Lecture 10: Power and Sample Size, Design Considerations, Emerging Issues

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Lecture Overview

Power/Sample Size calculation

- Power/Sample size calculation is essential to design future sequencing studies.
- Input information:
- Region information
  - LD structure and MAF spectrum.
  - Region size to test.

Power/Sample Size calculation

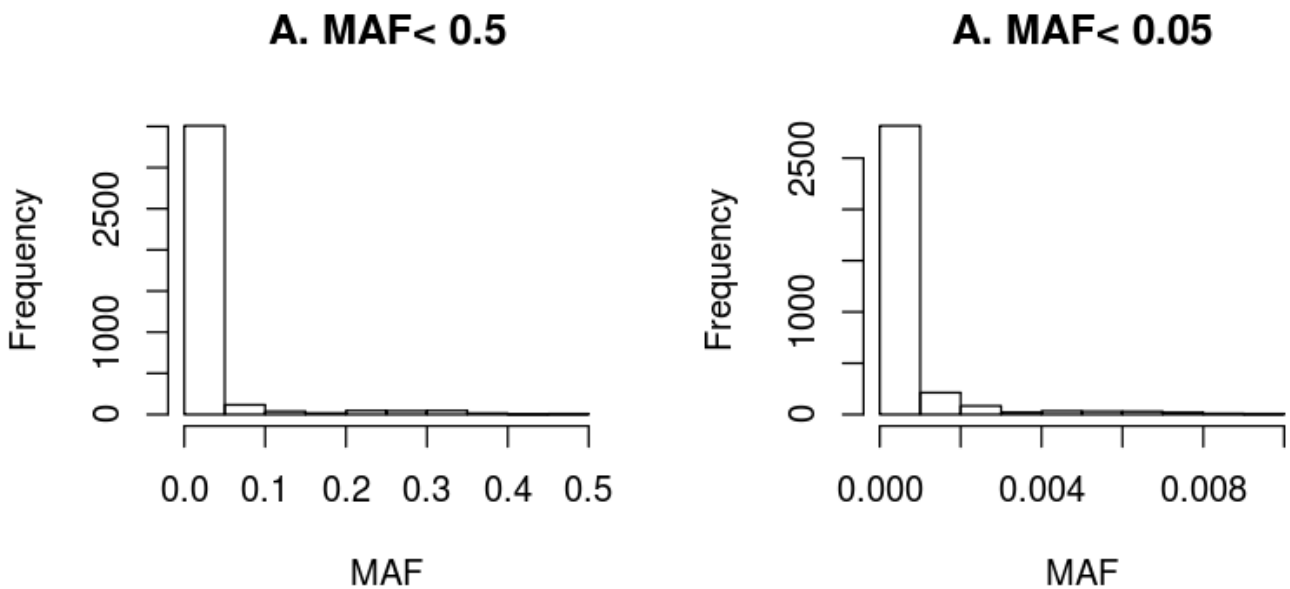
- Causal variant Information
  - Effect size (continuous traits), or Odds ratio (binary traits).
  - % of rare variants be causal.
  - % of causal variants with negative association direction.
- Binary traits
  - Case/Control Ratio.
  - Prevalence

Practical Points: SKAT Power Calculations

- Region information
  - Either simulated haplotypes or sample haplotypes from preliminary data.
  - The SKAT package provides 10,000 haplotypes over a 200 kb region generated by the coalescent simulator (COSI).

MAF spectrum

- MAF spectrum of the simulated haplotypes
- Most of SNPs have very low MAFs.



Practical Points: Power/Sample Size calculations

- Causal Variant Information:
  - To use $\log_{10}$ function $(-c \log_{10}(MAF))$ for the effect sizes or log odds ratio.
  - c is a parameter to determine the strength of association.
    - Ex: $c = 1$
      $\beta = 2$ or $\log(OR) = 2$ for a variant with MAF=0.01
      $\beta = 4$ or $\log(OR) = 4$ for a variant with MAF=10^{-4}.

