/DQ8
DQ2.2/DQ2.2
DQ7.5/DQ8
DQ8/X
DQ2.2/X
DQ7.5/X
DQ7.5/DQ7.5
X/X

Analysis overview

GRS analysis overview

		Key outputs
GRS	**Logistic regression:** Celiac (0/1) ~ GRS	• ROC curve • AUC for comparison to Sharp et al., 2019.
Additive genetic models	**Logistic regression:** Celiac (0/1) ~ HLA-DQ **genotype** dosage (0/1) + PC1 + … + PC5	• OR barplots • Frequency stacked barplots • Volcano plot • Comparison to Sharp et al., 2019
	Logistic regression: Celiac (0/1) ~ GRS risk **allele** dosage (0/1/2) + PC1 + … + PC5	• OR barplots • Volcano plot • Comparison to Sharp et al., 2019
	Logistic regression: Celiac (0/1) ~ HLA-DQ **allele group** dosage (0/1/2) + PC1 + … + PC5	• OR barplots • Volcano plot • Comparison to Sharp et al., 2019

Analysis data pipeline
GRS component: Non-HLA-DQ SNPs

Non-HLA-DQ, non-chromosome 21 SNPs captured by Illumina MEGA

Illumina MEGA genome-wide SNP data (*.bed/*.bim/*.fam)

Extract non-HLA-DQ, non-chr21 GRS SNPs
R/bash/UCSC Genome Browser/Plink

Non-HLA-DQ, non-chr21 GRS SNP genotypes

Infer strand and derive GRS allele dosages
R

Non-HLA-DQ, non-chr21 GRS SNP dosages (0/1/2)

Chromosome 21 SNP captured by Illumina MEGA

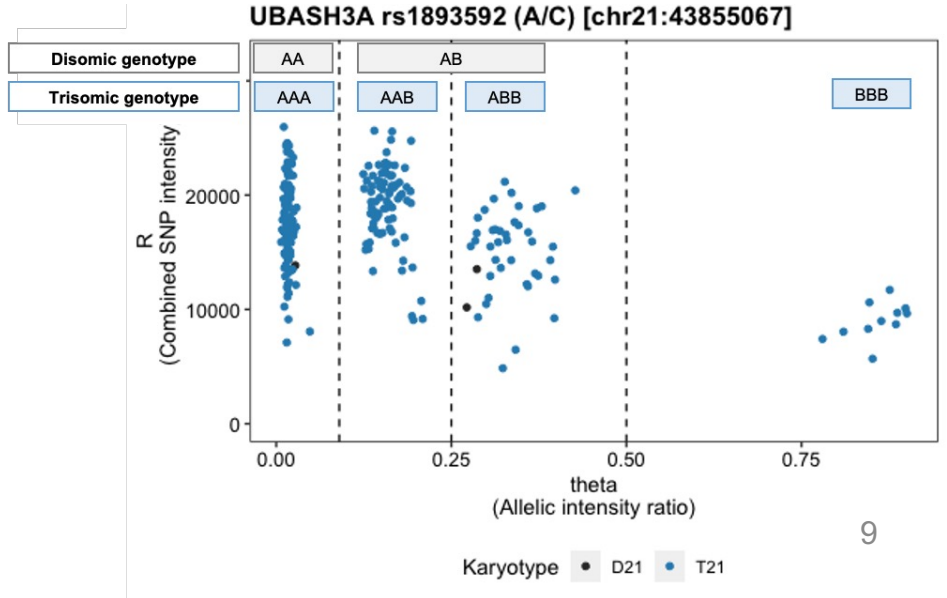
Genome-wide allelic intensity data (*.idat)

Extract chr21 SNP (rs1893592)
R/illuminaio/ bash

chr21 SNP intensity data (*.idat)

