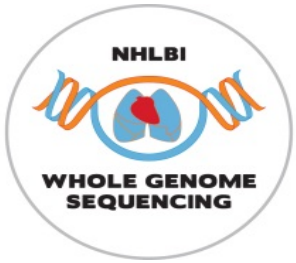


Module 12: Computational Pipeline for WGS Data

TOPMed Data Coordinating Center

July 18-20, 2018

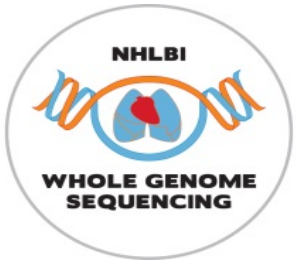
Introduction

Schedule

Each day:

8.30	- 10.00am	Session 1
10.00am	- 10.30am	break (snacks in South Campus)
10.30am	- noon	Session 2
noon	- 1.30pm	lunch on your own
1.30pm	- 3.00pm	Session 3
3.00pm	- 3.30pm	break (snacks in South Campus)
3.30pm	- 5.00pm	Session 4

Weds 5-6pm: Social hour, South Campus Center



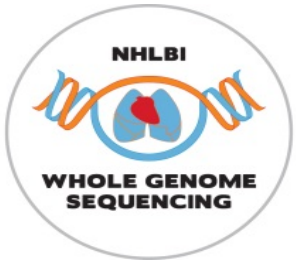
Schedule

Wednesday (3 hours)

- Introduction
- Data formats
- Intro to Genomic Data Storage
- Population structure and relatedness
  - Inference on this, and allowing for it

Thursday (6 hours)

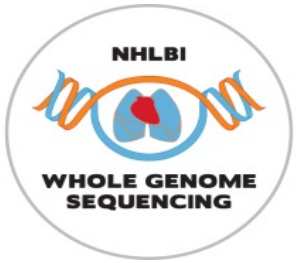
- Phenotype harmonization
- Association tests
  - Methods and motivation
  - GENESIS for association tests
- Variant annotation



Schedule

Friday (6 hours)

- Variant annotation (again)
- Pipeline design and examples
  - Analysis pipeline design
- Cloud platforms
  - Seven Bridges Genomics
  - Analysis Commons
- Hands-on cloud computing (optional, small groups)

