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

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Lecture Overview

Power/Sample Size calculation for GWAS

- Power/Sample size calculation is essential to design future studies
- What does power depend on?
  - α-level (# of SNPs or Genome Wide Significance)
  - MAF of causal variants
  - Sample size
  - Effect size:
    - Continuous: r^2 or β
    - Dichotomous: OR or RR
    - Others: more complicated
  - Design:
    - e.g. Case-control vs. cohort vs Repeated measures vs Fancier designs
  - Trait characteristics:
    - Standard deviation, Population Risk, etc.

Power/Sample Size calculation for GWAS

- Quanto is great software for doing power calculations for GWAS (https://pphs.usc.edu/download-quanto/)
- There are a lot of other software
- For fancier designs:
  - Simulations
  - "Trick" existing software: for longitudinally collected traits, we use univariate (usual methods) with "effective sample size" inflated to accommodate multivariate outcome

Basic Quanto Demo

Suppose:

- Thornton's Disease is characterized by excessive interest in statistical genetics which can have adverse impact on the pay of statisticians who would otherwise join Amazon
- We are interested in conducting a GWAS to understand the genetic underpinnings of this devastating condition
- Existing cohort of 3000 affected individuals and 6000 unaffected individuals (all unrelated)
- Want to apply for a grant from NIH which requires power justification
- Parameters of interest:
  - $\alpha = 10^{-8}$
  - Population risk of Thornton's Disease is 0.2%

Possible Write-Up

We assess the power of our proposed study assuming a genome wide significance level of $\alpha = 10^{-8}$ and population risk of 0.2%. Then with a sample size of 3000 cases and 6000 controls, under and additive model, we anticipate 80% power to detect an OR of 1.32, 1.25, and 1.24 for a SNP with MAF of 0.15, 0.30, and 0.45, respectively. These OR are well within the range of anticipated effect sizes based on prior literature indicating that our study has adequate power.

Power/Sample Size calculation for Rare Variants

- 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

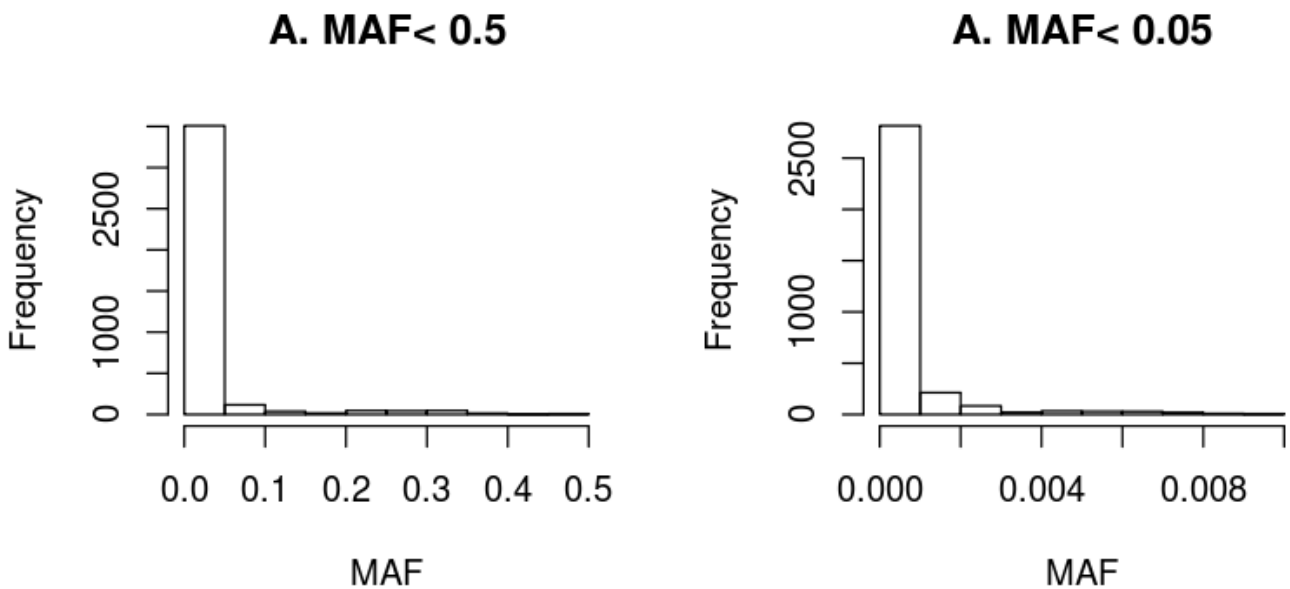
- Causal variant Information
  - Effect size (continuous traits), or Odds ratio (binary traits).
  - % of rare variants be causal.
  - % of causal variants with negative association direction.
- Binary traits
  - Case/Control Ratio.
  - Prevalence

