Power Analysis for Single and Rare Variant Aggregate Association Analyses

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Why Estimate Sample Sizes and/or Power?

- To avoid wasting time and money
  - Does not make sense to perform an inadequately powered study for which it is unlikely to to correctly reject the null hypothesis due to inadequate sample size
    - Collaborations can aid in increasing sample sizes
      - Caveats
        - » Disease definition may not be the same between studies
        - » Study subjects may be drawn for different populations
        - » Processing of genetic material maybe not be consistent
- Almost always necessary for grant proposals
  - Can be denied funding if unable to demonstrate planned study has adequate power
    - Realistic disease models are necessary when performing power calculations
    - Correctly adjust alpha for multiple testing which will be performed
      - e.g., use genome-wide significant level of 5×10^{-8} for GWAS studies

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Power and Sample Size Estimation for Case-Control Data

- The correct α must be use for sample size estimation/power analysis
- Type I (α) the probability of rejecting the null hypothesis of no association when it is true
- Due to multiple testing a more stringent value than α=0.05 is used in order to control the Family Wise Error Rate

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Power and Sample Size Estimation for Case-Control Data

- GWAS of common variants where each variant is test separately
  - $\alpha=5 \times 10^{-8}$ (Bonferroni Correction for testing 1,000,000 variant sites)
  - Shown to be a good approximation for the effective number of tests
    - Valid even when more than 1,000,000 variant sites tested
  - Effective number of tests is dependent of the linkage disequilibrium (LD) structure
- Single variant tests using whole genome sequence data
  - Many more rare variants than common variants
    - Lower levels of LD between rare variants than between common variants
  - The number of effective tests for rare variants is higher than for analysis limited to common variants
  - α is yet to be determined for association analysis of whole genome sequence data

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An Example of Determining Genome-wide Significance Levels for Common Variants

- Using genotypes from the Wellcome Trust Case-Control Consortium
- Dudbridge and Gusnato, Genet Epidemiol 2008
- Estimated a genome-wide significance threshold for the UK European population
- By sub-sampling genotypes at increasing densities and using permutation to estimate the nominal p-value for a 5% family-wise error
- Then extrapolating to infinite density
- The genome wide significance threshold estimate $\sim 7.2 \times 10^{-8}$
- Estimate is based on LD structure for Europeans
  - Not sufficiently stringent for populations of African Ancestry

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Power and Sample Size Estimation for Aggregate Rare Variant Tests

- For gene-based rare variant aggregate methods a Bonferroni correction for the number of genes/regions tested is used
  - e.g., 20,000 genes significance level $\alpha=2.5 \times 10^{-6}$
    - Can use a less stringent criteria
      - Not all genes have two or more variants
        - » Divide 0.05 by number of genes tested
    - If units other than genes are used
      - A more stringent criteria may be necessary
- For rare variants – very low levels of LD between variants in separate genes
  - Therefore, a Bonferroni correction is not overly stringent
    - The number of tests ≅ effective number tests
      - This would not be the case for variants in LD

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Power and Sample Size Estimation for Replication Studies

- For replication studies can base the significance level (α)
- On the number of genes/variants being brought from the discovery (stage I) study
- To replication (stage II)
- For example, if it is hypothesized that 20 genes and 80 independent variants will be brought to stage II (replication)
  - A Bonferroni correct can be made for performing 100 tests
    - An $\alpha = 5.0 \times 10^{-3}$ can be used for a family wise error rate of 0.05

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Estimating Power/Sample Sizes For Single Variant Tests

- Can be obtained analytically
- Information necessary
  - Prevalence
  - Risk allele frequency
  - Effect size (odds ratio-for case control data)
  - Genetic model for the susceptibility variant
    - Recessive ($\gamma_1=1$)
    - Dominant ($\gamma_2=\gamma_1$)
    - Additive ($\gamma_2=2\gamma_1-1$)
    - Multiplicative ($\gamma_2=\gamma_1^2$)

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Estimating Power/Sample Sizes For Individual Variants

