# Cardiac ACMG-ClinVar Penetrance Estimation

*James Diao, under the supervision of Arjun Manrai*

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## Contents



**Working Directory**: /Users/jamesdiao/Documents/Kohane_Lab/2017-ACMG-penetrance/ACMG_Penetrance

# 1 Download, Transform, and Load Data

## 1.1 Collect ACMG Gene Panel

http://www.ncbi.nlm.nih.gov/clinvar/docs/acmg/

```
## Table from ACMG SF v2.0 Paper 60 x 8 (selected rows):
```

| | Phenotype | MIM_disorder | PMID_Gene_Reviews_entry |
|---|---|---|---|
| **N1** | Hereditary breast and ovarian cancer | 604370\|612555 | 20301425 |
| **N2** | Hereditary breast and ovarian cancer | 604370\|612555 | 20301425 |
| **N3** | Li-Fraumeni syndrome | 151623 | 20301488 |
| **N4** | Peutz-Jeghers syndrome | 175200 | 20301443 |
| **N5** | Lynch syndrome | 120435 | 20301390 |

Table continues below

| | Typical_age_of_onset | Gene | MIM_gene | Inheritance | Variants_to_report |
|---|---|---|---|---|---|
| **N1** | Adult | BRCA1 | 113705 | AD | KP&EP |
| **N2** | Adult | BRCA2 | 600185 | AD | KP&EP |
| **N3** | Child/Adult | TP53 | 191170 | AD | KP&EP |
| **N4** | Child/Adult | STK11 | 602216 | AD | KP&EP |
| **N5** | Adult | MLH1 | 120436 | AD | KP&EP |

```
## ACMG-59 Genes:
```

```
##  [1] BRCA1   BRCA2   TP53    STK11   MLH1    MSH2    MSH6    PMS2
##  [9] APC     MUTYH   BMPR1A  SMAD4   VHL     MEN1    RET     PTEN
## [17] RB1     SDHD    SDHAF2  SDHC    SDHB    TSC1    TSC2    WT1
## [25] NF2     COL3A1  FBN1    TGFBR1  TGFBR2  SMAD3   ACTA2   MYH11
## [33] MYBPC3  MYH7    TNNT2   TNNI3   TPM1    MYL3    ACTC1   PRKAG2
## [41] GLA     MYL2    LMNA    RYR2    PKP2    DSP     DSC2    TMEM43
## [49] DSG2    KCNQ1   KCNH2   SCN5A   LDLR    APOB    PCSK9   ATP7B
## [57] OTC     RYR1    CACNA1S
```

## 1.2 Download ClinVar VCF

ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/clinvar.vcf.gz
ClinVar is the central repository for variant interpretations. Relevant information from the VCF includes:
(a) CLNSIG = "Variant Clinical Significance, 0 - Uncertain, 1 - Not provided, 2 - Benign, 3 - Likely benign, 4 - Likely pathogenic, 5 - Pathogenic, 6 - Drug response, 7 - Histocompatibility, 255 - Other"
(b) CLNDBN = "Variant disease name"
(c) CLNDSDBID = "Variant disease database ID"
(d) CLNREVSTAT = "Review Status, no_assertion, no_criteria, single - criterion provided single submitter, mult - criteria provided multiple submitters no conflicts, conf - criteria provided conflicting interpretations, exp - Reviewed by expert panel, guideline - Practice guideline"
(e) INTERP = Pathogenicity (likely pathogenic or pathogenic; CLNSIG = 4 or 5)

## 1.3 Download 1000 Genomes VCFs

ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/ALL.[chrom].phase3_[version].20130502.genotypes.vcf.gz
Downloaded 1000 Genomes VCFs are saved in: /Users/jamesdiao/Documents/Kohane_Lab/2017-ACMG-penetrance/1000G/

| gene | name | chrom | start | end | downloaded |
|---|---|---|---|---|---|
| BRCA1 | NM_007294 | 17 | 41196311 | 41277500 | TRUE |
| BRCA2 | NM_000059 | 13 | 32889616 | 32973809 | TRUE |
| TP53 | NM_000546 | 17 | 7571719 | 7590868 | TRUE |
| STK11 | NM_000455 | 19 | 1205797 | 1228434 | TRUE |
| MLH1 | NM_000249 | 3 | 37034840 | 37092337 | TRUE |