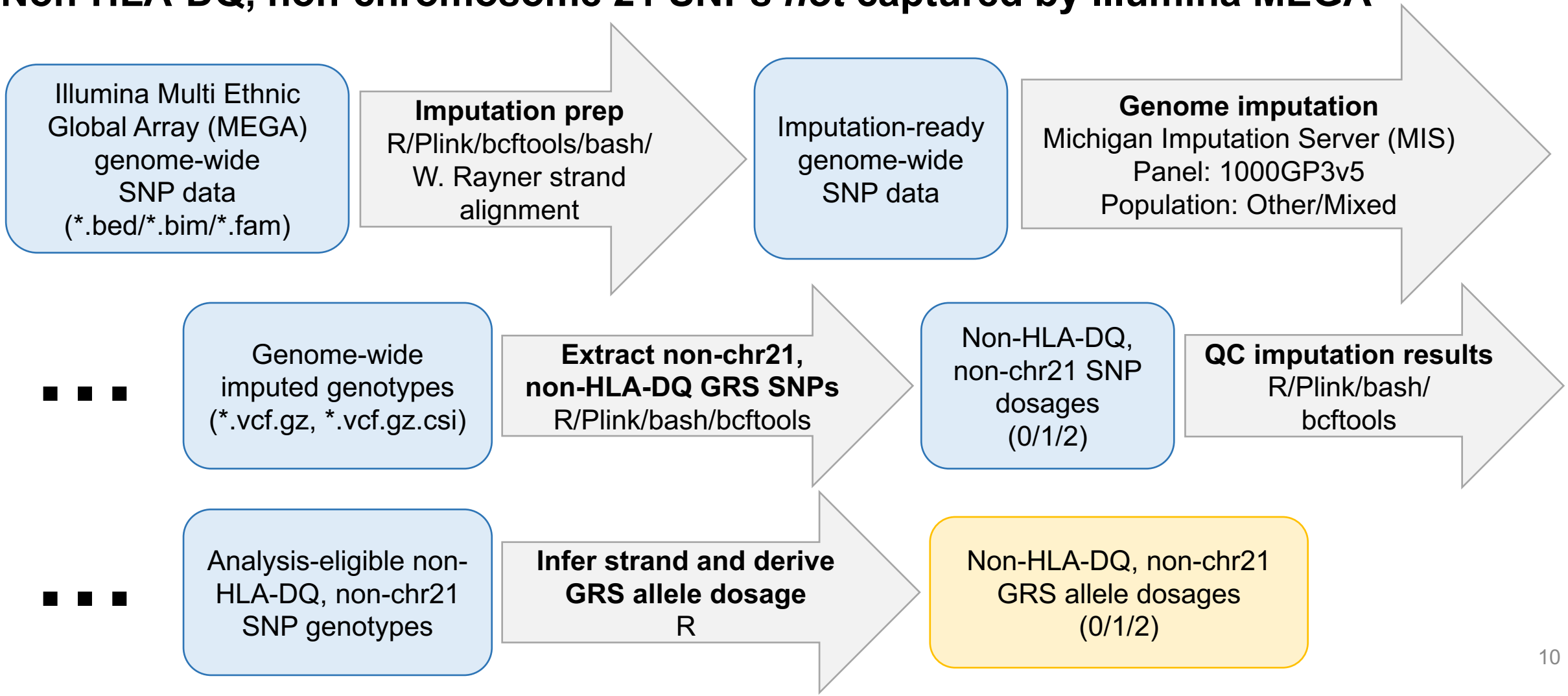
Manual genotype clustering
R

chr21 SNP dosages (0/1/2/**3**)



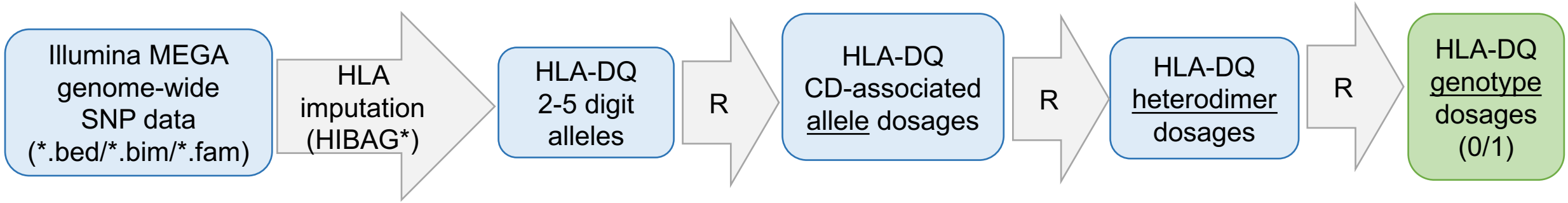
Analysis data pipeline
GRS component: Non-HLA-DQ SNPs (*continued*)

Non-HLA-DQ, non-chromosome 21 SNPs *not* captured by Illumina MEGA



Analysis data pipeline
GRS component: HLA-DQ genotypes

HLA-DQ genotypes *not* captured by Illumina MEGA



*Performed by Paul Norman et al.

Analysis data pipeline
Celiac GRS calculation

- Weight for each HLA-DQ genotype given in Sharp et al., 2019, Table S2
- Weights for each non-HLA-DQ SNP genotype given in Sharp et al., 2019 Table S3
- Score allele dosage extracted from MEGA genotype data (dosage 0/1/2), extracted from genome-wide imputation results (dosage 0/1/2), or derived after manual genotype clustering to account for trisomy 21 (dosage 0/1/2/3)