- Usually, information on disease prevalence is known from epidemiological data
- A range of risk allele allele frequencies and effect sizes are used
- A variety of genetic models can also used
  - Dominant
  - Additive
  - Multiplicative

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Armitage Trend Test

- Power and Sample size
  - Calculated under different models
    - Where γ is the relative risk
      - Multiplicative
        - » $\gamma_2=\gamma_1^2$
      - Additive
        - » $\gamma_2=2\gamma_1-1$
      - Dominant
        - » $\gamma_2=\gamma_1$
      - Recessive
        - » $\gamma_1=1$

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Gamma is the Relative Risk not the Odd Ratio

- Most software for power calculations/sample size estimation use the relative risk (γ) and not the odds ratio
- The relative risk only approximates the odds ratio when disease is rare (Prevalence ~< 0.1%)
  - The relative risk is not appropriate for common traits when a case-control design is used

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Correspondence Between the Odds Ratio and Relative Risk

Dominant Model

Disease Prevalence	1/2* RR=1.5	2/2** RR=1.5
0.01	1.51	1.51
0.10	1.59	1.59
0.20	1.71	1.71

Multiplicative Model

Disease Prevalence	1/2 RR=1.5	2/2 RR=2.25
0.01	1.51	2.28
0.10	1.59	2.61
0.20	1.71	3.25

Marker minor allele and disease allele frequency 0.01
D' and $r^2=1$
*1/2 genotype – heterozygous (one copy of the alternative allele)
**2/2 genotype - homozygous for the alternative allele

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Armitage Trend Test - Power Calculations

- Information need
  - Population prevalence
  - Genetic Model
  - Risk allele frequency
- Tools
  - http://ihg.gsf.de/cgi-bin/hw/power2.pl
  - Reference Slager and Schaid 2001

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Armitage Test for Trend

Sample size approximations for Armitage's test for trend:

Disease prevalence	0.01
High risk allele frequency	0.05
Type 1 error (alpha)	0.00000005
Power (1- beta)	0.8
Gamma 1	2
Gamma 2	2
Cases / (cases + controls)	0.5

Cases necessary = 1502

Controls necessary = 1502

Cases and controls necessary = 3004

submit Reset

Gamma (genotypic relative risk):
Under a multiplicative model, gamma2 = gamma1^2; under a additive model, gamma2 = 2 * gamma1 - 1;
under a dominant model, gamma2 = gamma1; under a recessive model, gamma1 = 1.

Adapted from:
Slager SL, Schaid DJ: Case-control studies of genetic markers:
Power and sample size approximations for Armitage's test for trend.
Hum Hered 52, 149-153 (2001).
and
Freidlin B, Zheng G, Li Z, Gastwirth JL:
Trend tests for case-control studies of genetic markers:
Power, sample size and robustness.
Hum Hered 53, 146-152 (2002).

Tim M. Strom

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Genetic Association Study (GAS) Power Calculator

- http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html
- A one-stage study power calculator
  - Which was derived from CaTs
    - Which is to perform two-stage genome wide association studies
      - Skol et al. 2006
- Cochran Armitage Trend Test
- Displays graphs of the results

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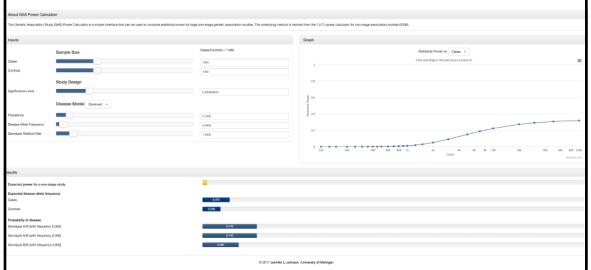
GAS Power Calculator



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Genetic Power Calculator

- http://zzz.bwh.harvard.edu/gpc/
- S Purcell & P Sham
- Uses the methods described in Sham PC et al. (2000) Am J Hum Genet 66:1616-1630
  - VC QTL linkage for sibships
  - VC QTL association for sibships
  - VC QTL linkage for sibships conditional on the trait
  - TDT for discrete traits
  - Case-Control for discrete traits
  - TDT for quantitative traits
  - Case-Control quantitative traits
- Although input is the relative risk
  - Displays odds ratios

