

The importance of red blood cell hydration in sickle cell disease

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ICM Seminar

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MHI-Human Genetics

Overview

- Introduction of Sickle Cell Disease (SCD)
- Overall strategies for finding hydration genes
 - Genome-wide association study (GWAS)
 - Expression quantitative trait loci (eQTL) Analysis
- Summary of Results

Sickle Cell Disease History



James B. Herrick
a cardiologist in Chicago
makes the reports of
sickle cell anemia in
Internal Medicine in
1910



**Linus Pauling et al. in
*Science***
linked the altered
hemoglobin to the sickling
phenomenon in 1949.
making SCD first molecular
disorder

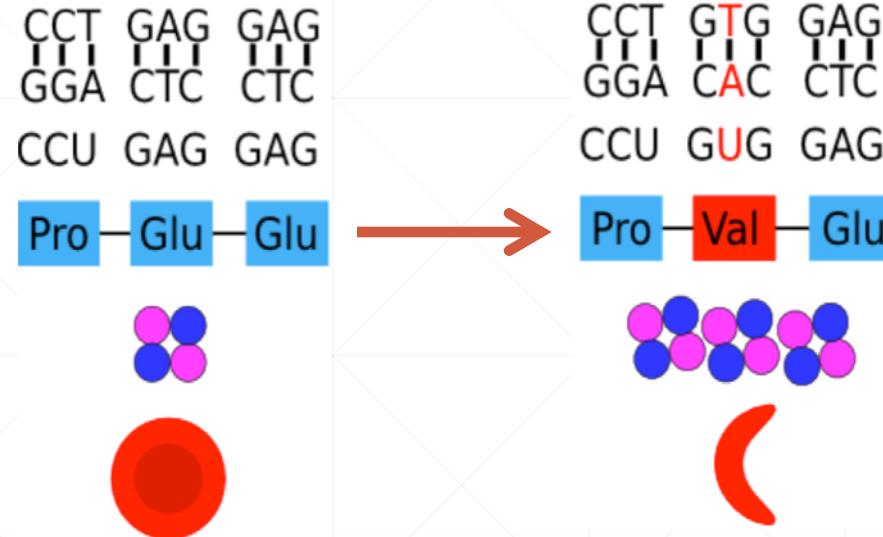


Anthony Allison
discovered the link
between the protective
effect of sickle cell trait
and malaria in 1956 in
Scientific American



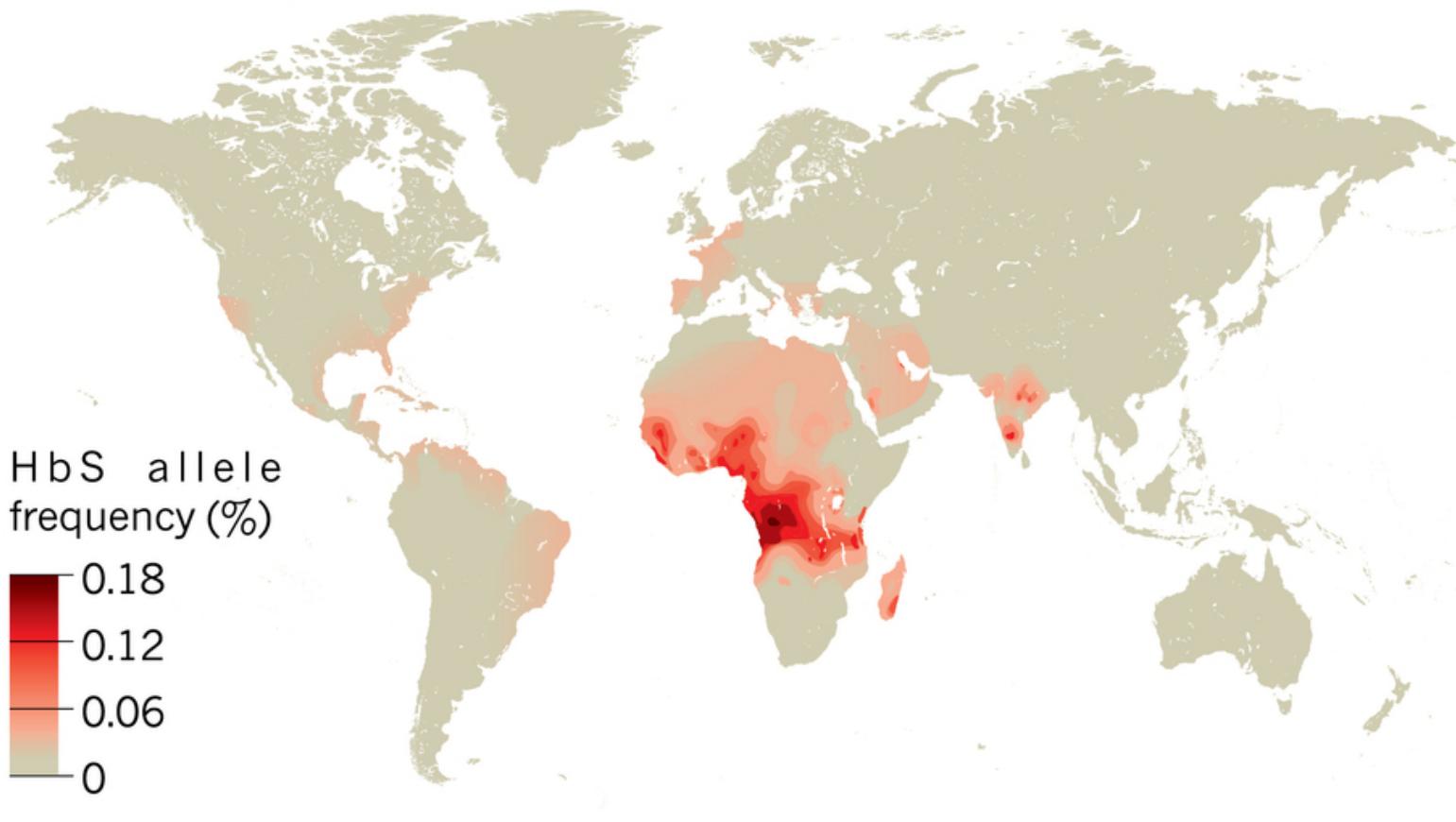
In 1958, **Vernon Ingram**,
found that a single mutation
is at the origin of a protein
change in *Biochim.
Biophys. Acta*.