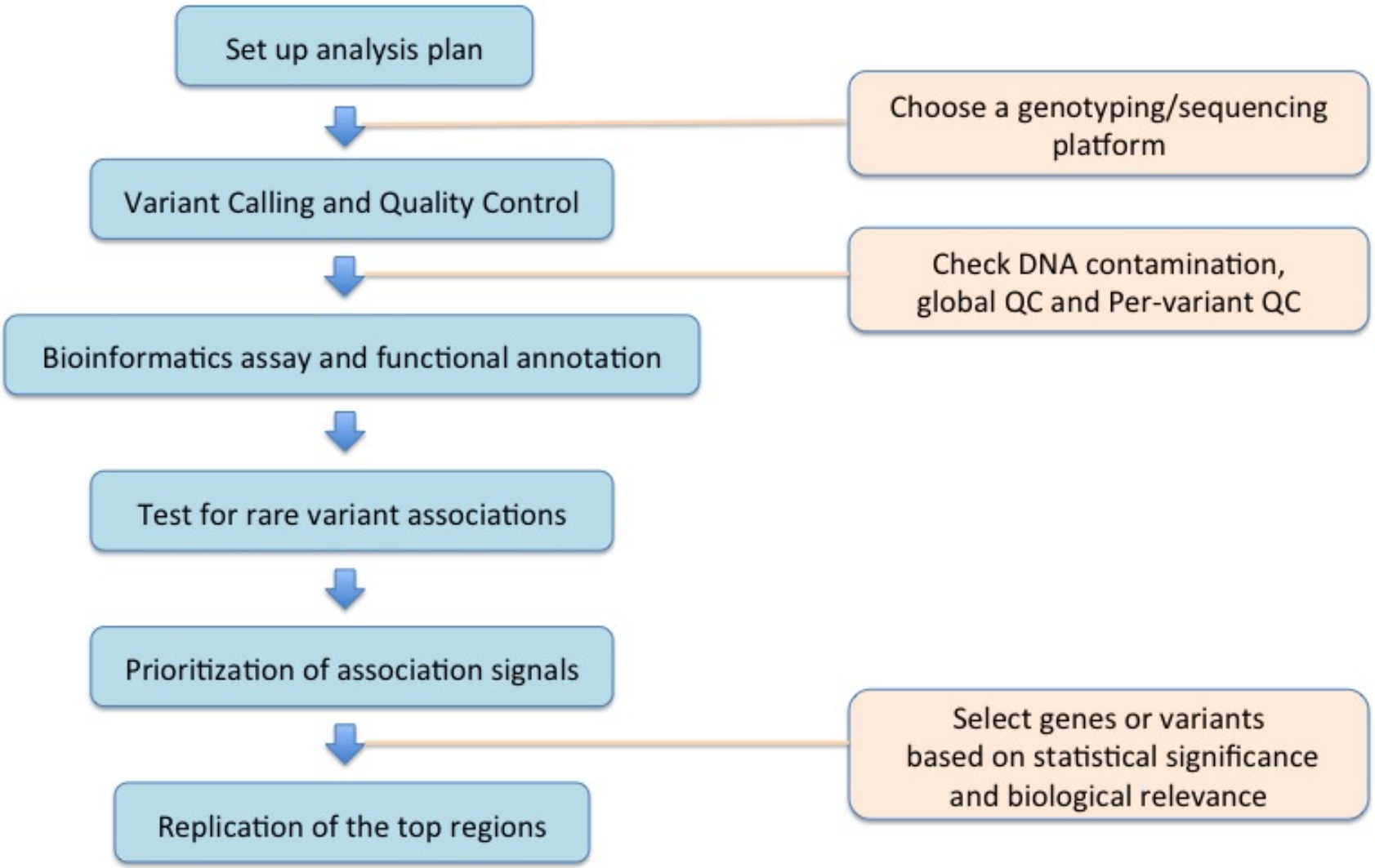
Practical Points: Power/Sample Size calculations

- In SKAT package, you can set c using the MaxOR (OR for MAF $= 10^{-4}$) or MaxBeta (β for MAF $= 10^{-4}$).

Practical Points: Power/Sample Size calculations

- Power depends on LD structure of the region and MAFs of the causal variants.
- We are interested in estimating power in multiple regions and multiple sets of causal variants selected from a certain disease model.
  - We estimate an average power.
  - Approximately 100 $\sim$ 500 sets of regions/causal variants are needed to estimate the average power stably.