## 1.4 Import and Process 1000 Genomes VCFs

(a) Unnest the data frames to 1 row per variant_ID key (CHROM_POSITION_REF_ALT).
(b) Remove all insertions, deletions, CNV, etc, and keep only missense variants (1 REF, 1 ALT)
(c) For 1000 Genomes: convert genomes to allele counts. For example: (0|1) becomes 1, (1|1) becomes 2. Multiple alleles are unnested into multiple counts. For example: (0|2) becomes 0 for the first allele (no 1s) and 1 for the second allele (one 2).

```
## Processed 1000 Genomes VCFs: 141467 x 2516 (selected rows/columns):
```

| GENE | AF_1000G | VAR_ID | CHROM | POS | ID | REF | ALT |
|---|---|---|---|---|---|---|---|
| BRCA1 | 0.004193290 | 17_41196363_C_T | 17 | 41196363 | rs8176320 | C | T |
| BRCA1 | 0.008386580 | 17_41196368_C_T | 17 | 41196368 | rs184237074 | C | T |
| BRCA1 | 0.000998403 | 17_41196372_T_C | 17 | 41196372 | rs189382442 | T | C |
| BRCA1 | 0.342252000 | 17_41196408_G_A | 17 | 41196408 | rs12516 | G | A |
| BRCA1 | 0.000399361 | 17_41196409_G_C | 17 | 41196409 | rs548275991 | G | C |

Table continues below

| HG00096 | HG00097 | HG00099 | HG00100 | HG00101 | HG00102 |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 1 | 1 | 0 | 2 |
| 0 | 0 | 0 | 0 | 0 | 0 |

## 1.5 Import and Process gnomAD/ExAC VCFs

(a) Unnest the data frames to 1 row per variant_ID key (CHROM_POSITION_REF_ALT).
(b) Remove all insertions, deletions, CNV, etc, and keep only missense variants (1 REF, 1 ALT)
(c) Collect superpopulation-level allele frequencies: African = AFR, Latino = AMR, European (Finnish + Non-Finnish) = EUR, East.Asian = EAS, South.Asian = SAS.

```
## Processed gnomAD VCFs: 31729 x 49 (selected rows/columns):
```

| | GENE | AF_GNOMAD | AF_GNOMAD_NFE |
|---|---|---|---|
| **22531** | MYH7 | 0.000004119 | 0.000000000000 |
| **24995** | TPM1 | 0.000032280 | 0.000000000000 |
| **6720** | DSG2 | 0.000004063 | 0.000008958486 |
| **14845** | LDLR | 0.000004061 | 0.000000000000 |
| **541** | PKP2 | 0.000004066 | 0.000000000000 |

## 1.6 Collect 1000 Genomes Phase 3 Populations Map

This will allow us to assign genotypes from the 1000 Genomes VCF to ancestral groups.
From: ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/integrated_call_samples_v3.20130502.ALL.panel

```
## Phase 3 Populations Map Table: 2504 x 4 (selected rows)
```

| sample | pop | super_pop | gender |
|---|---|---|---|
| NA19027 | LWK | AFR | male |
| HG02028 | KHV | EAS | female |
| HG01524 | IBS | EUR | male |
| NA20514 | TSI | EUR | female |
| HG00362 | FIN | EUR | female |
| HG03788 | ITU | SAS | male |

## 1.7 Merge ClinVar with gnomAD, ExAC, and 1000 Genomes

```
## Breakdown of ClinVar Variants
```

| Subset_ClinVar | Number_of_Variants |
|---|---|
| Total ClinVar | 224657 |
| LP/P | 42826 |
| ACMG LP/P | 9139 |
| ACMG LP/P in gnomAD | 662 |
| ACMG LP/P in 1000 Genomes | 53 |

```
## Breakdown of ACMG-gnomAD Variants
```

| Subset_gnomAD | Number_of_Variants |
|---|---|
| ACMG in gnomAD | 31729 |
| ClinVar-ACMG in gnomAD | 4089 |
| LP/P-ACMG in gnomAD | 662 |

## 1.8 Overall Non-Reference Sites

#### 1.8.0.1 For 1000 Genomes

Each individual has $n$ non-reference sites, which can be found by counting. The mean number is computed for each population.

