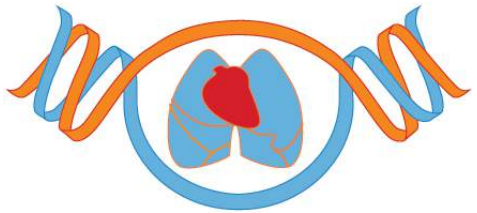
Pushing the boundaries of whole genome sequencing: From genotype to phenotype with a few extras in between.

Symposium on Advances in Genomics, Epidemiology, and Statistics.

Rasika Mathias
Johns Hopkins University
June 7th, 2019



GeneSTAR Research Center
Genetic Studies of Atherosclerosis Risk

JOHNS HOPKINS
MEDICINE

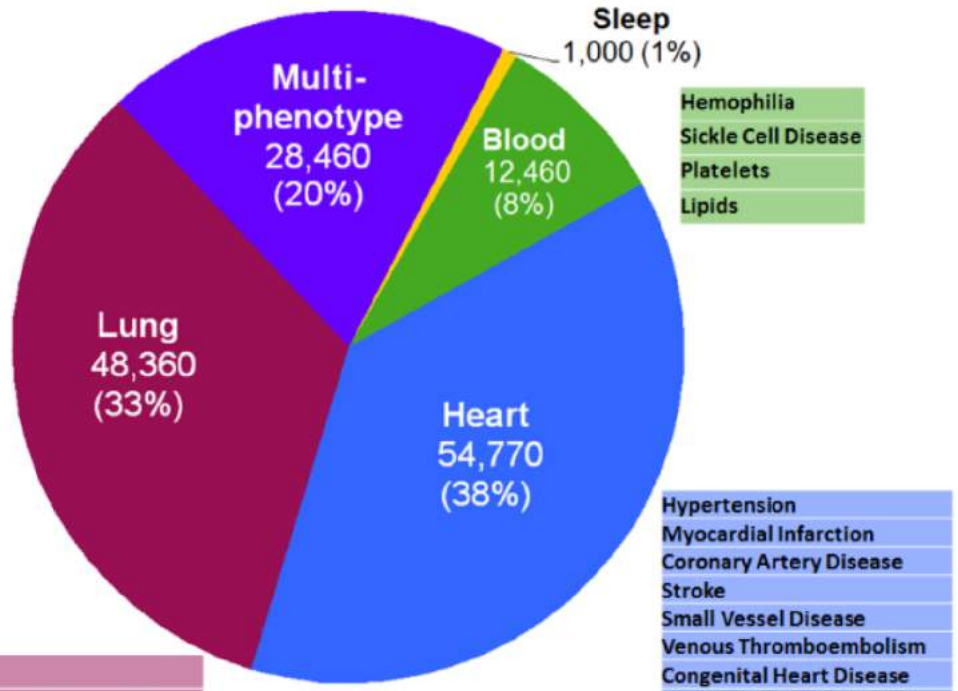
Leveraging whole genome sequencing to identify novel determinants of platelet function.

Novel genetic loci identified for telomere length.

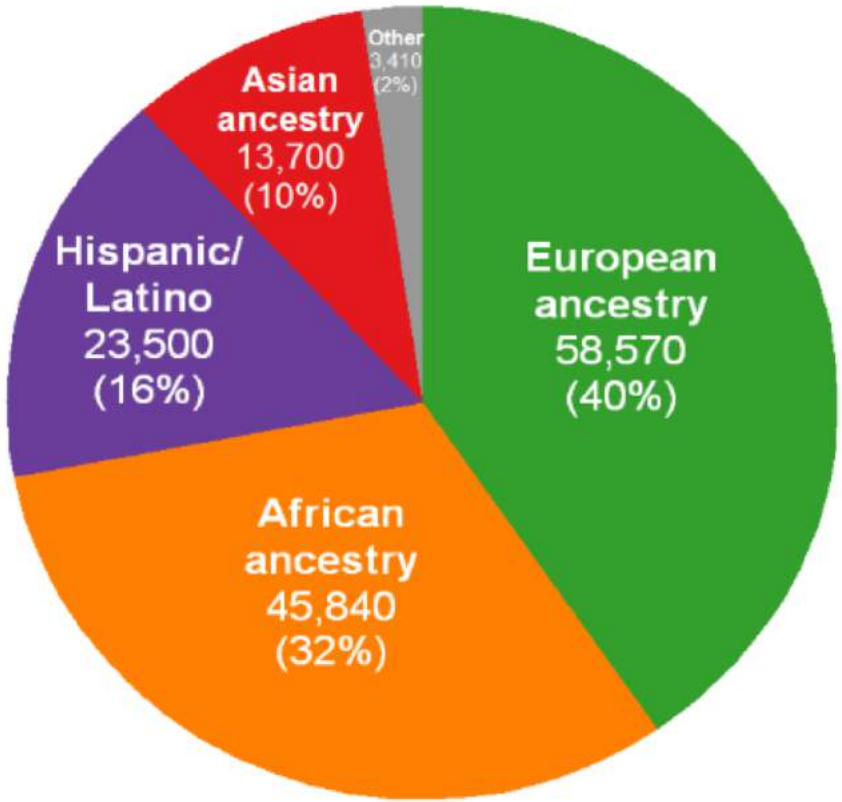
NHLBI Trans-Omics for Precision Medicine

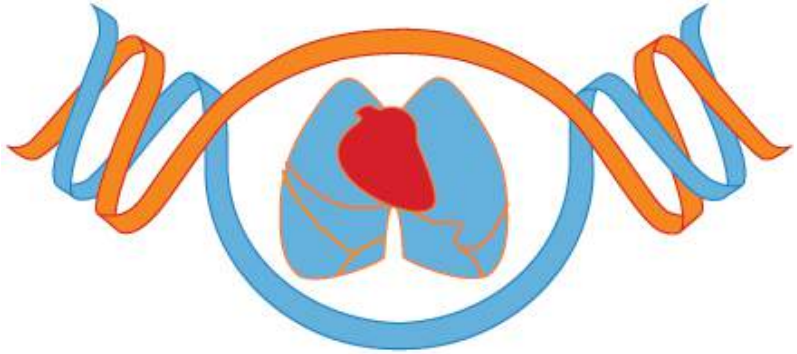
Centers | Projects/Studies | Working Groups | Data | Publications | EAP | ELSI | Workshops

Sample numbers by phenotype area (N=144k total)