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Genetic Power Calculator

Case - control for discrete traits

```
High risk allele frequency (A)   : 0.01   (0 - 1)
Prevalence                       : 0.2    (0.0001 - 0.9999)
Genotype relative risk Aa        : 1.5    ( >1 )
Genotype relative risk AA        : 1.5    ( >1 )

D-prime                          : 1      (0 - 1)
Marker allele frequency (B)      : 0.01   (0 - 1)

Number of cases                  : 10000  (0 - 10000000)
Control : case ratio             : 1      ( >0 )
                                          ( 1 = equal number of cases and controls)
                                   Unselected controls? (* see below)

User-defined type I error rate   : 0.00000005 (0.00000001 - 0.5)
User-defined power: determine N  : 0.80   (0 - 1)
(1 - type II error rate)
```

Process Reset

Created by Shaun Purcell 24.Oct.2008

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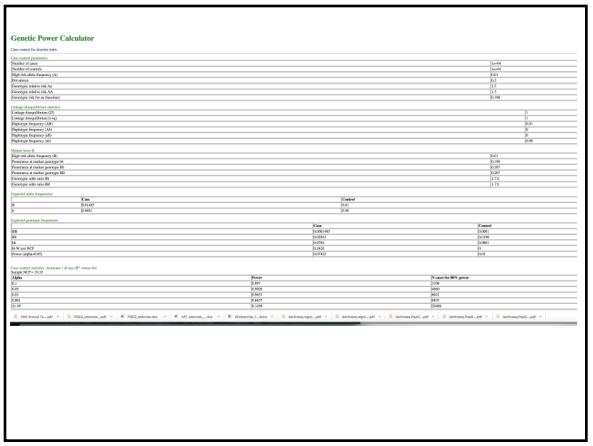

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Power Association With Errors (PAWE)

- http://compgen.rutgers.edu/pawe/
- Implements the linear trend test
- Four different error models can be used
  - See online documentation for complete explanation
- Can either perform:
  - Power calculations for a fixed sample size
  - Sample size calculations for a fixed power
- The genotype frequencies can be generated either using a:
  - Genetic model free method or
  - Genetic model-based method

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Quanto

- Provides sample size and power calculations for
- Genetic and environmental main effects
- Interactions
  - Gene x gene
  - Gene x environment
- Sample & power calculations can be carried for:
  - Case-control
    - Unmatched
    - Matched
  - Case-sibling
  - Case-parent (trios)
    - Quantitative
    - Qualitative
  - Independent sample of individuals
    - Quantitative traits
      - Assumption sampled from a random population
- Can only be run under windows
  - https://pphs.usc.edu/download-quanto/

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Linkage Disequilibrium (LD)

- Power will be reduced if causal variant is not in perfect LD (r^2=1) with the tag SNP
- Can adjust sample size when r^2 <1 to increase power to the same level as when r^2=1
- Can estimate sample size when $r^2 \neq 1$
  - $N/r^2=N'$
  - Valid only for multiplicative model
  - (Pritchard and Przeworski, 2001)
- Power calculation almost always assume that r^2=1
- For whole genome sequence data this should be the case since usually the causal variant would be included in the data

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Power Analysis for Rare Variant Aggregate Association Tests

- Many unknown parameters must be modeled
  - Allelic architecture within a genetic region
    - Varied across genes and populations
  - Effects of variants within a region
    - Fixed or varied effect sizes of causal variants
    - Bidirectional effect of variants
    - Proportion of non-causal variants
- Power estimated empirically
- Simplified assumptions can be made to obtain analytical estimates
  - All variants have the same effect size
  - No non-causal variants within a region that is analyzed in aggregate

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Simplistic Analytical Power Calculation for Rare-variant Aggregate Association Analysis

- Assumption
  - All rare variants are causal and have the same effect size
- Although usual not be correct
  - Provides a gestalt of the power for a given samples or sample size for a given power
- Use aggregate of allele frequencies
  - For example, assume a cumulative allele frequency of 0.025
  - Use an exome-wide significant level e.g., 2.5×10^{-6}
- Provide disease prevalence and penetrance model
- Perform calculations in the same manner as was described for single variants