Beta-globin gene



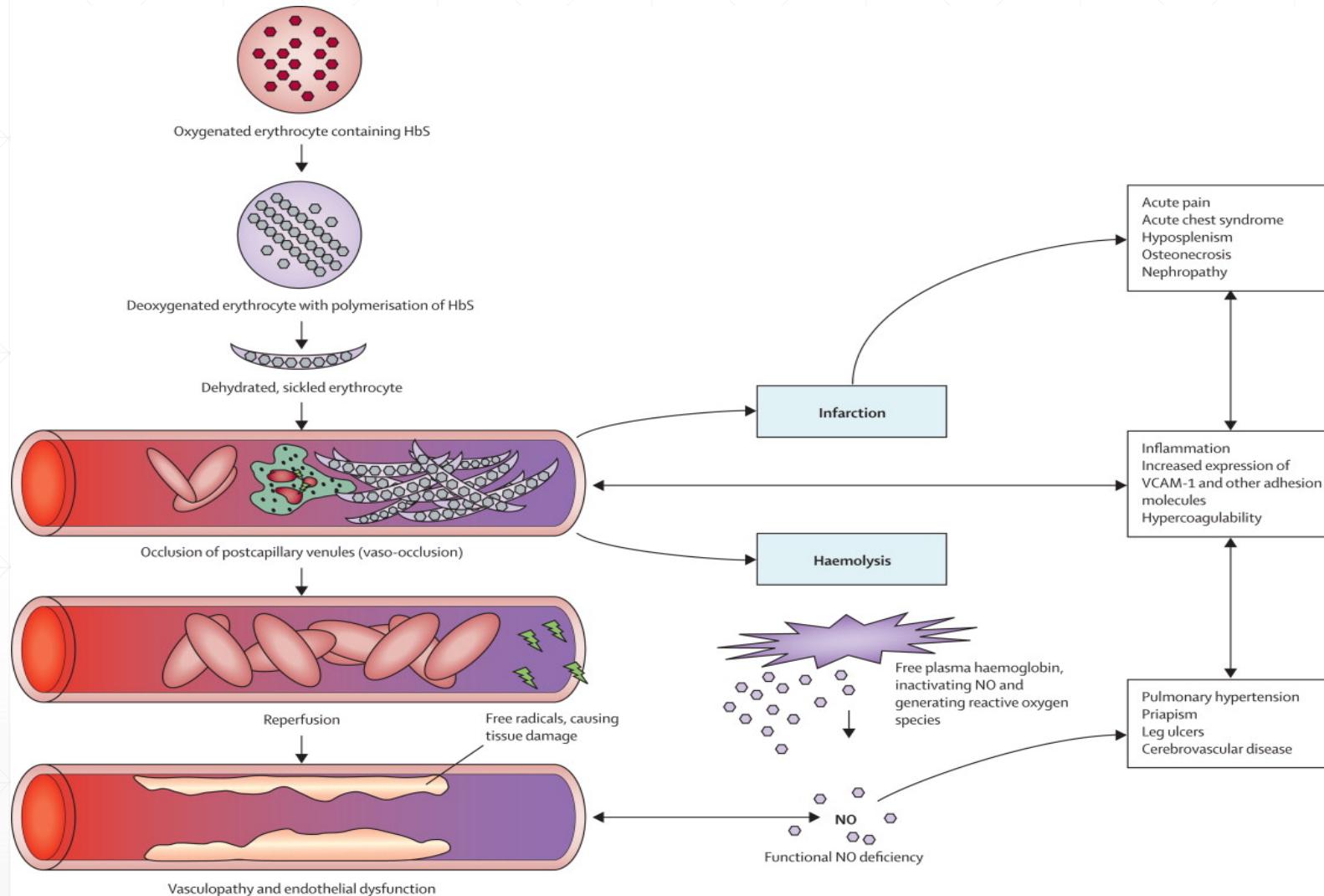
Demographic Impact of SCD

HbS allele frequency



- World Health Organization, estimates that as of 2011, about 5% of the global population carries the mutation causing the disease
- 200,000 SCD babies are born each year in Africa, 50% will die before reaching 1 year old (third-leading cause of death)

