The Discovery of Genetic Loci Associated with Fetal Hemoglobin Levels in Sickle Cell Disease Patients through Epigenomic Prioritization

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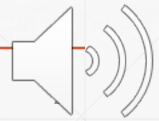
MHI-Human Genetics

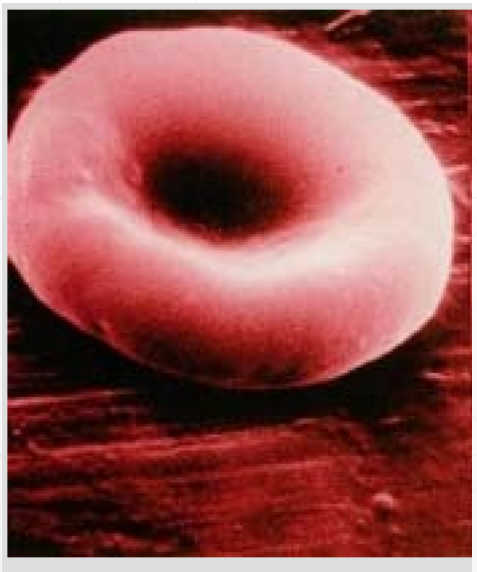
Presentation Outline

- Introduction of sickle cell disease.
  - Historical perspective.
  - Demographic burden.
  - What is fetal hemoglobin? Why we studied it?
- Method
  - Conditional Meta-analysis of ~10,000 patients.
- Results
  - Focus on results at *KLF1*, on Chr19.
- Conclusion



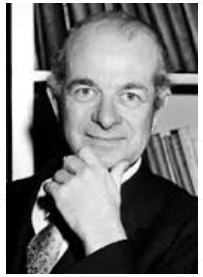
Sickle Cell Disease – In a Nutshell

- Blood disorder that causes red blood cell to be change their shape:
- ‘Croissant’ **AS OPPOSED TO** ‘Doughnut’



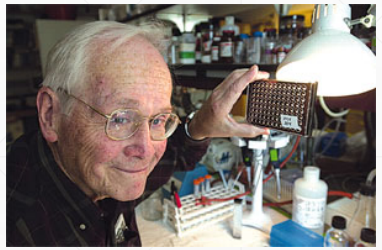
Sickle Cell Disease History

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



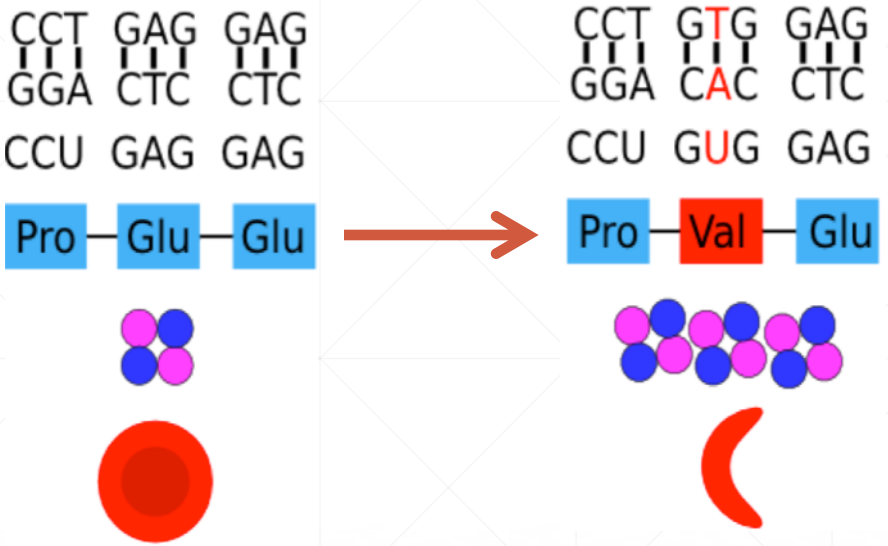
Linus Pauling et al. in *Science*
linked the altered hemoglobin to the sickling phenomenon in 1949. making SCD first molecular disorder