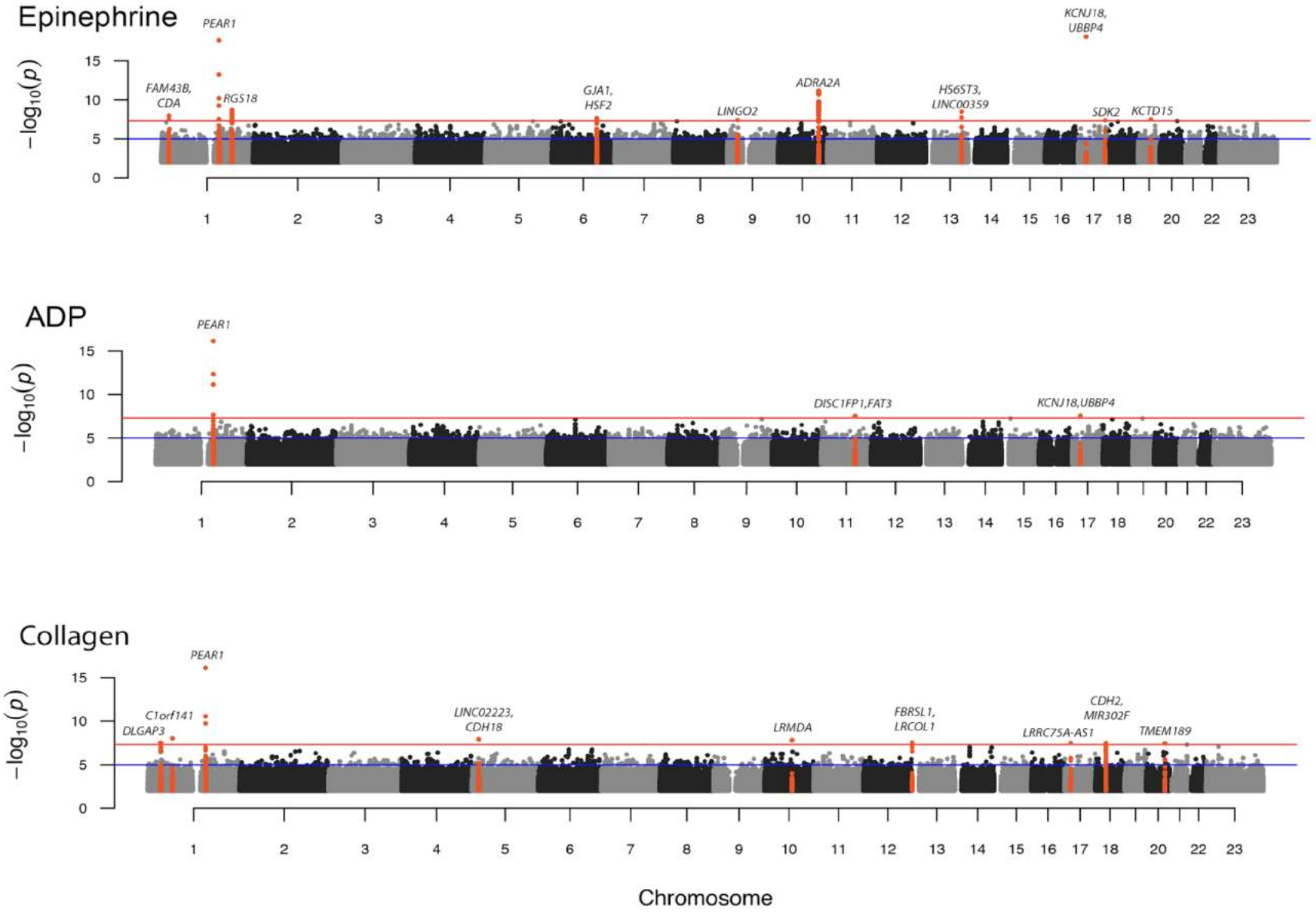
Sample numbers by ancestry/ethnicity (N=144k total)





Leveraging whole genome sequencing to identify novel determinants of platelet function

Genome-wide single variant tests for association were performed on ~76 million single nucleotide variants (SNV) in 3,125 European Americans (EA) and 730 African Americans (AA) from the Framingham Heart Study (FHS), Older Order Amish Study (OAA), and the Genetic Study of Atherosclerosis Risk (GeneSTAR) Study.



104 variants associated with platelet aggregation in response to ADP, epinephrine, or collagen (P-value<$5x10^{-8}$)

Iterative conditional analyses refines 16 independent loci

Known Loci
(N = 2)

Novel Loci
(N = 14)

	MAF	hg38	rsID	ref/alt	Nearest Gene	ADP	Epinephrine	Collagen
known		Chr10:111139289	rs7097060	T/A	*ADRA2A,GPAM*			
known		Chr1:156899922	rs12041331	G/A	*PEAR1*			
novel		Chr17:16451482	rs575524466	G/A	*LRRC75A-AS1*			
novel		Chr20:50142397	rs542707094	CTG/C	*TMEM189,TMEM189-UBE2V1*			
novel		Chr9:28873884	rs185159562	T/A	*LINGO2*			
novel		Chr10:75490891	rs138028657	A/G	*LRMDA*			
novel		Chr12:132589485	rs140148392	G/A	*FBRSL1,LRCOL1*			
novel		Chr11:92185065	rs183146849	A/T	*DISC1FP1,FAT3*			
novel		Chr1:67128641	rs142001088	C/T	*C1orf141*			
novel		Chr5:19109993	rs112157462	T/C	*LINC02223,CDH18*			
novel		Chr13:96912429	rs61974290	A/G	*HS6ST3,LINC00359*			
novel		Chr1:20567949	rs12137738	A/T	*FAM43B,CDA*			
novel		Chr18:29059923	rs138845468	TAAATA/T	*CDH2,MIR302F*			
novel		Chr6:121921871	rs58250884	A/G	*GJA1,HSF2*			
novel		Chr17:21960955	.	A/T	*KCNJ18,UBBP4*			
novel		Chr1:192194880	rs1175170	G/C	*RGS18,RGS21*			

MAF

C > 0.05

L 0.01 – 0.05

R < 0.01

$P < 5x10^{-8}$

RGS18 controls platelet generation and function

Regulator of G-Protein Signaling 18 Controls Both Platelet Generation and Function

Nathalie Delesque-Touchard[1]*, Caroline Pendar
Véronique Salel[1], Caroline Hervé[1], Anne-Marie
Tania Sorg[3], Jean-Marc Herbert[1], Pierre Savi[1], F

1 Early to Candidate (E2C), Sanofi, Toulouse, France, 2 SCP Biologics, Sanofi
Institute (MCI), Strasbourg, France

Abstract

RGS18 is a myeloerythroid lineage-specific regulator of
platelets. In the present study, we describe the first ger
phenotypic differences between RGS18-/- and wild-type

Regulator of G-protein signaling 1
in platelets

Kristina Gegenbauer,[1,2] Giuliano Elia,[3] Alfonso Blan

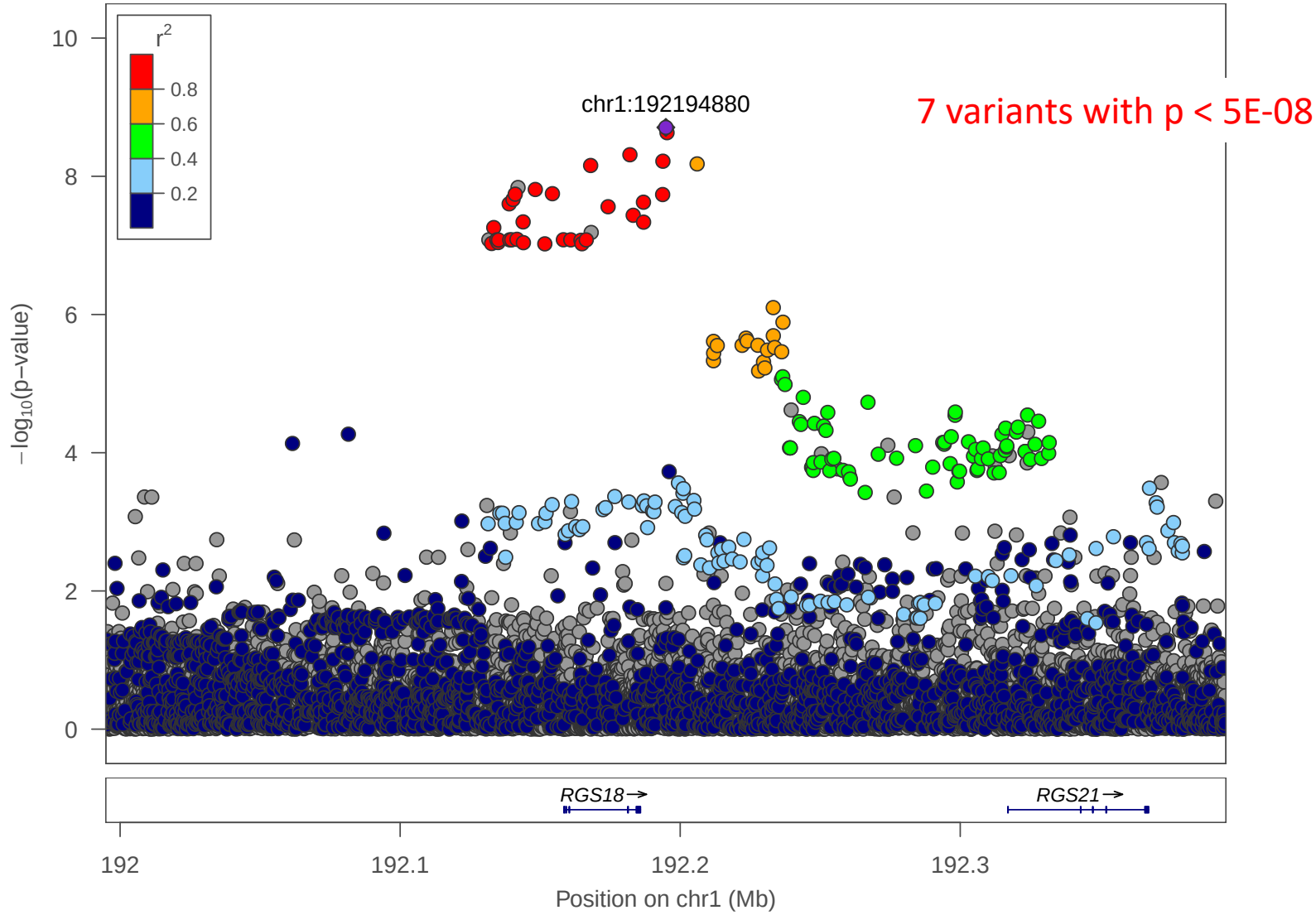
[1]Conway Institute, University College Dublin, Dublin, Ireland; [2]Sch
[3]Mass Spectrometry Resource, Conway Institute, University Colle