Pathophysiology of sickled cells



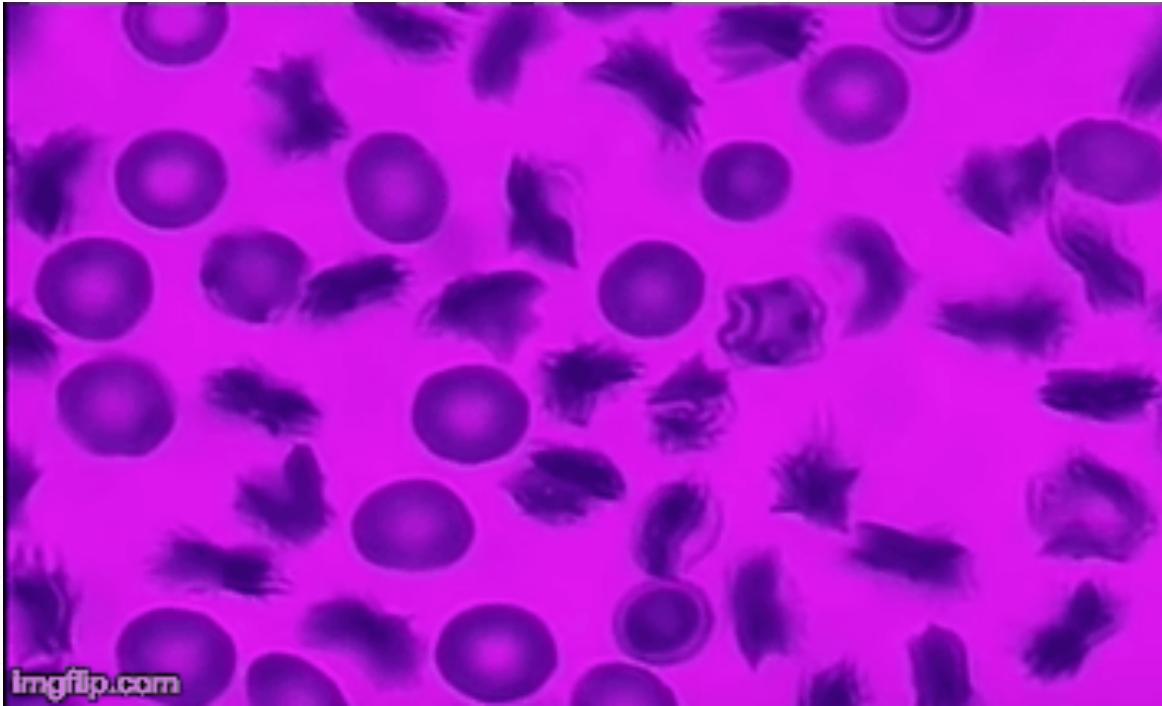
A few therapies exist for curing/dealing with SCD complications

- Only FDA approved
- It's goal is to prevent the polymerization of abnormal hemoglobin
- The response is amongst patients is wide, and taking has many negative side effects



Our approach:

Rehydrate Dense Red Blood cells



What are Dense Red Blood Cell? (DRBC)

- Cells with:
 - Decreased water content
 - Increased hemoglobin content (MCH)
- Several studies have tried to link DRBC with SCD complications

The percentage of dense red cells does not predict incidence of sickle cell painful crisis.

Billet HH et al.

Blood. 1986 8(1):301-303.

Rheology predictors of the severity of the painful sickle cell crisis.

Ballas SK et al.

Blood Cells. 1988 72(4):1216-1223.

Dense Red Blood Cell (DRBC) linked to SCD complications

Erythrocyte density in sickle cell syndromes is associated with specific clinical manifestations and hemolysis.

Pablo Bartolucci,¹⁻³ Carlo Brugnara,⁴ Armando Teixeira-Pinto,⁵ Serge Pissard,⁶ Kamran Moradkhani,⁶ Hélène Jouault,⁷ and Frederic Galacteros¹⁻³

Blood. 2012 Oct 11;120(15):3136-41.

- Sample size of 500 patients
- DRBC highly predictable traits of SCD
- DRBC significantly higher for patients:
 - Renal dysfunction
 - Priapism
 - Leg ulcer
- DRBCs is decreased by 34% within 6 months of starting hydroxyurea treatment.

Cohort Description

Cohort Name	Gen-Mod
Regions of Origins	West Africa, Central Africa, Caribbean
N by gender (M/F)	185/223
Age (years, mean(SD))	31(9)
Genotyped SNPs/Imputed SNPs	2.4 Millions/ 31 Millions
Complications available	Leg Ulcer, Priapism, Stroke, Retinopathy, Hydroxyurea Usage
Blood traits available	MCV, MCH, Retic, RBC Count, Platelet, and more