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

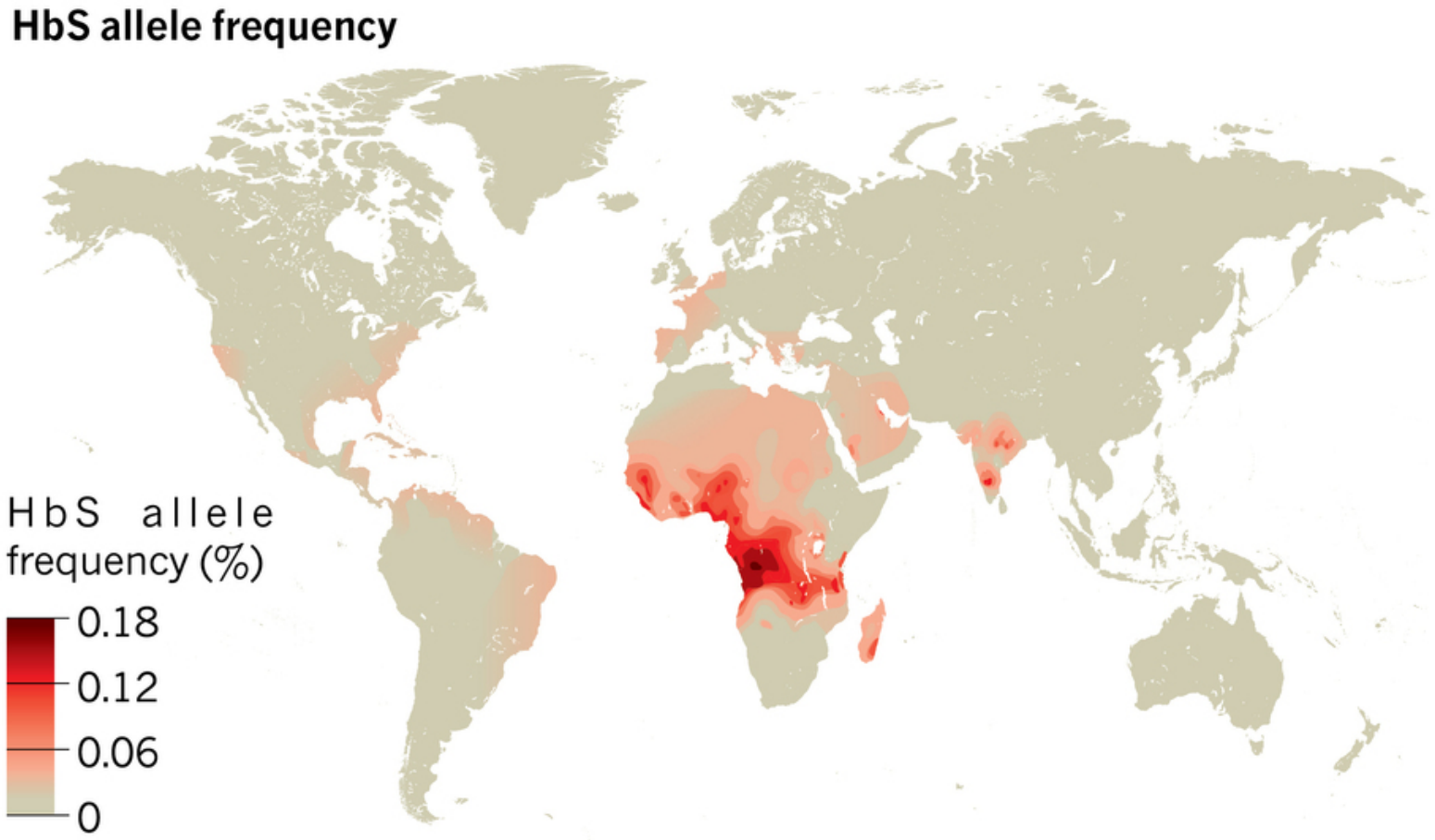


In 1958, **Vernom Ingram**, found that a single mutation is at the origin of a protein change in ***Biochim. Biophys. Acta***.

Beta-globin gene



Demographic Impact of SCD

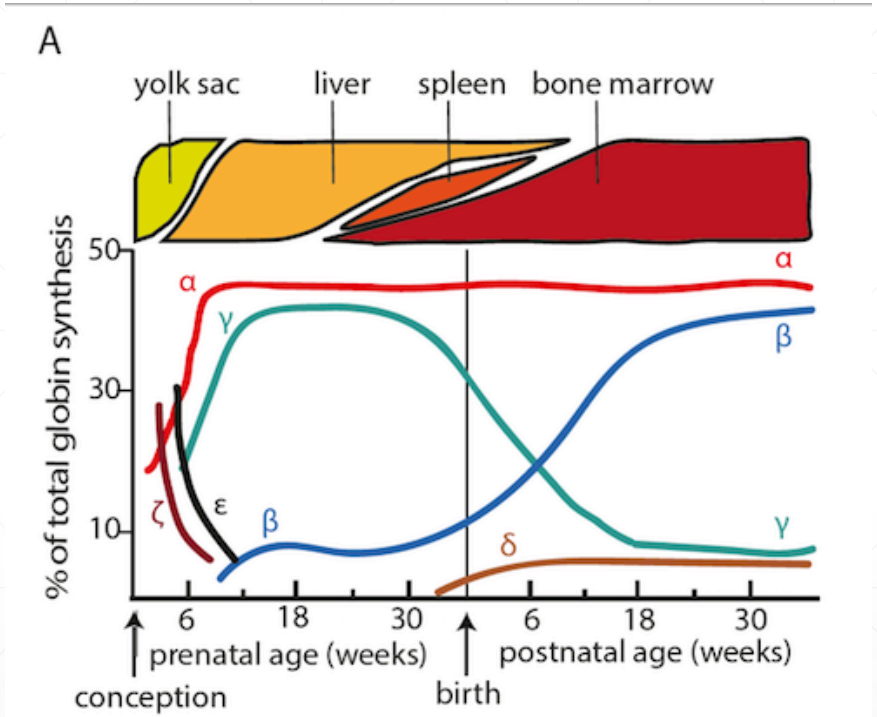


- World Health Organization, estimates that as of 2011, about 5% of the global population carries the mutation causing the disease
- 200,000 SCD babies are born each year in Africa, 50% will die before reaching 1 year old (third-leading cause of death)

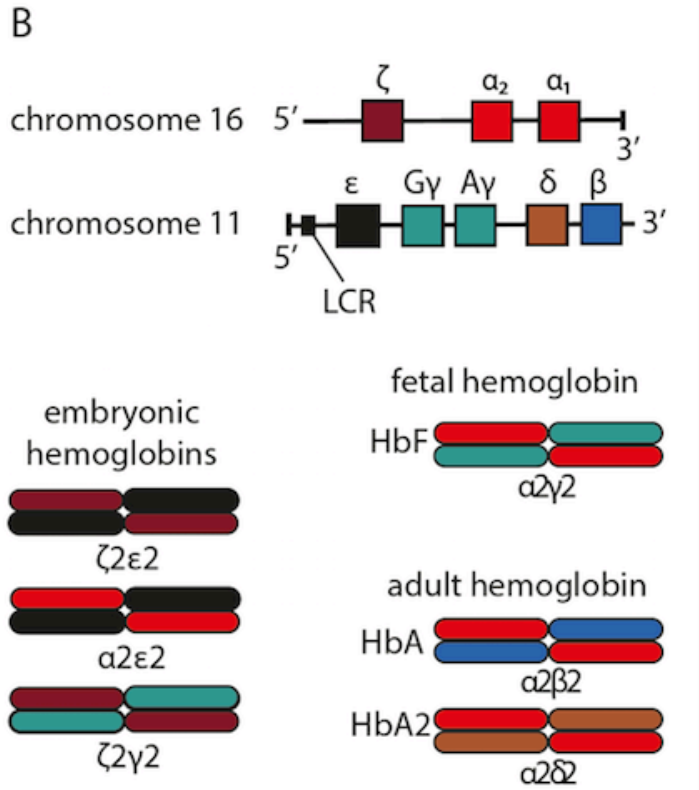
Simon Pleasants et al, Nature 515,S2(13) November 2014)doi:10.1038/515S2a

What is fetal hemoglobin (HbF)?