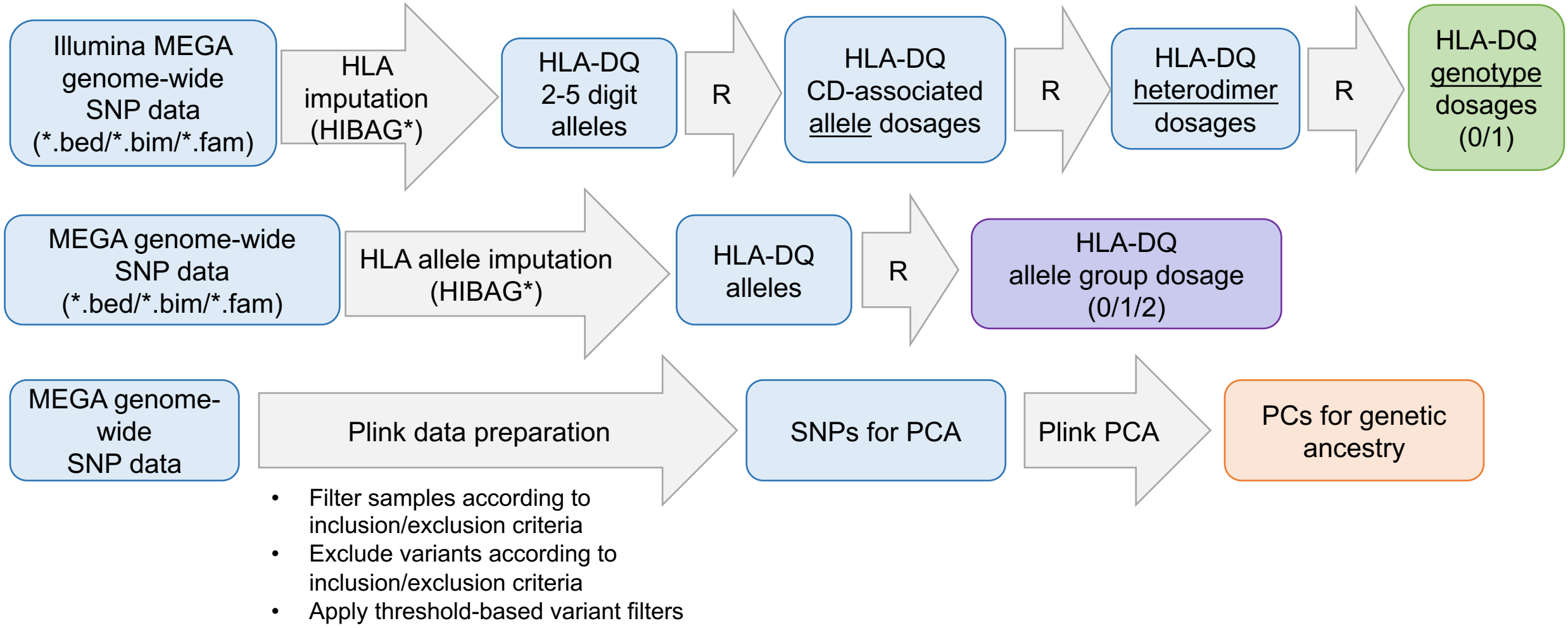
GRS = 4.97 x DQ2.5/DQ2.5 dosage (0/1) + 4.96 x DQ2.5/DQ2.2 dosage (0/1) + … + 0.0 x X/X dosage (0/1) +

0.425 x rs4143332 (C) dosage (0/1/2) + 0.372 x rs3128927 (T) dosage (0/1/2) + … +

-0.128 x rs1893592 (C) dosage (0/1/2/3) + … +

-0.083 x rs10167650 (G) dosage (0/1/2)

Analysis data pipeline
Additional data for variant-level analyses



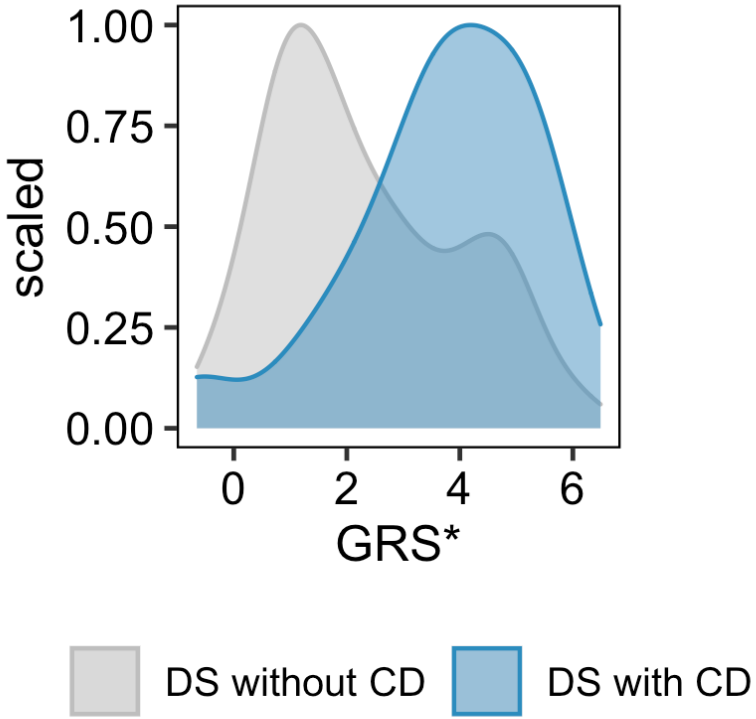
*Performed by Paul Norman et al.