**Are there any variants of small effects
associated with DRBC?**

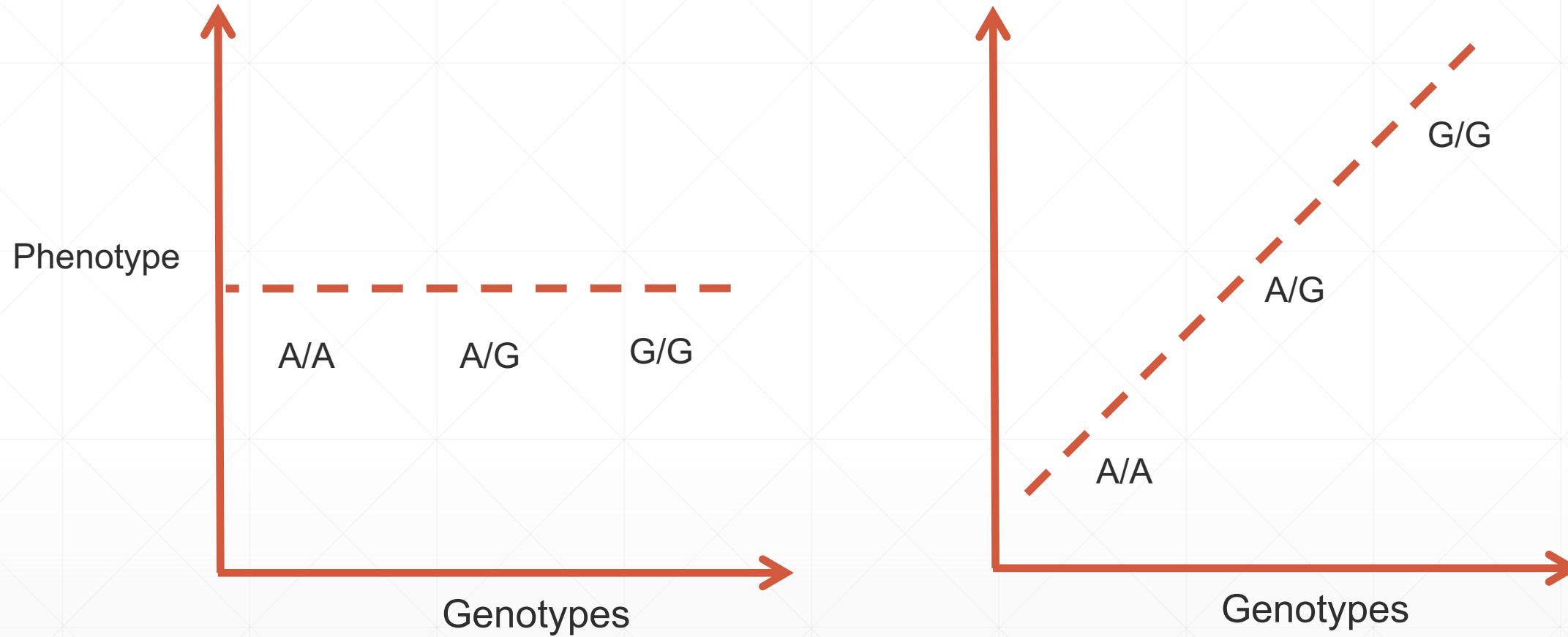
Genome Wide Association Study (GWAS)

- Powerful method to interrogate the whole genome
- We can do this thanks to large catalogs of human variation, such as 1000G project, Hapmap, more recently Haplotype Reference Consortium
- Arrays able to genotype up to 2M genetic variants

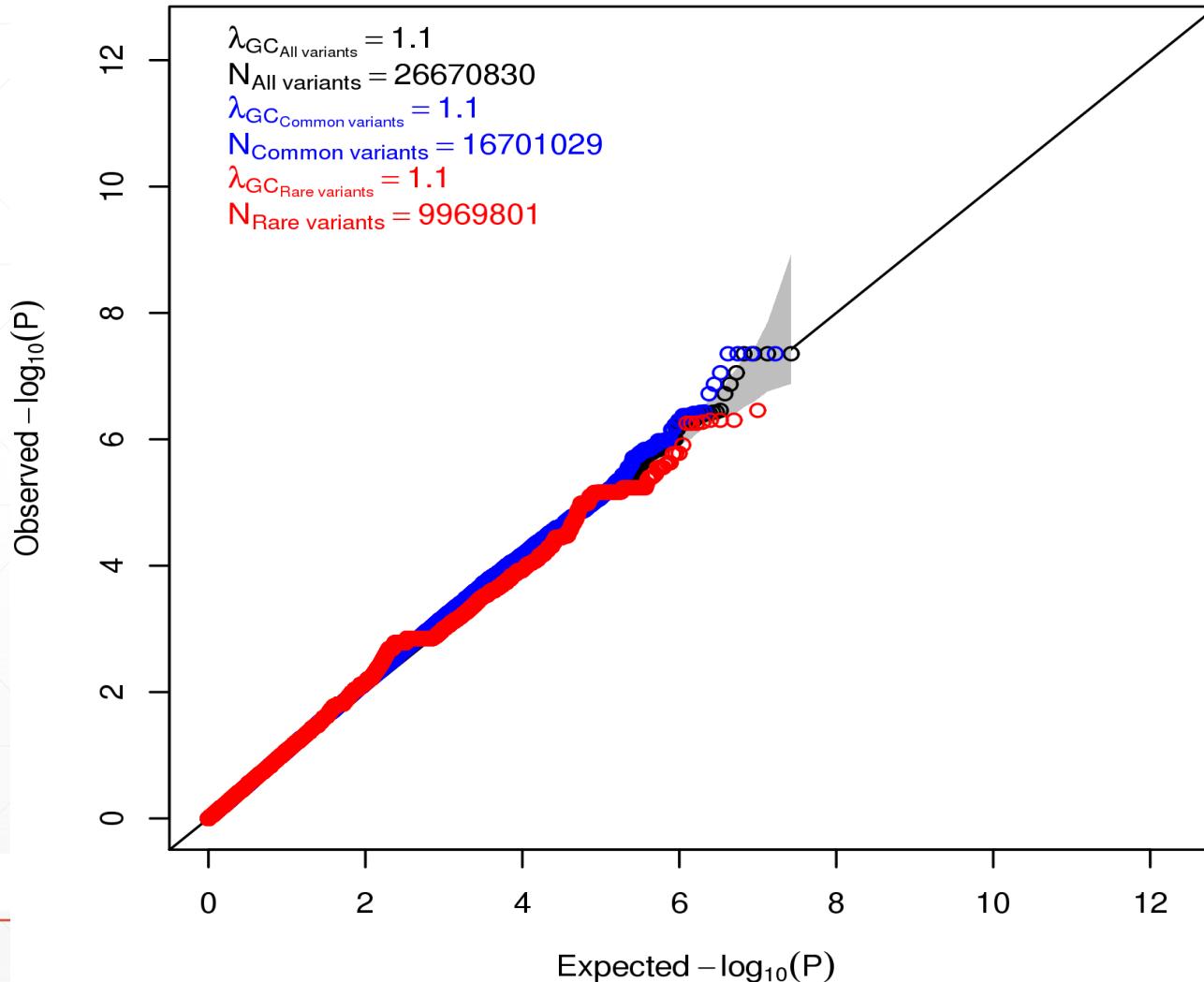
GWAS Study Design

- Discovery
 - Case vs. Control Study for binary trait (Having vs not having a disease)
 - Quantitative Trait Analysis (Numerical value)
 - Looking at extremes
- Replication
 - Essential to validate your findings