Regulator of G-protein signaling 18 (RGS18) is a GTPase-activating protein for the G-α-q and G-α-i subunits of heterotrimeric G-proteins that turns off signaling by G-protein coupled receptors. RGS18 is highly expressed in platelets. In the present study, we show that the 14-3-3γ protein binds to phosphorylated serines 49 and 218 of RGS18. Platelet activation by thrombin, thromboxane A2, or ADP stimulates the association of 14-3-3 and RGS18, probably by increasing the phosphorylation of serine 49. In contrast, trea
clin and n
cyclic nu
AMP-dep
cyclic GI
(PKGI), induces the phosphorylation of serine 216 of RGS18 and the detachment of 14-3-3. Serine 216 phosphorylation is able to block 14-3-3 binding to RGS18 even in the presence of thrombin, thromboxane A2, or ADP. 14-3-3–deficient RGS18 is more active compared with 14-3-
block Gq-dependent calcium signaling. These findings indicate cross-talk between platelet activation and inhibition pathways at the level of RGS18 and Gq. (*Blood*. 2012;119(16):3799-3807)

- RGS18 -/- mice :
  - lower number of bone marrow Megakaryocytes(MK).
  - peripheral platelets are more prone to be activated at baseline compared to wild type.
  - In presence of platelet agonists, platelets aggregate more compared to RGS18 wild types.

- Differential phosphorylation of RGS18 (Serine49 vs Serine216) modifies Calcium gradient in platelets. This change in gradient of calcium dictates the level of platelet activation.

RGS18 and platelet aggregation to Epinephrine



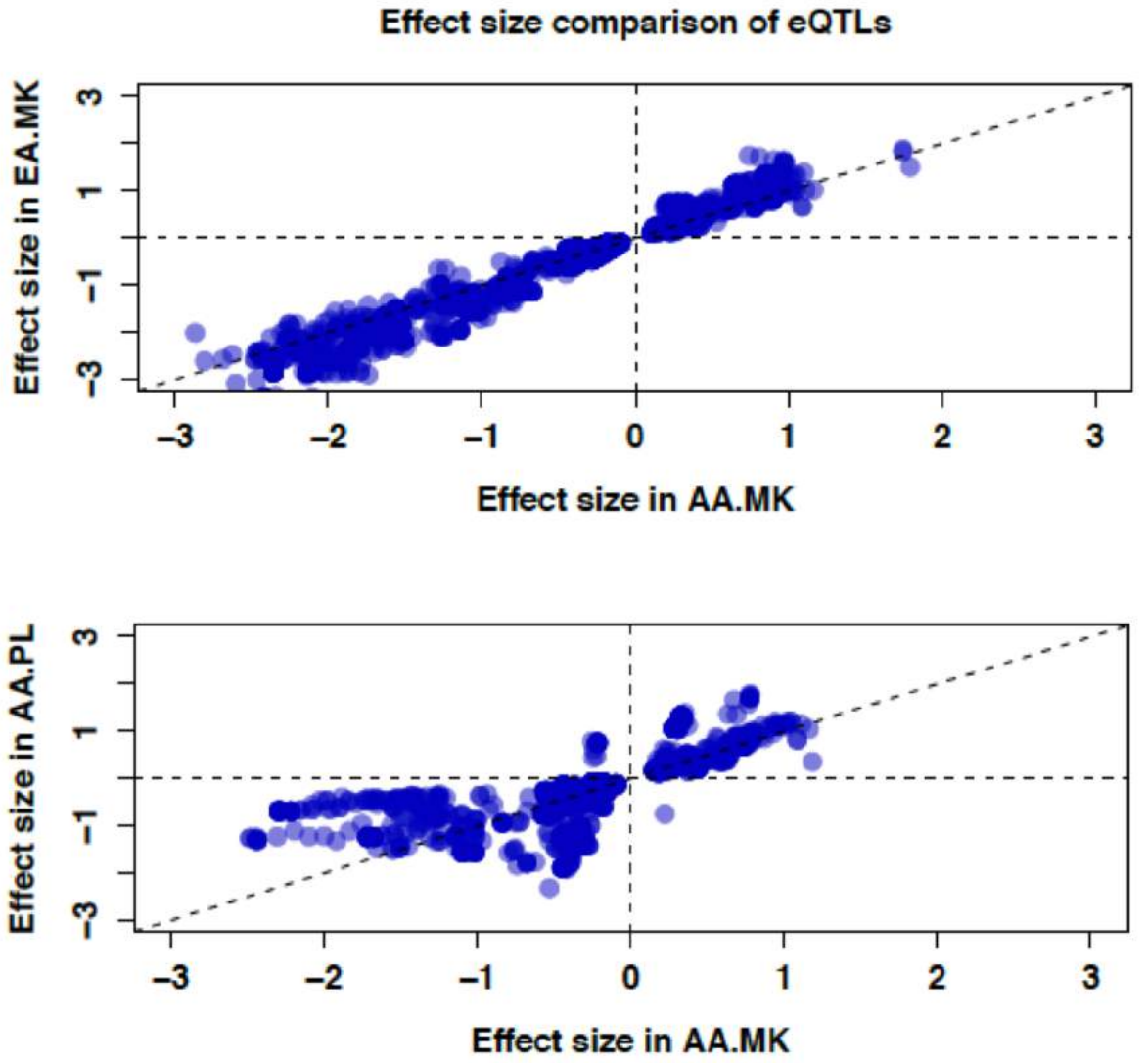
GeneSTAR Data available for eQTL analysis RNASeq + WGS

PLATELETS

- AA
  - N=84
  - 5,004,400 SNPs
  - 9500 genes
- EA:
  - N=101
  - 4,433,801 SNPs
  - 9,662

iPSC-derived MEGAKARYOCYTES

- AA
  - N=110
  - 5,500,942 SNPs
  - 4,998 genes
- EA:
  - N=180
  - 5,064,974 SNPs
  - 4,555 genes