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}.

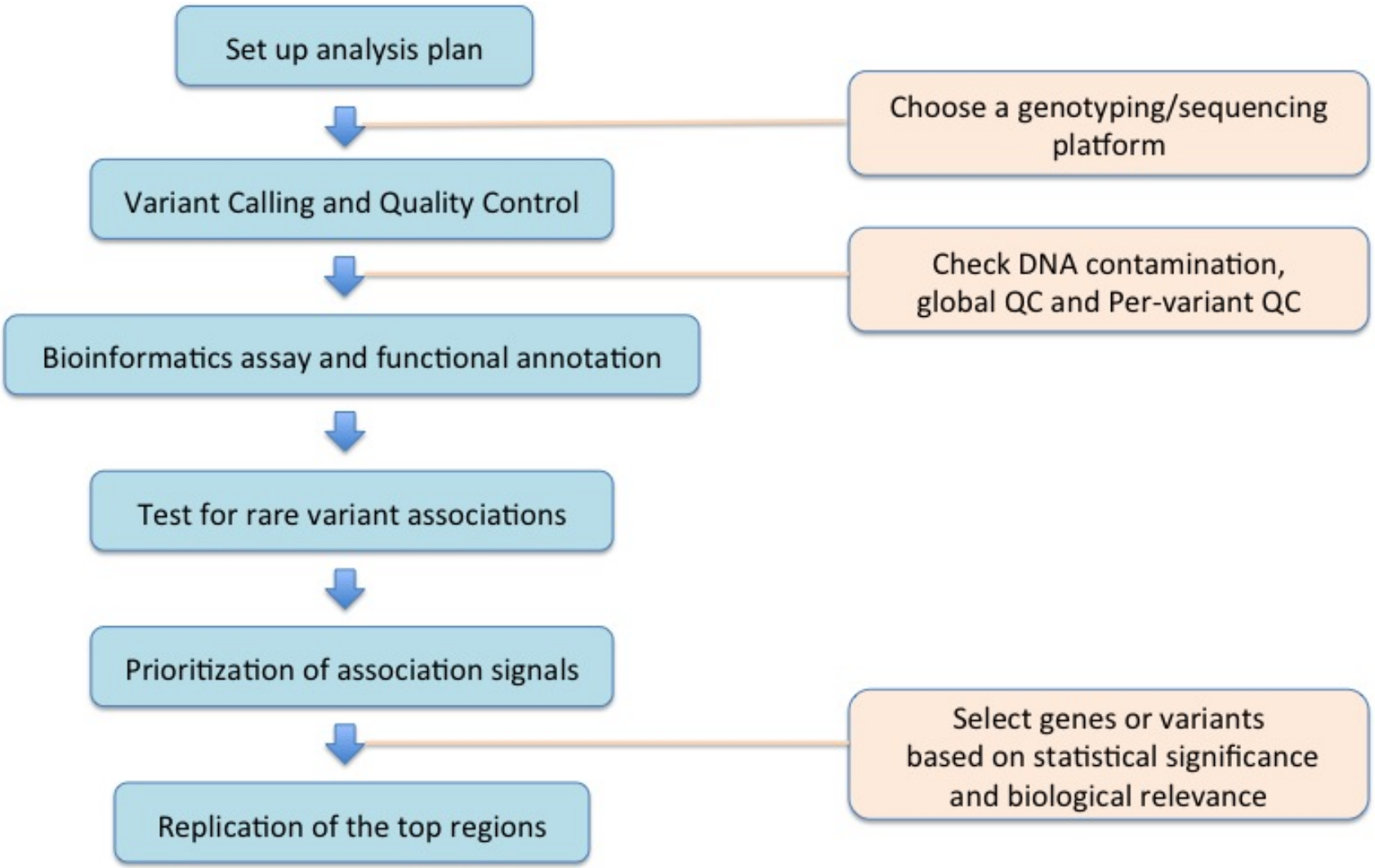
Practical Points: Power/Sample Size calculations