Tissues where red blood cells are produced



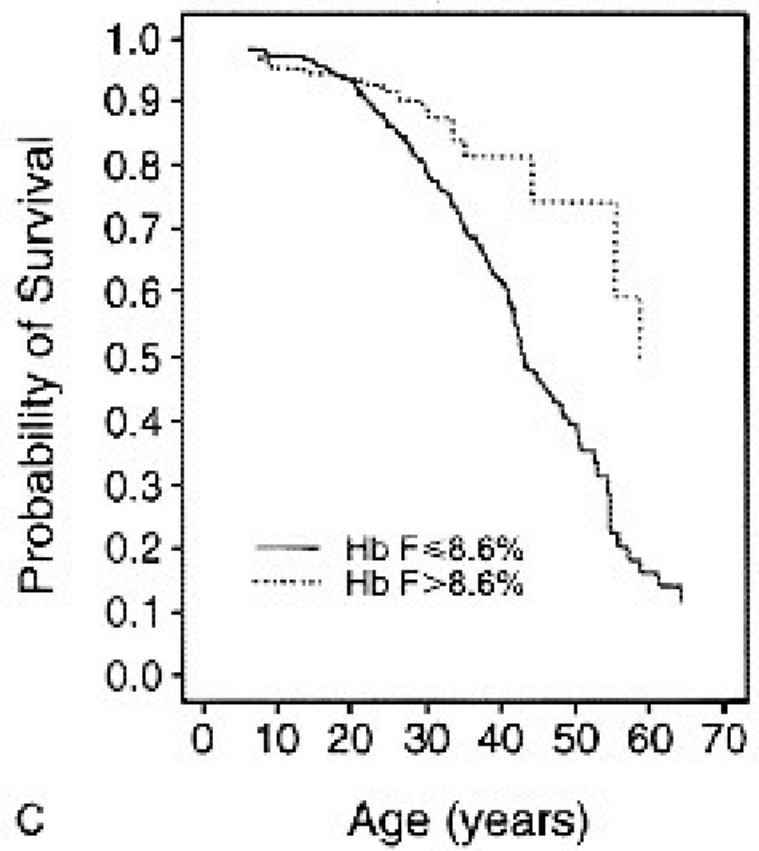
Time



Sankaran, V.G. & Nathan, D.G. Reversing the hemoglobin switch. N Engl J Med 363, 2258-60, 2010.
Fernandez, K.S. and P.A de Alarcon, Development of the hematopoietic system and disorders of hematopoiesis that present during infancy and early childhood. Pediatr Clin North Am, 2013
Maria Mikropoulou, An shRNA Screen for the Discovery of Suppressors of Fetal Hemoglobin, 2016

Why do we study fetal hemoglobin (HbF)?

Pediatric hematologist, Janet Watson (1948) first to observe HbF's benefit.



Later, studies demonstrated higher levels of HbF reduce many of known complications such as leg ulcers, pain crises, strokes, and to name a few.

Orah S. Platt, et. al, Original Article Mortality In Sickle Cell Disease -- Life Expectancy and Risk Factors for Early Death. N Engl J Med 1994
Rodgers GP, Steinberg MH. Pharmacologic treatment of sickle cell disease and thalassemia: the augmentation of fetal hemoglobin. Cambridge University Press; 2001

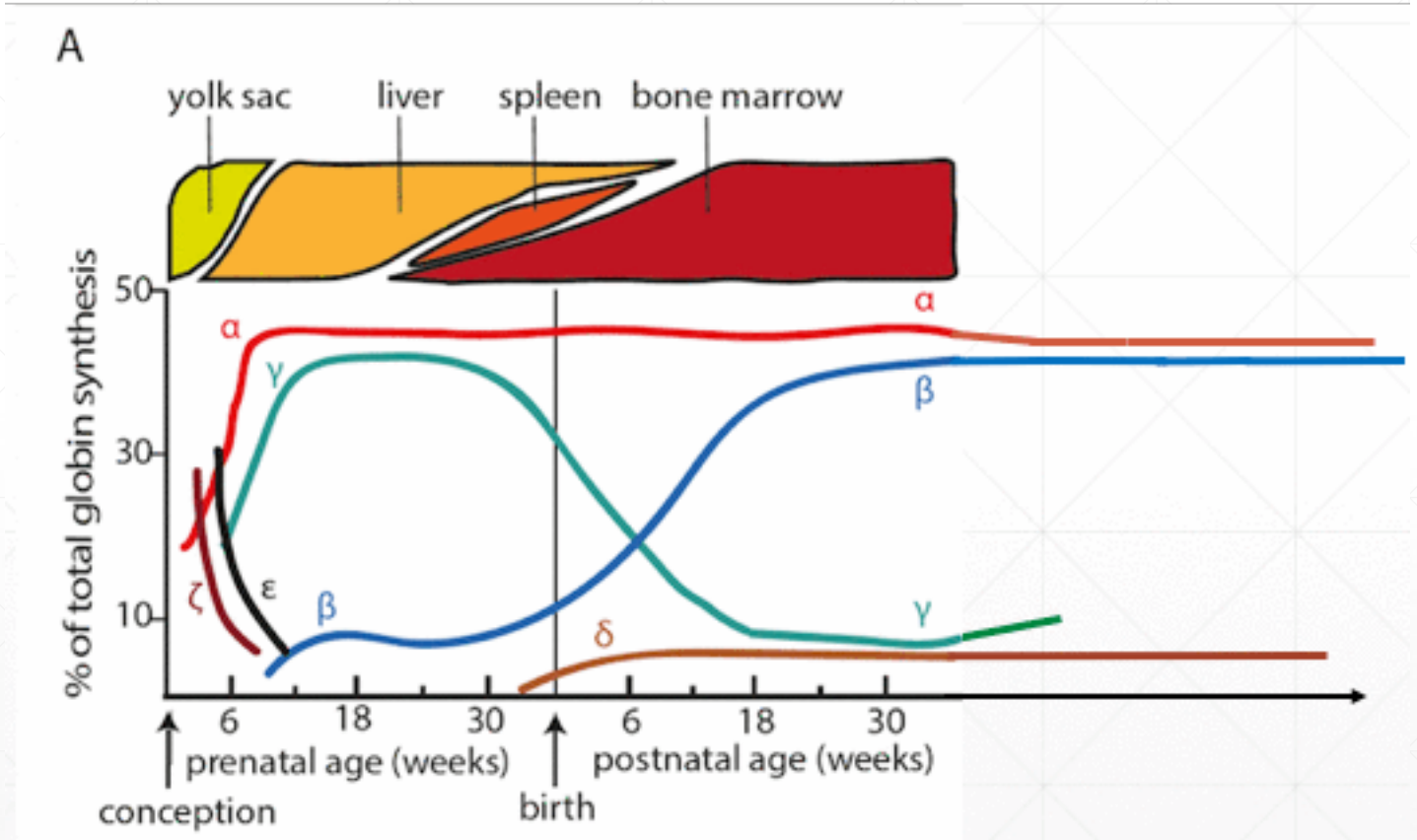
Drug that stimulates the production of HbF

- Only FDA approved and most prescribed drug.
- Benefits:
  - Allowed many infant to reach adulthood.
  - Reduces pain crises.
- Drawbacks:
  - Not as effective as blood transfusions in preventing strokes.
  - Patients responses is variable.
  - Patients complain about its many negative side effects.