Selected results/figures

GRS performance in DS (GRS version: Sharp et al., 2022)

Celiac GRS* in DS +/- CD

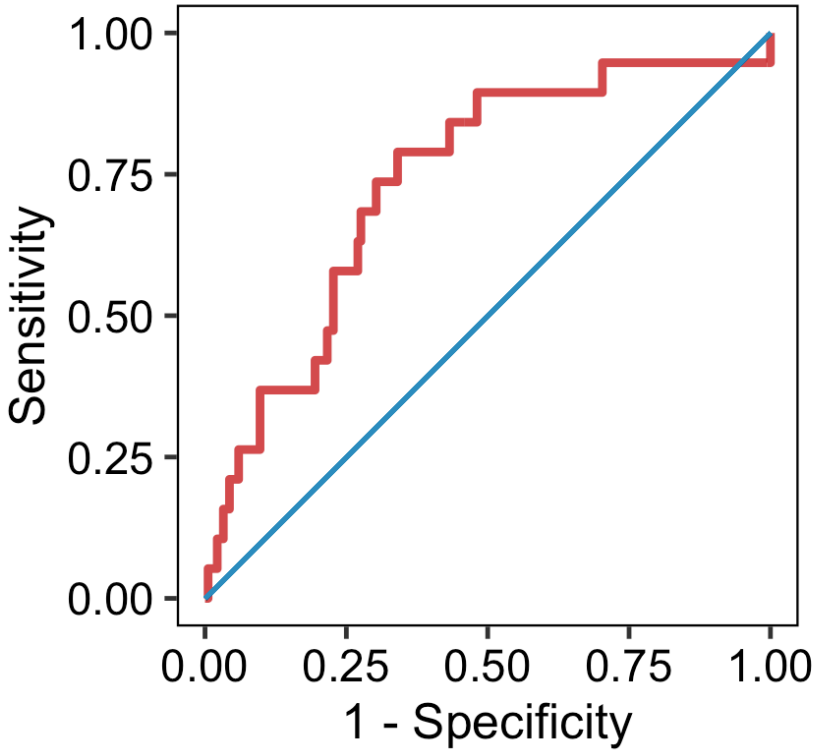
OR = 1.62 (1.22, 2.22)
P<0.0014



*Sharp et al., 2022

Predictive accuracy of GRS* in DS

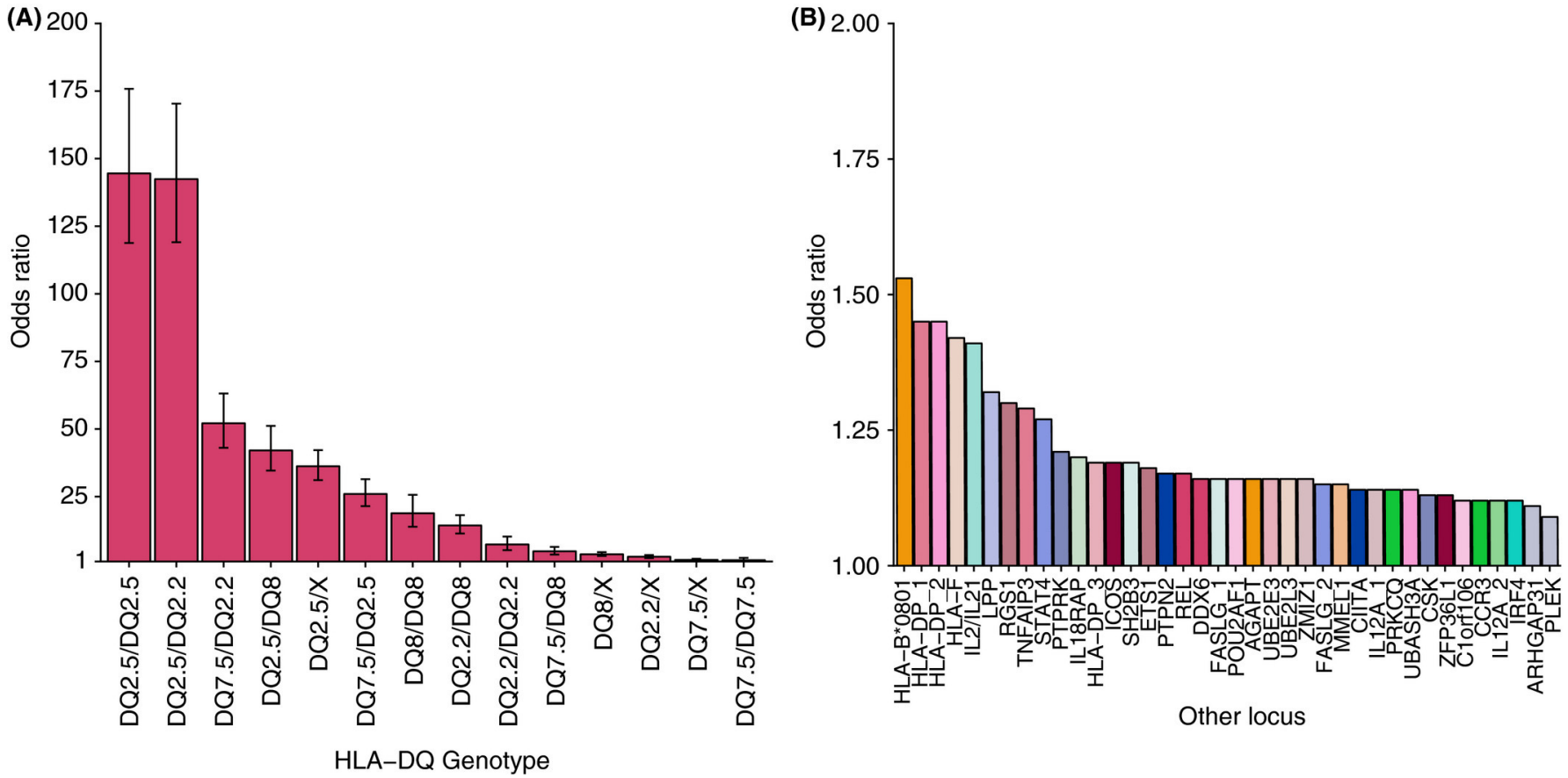