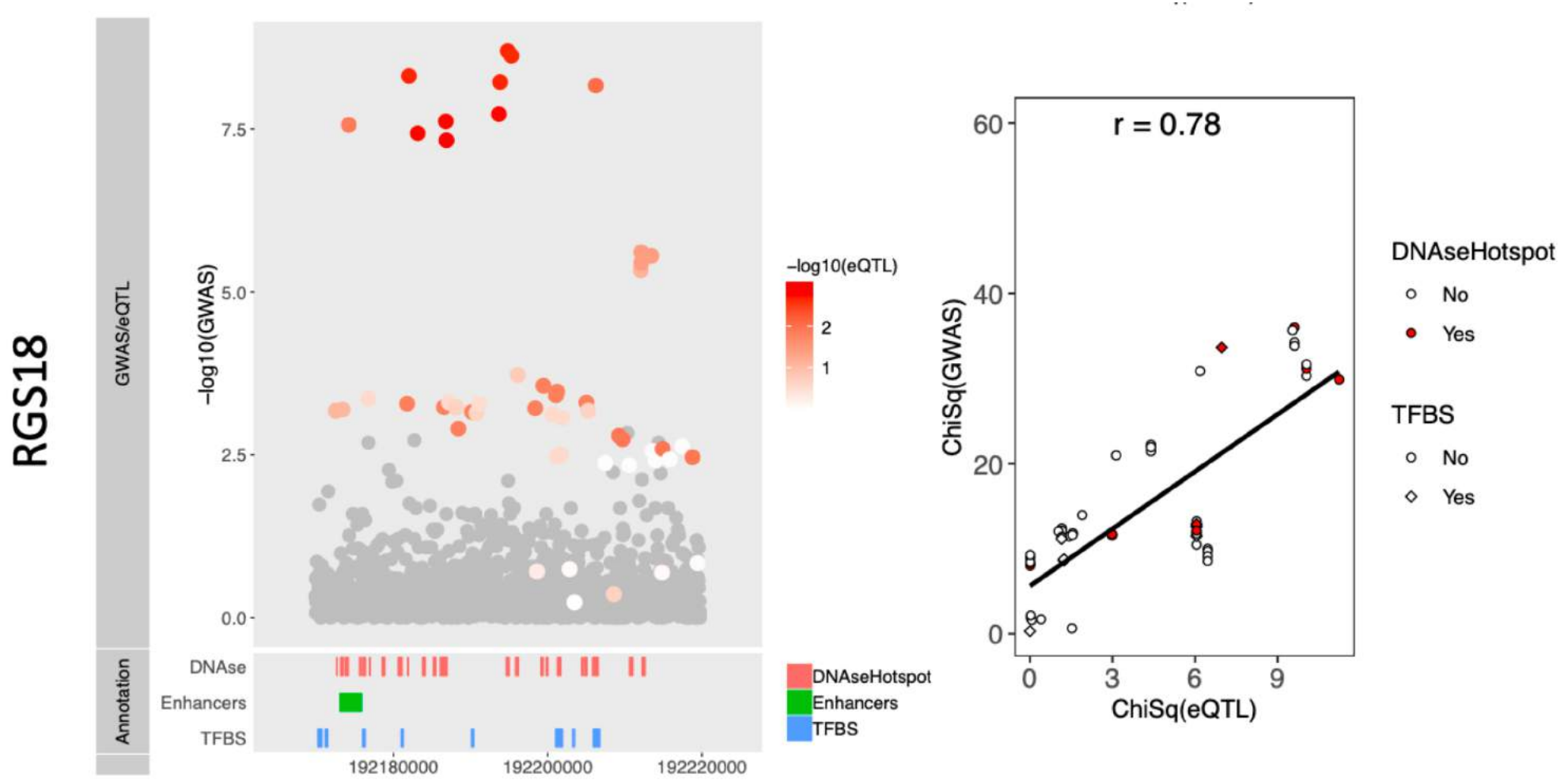


Overlap in platelet aggregation loci and eQTL for the top 22 loci.

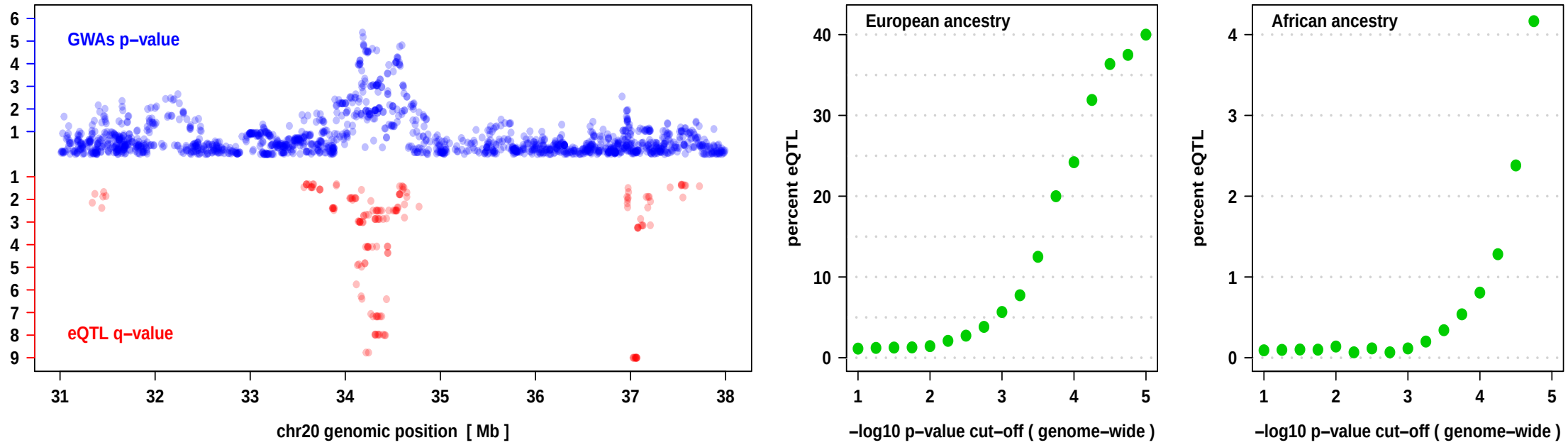
eQTL analysis of top variants in PLT RNAseq data:

		eQTL signal	
SNP	Gene	pvalue	beta
rs12041331	PEAR1	3.01E-06	0.16729668
rs1175170	RGS18	2.29E-03	0.08045384

Co-localization Approaches integrating GWAS and eQTLs



Co-localization also supports the role for multiple causal variants mapping to the GWAS peaks.



Left: EA GWAS p-values (blue) and eQTL q-values (red, only q<0.05 shown) on the –log10 scale near 34Mb on chr20, indicating obvious SNP/eQTL colocalization. **Right:** Percent eQTL among SNPs (y-axis) as a function of p-value cut-off shows an enrichment of eQTLs among stronger associated SNPs for both EAs and AAs. The much higher percentage among EAs indicates the inadequacy of SNP arrays to capture LD among AAs.

Poster # 23: Identifying SNP Associations in Under-Powered Whole-Genome Sequencing Association Studies Using eQTLs. Julius Ngwa.

https://www.biorxiv.org/content/10.1101/621565v1

Cold Spring Harbor Laboratory

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New Results

Comment on this paper

Genome Sequencing Unveils a New Regulatory Landscape of Platelet Reactivity

Ali R. Keramati, Ming-Huei Chen, Benjamin A.T. Rodriguez, Lisa R.Yanek, Brady J. Gaynor, Kathleen Ryan, Jennifer A. Brody, NHLBI Trans-Omics for Precision (TOPMed) Consortium, NHLBI TOPMed Hematology and Hemostasis Working Group, Kai Kammers, Kanika Kanchan, Kruthika Iyer, Madeline H. Kowalski, Achilleas N. Pitsillides, L. Adrienne Cupples, Alan R. Shuldiner, Jeffrey R. O'Connell, Braxton D. Mitchell, Nauder Faraday, Margaret A. Taub, Lewis C. Becker, Joshua P. Lewis, Rasika A. Mathias, Andrew D. Johnson

doi: https://doi.org/10.1101/621565

Novel genetic loci identified for telomere length.

NHLBI Trans-Omics for Precision Medicine
Whole Genome Sequencing Program

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of PUBLIC HEALTH

Estimating telomere length from WGS data

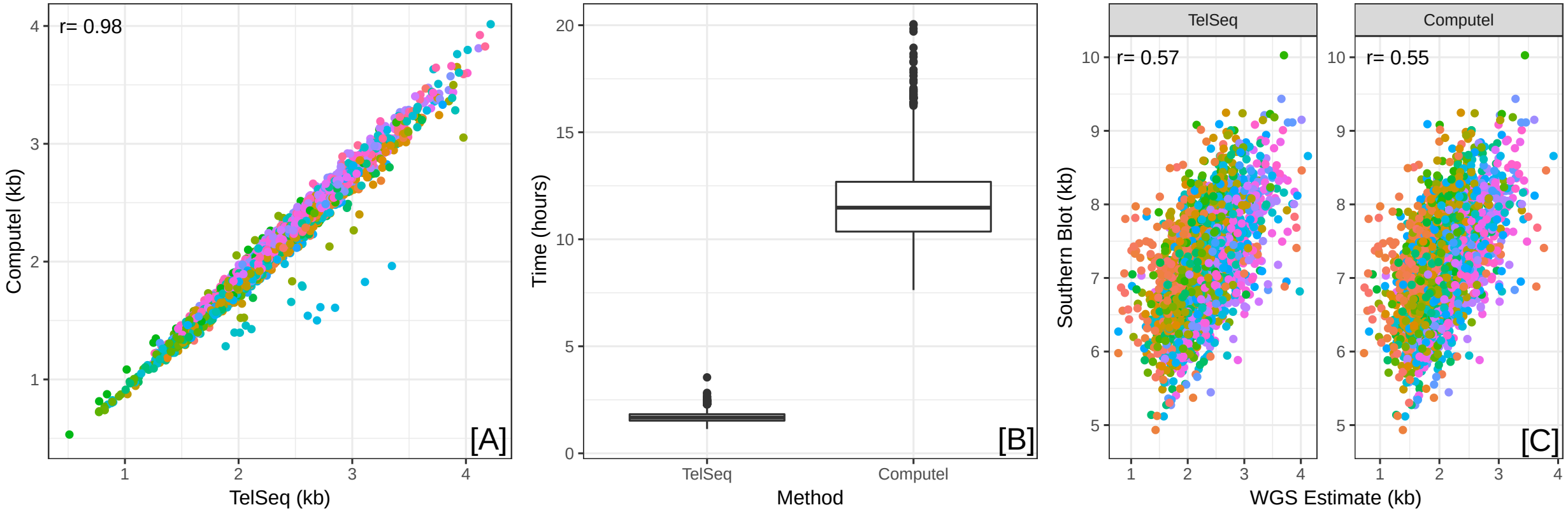
Telseq:

Filters WGS reads with a specified number of occurrences of the telomere hexamer TTAGGG, adjusting for counts of overall reads with similar GC content (Z. Ding et al., Nucl. Acids Res. 2014)

Computel:

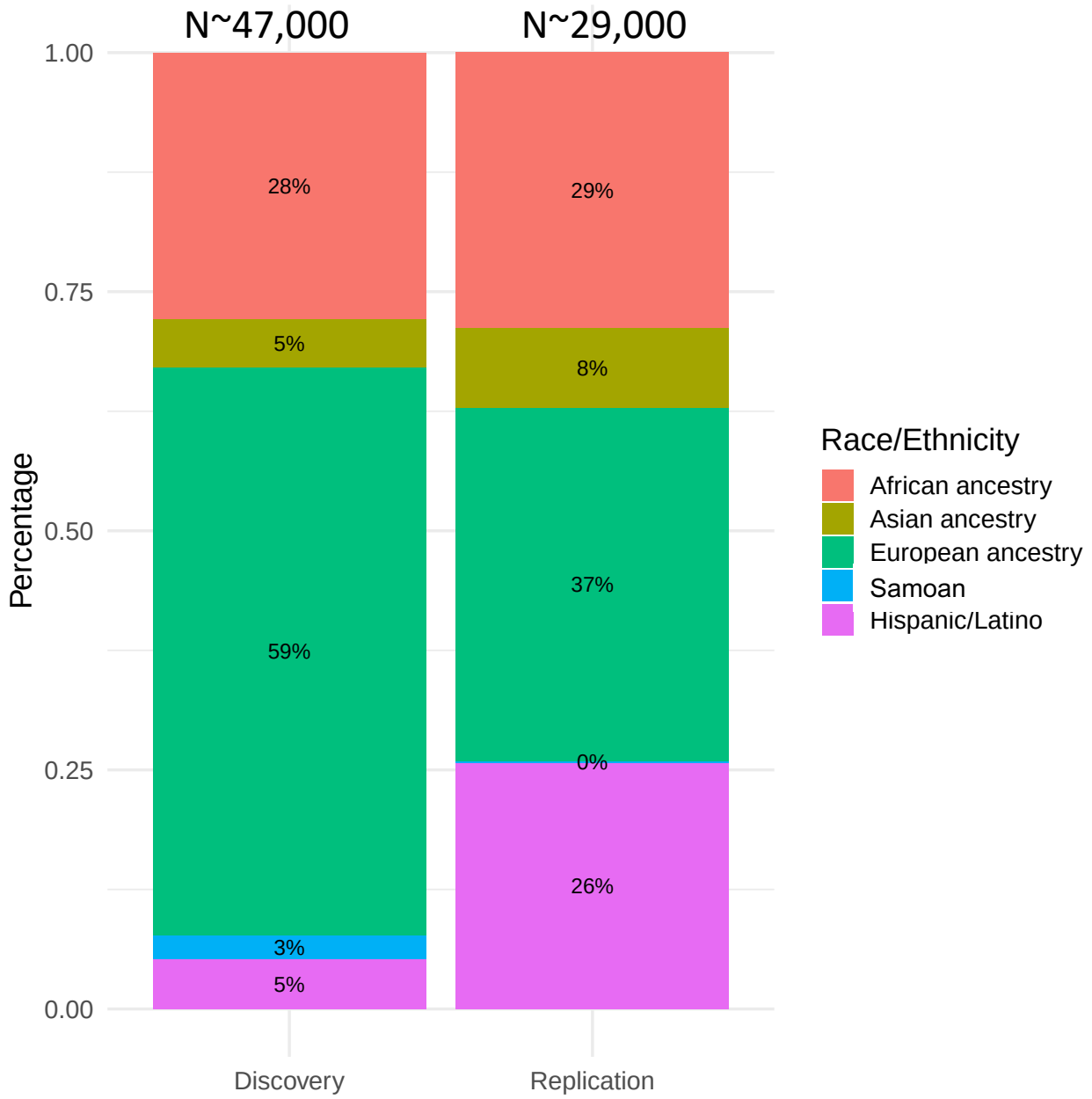
Realigns all reads to “telomere reference genome” using bowtie alignment software (L. Nersisyan & A. Arakelyan, PLoS One, 2015)