Segal JB et. al, Hydroxyurea for the treatment of sickle cell disease. Evid Rep Technol Assess (Full Rep). 2008 Mar;(165):1-95

Idea: Reverse the Normal Development of Globin Expression

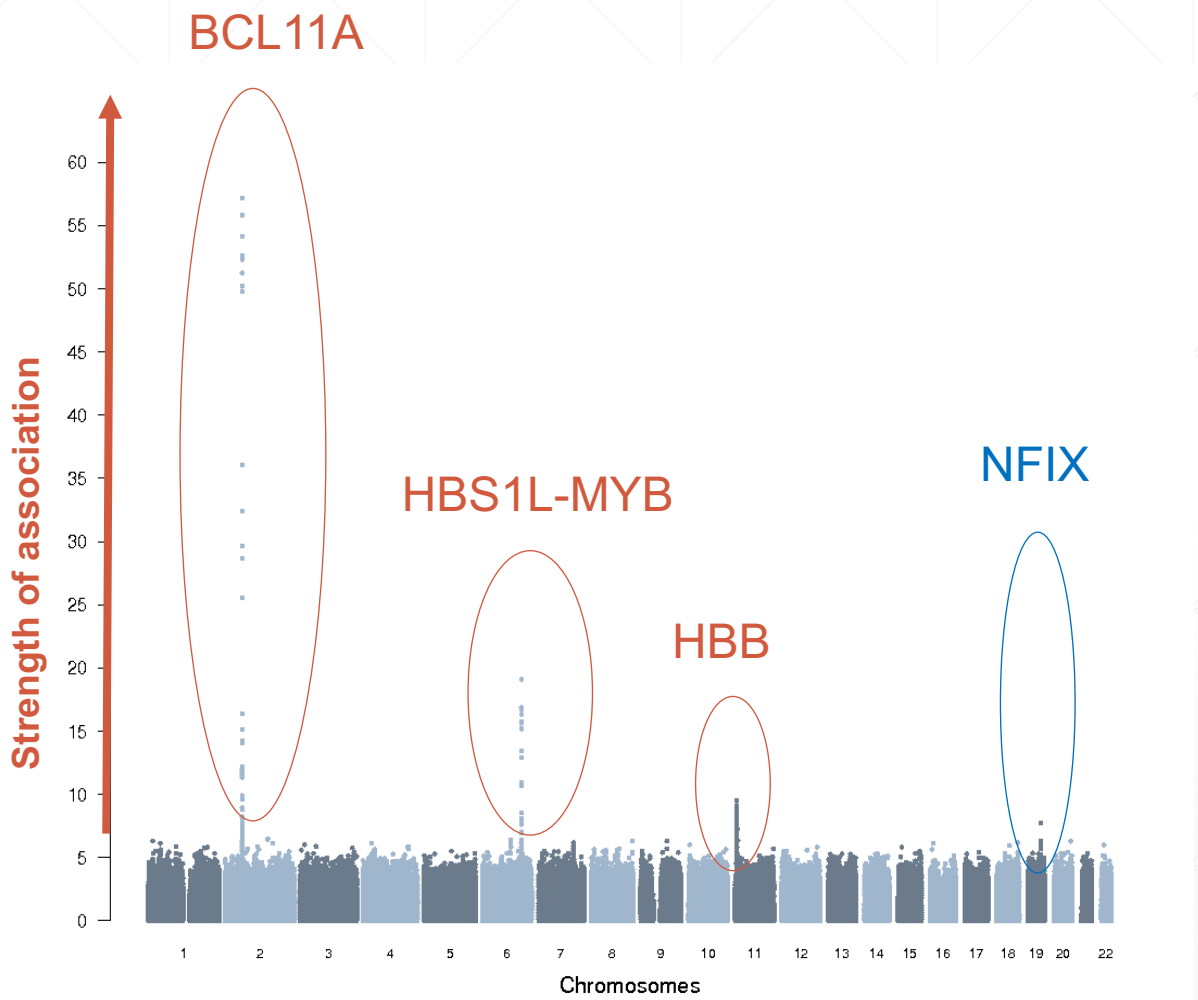
- Find genes or mutations that cause fetal hemoglobin to remain higher levels. Mutations such as hereditary persistency of fetal hemoglobin (HPFH).



Are there DNA sequences variants responsible for changing HbF levels?

Finding DNA Polymorphism of HbF Through GWAS

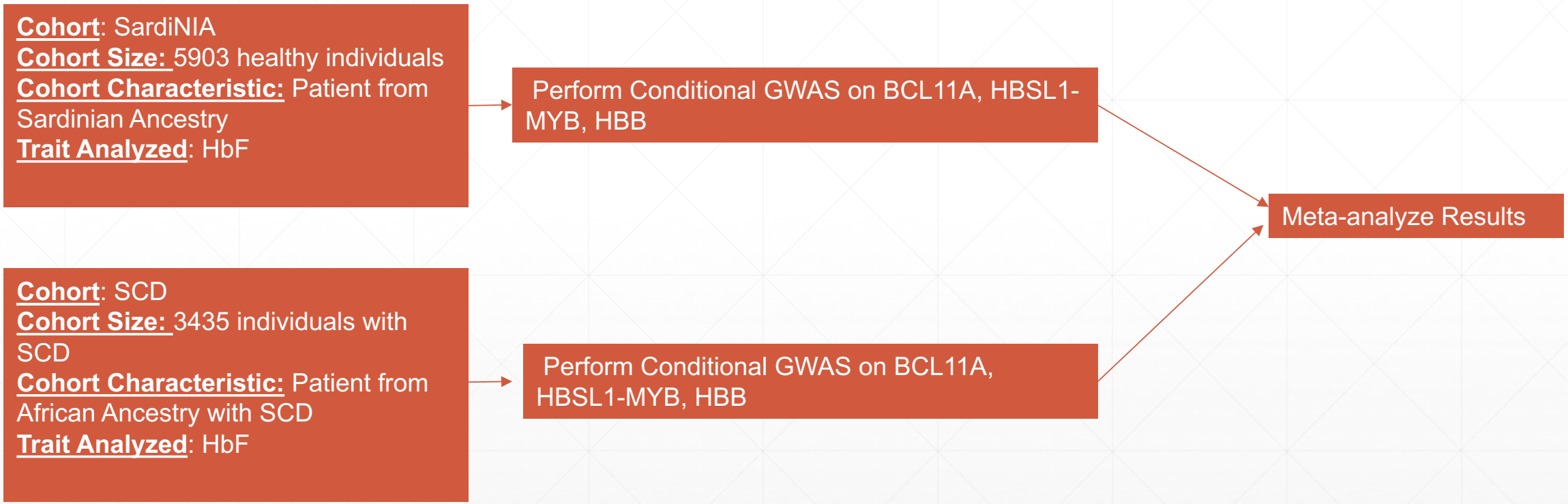
- Together BCL11A, HBS1L-MYB, HBB account for at least ~50% of variability of HbF in humans.
- More recently an *NFIX*-rs183437571 variant was identified on chr19 as the new locus for HbF.