- 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

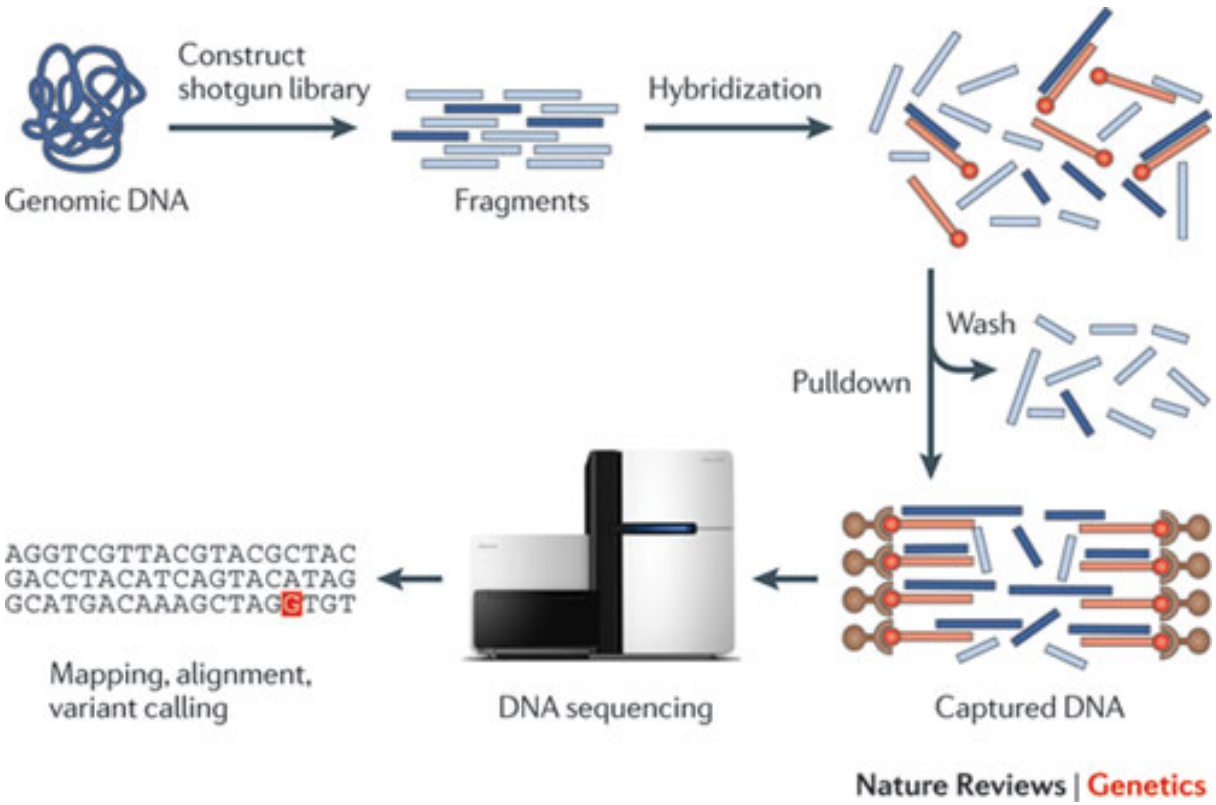
- High depth whole genome sequencing is the most informative, however it is currently expensive.
- Alternative sequencing designs and genotyping platforms
  - Low depth sequencing
  - Exome seqeuncing
  - High coverage microarrays (Exome chip)
  - Imputation