Estimating telomere length: TOPMed data

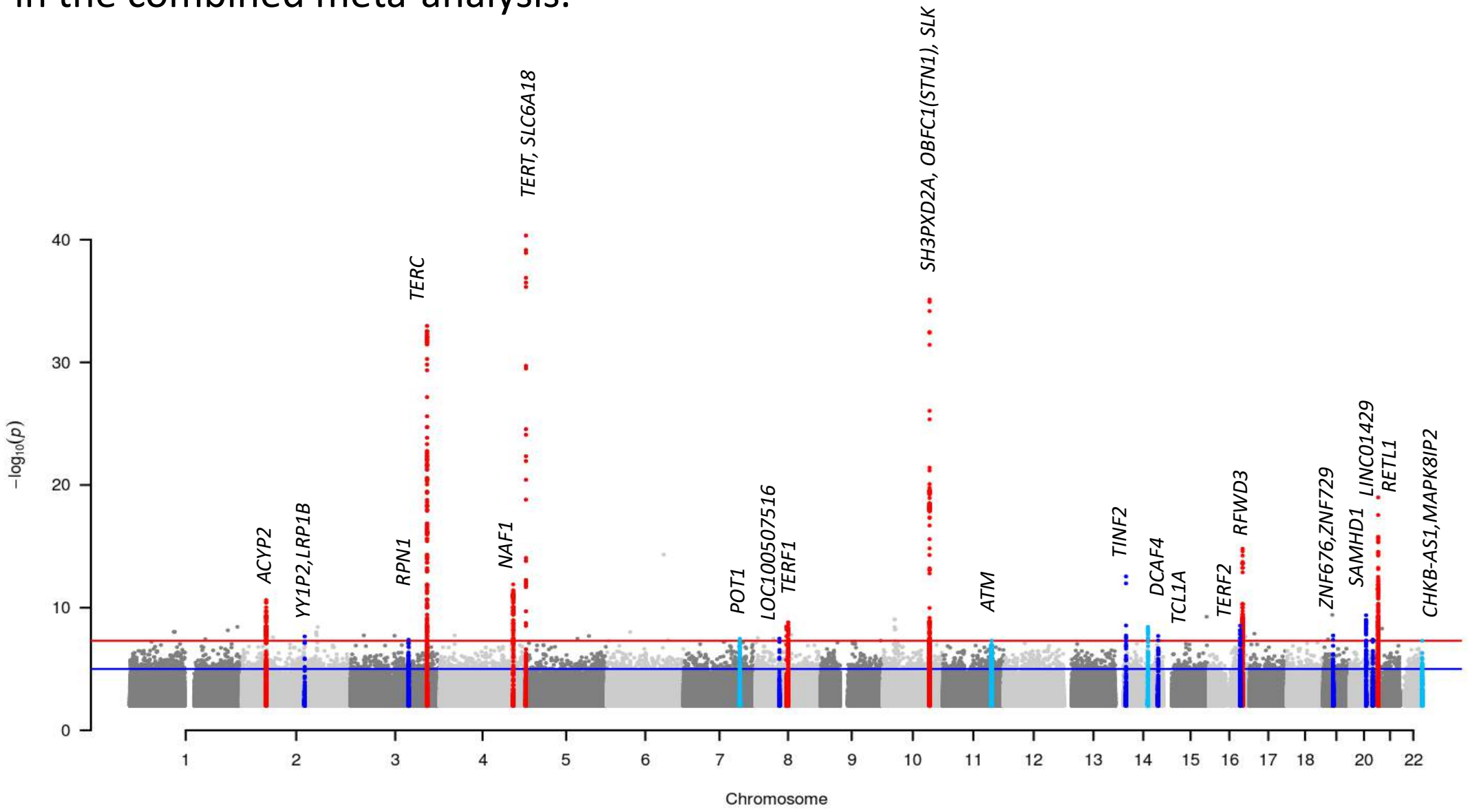


[A] Pearson correlation between TelSeq and Computel length estimates on 3362 TOPMed samples. **[B]** Comparison of computational times for TelSeq and Computel **[C]** Pearson correlation between TelSeq (left) and Computel (right) and Southern Blot TL estimates on 2429 samples from JHS. *Colors indicate sequencing plate in Panels A and C.*

TL analysis in TOPMed will be the single largest dataset to investigate genetics of TL in the most diverse sample available!



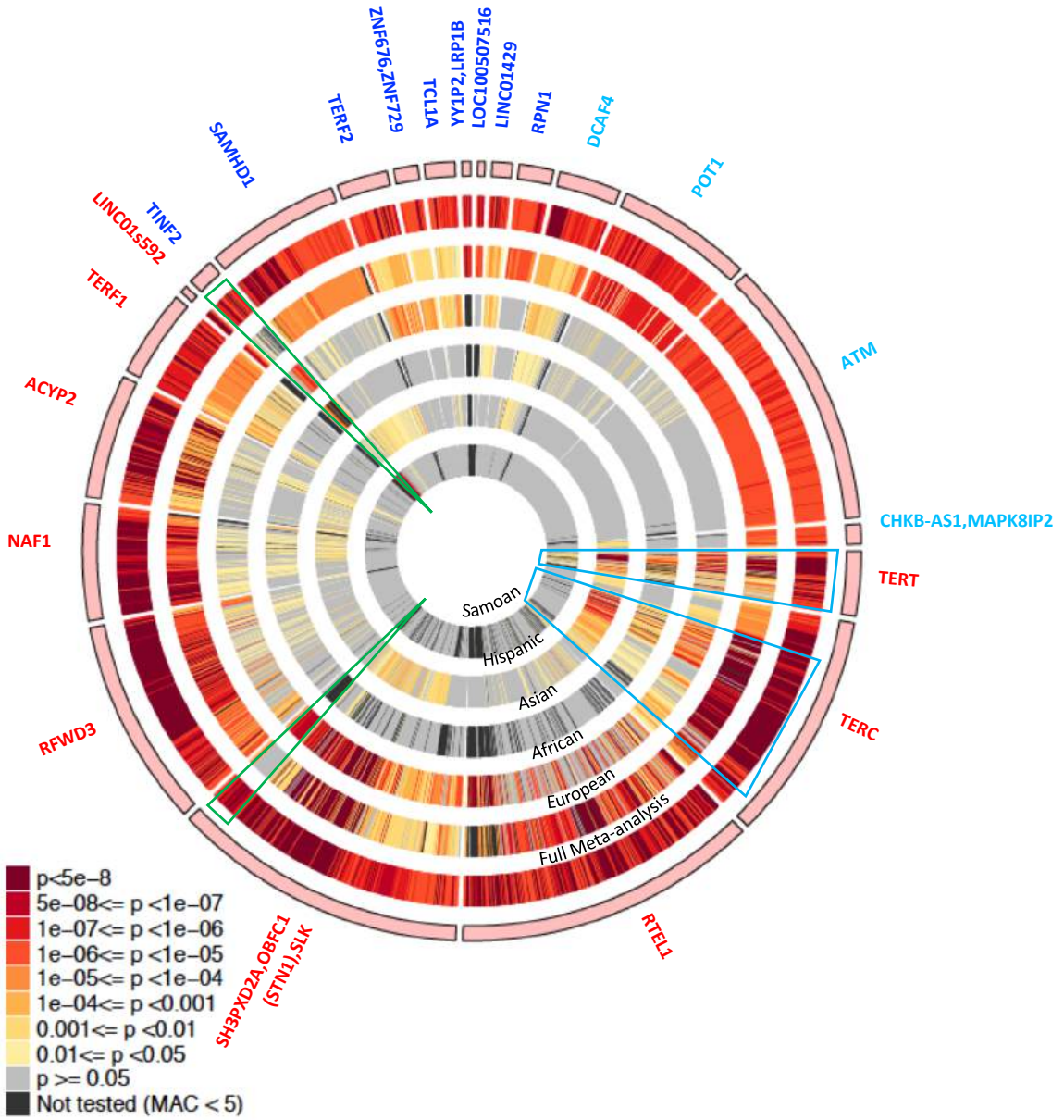
Multiethnic genomewide tests for association using 82M sequence identified variants on N=75,176 samples with sequence generate telomere length from TOPMed. All loci had a peak $p<5x10^{-8}$ in the combined meta-analysis.



Multiethnic genomewide tests for association in TOPMed: 22 identified loci

			Discovery (n=46458)		Replication (n=28718)		Meta-Analysis		Effect Size	
	Locus Name	Known vs Novel	AAF	P-Value	AAF	P-Value	P-Value	Direction	Variance	Basepairs
Tier 1	**TERT***		**31%**	**3.3E-24**	**28%**	**1.3E-18**	**4.6E-41**	**++**	**0.21%**	**61.6**
	TERC*		**21%**	**6.7E-19**	**22%**	**1.2E-16**	**1.1E-33**	**--**	**0.20%**	**-68.4**
	RTEL1*		**70%**	**1.7E-13**	**71%**	**1.0E-07**	**1.0E-19**	**--**	**0.10%**	**-42.7**
	SH3PXD2A,OBFC1(STN1),SLK*		**69%**	**1.8E-19**	**66%**	**2.2E-18**	**7.6E-36**	**--**	**0.21%**	**-65.0**
	RFWD3*	**Novel**	**44%**	**4.1E-15**	**43%**	**3.6E-03**	**1.6E-15**	**--**	**0.07%**	**-31.8**
	NAF1*		**78%**	**1.8E-09**	**78%**	**1.3E-04**	**1.3E-12**	**++**	**0.07%**	**37.2**
	ACYP2*		**17%**	**2.4E-08**	**17%**	**2.0E-04**	**2.4E-11**	**++**	**0.05%**	**34.2**
	TERF1*	**Novel**	**58%**	**1.4E-07**	**54%**	**2.1E-03**	**1.6E-09**	**--**	**0.05%**	**-27.8**
	LINC01592	Novel	0%	5.8E-09	0%	4.5E-02	6.1E-09	--	0.03%	-407.3
Tier 2	**TINF2**	**Novel**	**1%**	**1.1E-07**	**1%**	**4.3E-07**	**2.8E-13**	**++**	**0.09%**	**150.6**
	SAMHD1	**Novel**	**23%**	**7.6E-08**	**26%**	**1.1E-03**	**4.1E-10**	**--**	**0.06%**	**-34.0**
	TERF2	**Novel**	**31%**	**2.6E-06**	**30%**	**2.9E-04**	**2.9E-09**	**++**	**0.04%**	**26.7**
	ZNF676,ZNF729		59%	4.5E-07	57%	7.3E-03	1.9E-08	++	0.03%	21.3
	TCL1A	Novel	34%	3.8E-06	37%	1.4E-03	2.0E-08	++	0.03%	24.2
	YY1P2,LRP1B	Novel	0%	3.7E-07	0%	9.7E-03	2.2E-08	++	0.02%	651.2
	LOC100507516	Novel	0%	3.7E-06	0%	2.2E-03	3.3E-08	--	0.03%	-236.8
	LINC01429		14%	3.1E-06	15%	3.2E-03	4.0E-08	++	0.04%	32.8
	RPN1	Novel	26%	4.5E-06	23%	2.4E-03	4.2E-08	++	0.03%	26.2
Tier 3	DCAF4		10%	1.2E-05	10%	7.1E-05	3.6E-09	++	0.04%	39.5
	POT1	**Novel**	**21%**	**2.6E-04**	**19%**	**2.0E-05**	**3.6E-08**	**--**	**0.04%**	**-30.3**
	ATM	**Novel**	**50%**	**2.2E-05**	**49%**	**6.0E-04**	**4.9E-08**	**--**	**0.04%**	**-25.3**
	CHKB-AS1,MAPK8IP2	Novel	30%	9.5E-05	26%	1.2E-04	5.0E-08	--	0.04%	-26.9

Association signal for the 22 loci showing all variants having a $p<1x10^{-5}$ in the meta-analysis, and the ancestry specific signal at each of these variants.