Data Processing and Analysis Flowchart



Genotyping Platforms

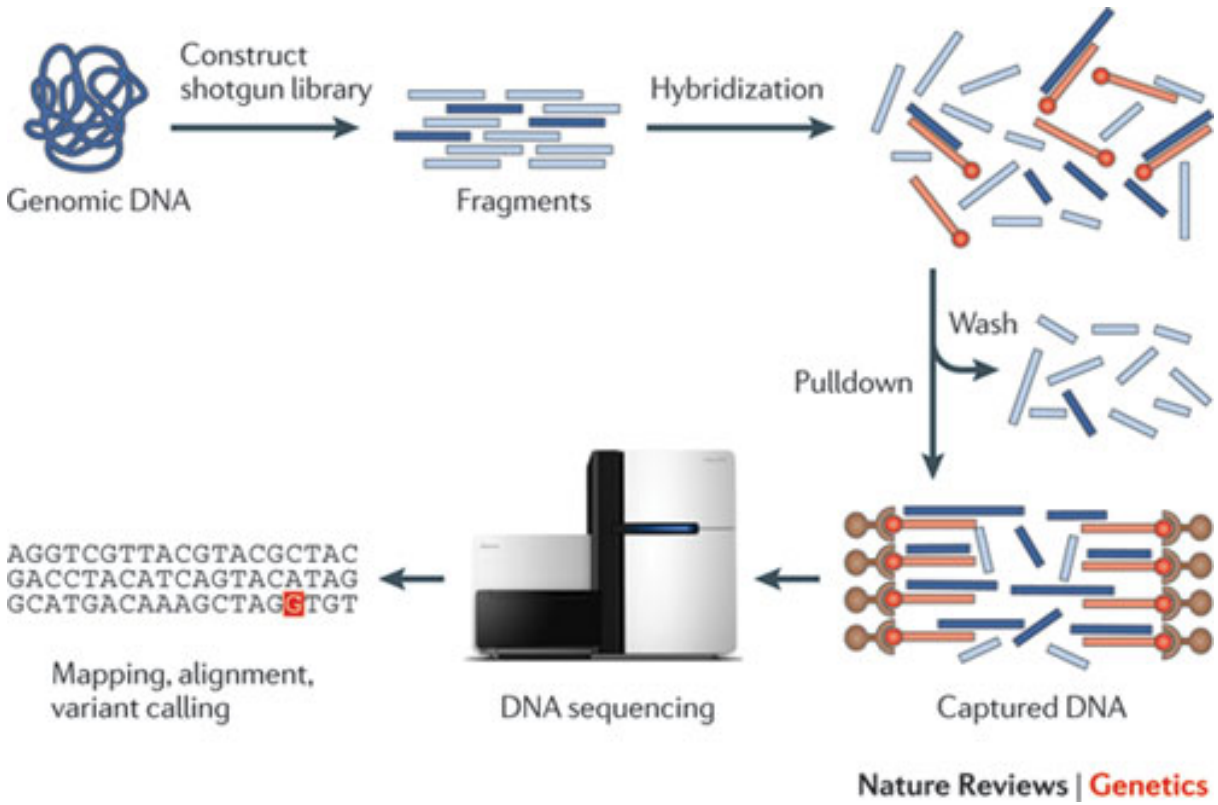
- High depth whole genome sequencing is the most informative, however it is currently expensive.
- Alternative sequencing designs and genotyping platforms
  - Low depth sequencing
  - Exome seqeuncing
  - High coverage microarrays (Exome chip)
  - Imputation

Low depth whole genome sequencing

- Sequencing $7 \sim 8$ samples at low depth (4x) instead of 1 sample at high depth (30x)
- Low depth sequencing
  - Relatively affordable
  - LD based genotyping: leverage information across individuals to improve genotype accuracy.
  - 1000 Genome (4x) and UK 10K (6x) originally used low depth sequencing.
- Cons:
  - Subject to appreciable sequencing errors

Exome sequencing

- Restrict to the protein coding region ($1 \sim 2\%$ of genome (30 Mbps)).