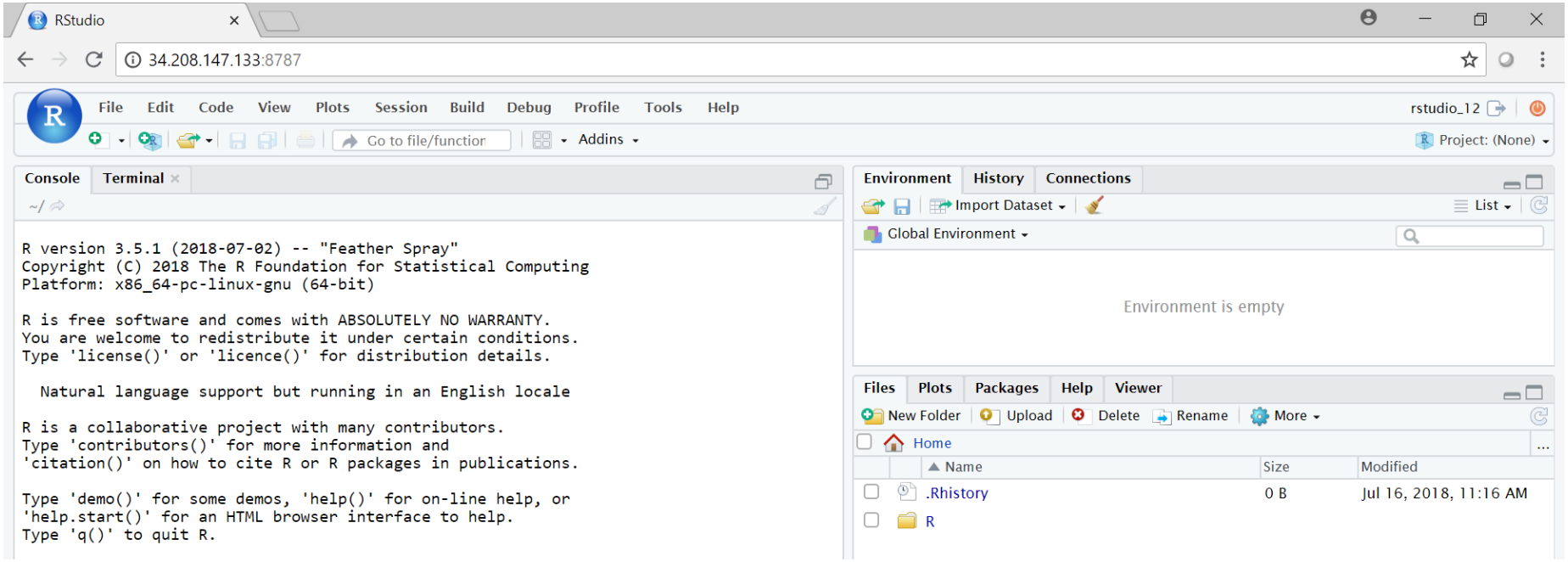


Connectivity

- Wireless Connection: TBA
- Slides and schedule: https://uw-gac.github.io/topmed_workshop_2018/index.html#schedule
- Hands-on exercises: https://uw-gac.github.io/topmed_workshop_2018/
- Slack channel: (sign up!)
- https://sisg2018module12.slack.com
- …contact bheavner@uw.edu for help with slack

Log-in to Amazon Web Services

- URL: http://34.208.147.133:8787
- user: `rstudio_`N, where $1 \leq N \leq 100$
- Pwd: `rstudioserver`N , for the same N
- Forget your number? See tinyurl, on the board



Workshop Outline and People

Introduction to TOPMed Data – Wednesday pm

a. Overview and data access

Ken Rice
Professor
TOPMed DCC PI

b. Genotypes and data formats

Stephanie Gogarten
Research Scientist

Workshop Outline and People

Population Structure and Relatedness – Weds pm

Tim Thornton
Associate Professor

Stephanie Gogarten
Research Scientist

Phenotype harmonization – Thurs am

Adrienne Stilp
Research Scientist

Workshop Outline and People

Association testing & GENESIS – Thursday am/pm

Ken Rice
Associate Professor
TOPMed DCC PI

Stephanie Gogarten
Research Scientist

Variant annotation – Thursday pm and Friday am

Deepti Jain
Research Scientist

Ben Heavner
Research Scientist

Workshop Outline and People

UW Genetic Analysis Center Pipeline – Fri am

Stephanie Gogarten
Research Scientist

Dave Levine
Research Scientist

Roy Kuraisa
Computer Scientist

Statistical Analysis in the Cloud – Fri

Jen Brody
Research Scientist

Milan Domazet
Analyst, Seven Bridges

Workshop Outline and People

Additional GAC faculty/staff on hand to help and advise:

Cecelia Laurie
Research Scientist

Leslie Emery
Research Scientist

Caitlin McHugh
Research Scientist

Prof Bruce Weir
TOPMed DCC PI

Xiuwen Zheng
Research Scientist

One more **fantastic** contact person…

Cathy Laurie
GAC Director

Workshop Outline and People

And you? Please tell us – very briefly:

- Who you are
- Where you work
- What you would like to get from the module

Other essentials

- This room will not be locked
- Restrooms are available down the hallway
- Lunch options – to follow! Or follow a local…
- Bags on final day (Light Rail to airport beats taxis…)
- Final session: please contact me/Stephanie
- Questions?

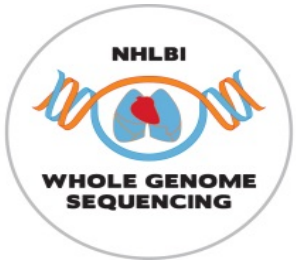
TOPMed overview

- Goals/structure of the TOPMed program
- What TOPMed data is available
- How to access it

Goals of the TOPMed program

- Sponsored by the NHLBI*; focus on heart, lung, blood and sleep traits
- Primary goal is to identify genetic variants with effects on subclinical-disease measures, clinical disease events, disease severity and response to treatment
- Facilitate personalized approaches to prevention, diagnosis and treatment of disease

* Some NHLBI leadership attending this module!