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

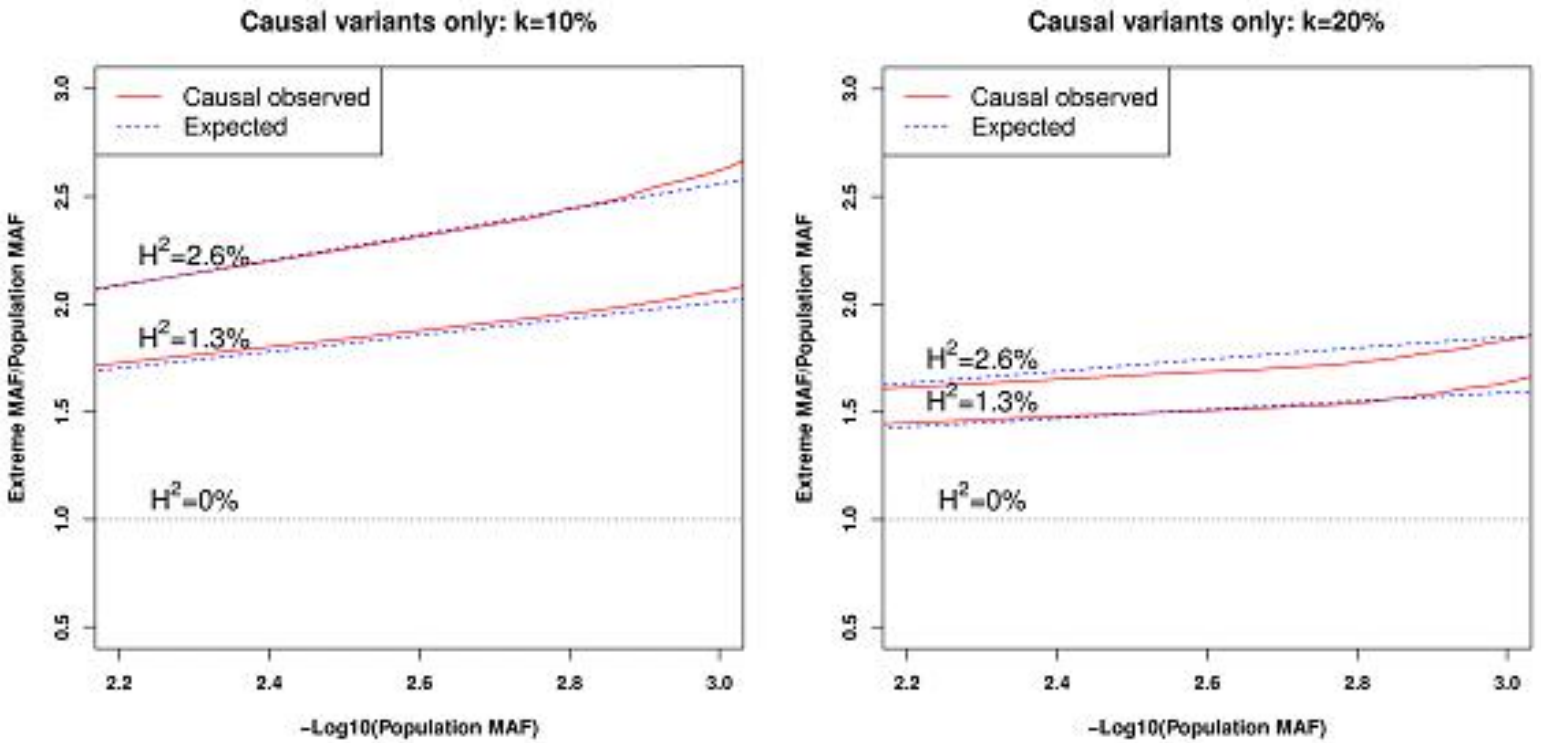
	Advantage	Disadvantage
High-depth WGS	Can identify nearly all variants in genome with high confidence.	Currently very expensive.
Low-depth WGS	Cost-effective, useful approach for association mapping.	Limited accuracy
Whole exome sequencing	Can identify all exomic variants; less expensive than WGS.	Limited 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