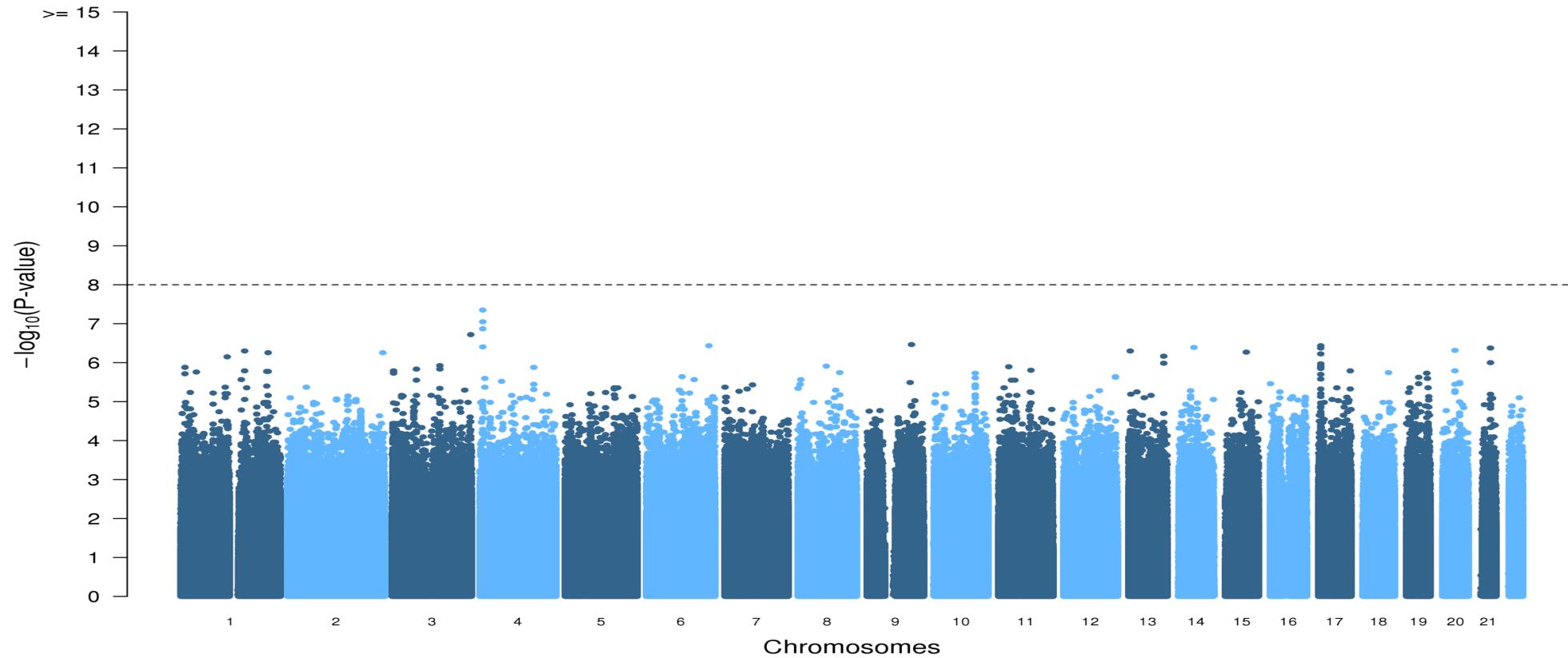
Association Tests Basic Principle



Genome-Wide Scan Results



Genome-Wide Scan Results



DRBC in other GWAS (Pvalue < 0.05)

Seventy-five genomic loci influencing the human red blood cell.

Van de Harst. et. Al.

Nature. 2012 Dec 20;492(429):369-75.