The TOPMed Program

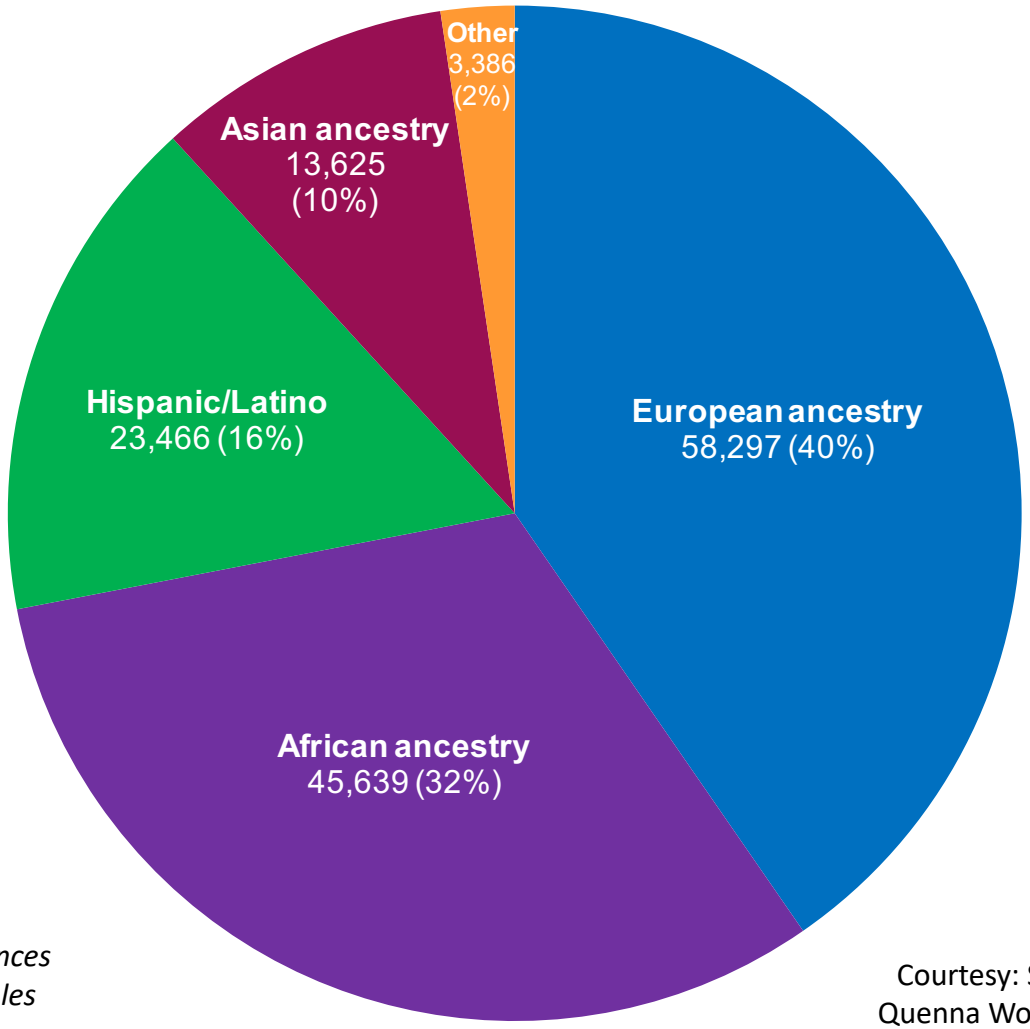
- Provide whole-genome sequencing (WGS) and other omics measures to pre-existing studies
- WGS well advanced, several datasets freely available via dbGaP/SRA
- Other omics assays just beginning, not yet available
- **Extensive** phenotypic and exposure data for participating studies available on dbGaP

Who's in TOPMed?