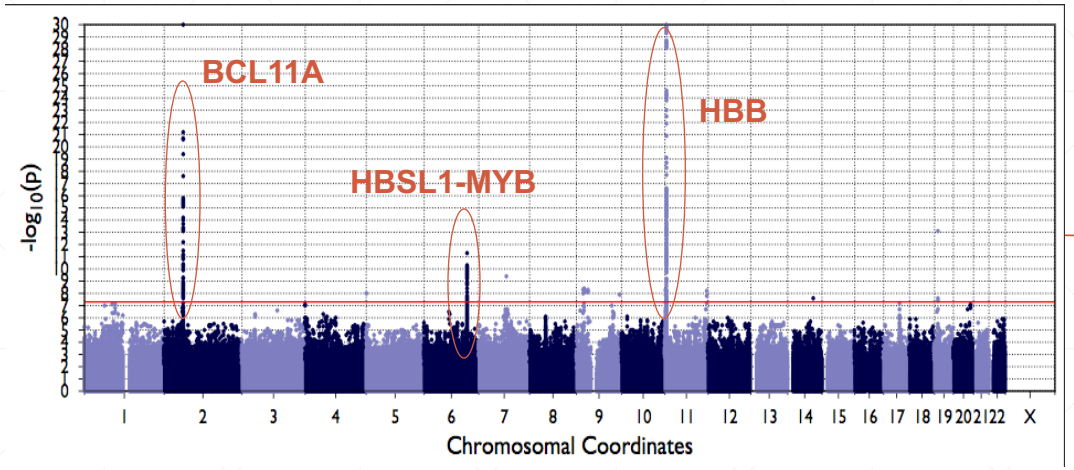
Lettre et. al, DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease, PNAS 2008
Uda et. al, Genome-wide association study shows *BCL11A* associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia, PNAS 2008
Danjou F et. al, Genome-wide association analyses based on whole-genome sequencing in Sardinia provide insights into regulation of hemoglobin levels, Nature 2015

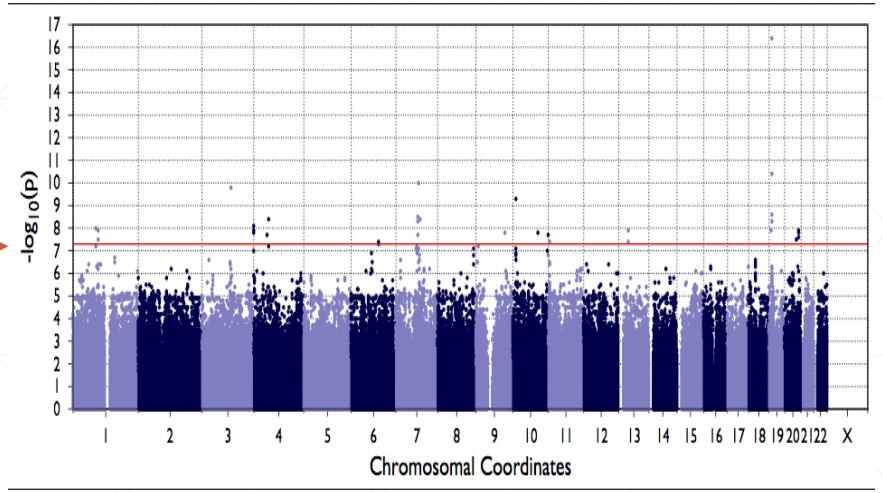
Knowing that ~50% of heritability comes from BCL11A, HBS1L-MYB, HBB, are there other DNA sequences variants responsible for changing HbF levels?

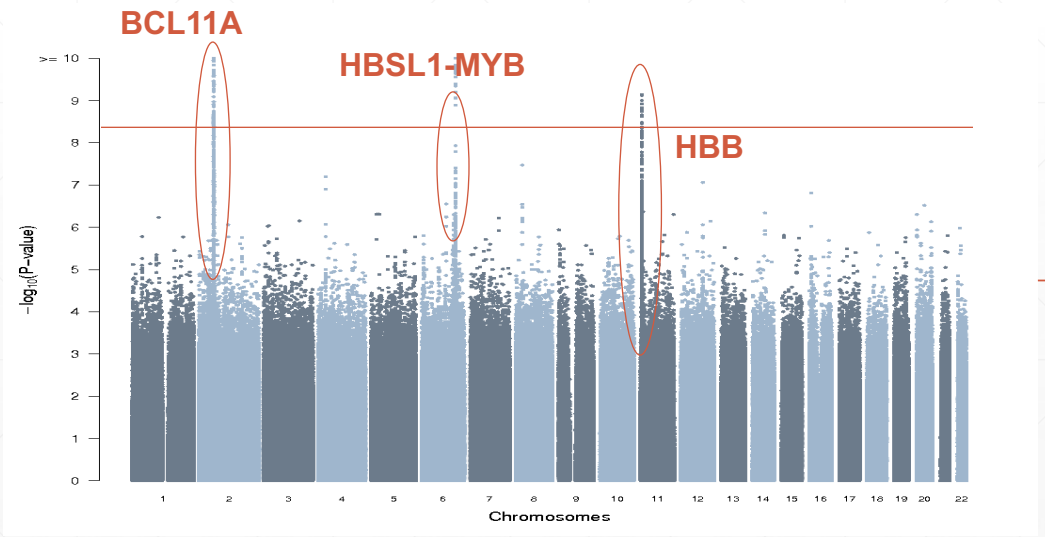
Conditional Meta-analysis of HbF in SCD and Sard