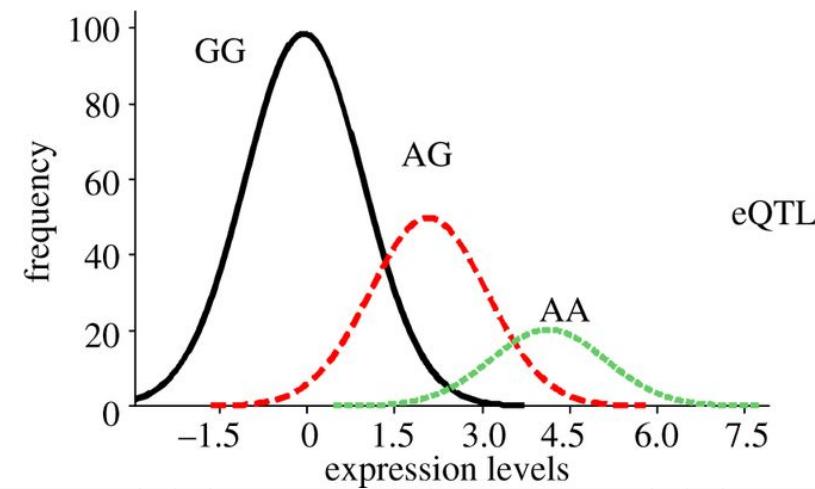
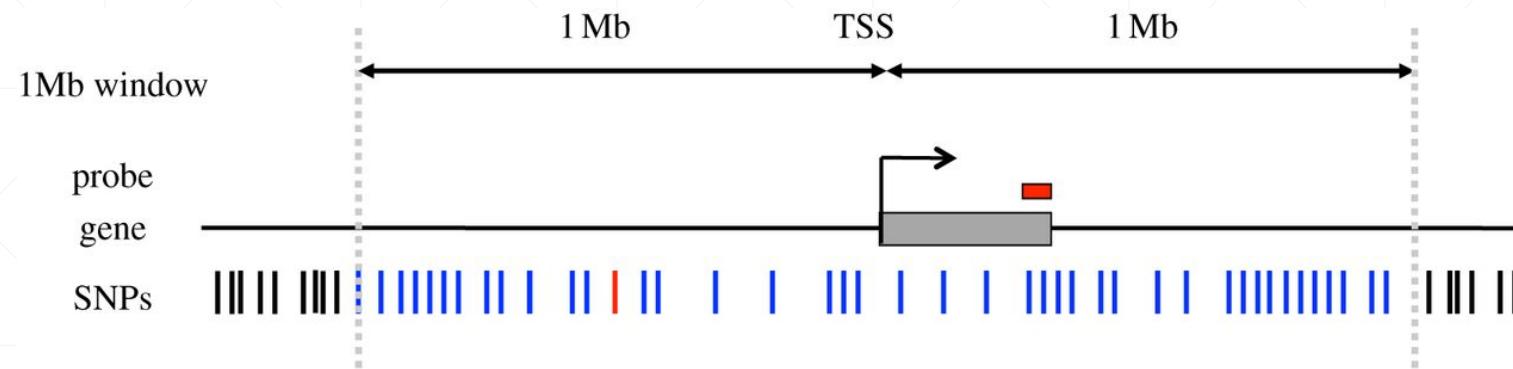
SNP	ALT	BETA (SE)	Annotation	Nearest gene	Associated with
rs2867932	G	-0.021	intron	DNAJA4	Associated with MCHC
rs7551442	A	-0.023	intron	OPTC	Associated with MCHC
rs888424	A	0.006	intron	AKAP10	Associated with MCH
rs7529925	C	0.014	intron	LINC01222	Associated with RBC count
rs3184504	T	0.051	missense	SH2B3	Associated with HGB
rs10159477	A	0.087	intron	HKDC1	Associated with HGB
rs1175550	G	0.008	intron	CCDC27	Associated with MCHC
rs855791	G	0.012	missense	KCTD17	Associated with MCH
rs13061823	T	-0.168	intron	GK5	Associated with MCV
rs2572207	C	0.153	intron	MIR4511	Associated with MCV
rs5754217	G	0.194	intron	RIMBP3C	Associated with MCV
rs9369427	A	0.042	intergenic	VEGFA	Associated with HGB

Conclusions on GWAS Scan

- Overall we do find some associations between DRBC and known variants.
- We are expecting an additional 500 samples which will help us replicate and thus confirm the validity of our findings.

**Are there eQTLs that affect the expression level
of hydration gene?**

What are eQTLs?



55 Candidate Genes (Sample Below)

Official Gene Symbol	Gene Name	Gene Annotation (From The Human Protein Atlas)
PKD2	Polycystic kidney disease 2 (autosomal dominant)	Disease related genes, Plasma proteins, Potential drug targets, Predicted membrane proteins, Transporters, Voltage-gated ion channel
SLC12A4	Solute carrier family 12 (potassium/chloride transporter), member 4	FDA approved drug targets, Predicted membrane proteins, Transporters
SLC12A7	Solute carrier family 12 (potassium/chloride transporter), member 7	Plasma proteins, Predicted intracellular proteins, Predicted membrane proteins, Transporters
ATP2B4	ATPase, Ca++ transporting, plasma membrane 4	Enzymes, Predicted intracellular proteins, Predicted membrane proteins, Transporters
KCNN4	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	FDA approved drug targets, Predicted intracellular proteins, Predicted membrane proteins, Predicted secreted proteins, Transporters, Voltage-gated ion channels
PIEZ01	Piezo-type mechanosensitive ion channel component 1	Disease related genes, Potential drug targets, Predicted intracellular proteins, Predicted membrane proteins, Transporters

eQTL Analysis

Methods

- Searched for eQTL in GTEx portal and in erythroblasts data generated in our lab
- For a specific gene identify which variation is the most significant
- Test those against SCD complications in GEN-MOD and CSSCD:
 - Priapism
 - Leg ulcer