At time of writing:

- Up to 144K participants – largest WGS resource
- 41 studies (may contribute >1 subject group)
- 7 sequencing centers
- Informatics Research Center (Umich) focusing on genotype data, e.g. joint calling & analysis
- Data Co-ordinating Center (UW) focusing on genotype data, e.g. harmonization & analysis

Who's in TOPMed?

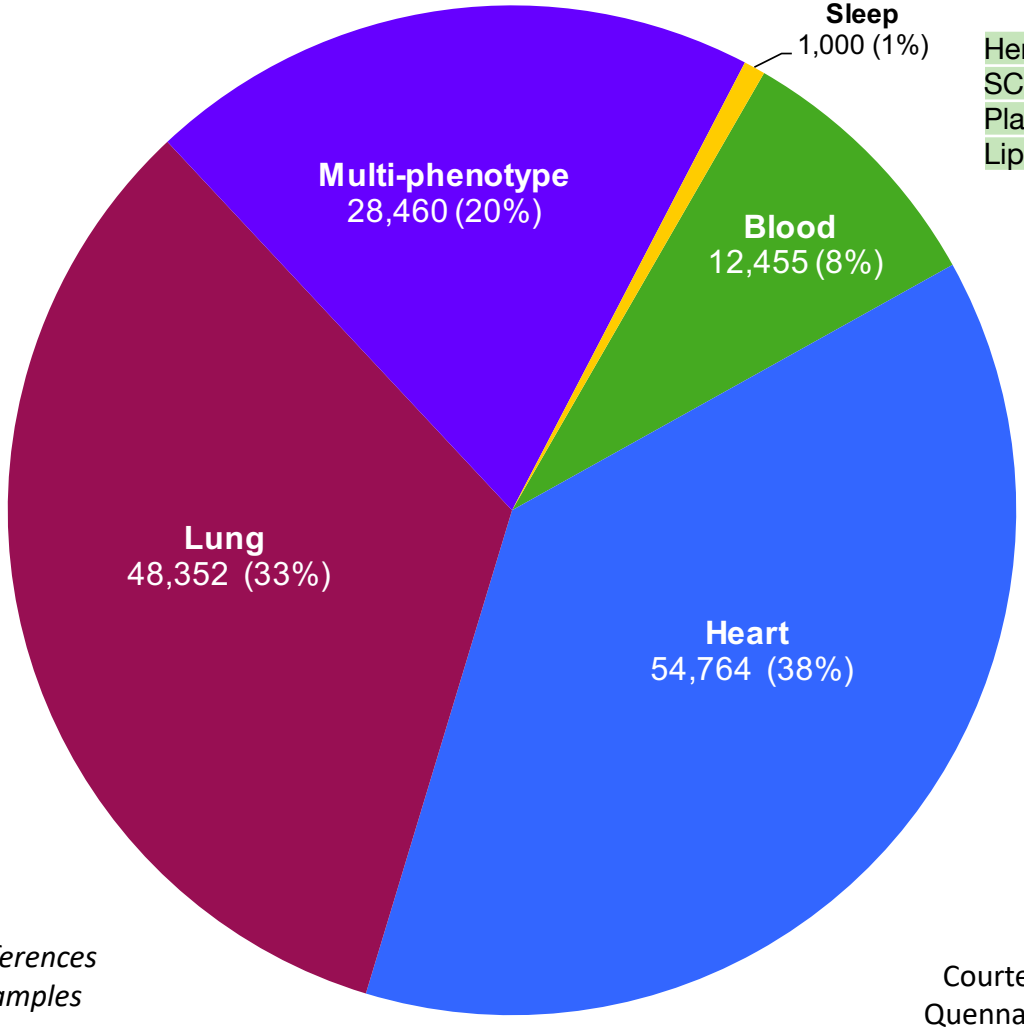


Counts may not reflect differences in planned vs sequenced samples

Courtesy: Sarah Nelson and Quenna Wong, TOPMed DCC

Who's in TOPMed?