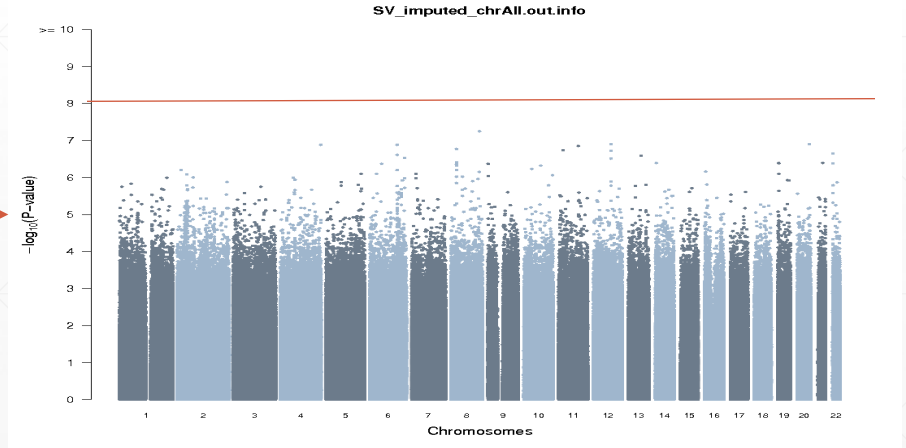


Conditional Meta-analysis of HbF in SCD and Sardinian



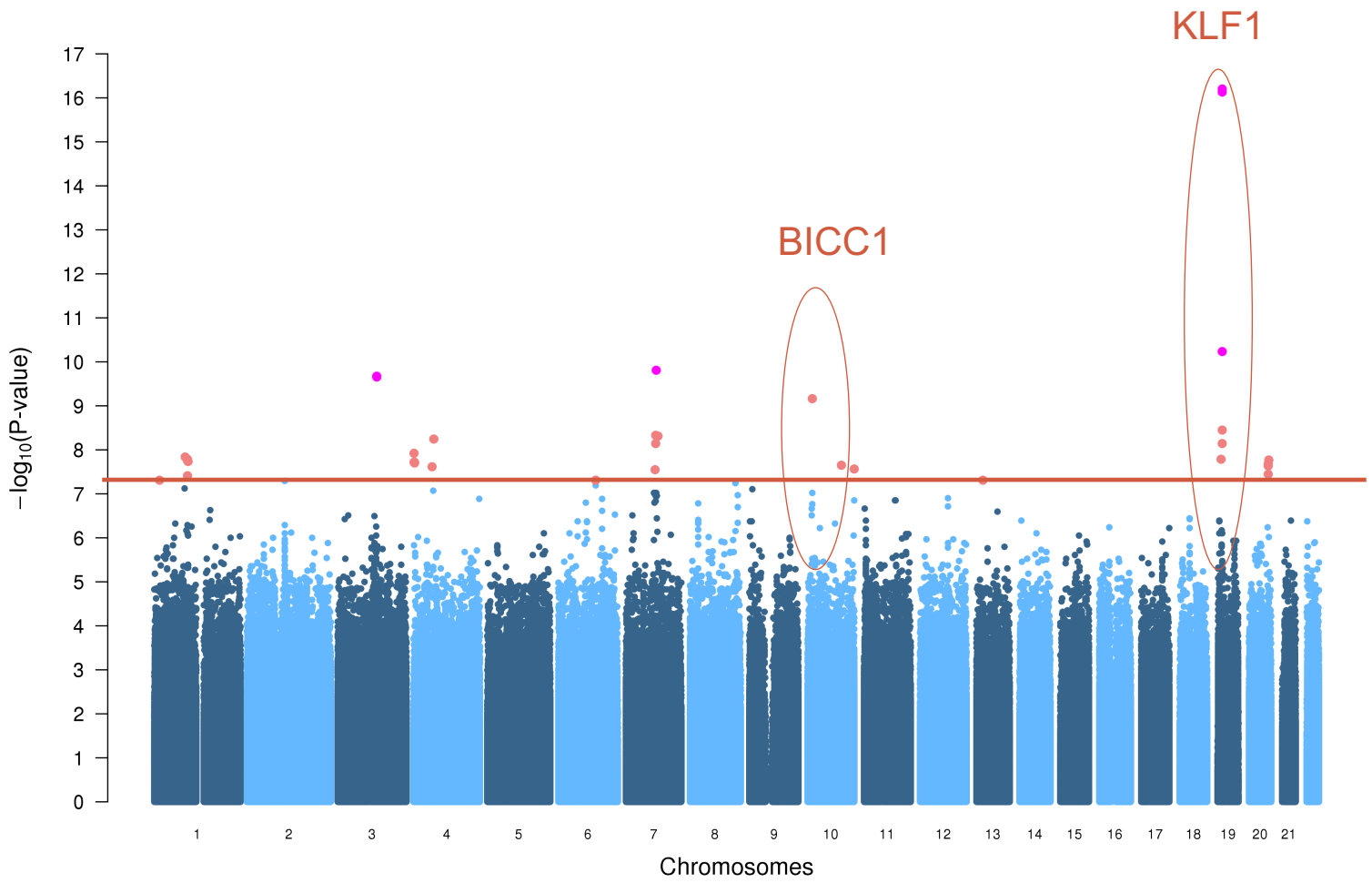






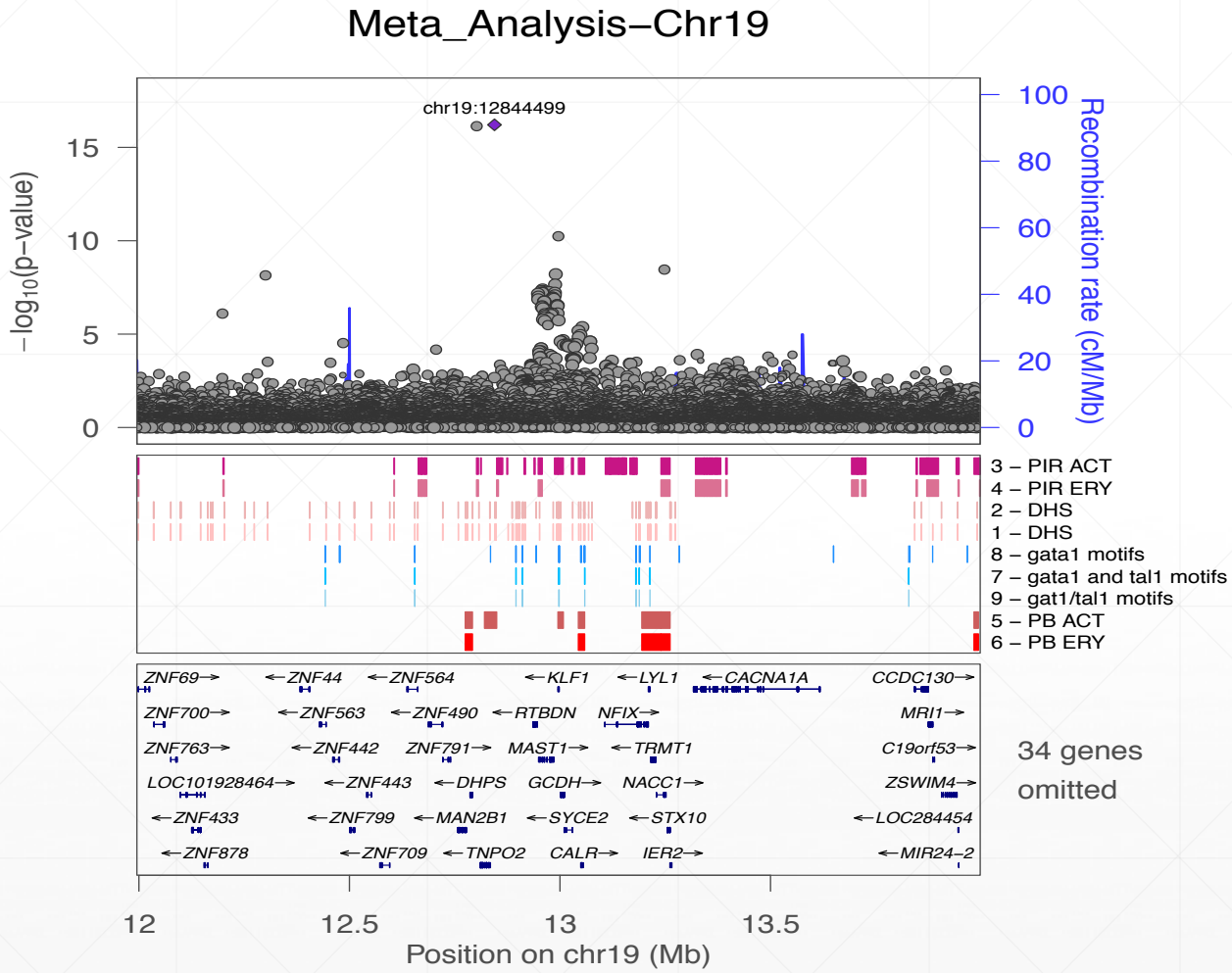
Conditional Meta-analysis of HbF in SCD and Sardinian

- Discovery of new gene modulating HbF *KLF1.*
  - *KLF1* never reported in GWAS before, and not in LD with *NFIX* variant.
- Approach yielded many synonymous variants as expected with GWAS.
- Several rare variants and several variants present in either just Sardinians or just SCD.