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Empirical Power Calculations

- A variety of methods can be used to generate variant data to empirically estimate power
- Variant data is generated
  - Based upon a penetrance model samples of cases and controls are generated
  - Or a quantitative trait is generated based upon the genetic variance
- Multiple replicates are generated and analyzed
  - To determine the power

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Empirical Power Calculations

- Examples
  - 5,000 replicates are generated each with 20,000 cases and 20,000 controls
    - The power is the proportion of replicates with p-value less than the specified threshold, e.g., $5x10^{-8}$
  - For rare-variant aggregate tests all autosomal genes are generated and those genes with more than two rare variants (e.g., predicted loss of function) are analyzed
    - The power is the proportion of genes that were tested with p-value which is below a specified threshold, e.g., $2.5x10^{-6}$

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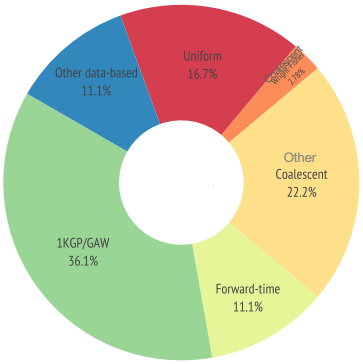
Simulation Methods



Note: Not all methods give a realistic distribution of variants & in particular for rare variants

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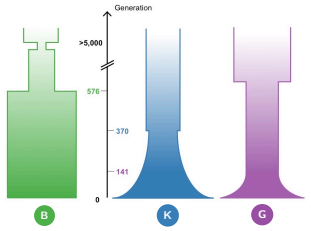
Generating Exome Sequence Data Sets
Forward-time Simulation

Data	Haplotype Counts	Demographics
Boyko	105,814*	
Kyrukov	1,800,000*	
Gazave	1,308,000*	

*Selection coefficients used to define "variant type"
-"Missense" ($1.0 \times 10^{-8} - 1.8 \times 10^{-2}$)
-"Nonsense, splice site and frameshift" (>1.8×10^{-2})

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SKAT Power Calculator

- R Library
- Provides a haplotype matrix
  - 10,000 haplotypes over 200kb region
  - Simulated using a calibrated coalescent model (cosi)
  - Mimicking linkage disequilibrium structure of European ancestry
  - User can also provide haplotype data
- Power and sample size calculations for binary and quantitative traits
- User specify proportion of variants that increase or lower risk

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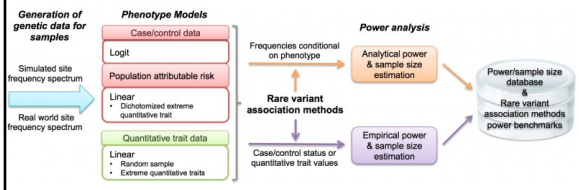
SEQPower

http://www.bioinformatics.org/spower/



Wang et al. 2014 Bioinformatics

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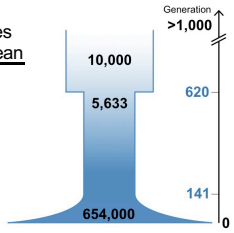
Generating Variants: Using a European Demographic Model and Exome Sequence Data

- Variant data generated on 18,397 genes
- Variant data simulated using a European population demographic model
  - Gazave et al. 2013



- Variants generated using exome sequence data
  - 4332 Exomes obtained from European American

Which method performs better and why?

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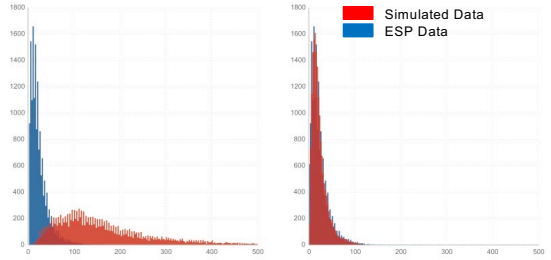
Does Generating Variant Data Using the European Population Demographic Model Perform Well?