Phase 1-4: 144K Study Participants



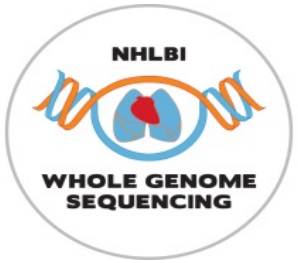
Asthma	26,587 (18%)
COPD	18,931 (13%)
IPF	1,500 (1%)
Sarcoidosis	636 (0%)
Other	450 (0%)
ILD	248 (0%)

Hemophilia	5,147 (4%)
SCD	4,944 (3%)
Platelets	1,399 (1%)
Lipids	965 (1%)

Hypertension	10,742 (7%)
MI	7,710 (5%)
Other	7,500 (5%)
CAD	7,176 (5%)
Stroke	4,900 (3%)
SVD	3,622 (3%)
VTE	3,343 (2%)
CHD	3,230 (2%)
Afib	2,799 (2%)
CAC	1,368 (1%)
Adiposity	1,296 (1%)
CHF	1,078 (1%)

Counts may not reflect differences in planned vs sequenced samples

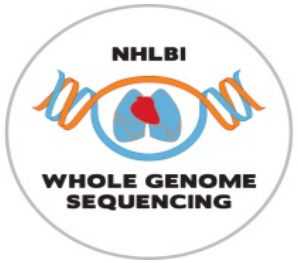
Courtesy: Sarah Nelson and Quenna Wong, TOPMed DCC



TOPMed data availability

TOPMed data are made available to the scientific community via the database for **Genotypes and Phenotypes (dbGaP)** and the **Sequence Read Archive (SRA)**

- The SRA and dbGaP are separate data archives. Both have controlled-access and open-access components. Controlled-access SRA data are restricted to approved dbGaP users.
- SRA contains DNA sequence data (CRAM files) and single-sample genotype calls (VCF) – more on these later
- dbGaP contains phenotypic data and various types of molecular data (including multi-sample VCF files)
- Today we will focus on dbGaP and SRA data structures



TOPMed data availability

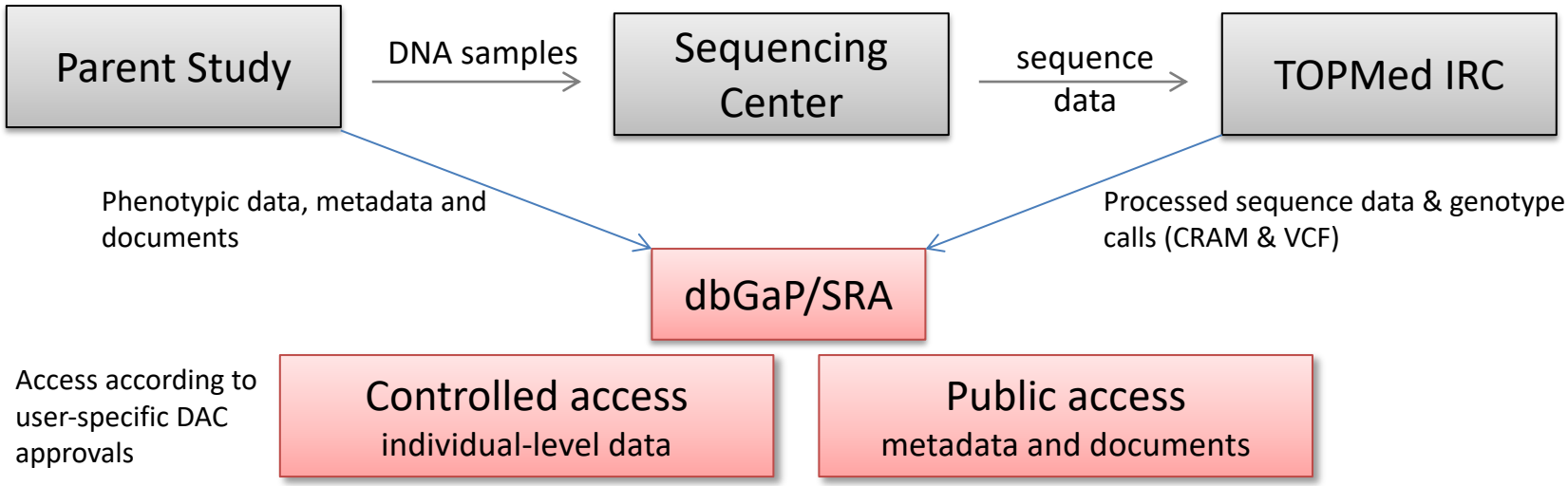
- Individual-level TOPMed data are **controlled-access** – i.e. must apply to NIH Data Access Committee, and get approval
- **Exactly** which data provided depends on **what it is used for**, because participants consent to some uses and not others
- Access via various "Data Commons" systems (data & compute resources) is coming
- CRAMs only available in SRA for Phase I, for now
- Our examples use simulated/1000G data, and (for speed) are smaller than real WGS