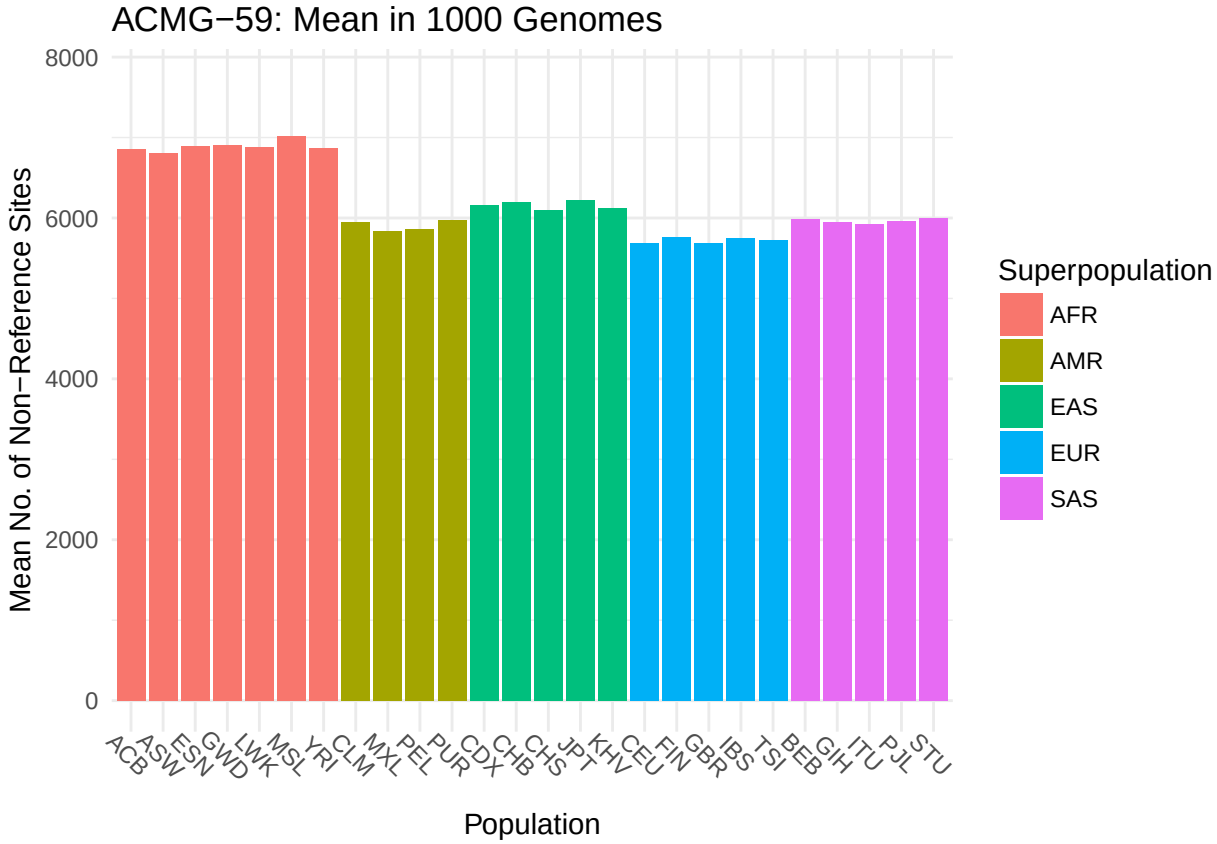
Ex: the genotype of 3 variants in 3 people looks like this:

| | HG00366 | HG00367 | HG00368 |
|---|---|---|---|
| **Variant 1** | 2 | 1 | 1 |
| **Variant 2** | 2 | 1 | 1 |
| **Variant 3** | 1 | 0 | 0 |

Count the number of non-reference sites per individual:

| HG00366 | HG00367 | HG00368 |
|---|---|---|
| 3 | 2 | 2 |

```
## Mean = 2.33
```




Note: the error bars denote standard deviation, not standard error.