Area under the curve: 0.7354



Logistic: Celiac ~ GRS*
*Sharp et al., 2022

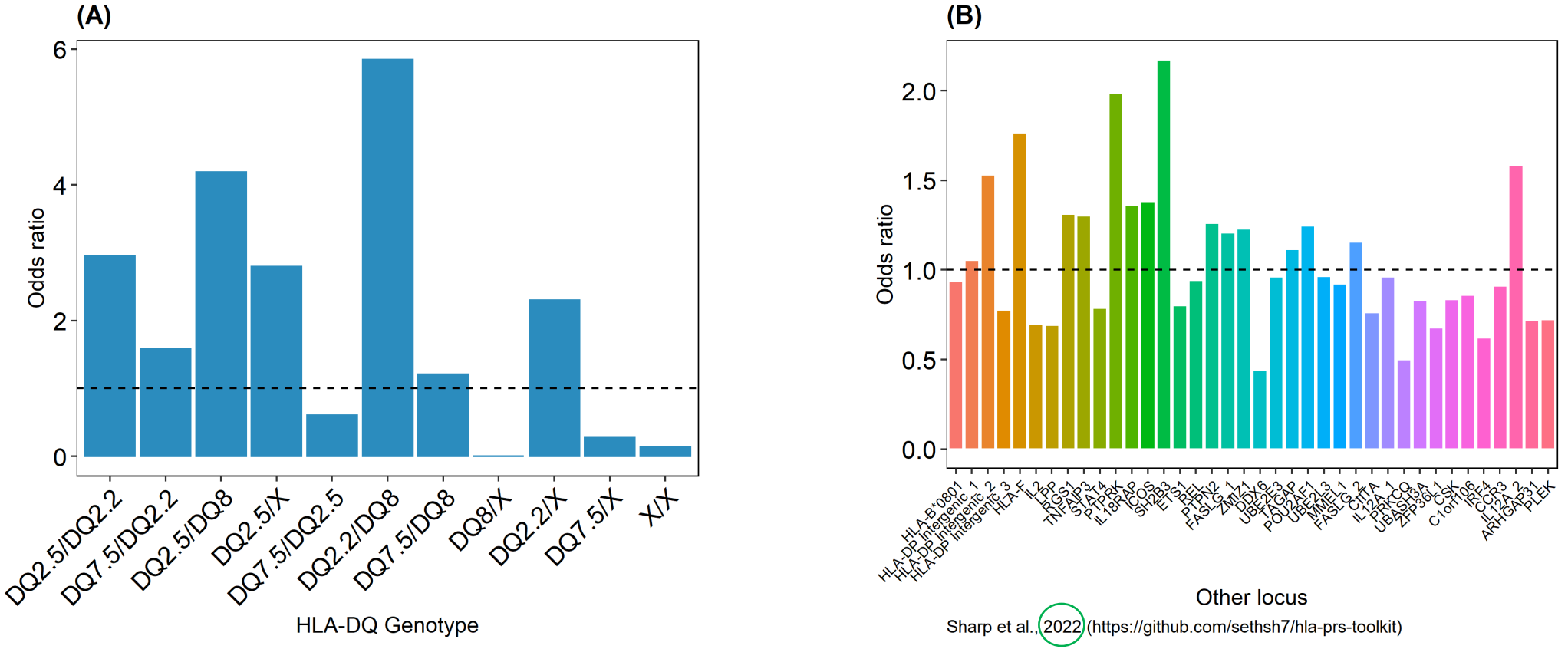
Figure 1 of Sharp et al., 2019

A single nucleotide polymorphism genetic risk score to aid diagnosis of coeliac disease: a pilot study in clinical care