Bamshad et al. (2011), NRG

Exome sequencing

- Focus on the high value portion of the genome
- Relatively cost effective
- Cons: Only focus on the exome
  - Most of GWAS hits lie in non-exomic regions
  - Many non-coding regions have biological functions

Exome array

- Using variants discovered in 12,000 sequenced exome
- Low cost ($10 \sim 20x$ less than Exome sequencing)
  - 250K non-synonymous variants
  - 12K splicing variants
  - 7K stop altering variants
- Cons:
  - Cannot investigate very rare variants.
  - Limited coverages for non-European populations

GWAS chip + Imputation

- Imputation: Estimate genotypes using reference samples
  - Imputation accuracy increases as the number of reference samples increases
- No additional experiment cost
- Cons:
  - Low accuracy of imputed rare variants

Summary

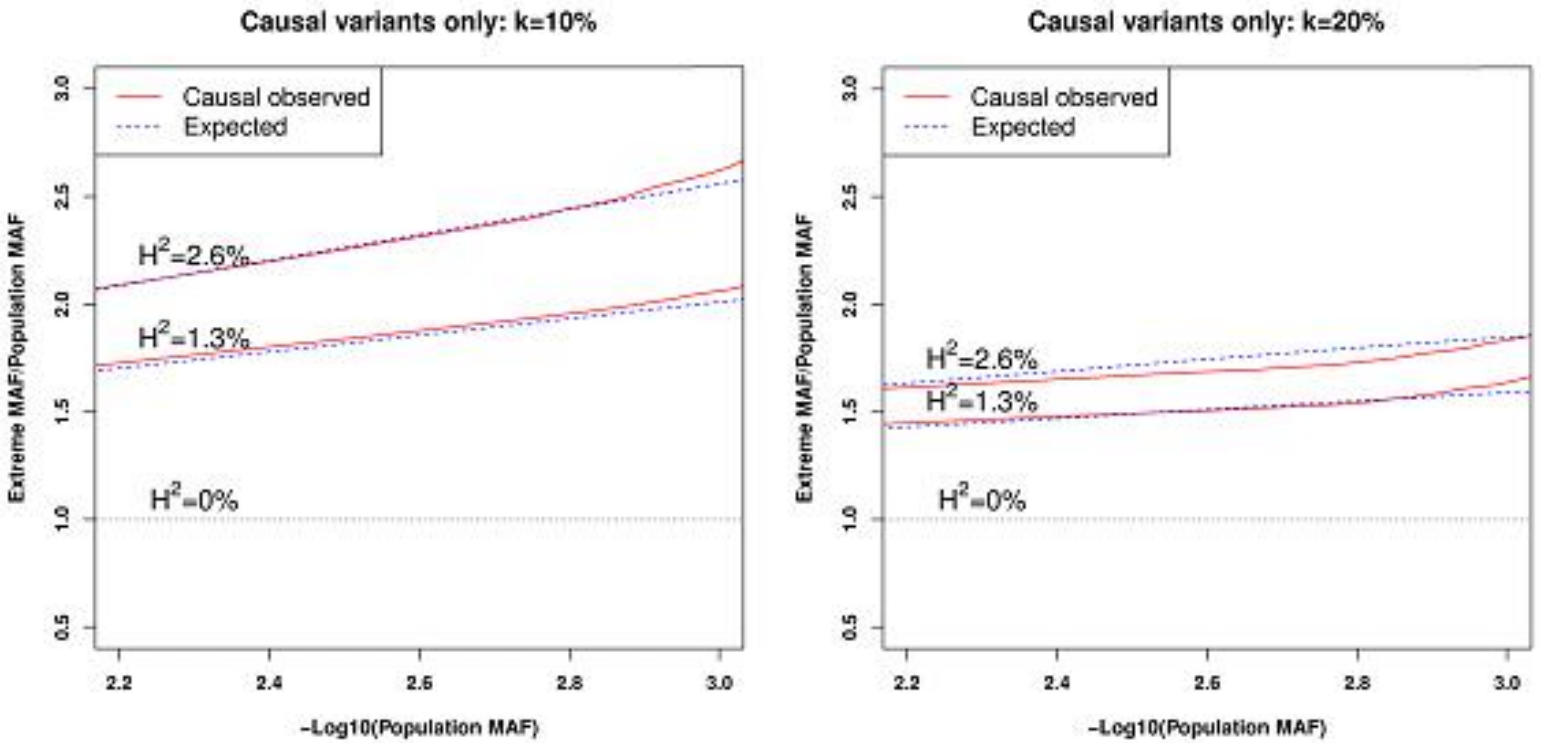
	Advantage	Disadvantage
High-depth WGS	Can identify nearly all variants in genome with high confidence.	Currently very expensive.
Low-depth WGS	Cost-effective, useful approach for association mapping.	Limited accuracy
Whole exome sequencing	Can identify all exomic variants; less expensive than WGS.	Limited to the exome.
GWAS chip + Imputation	Low cost.	Lower accuracy of imputed rare variants.
Exome chip (custom array)	Much cheaper than exome sequencing.	Limited coverage for very rare variants and for non-Europeans. Limited to target regions.