#### 1.8.0.2 For gnomAD/ExAC

The mean number of non-reference sites is $E(V)$, where $V = \sum_{i=1}^{n} v_i$ is the number of non-reference sites at all variant positions $v_1$ through $v_n$.

At each variant site, the probability of having at least 1 non-reference allele is $P(v_i) = P(v_{i,a} \cup v_{i,b})$, where $a$ and $b$ indicate the 1st and 2nd allele at each site.

If the two alleles are independent, $P(v_{i,a} \cup v_{i,b}) = 1 - (1 - P(v_{i,a}))(1 - P(v_{i,b})) = 1 - (1 - AF(v_i))^2$

If all variants are independent, $E(V) = \sum_{i=1}^{n} 1 - (1 - AF(v_i))^2$ for any set of allele frequencies.

Ex: the allele frequencies of 3 variants across the 5 superpopulations looks like this:

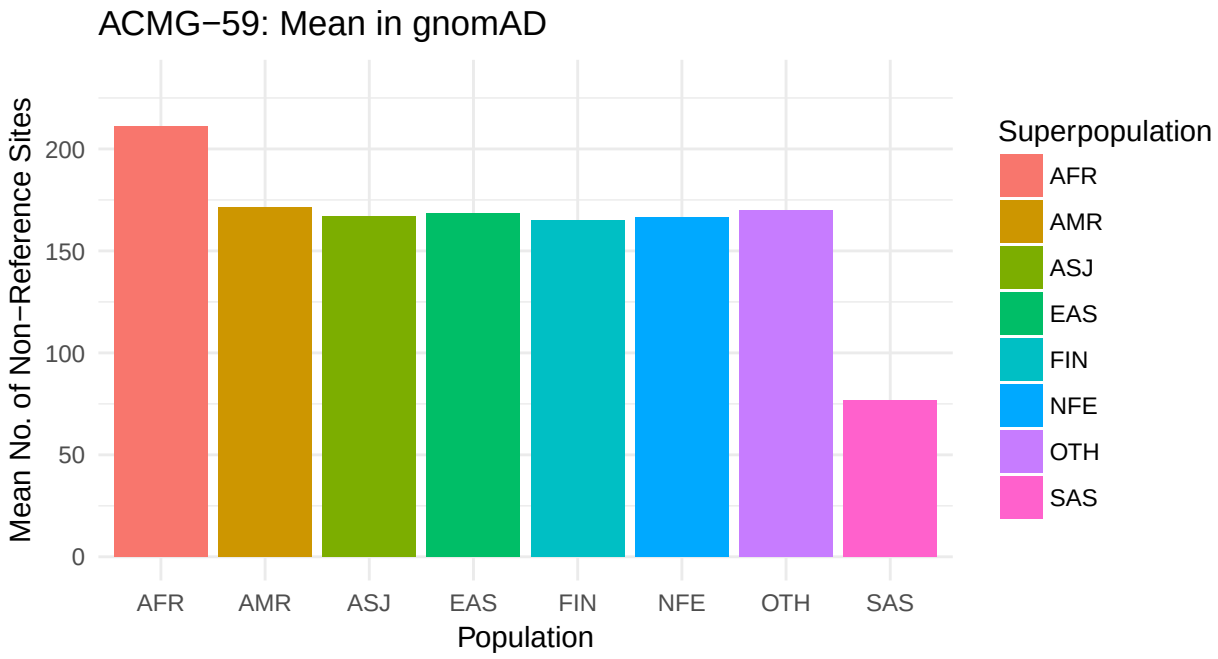
| | AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|---|
| **Variant 1** | 0.1 | 0.2 | 0 | 0 | 0.3 |
| **Variant 2** | 0.2 | 0 | 0.3 | 0 | 0.1 |

The probability of having at least 1 non-reference site at each variant - (0|1) (1|0) or (1|1) is given by $1 - (1 - AF)^2$. Note that this is approximately $2 * AF$ when $AF$ is small:

| | AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|---|
| **Variant 1** | 0.19 | 0.36 | 0 | 0 | 0.51 |
| **Variant 2** | 0.36 | 0 | 0.51 | 0 | 0.19 |

By linearity of expectation, the expected (mean) number of non-reference sites is $\sum E(V_i) = \sum(columns)$.

| AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|
| 0.55 | 0.36 | 0.51 | 0 | 0.7 |



## 1.9 Fraction of Individuals with Pathogenic Sites

#### 1.9.0.1 For 1000 Genomes

We can count up the fraction of individuals with 1+ non-reference site(s) in each population. This is the fraction of individuals who would receive a positive genetic test result in at least 1 of the ACMG-59 genes.