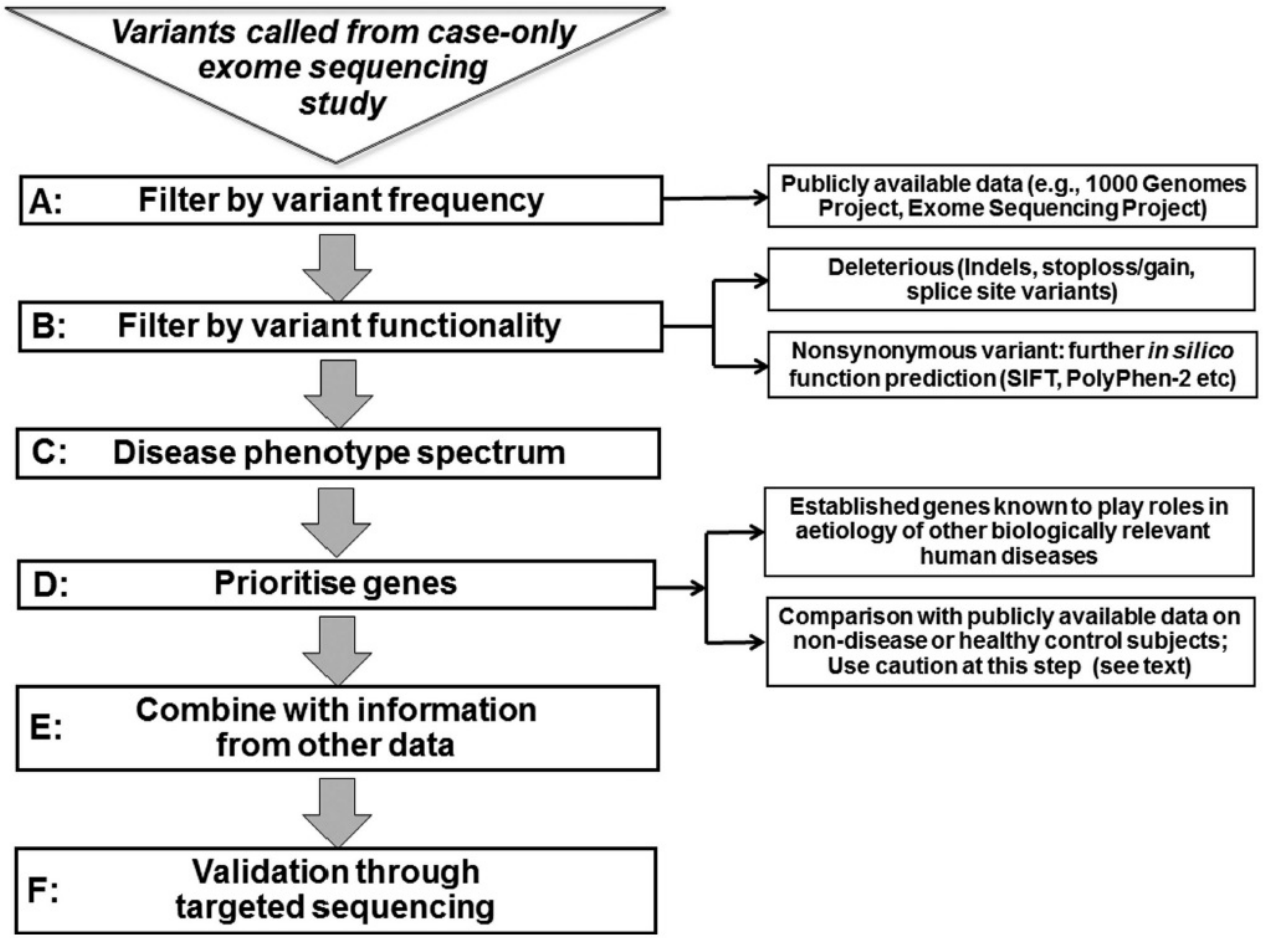
Variant Functionality Filtering

- 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
- Biobanks and Huge Consortia
  - Meta analysis
  - Computational costs and storage
  - Unbalanced designs
- 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?

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
- (Statistically) complex phenotypes