TOPMed Study/Parent Study

These are currently organized as separate dbGaP accessions:

- **Parent study** = pre-existing study that recruited subjects, obtained informed consent, collected biosamples and data (including phenotypic data and various types of molecular data); provides DNA samples for TOPMed WGS. Some have been collecting data for decades.
- **TOPMed Study** = TOPMed-funded study consisting of DNA samples and phenotypic data from one or more Parent studies; some are focused on a specific disease area, while others are very broad in phenotypic characterization.

TOPMed Data Flow



- Phenotypes available through dbGaP…
- …also within-study, we are harmonizing across TOPmed (more later)

Parent study designs

Study designs reflect original “Epi” goals:

- Prospective cohort studies – focus on risk factors, longitudinal trends and incident disease
- Case-control studies – usually cross-sectional, cases and controls from the same population(s)
- Randomized trials for interventions (causation)
- Family-based genetic studies
- Case-only studies – disease severity and/or response to treatment

TOPMed study designs

- Some derived from **single Parent study** by selecting according to various criteria – e.g. relatedness, having phenotypes of interest or extent of phenotypic characterization
- Some are a **consortium of multiple Parent studies** that each contribute a common phenotype of interest – e.g. atrial fibrillation cases from several parent studies, along with controls from same/other studies

Yes, this all gets complex! But designs ***do*** matter when choosing appropriate analyses.

Watch out for…

- *dbGaP accession numbers* identify studies, sub-studies and their subordinate digital objects
- *SRA RUN identifiers* for each DNA sample as a set of CRAM, CRAI and sample-specific VCF files
- A *subject* is a person, a *sample* is an analyte or biological specimen sampled from a subject (e.g. DNA from blood)
- One subject in one study can have multiple samples! Mappings are available…

dbGaP file types (controlled access)

- Subject consent – submitted Subject IDs with associated consent group types
- Subject-Sample mapping – correspondence between subject and sample IDs
- Sample attributes – e.g. analyte type, specimen body site
- Pedigree – documented familial relationships
- Subject phenotype data
- Molecular data
- Medical imaging
- Phenotype-genotype association test results

dbGaP file types (public access)

- Data dictionaries – variable names, descriptions, encoded values, etc
- Variable reports – generated by dbGaP – variable summaries (counts, ranges, etc)
- Study documents – e.g. study design, methods of molecular data acquisition, methods of phenotypic data acquisition (including protocols and questionnaires)
- These files can be downloaded from dbGaP's ftp site

dbGaP file structure

No one format is specified by dbGaP (!) – here are two very different examples

Figure 7. Examples of variation in structure of phenotypic data set: Multiple observations per phenotype per subject.

a. Multiple observations per phenotype per subject, wide format: Concentrations of lipids in blood for Clinic Visits 1, 2 and 3. In this case, clinic visit identifier is provided in a clinic visit variable. Age is given explicitly as a variable with units of "years old".

SUBJECT_ID	CLINIC_VISIT	AGE	LDL	HDL	TC
A10356	1	45	89	72	150
A10356	2	49	92	70	148
A10356	3	53	90	71	151
A30865	1	62	94	65	145
A30865	2	66	105	62	1
A30865	3	70	98	66	152
A48765	1	58	105	55	160
A48765	2	62	110	53	165
A48765	3	66	111	54	166

b. Multiple observations per phenotype per subject, wide format: Concentrations of lipids in blood for Clinic Visits 1 and 2. In this case, clinic visit identifier is embedded in the phenotypic variable names. Age is not given in this data set; must be inferred from other data set(s).