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https://www.biorxiv.org/content/10.1101/749010v1

New Results

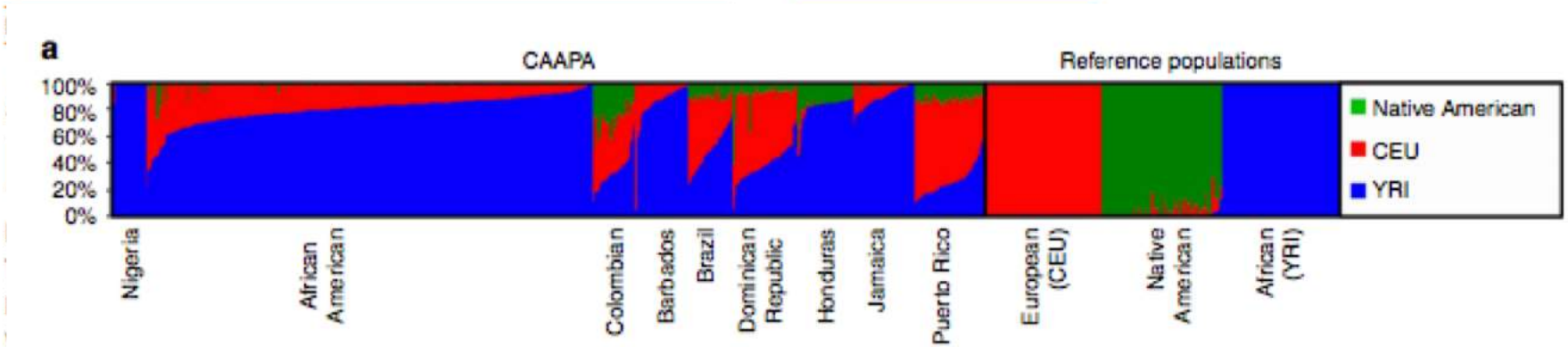
Novel genetic determinants of telomere length from a multi-ethnic analysis of 75,000 whole genome sequences in TOPMed

Margaret A Taub, Joshua S Weinstock, Kruthika R Iyer, Lisa R Yanek, Matthew P Conomos, Jennifer A Brody, Ali Keramati, Cecelia A Laurie, Marios Arvanitis, Albert V Smith, John Lane, Lewis C Becker, Joshua C Bis, John Blangero, Eugene R Bleecker, Esteban G Burchard, Juan C Celedon, Yen Pei C Chang, Brian Custer, Dawood Darbar, Lisa de las Fuentes, Dawn L DeMeo, Barry I Freedman, Melanie E Garrett, Mark T Gladwin, Susan R Heckbert, Bertha A Hidalgo, Christie Ingram, Marguerite R Irvin, W Craig Johnson, Stefan Kaab, Lenore Launer, Jiwon Lee, Simin Liu, Arden Moscati, Kari E North, Patricia A Peyser, Nicholas Rafaels, Laura M Raffield, Daniel E Weeks, Marsha M Wheeler, L. Keoki Williams, Wei Zhao, Mary Armanios, Stella Aslibekyan, Paul L Auer, Donald W Bowden, Brian E Cade, Ida Yii-Der Chen, Michael H Cho, L Adrienne Cupples, Joanne E Curran, Michelle Daya, Ranjan Deka, Xiuqing Guo, Lifang Hou, Shih-Jen Hwang, Jill M Johnsen, Eimear E Kenny, Albert M Levin, Chunyu Liu, Ryan L Minster, Mehdi Nouraie, Ester C Sabino, Jennifer A Smith, Nicholas L Smith, Jessica Lasky Su, Marilyn J Telen, Hemant K Tiwari, Russell P Tracy, Marquitta J White, Yingze Zhang, Kerri L Wiggins, Scott T Weiss, Ramachandran S Vasan, Kent D Taylor, Moritz F Sinner, Edwin K Silverman, M. Benjamin Shoemaker, Wayne H-H Sheu, Jerome I Rotter, Susan Redline, Bruce M Psaty, Juan M Peralta, Nicholette D Palmer, Ruth JF Loos, Courtney G Montgomery, Braxton D Mitchell, Deborah A Meyers, Stephen T McGarvey, Angel CY Mak, Rajesh Kumar, Charles Kooperberg, Barbara A Konkle, Shannon Kelly, Sharon LR Kardia, Robert Kaplan, Jiang He, Hongsheng Gui, Myriam Fornage, Patrick T Ellinor, Mariza de Andrade, Adolfo Correa, Eric Boerwinkle, Kathleen C Barnes, Allison E Ashley-Koch, Donna K Arnett, Christine Albert, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, TOPMed Hematology and Hemostasis Working Group, TOPMed Structural Variation Working Group, Cathy C Laurie, Goncalo Abecasis, Abraham Aviv, Deborah A Nickerson, James G Wilson, Stephen S Rich, Daniel Levy, Alexis Battle, Thomas W Blackwell, Ingo Ruczinski, Timothy Thornton, Jeff O'Connell, James A Perry, Nathan Pankratz, Alexander P Reiner, Rasika A Mathias