KLF1 – Strong evidence of HbF modulation

- Recent studies in a Maltese family with HPFH found a nonsense mutation in *KLF1*.
- Additionally, *KLF1* reduces *BCL11A* expression, upregulating gamma-globin and beta-globin gene expression.



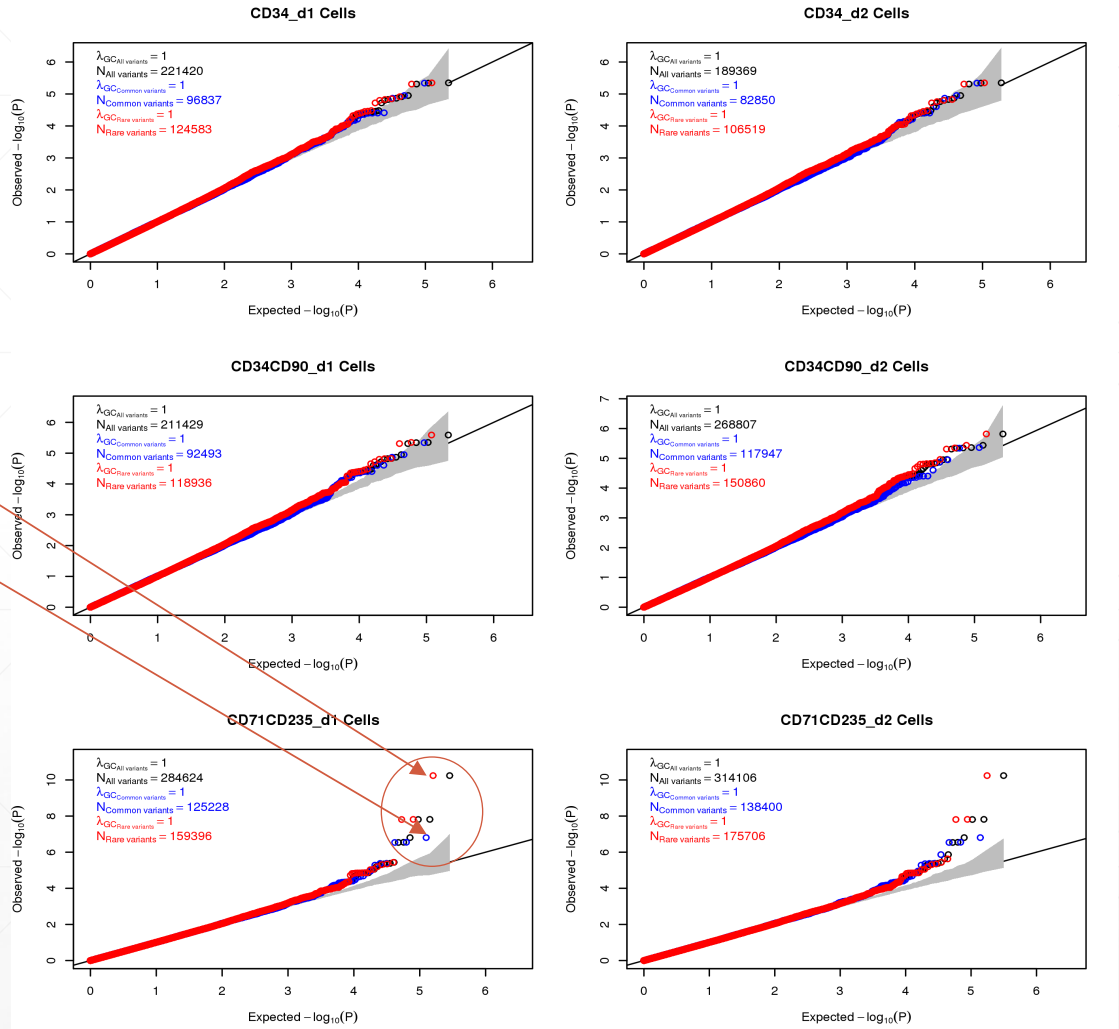
Borg, J., et al., Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. Nat Genet, 2010. 42(9): p. 801-5
Zhou, D., et al., KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching. Nat Genet, 2010. 42(9): p. 742-4.