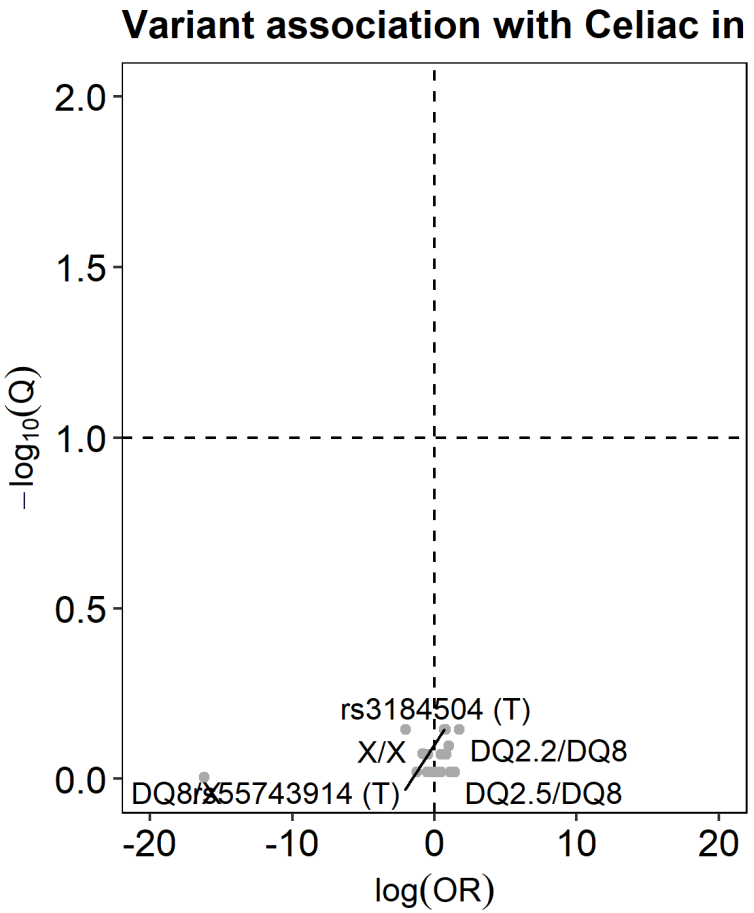


Aliment Pharmacol Ther, Volume: 52, Issue: 7, Pages: 1165-1173, First published: 13 August 2020, DOI: (10.1111/apt.15826)

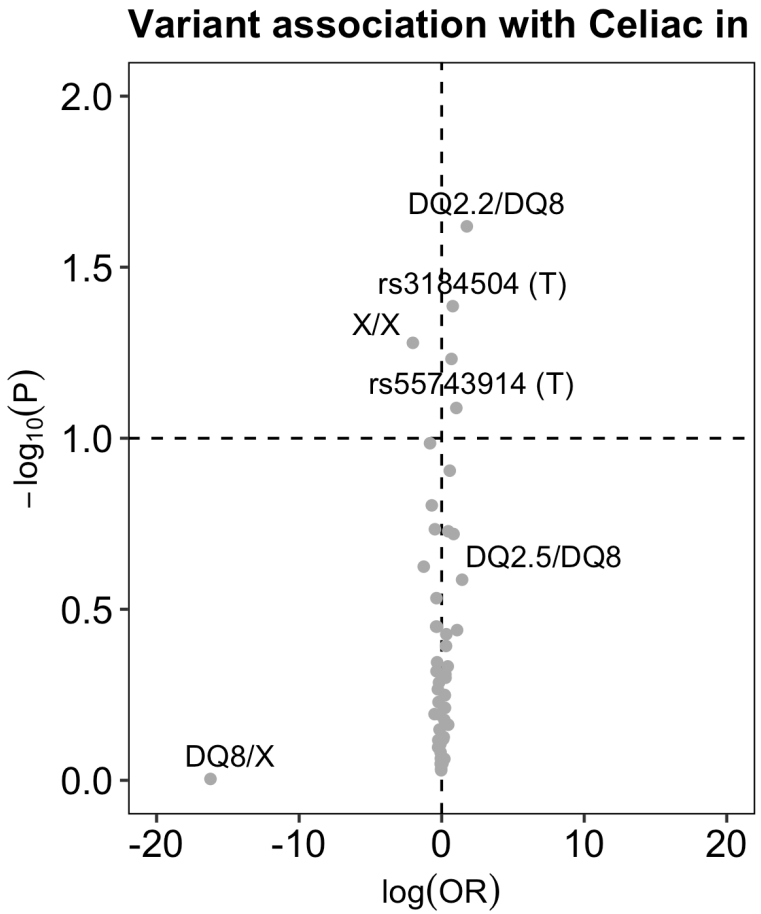
Odds ratios observed in the HTP Revised GRS (Sharp et al., 2022)



Results of single-variant logistic regression models for CD (0/1) versus dosage of score allele (GRS version: Sharp et al., 2022)



+ PC1 + PC2 + PC3 + PC4 + PC5, family = binomial(link = "logit"), data = .)



+ PC1 + PC2 + PC3 + PC4 + PC5, family = binomial(link = "logit"), data = .)