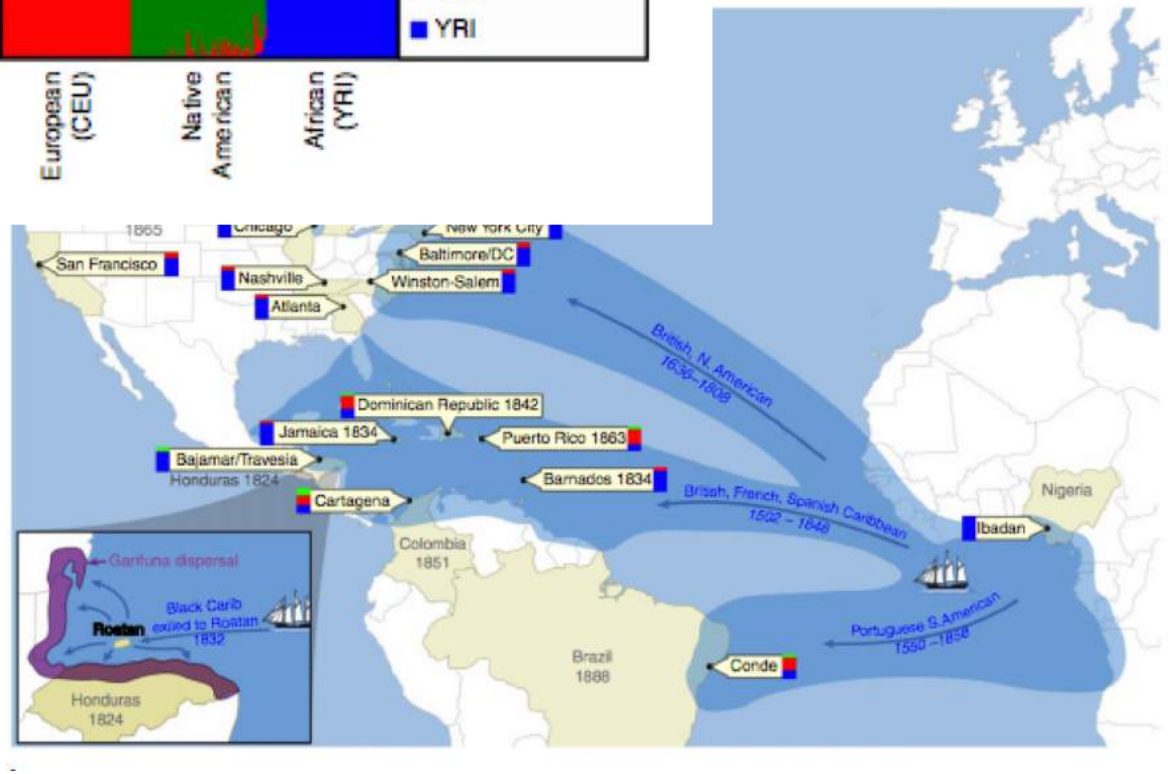
doi: https://doi.org/10.1101/749010

nature COMMUNICATIONS

ARTICLE



Zhaohui S. Qin[11], Alan F. Scott[1], Maria Yazdanbakhsh[19], James G. Wilson[20], Javier M
Kumar[23,24], Pedro C. Avila[25], L. Keoki Williams[15,26], Harold Watson[27,28], Lorraine B.
Olufunmilayo Olopade[32], Ricardo Oliveira[33], Carole Ober[34], Dan L. Nicolae[32,35], Debo
Jennifer Knight-Madden[18], Tina Hartert[29], Nadia N. Hansel[1], Marilyn G. Foreman[36], Jea
Georgia M. Dunston[38,39], Luis Caraballo[40], Esteban G. Burchard[9,41], Eugene Bleecker
Francisco Herrera-Paz[16,17,43], Kimberly Gietzen[44], Wendy E. Grus[45], Michael Bamshad
Kenny[4,47], Ryan D. Hernandez[41,48,49], Terri H. Beaty[2], Ingo Ruczinski[3], Joshua Akey[5],



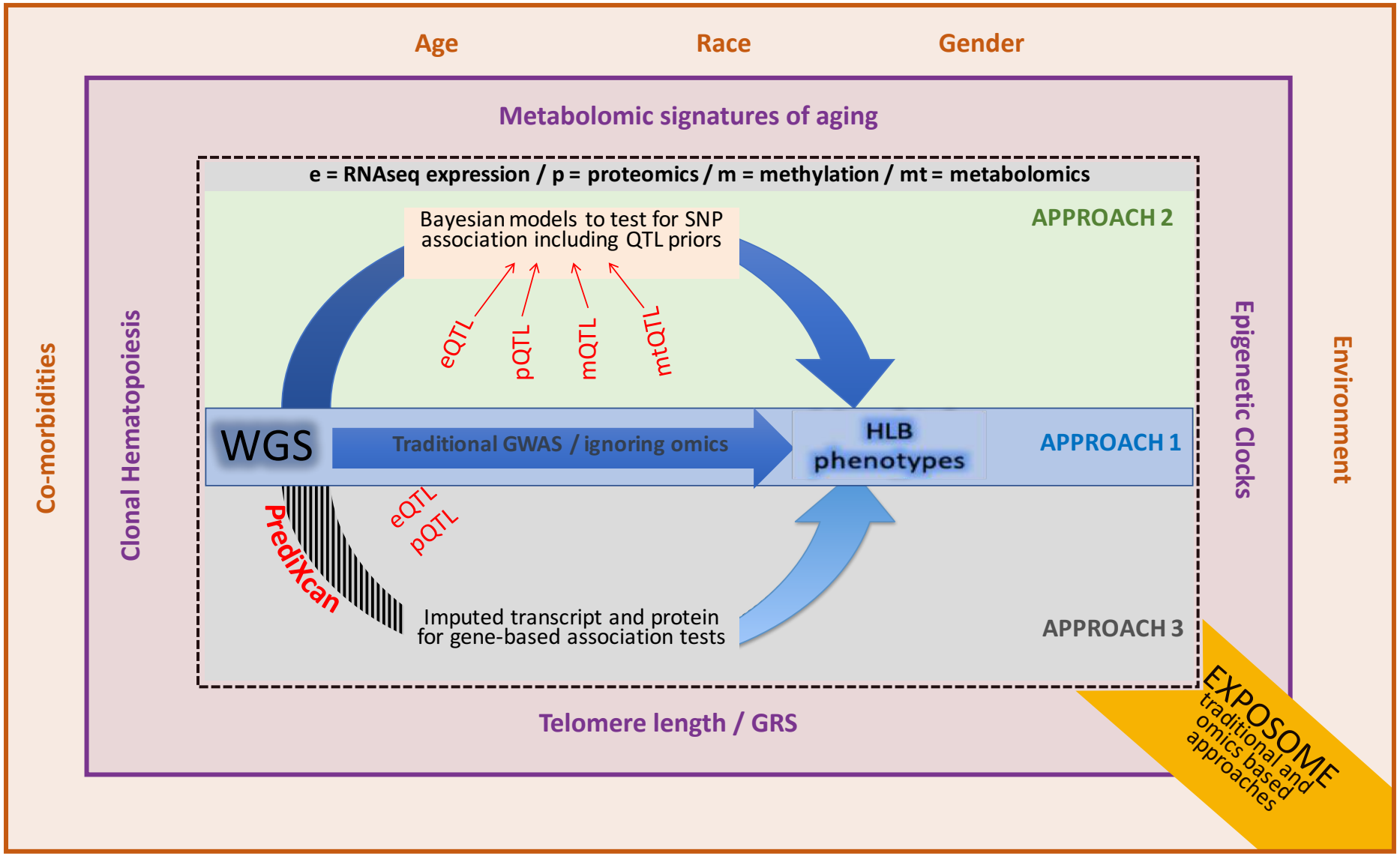
Poster # 12: The association between African ancestry and telomere length across the African diaspora: evidence from the CAAPA study. Kruthika Iyer.

Lessons from the TOPMed illustration

High success of the opportunity created to call TL and to understand the genetics of TL.

Multiple novel loci with strong biological plausibility.

New opportunity created to examine TL-phenotype associations for HLB disorders.


Age
Race
Gender
Metabolomic signatures of aging
e = RNAseq expression / p = proteomics / m = methylation / mt = metabolomics
Bayesian models to test for SNP association including QTL priors
APPROACH 2
eQTL
pQTL
mQTL
mtQTL
WGS
Traditional GWAS / ignoring omics
HLB phenotypes
APPROACH 1
eQTL
pQTL
PrediXcan
Imputed transcript and protein for gene-based association tests
APPROACH 3
Co-morbidities
Clonal Hematopoiesis
Epigenetic Clocks
Environment
Telomere length / GRS
EXPOSOME
traditional and omics based approaches

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Framingham Heart Study

Andrew Johnson

Ming-huei Chen

Benjamin Rodriguez

Jennifer Huffman

Older Order Amish Study

Joshua Lewis

Braxton Mitchell

Brady Gaynor

Kathleen Ryan

GeneSTAR

Ali R. Keramati

Lew Becker

Nauder Faraday

Lisa Yanek

Kruthika Iyer

Margaret Taub

Kai Kammers

Grants and Funding:

The Framingham Heart Study is conducted and supported by the NHLBI in collaboration with Boston University (Contract No. N01-HC-25195). M.H.C., J.D.E. and A.D.J. were supported by National Heart, Lung and Blood Institute Division of Intramural Research funds.

GeneSTAR was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute (U01 HL72518, HL087698, and HL112064) and by a grant from the National Institutes of Health/ National Center for Research Resources (M01-RR000052) to the Johns Hopkins General Clinical Research Center. Genotyping services were provided through the RS&G Service by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number HHSN268201100037C from the National Heart, Lung, and Blood Institute.

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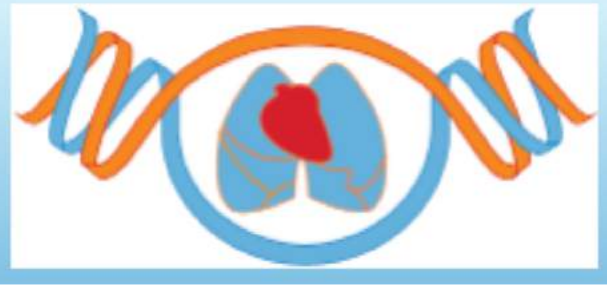

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