KLF1 – Strong evidence of HbF modulation

- Annotation with transposase access chromatin sites specific to different cell maturation stages.

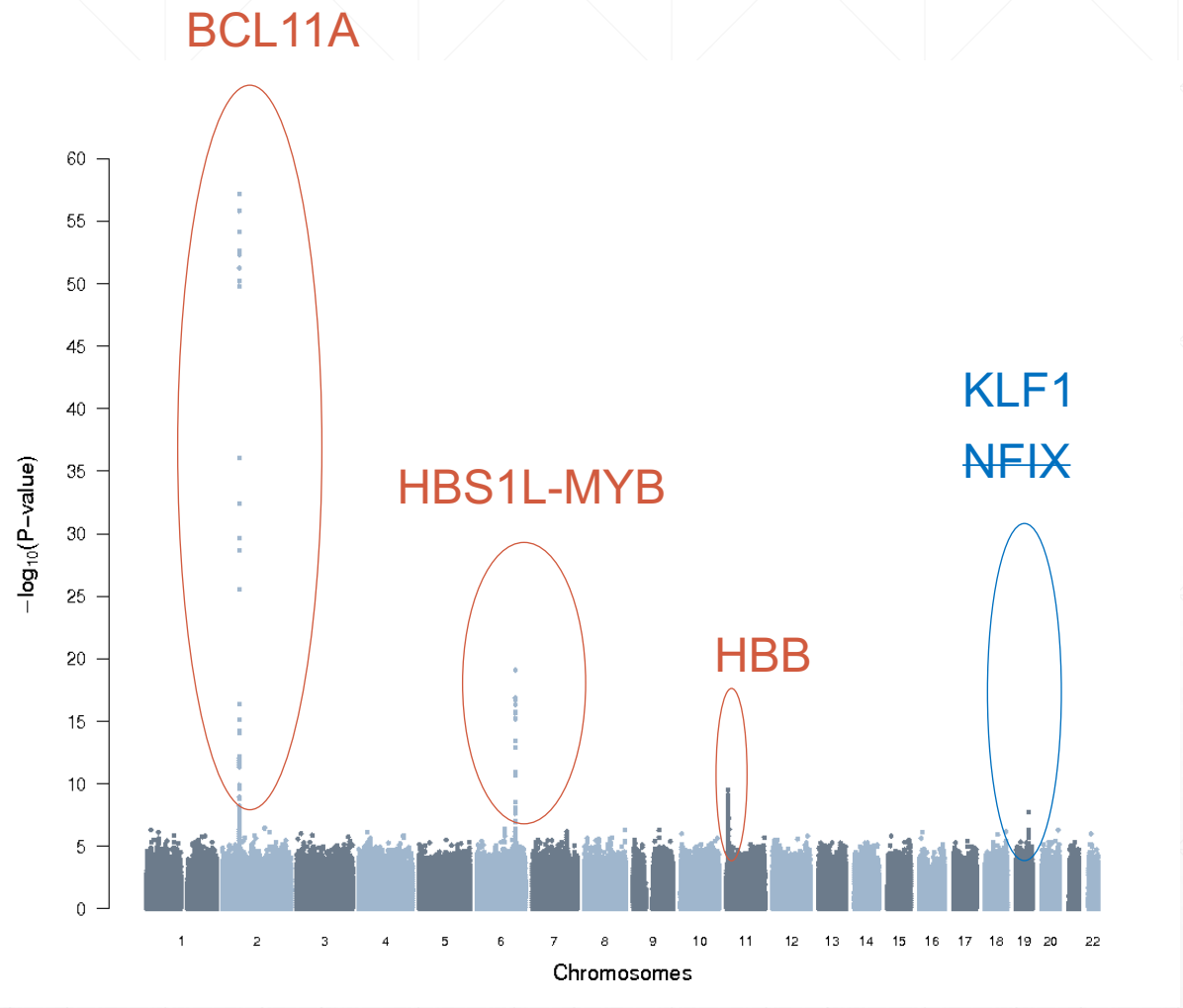
SNP	Freq	Present in both cohorts
rs558942739	Rare	Only in Sardanians
rs2280742	Common	Yes

- We found several proxies for the common variant, and several eQTL effects on *DNASE2* and *KLF1*.
- This mutation could represent an HPFH mutation.



Conclusion

- Together *BCL11A*, *HBS1L-MYB*, *HBB* account for at least ~50% of variability of HbF in humans. We are looking for loci explaining the remaining heritability:
  - Approach: Using conditional meta-analysis in ~10,000 individuals from Sardinian and African ancestry.
- We have potentially identified two new loci that modulates HbF Chr19.
- Our annotation provided additional evidence that polymorphism at *KLF1* is the causal variant rather than *NFIX*.



Perspectives

- Fine-map association results on Chr19 and Chr10.
  - Goal: Identifying the causal variant.
- Perform enrichment analysis to strengthen our current hypothesis.
- Identify variant present in a single cohort to genotype.
- Design functional experiments to test the consequence of causal mutations.

Acknowledgements

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Questions?