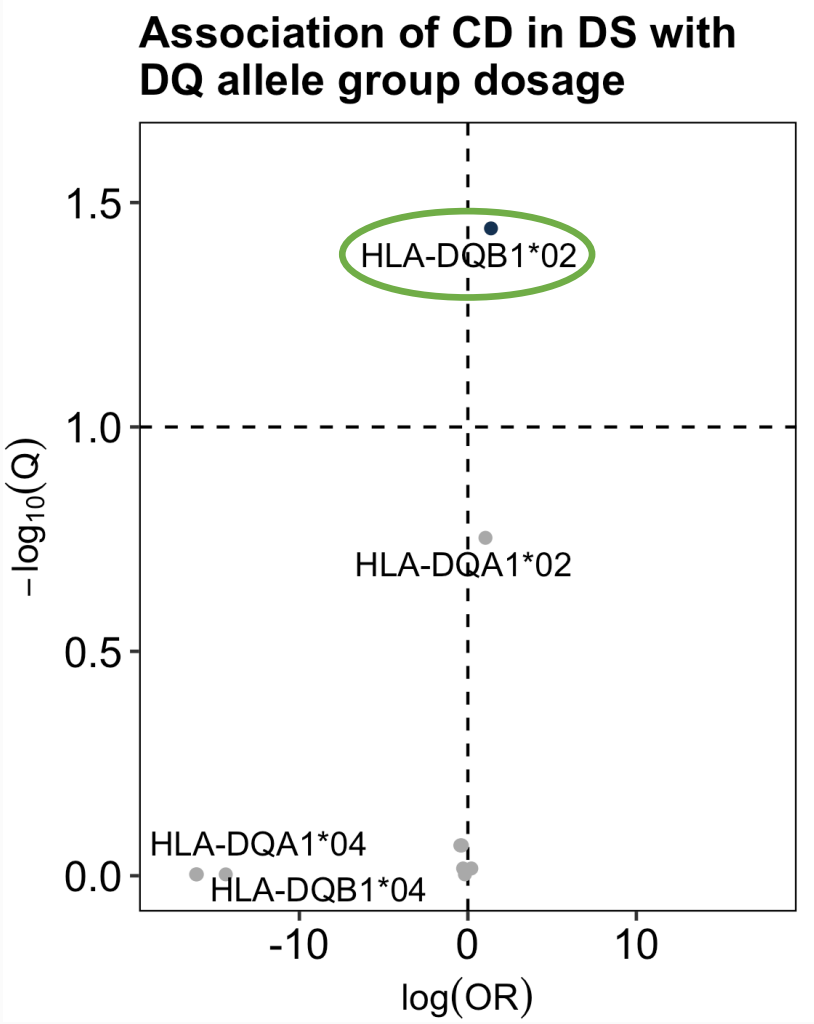
Dosage of DQB1*02 alleles is significantly associated with CD in DS

Alleles encoding CD-associated heterodimers

Heterodimer	DQA1*	DQB1*
DQ2.5	*05*	*0201*
DQ2.2	*0201*	*0202*
DQ8	*03*	*0302*
DQ7.5	*05*	*03*

HLA-DQB1*02

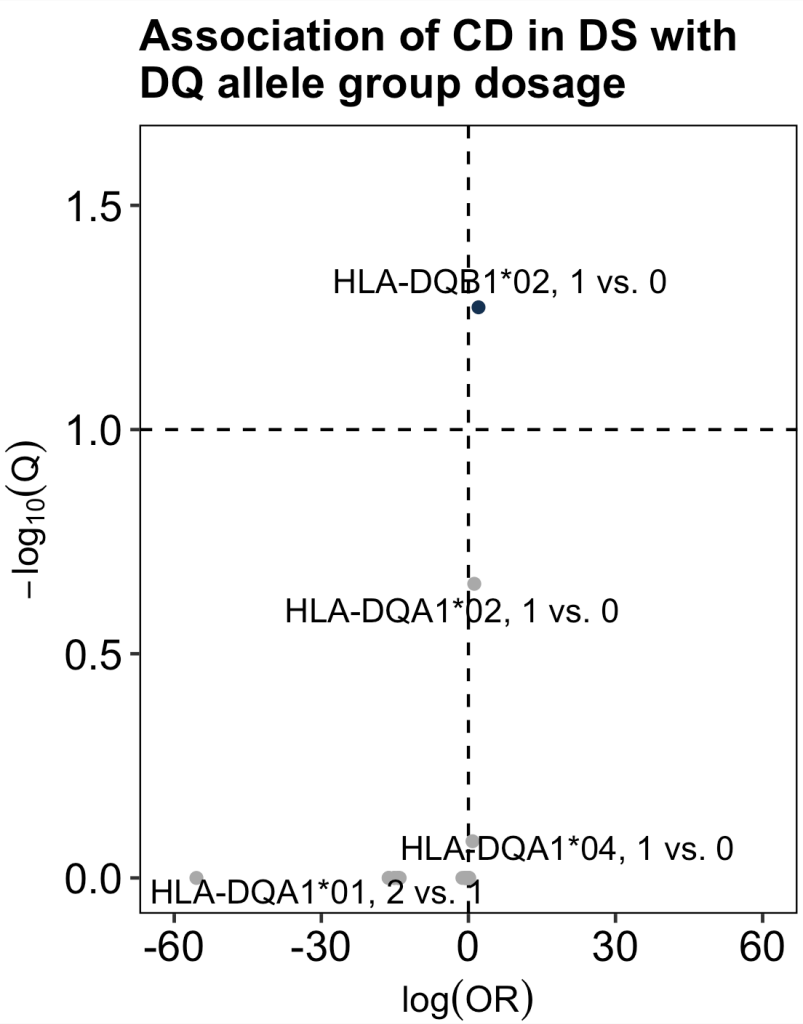
- In an **additive genetic model**, dosage of **DQB1*02** alleles (e.g., DQB1*02:01, DQB1*02:02) was significantly associated with Celiac disease in Down syndrome **(OR = 3.95, Q<0.0361).**
- **On average, each additional DQB1*02 allele corresponded to a 3.95-fold increase in the odds of CD in DS.**



Celiac ~ Dosage + PC1 + PC2 + PC3 + PC4 + PC5

Possible dominant association of DQB1*02 with a 7.81-fold increase in the odds of CD in DS