Extreme phenotype sampling

- Rare causal variants can be enriched in extreme phenotypic samples
- Given the fixed budget, increase power by sequencing extreme phenotypic samples.

Enrichment of causal rare variants in phenotypic extremes

- Estimated folds increase of the observed MAFs of causal variants (k% high/low sampling, H^2=Heritability).



Extreme phenotypic sampling

- Continuous traits:
  Select individuals with extreme trait values after adjusting for covariates.
- Binary traits:
  Select individuals on the basis of known risk factors
  - Ex. T2D : family history, early onset, low BMI

Extreme phenotypic sampling

- Extreme continuous phenotype (ECP) can be dichotomized, and then any testing methods for binary traits can be used.
- But dichotomization can cause a loss of information and can decrease the power.
- Methods modeling ECP as truncated normal distribution has been developed (Barnett, et al, 2013, Gen. Epid).

Case Only Analysis

- Case only analysis: sequencing only cases (sporadic or familial)
- Rationale:
  - Expense
- Typical n:
  - 100 – 1000
  - $<$ 100 or even $<$ 50

When Sample Size "Sufficient"

- Can use reference controls: 1000 Genomes, exome sequencing project, etc.
- Caution:
  - Batch effects, sequencing artifacts, processing differences
  - Relevant population: must be comparable
  - Covariate adjustment
  - Potential cases among reference

Case Only Analysis with Modest n

- Small sample sizes: $n = 25$
- Potentially strong effects? High penetrance? Extremes?
- Standard case control testing may be under powered
- **Basic strategy**: Screening, filtering and bioinformatics

Reference: L. Wu, et al. (2015) *J. Med. Genet.*.

Modest *n*: Variant Filtering

Idea: Prioritize variants from large scale screen

Variant Frequency Filtering

- Use reference data, e.g. 1000 Genomes
- Remove variants with higher MAF:
  - MAF $\geq$1%
  - or Variants that appear at all in reference
- Rationale: 85% of non-synonymous and 90% of stop-gain/splice-disrupting variants are rare