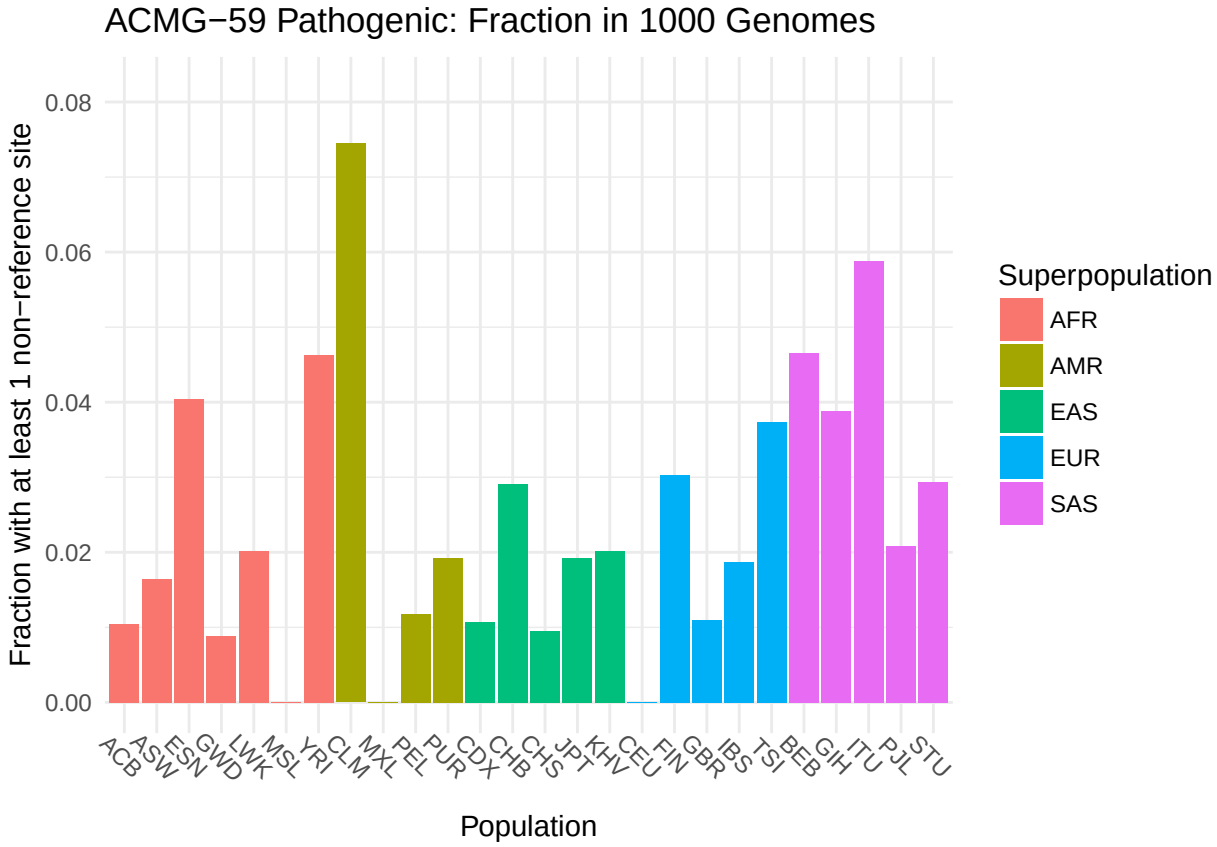
Ex: the genotype of 3 variants in 3 people looks like this:

| | HG00366 | HG00367 | HG00368 |
|---|---|---|---|
| **Variant 1** | 2 | 1 | 1 |
| **Variant 2** | 2 | 1 | 1 |
| **Variant 3** | 1 | 0 | 0 |

Count each individual as having a non-reference site (1) or having only reference sites (0):

| HG00366 | HG00367 | HG00368 |
|---|---|---|
| 1 | 1 | 1 |

```
## Mean = 1
```



#### 1.9.0.2 For gnomAD/ExAC

The probability of having at least 1 non-reference site is $P(X)$, where $X$ indicates a non-reference site at any variant position $v_1$ through $v_n$.