Distribution of number of variants per gene



- Simulated variant counts based on the entire simulated population
- Simulated variant counts based on haplotype pool down-sampled to ESP size

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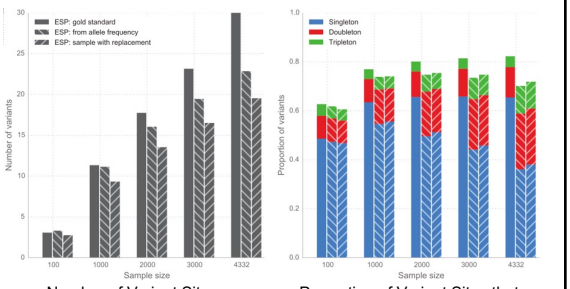
Simulating Data Using Sequence Data (ESP)



Number of Variant Sites

Proportion of Variant Sites that are Singletons, Doubletons and Tripletons

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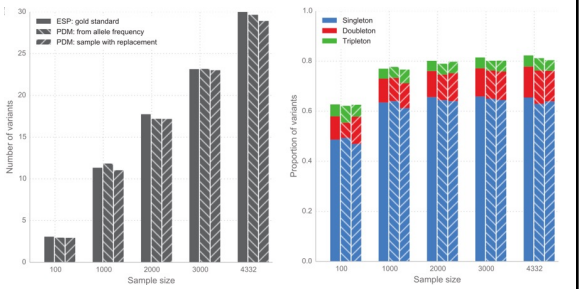
Simulating Data: Using Population Demographic Models (PDM)



Number of Variant Sites

Proportion of Variant Sites that are Singletons, Doubletons and Tripletons

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Simulation Studies to Evaluate Power for Rare Variant Association Studies

- It is unknown which genes are important in disease etiology
  - Correct allelic architecture is unknown
- Can get a better understanding of power to detect associations by generating variants for the entire exome
- Use a variety of disease models
  - Odds ratios
  - Proportion of pathogenic variants
- Analyze of all genes
  - e.g., those with 2 or more variant sites
- Determine power as the proportion of genes that meet exome-wide significance (e.g., alpha=2.5×10^{-6})

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Power Analysis

- For tests of individual variants
  - Power depended on sample size, disease prevalence, minor allele frequency, genetic model and variant effect size
- For rare variants (aggregate association tests)
  - Also dependent on the allelic architecture
    - Cumulative variant frequency within analyzed region
    - Proportion of causal variants
      - How much contamination from non-causal variants
    - Effect sizes the same the same or different across gene regions
      - Effects of variants in the same or different directions
        - » Protective and detrimental for binary traits
        - » Increase and decrease quantitative trait values

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Power Analysis Rare Variants (Aggregate Association Tests)

- Power will not only vary between traits greatly
- The power to detect an association will also vary drastically between genes for the same complex trait
  - For some causal genes even with hundreds of thousands of samples power will be low
  - While for other causal genes a few thousand samples may be sufficient

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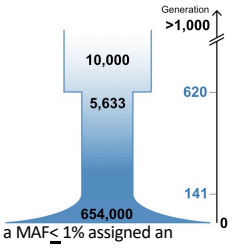
How Large of a Sample Size is Necessary to Detect Rare Variant Associations?

- Data generated on 18,397 genes
- Variant data simulated using a European population demographic model
  - Gazave et al. 2013



- Every missense, nonsense and splice with a MAF< 1% assigned an odds ratio of 1.5
- Sample sizes to detect X number of genes determined for
  - $\alpha = 2.5 \times 10^{-6}$
  - power=0.8

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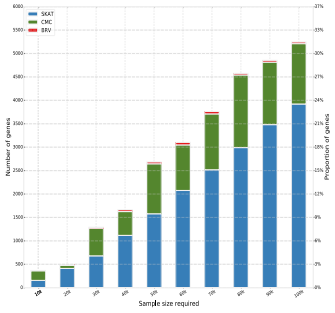
Sample Sizes Necessary to Detect an Association (Case-Control Data)



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