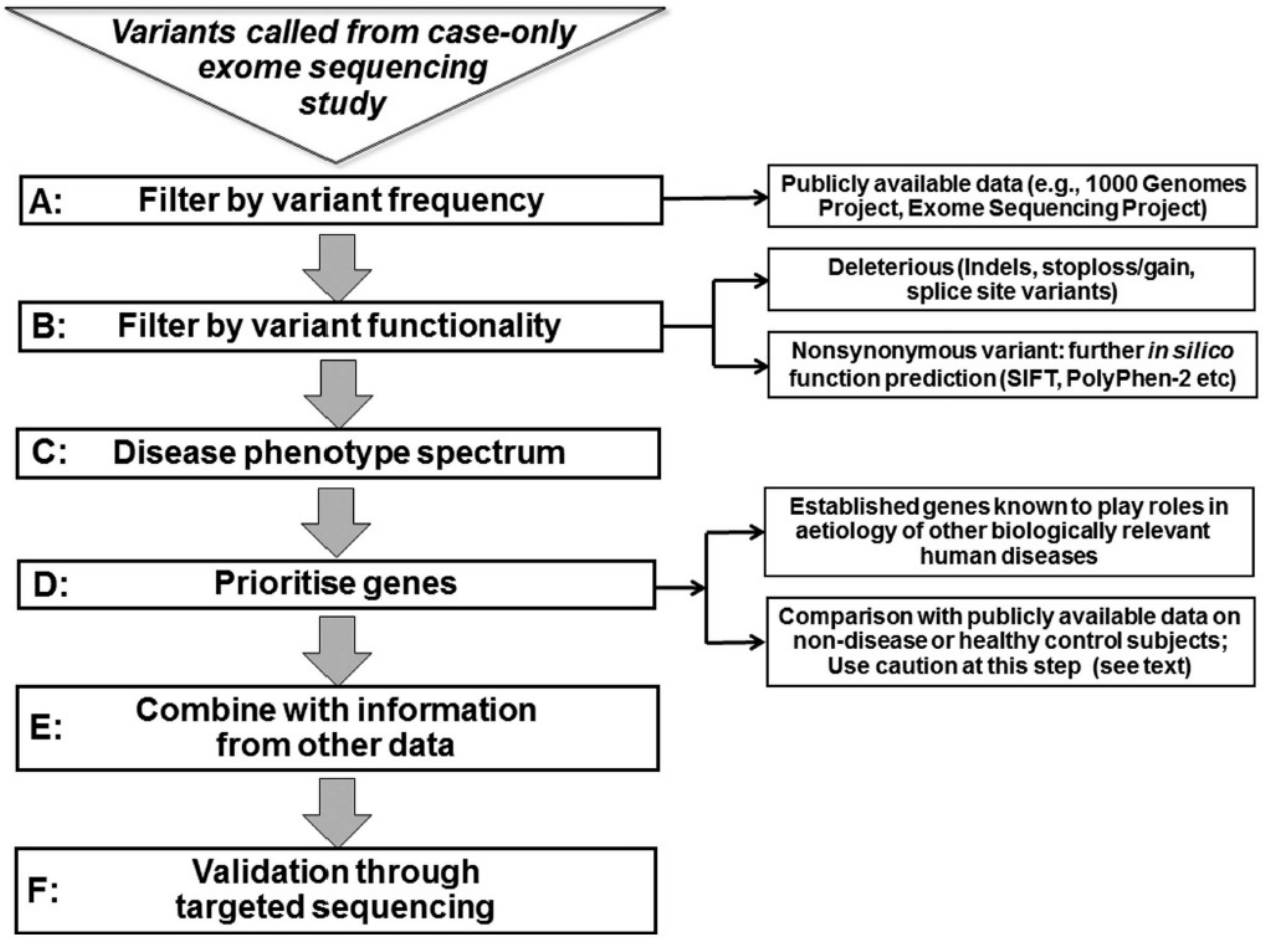
Variant Functionality Filtering

- Functionality scores for individual variants
- SIFT PolyPhen-2, others.
- High sensitivity, but low specificity

Modest *n*: Further Prioritization

- **Disease Phenotype Spectrum:**
- **Gene Prioritization:** knowledge on which genes play role in etiology of same or related disease
- **Publicly Available Controls:** Similar to reference data, but actual association analysis; same cautions
- **Other Genomic Data:** Integration with multiple sources of evidence
- **Validation:** Targeted sequencing of new cases and controls is only way to statistically validate findings

Filtering Summary



Reference: L. Wu, et al. (2015) *J. Med. Genet.*.

Additional Concerns

- Quality control:
  - Are the observed variants really variants?
  - Batch effects
  - Some standard pipelines now in place
- Population stratification:
  - Common strategy: just use same PCs from common variant analysis to correct for PS
  - Some evidence that rare variants require special accommodation (much larger number of PCs)
- Accommodating common variants:
  - What do you do with common variants?
  - (a) Assess joint effect with rare variants
  - (b) Adjust for effect of common variants

Additional Concerns

- Prediction
  - In a new population (sample), we're unlikely to see the same variants and we're likely to see a lot of variants not previously observed
- Prioritization of individual variants
  - How to choose individual causal variants?
  - Some work on variable selection methods, but no ability to control type I error.
  - Bioinformatics and functionality tools may be useful
- Incorporation of functional information and other genomic data

Additional Concerns

- Design Choices
  - Want to enrich for variants (extreme phenotypes)
  - Some of these designs require specialized methods
  - Stuck with the design chosen
- Dealing with admixed populations
- Related individuals
- Tim: what is a "rare variant"?
- (Statistically) complex phenotypes