- To identify non-additive associations (i.e., dominant, recessive), we performed a follow-up analysis of DQ allele group dosage as a categorical predictor, rather than numeric.
- **Carrying one DQB1*02 allele was associated with a 7.81-fold increase in the odds of CD in DS (OR = 7.81, Q< 0.0534),** as compared to carrying zero DQB1*02 alleles.
- By contrast, we found no significant difference in the odds of Celiac given two **DQB1*02** alleles compared to one (OR = 0.80, Q<0.9999).
- **These results may indicate a dominant effect of DQB1*02 in the pathogenesis of CD in individuals with DS (OR = 7.81, Q<0.0534).**



Celiac ~ Dosage.DQalleleGroup + PC1 + PC2 + PC3 + PC4 + PC5

Dominant association of DQB1*02 with a 7.81-fold increase in the odds of Celiac disease in Down syndrome

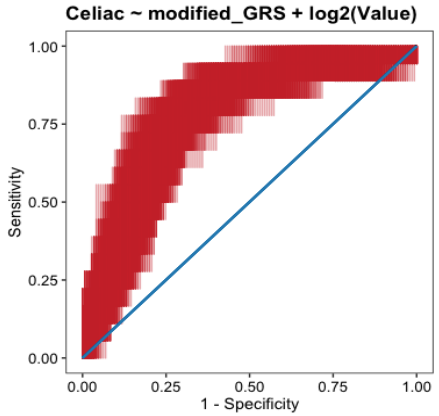
- **84.2% of Celiac cases (16/19) carried one or more DQB1*02 allele**, with the DQ genotypes:
  - **DQ2.5**/X (n=5)
  - **DQ2.2**/X (n=4)
  - **DQ2.2**/DQ8 (n=3)
  - **DQ2.5**/**DQ2.2** (n=1)
  - **DQ2.5**/DQ8 (n=1)
  - DQ7.5/**DQ2.2** (n=1)
  - DQ7.5/**DQ2.5** (n=1)
- Only three Celiac cases were not carriers of DQB1*02, instead presenting with the genotypes:
  - DQ7.5/DQ8 (n=1)
  - DQ7.5/X (n=1)
  - X/X (n=1)

Alleles encoding traditionally CD-associated heterodimers

Heterodimer	DQA1*	DQB1*
DQ2.5	*05*	*0201*
DQ2.2	*0201*	*0202*
DQ8	*03*	*0302*
DQ7.5	*05*	*03*

Models involving both GRS and single **protein abundance** improve prediction of celiac disease in Down syndrome (Please email Jessica.rose.shaw@gmail.com for details)

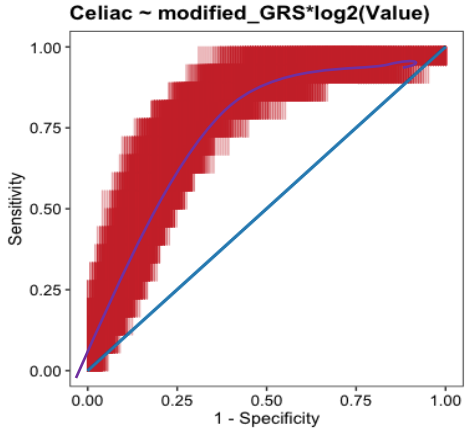
Prediction of celiac status using GRS* and abundance of any one protein as precision covariate



AUC values

UID <chr>	Celiac ~ modified_GRS <dbl>	Celiac ~ modified_GRS + log2(Value) <dbl>
WAP four-disulfide core domain protein 6 (WFDC6.13412.5)	75.2	84.5
4-hydroxy-2-oxoglutarate aldolase, mitochondrial (HOGA1.10024.44)	75.2	83.9
Tumor necrosis factor receptor superfamily member 17 (TNFRSF17.2665.26)	75.2	83.7
Membrane-associated progesterone receptor component 2 (PGRMC2.8681.93)	75.2	83.5
Unique cartilage matrix-associated protein (UCMA.10977.55)	75.2	83.1
Zinc finger protein 134 (ZNF134.12787.47)	75.2	82.9
Marginal zone B- and B1-cell-specific protein (PACAP.16322.10)	75.2	82.8
Furin (FURIN.6276.16)	75.2	82.7
GRB2-related adapter protein (GRAP.12820.1)	75.2	82.6
Brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2.19331.18)	75.2	82.3

Prediction of celiac status with interaction of GRS* and abundance of any one protein



AUC values

UID <chr>	Celiac ~ modified_GRS <dbl>	Celiac ~ modified_GRS*log2(Value) <dbl>
SUN domain-containing protein 3 (SUN3.8852.10)	75.2	86.7
F-box/LRR-repeat protein 5 (FBXL5.12846.3)	75.2	85.7
Regulator of G-protein signaling 4 (RGS4.17855.28)	75.2	85.4
Macrophage-capping protein (CAPG.4968.50)	75.2	85.2
Tumor necrosis factor receptor superfamily member 17 (TNFRSF17.2665.26)	75.2	85.2
Polyadenylate-binding protein 3 (PABPC3.10447.18)	75.2	84.6
Polyadenylate-binding protein 4 (PABPC4.12603.87)	75.2	84.6
WAP four-disulfide core domain protein 6 (WFDC6.13412.5)	75.2	84.6
Mitogen-activated protein kinase 14 (MAPK14.5007.1)	75.2	84.3
ADP-dependent glucokinase (ADPGK.6221.1)	75.2	84.2

Questions?