SUBJECT_ID	LDL_VISIT1	LDL_VISIT2	HDL_VISIT1	HDL_VISIT2
A10356	89	92	72	70
A30865	94	105	65	62
A48765	105	110	55	53

dbGaP file structure

- This may seem messy/awkward
- It is, but most of those cleaning it up are volunteers, and resources are limited. If you're a trait expert affiliated to a TOPMed study, **please** join the relevant TOPMed Working Group
- More on DCC's harmonization work with Adrienne, tomorrow

Discovering genetic risk factors for disease

- This is a primary goal of TOPMed
- TOPMed investigators are performing Genome-Wide Association Studies (GWAS) using genotype calls from whole-genome sequencing across multiple studies
- The process consists of several steps outlined in the following slides

TOPMed GWAS: Step 1 – Planning

Develop analysis plan, including specification of all variables needed, specifically:

- Primary outcome phenotype (e.g. HDL level in serum); if a derived variable is to be used (e.g. diabetes status), define derivation algorithm and required component variables
- Covariates to be adjusted for (e.g. age at measurement, sex, and study) or otherwise allowed for (relatedness, measurement accuracy info)
- Ancillary variables for modifying phenotypes in the model (e.g. medication use) and/or selection of subjects to include/exclude (e.g. fasting status)

TOPMed GWAS: Step 2 – Prepare the data

- Identify the necessary variables in dbGaP and construct data sets
  - Search dbGaP phenotype files for variables related to the required phenotypes
  - Decide which ones are relevant
  - Determine which subjects have both relevant phenotypes and genotypic data (from TOPMed WGS)
  - Determine which subjects with pheno/genotype also gave **consent for this analysis**
- Harmonize phenotypes across studies
  - Evaluate similarities and differences among studies and develop harmonization plan
  - QC source variables
  - Write and run harmonization code on each study
  - QC harmonized phenotypes
  - Identify subject exclusions (e.g. non-fasting, <18 years old, outliers, etc.)

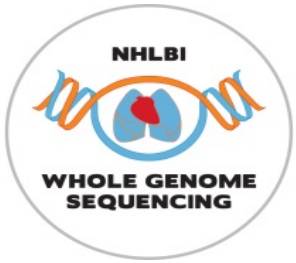
TOPMed GWAS: Step 3 – Prepare genotypes

Assuming genotype QC has been done, still need to:

- Start with a genotype call set constructed from joint calling of all subjects to be included in the analysis
- For subjects to be analyzed, calculate and analyze relatedness and population structure; decide on any further exclusions
- Calculate Genetic Relatedness Matrix for samples to be included
- Define genomic aggregation units (i.e. genomic ranges for genes, regulatory elements, etc.)
- Define variant filtering (e.g. minor allele count, conservation score, loss-of function, etc.)

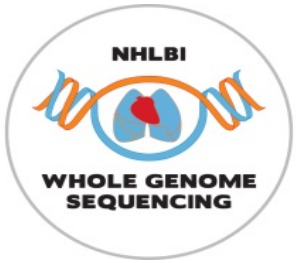
TOPMed GWAS: Step 4 – analyze & interpret

- Select analysis pipeline (e.g. ENCORE, GENESIS, etc.)
- Select computing environment for the analysis pipeline, including I/O, memory requirements and parallelization strategy
- Perform association tests, visualize results
- Evaluate and interpret association test results
  - Evaluate model fit, type I error rate control, heteroscedasticity
  - Modify analysis plan as needed – possibly rerun, or filter out worst behavior
  - Check for novel hits (typically using follow-up conditional analysis)
  - Develop hypotheses about causal variants and affected gene(s)
    - Compare results to genomic annotations for variants, including eQTL (e.g. using GTEx)
    - Examine possible functions of implicated genes (e.g. using MODs)



Questions?

- Ask one of us, or use the Slack channel
- Visit the TOPMed website (some material restricted to TOPMed investigators)



Acknowledgments

- The TOPMed program supported by NHLBI
- TOPMed investigators and their Parent Studies
- Participants of Parent studies
- TOPMed sequencing centers
- Members of the TOPMed DCC and IRC