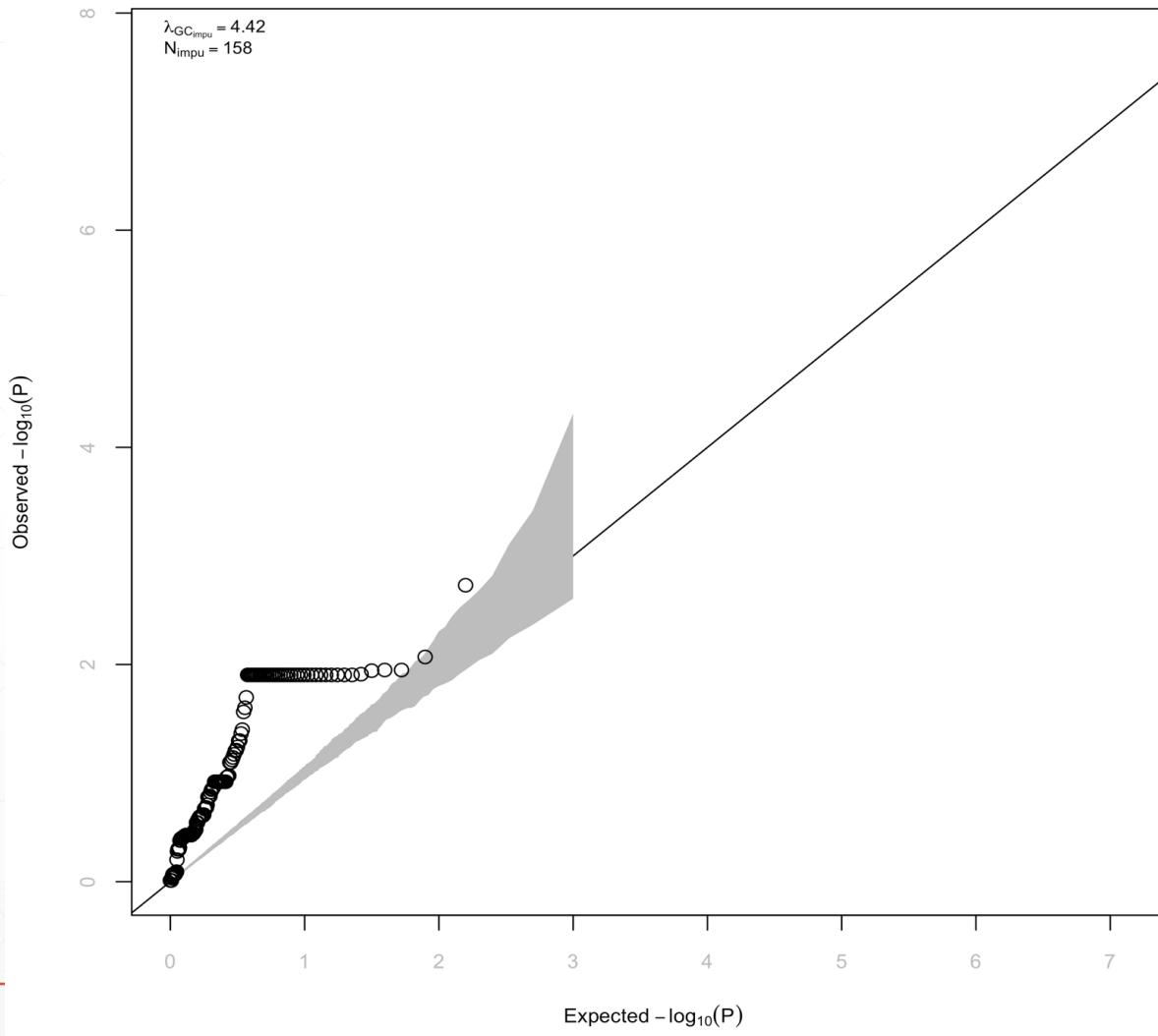


www.gtexportal.org/home

eQTL Analysis - Results

Methods

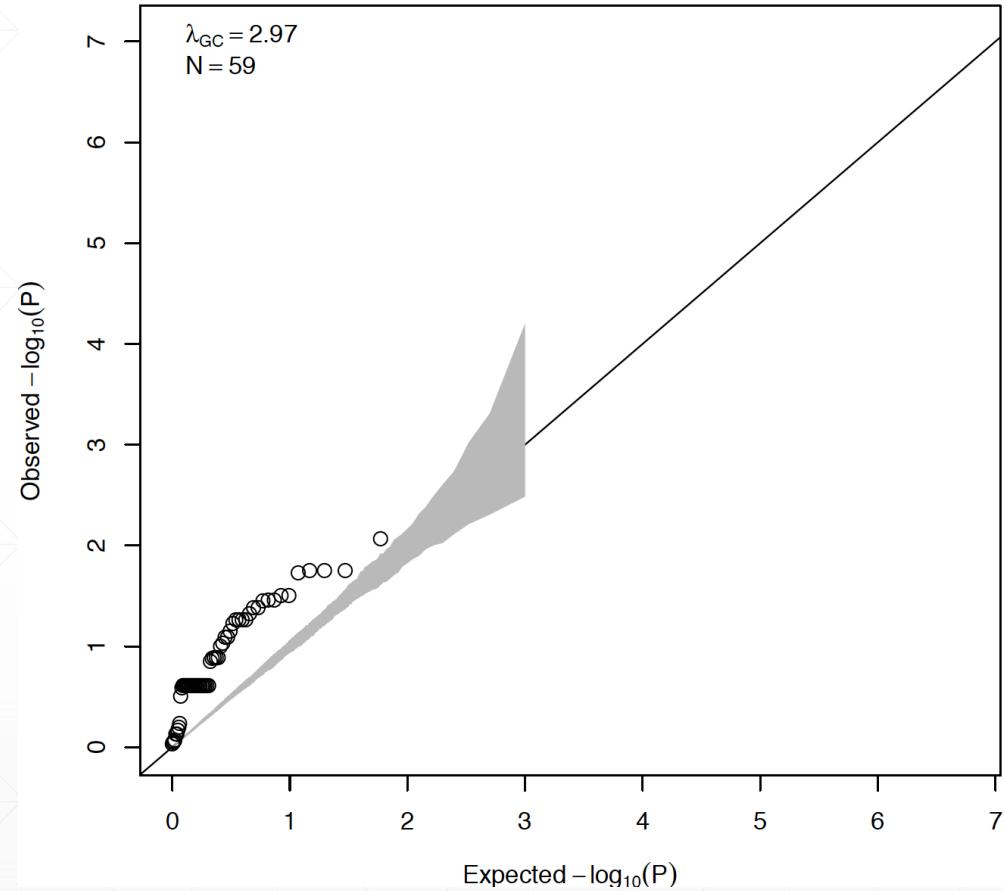
Erythroblasts



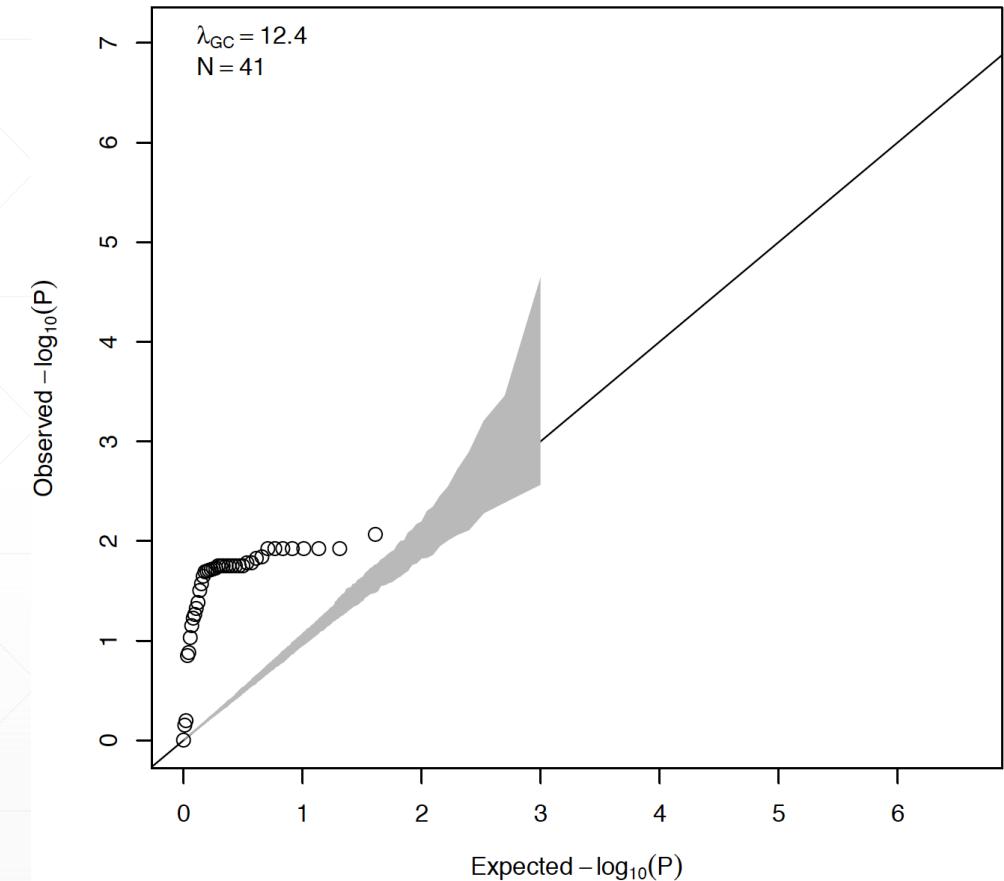
eQTL Analysis - Results

Results

Artery_Coronary



Pituitary



eQTL Analysis - Results

Methods

Leg Ulcer

eQTL Gene	Pvalue	Tissue	Odds Ratio	Annotation	N
PKD2	1e-4	Testis	1.89	intron	751
SLC12A6	0.04	Thyroid	1.33	intron	751

Priapism

eQTL Gene	Pvalue	Tissue	Odds Ratio	Annotation	N
SLC41A2	4 x 1e-3	Testis	2.9	intergenic	333
GRIN2D	0.02	Nerve Tibial	1.55	intron	333

Conclusion eQTL

- We found several significantly associated eQTLs for DRBC
- We were able to link those associations with sickle cell disease specific complications through our candidates genes
- We are planning a pseudo replication of these promising results in UKBiobank by testing whether these SNPs are positively associated and if they increase MCHC content

Overall Conclusion

- Results from our GWAS analysis, didn't yield much but this is the first GWAS of a phenotype that is not commonly measured in sickle cell patients or generally by hematologist.
- Our eQTL analysis provided the strongest evidence particularly for the PKD2 genes for the role of hydration in SCD as genotype is strongly linked to the odds of leg ulcer. However more functional work is needed to confirm this results
- The future is still bright, we'll add 500 patients to our GWAS analysis, we are waiting on whole-exomes sequencing data of 100 patients SCD patients

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 - Simon Lalonde
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 - Carlos Brugnara
 - Frederic Galacteros
 - Pablo Bartolucci

Appendix

Density-based separation in multiphase systems provides a simple method to identify sickle cell disease.

Ashok A. Kumara et al.
PNAS, August 1, 2014

Measuring single-cell density.

William H. Grover et al.
PNAS, March 24, 2011

Complications by genotypes

Clinical and hematological features of the most common genotypes of sickle cell disease

Genotype	Incidence in African Americans	PCV	Retic	MCV	HbF	% Variant	Severity
Sickle cell anemia (HbS homozygotes)	1/600	25	8	90	5	>90% HbS	4
HbSC disease	1/800	35	3	80	2	50% HbS and HbC	2
S-β ⁰ thalassemia	1/1500	27	7	82	7	90% HbS	4
S-β ⁺ thalassemia		38	2	70	2	5%-30% HbA	2
HbSE disease	rare	35	3	75	2	~30% HbE	2
Sickle cell anemia-α thalassemia	30% of all cases	30	6	78	5	>90% HbS	3