Recall that $P(v_i) = P(v_{i,a} \cup v_{i,b}) = 1 - (1 - AF(v))^2$ when alleles are independent.

If all alleles are independent, $P(X) = P(\bigcup_{i=1}^{n} v_i) = 1 - \prod_{i=1}^{n}(1 - AF(v_i))^2$

Ex: the allele frequencies of 3 variants across the 5 superpopulations looks like this:

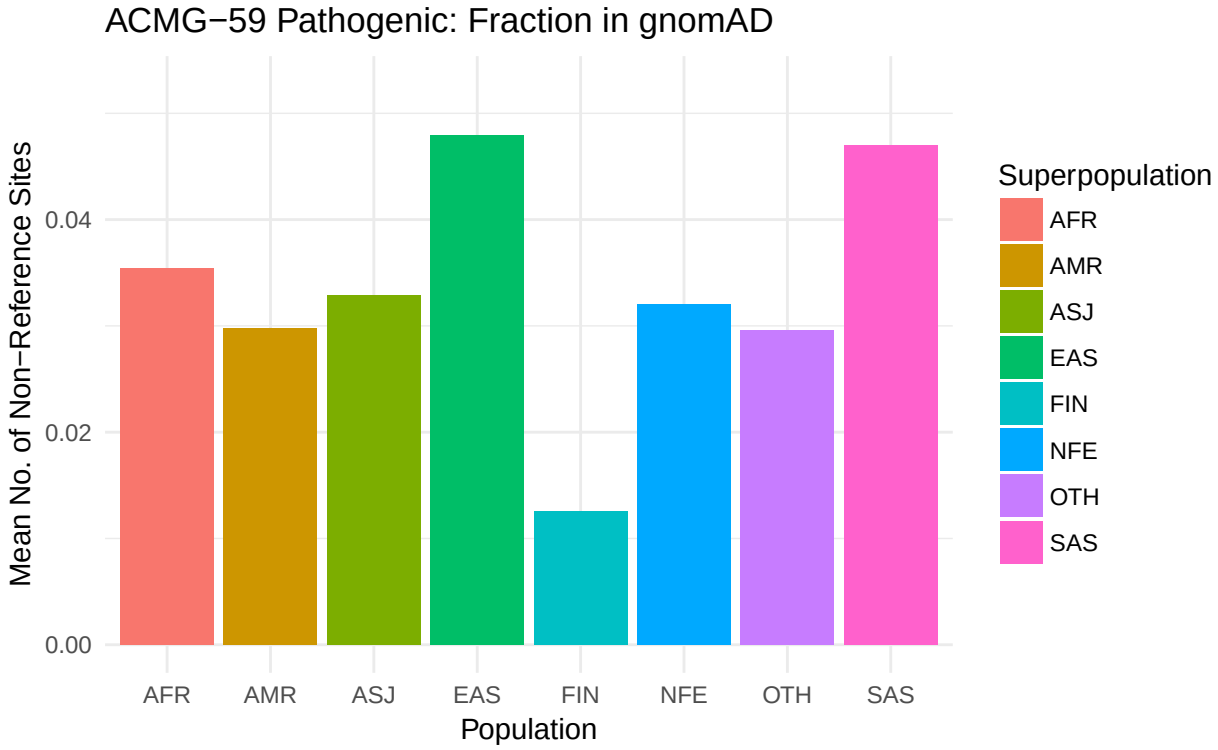
| | AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|---|
| **Variant 1** | 0.1 | 0.2 | 0 | 0 | 0.3 |
| **Variant 2** | 0.2 | 0 | 0.3 | 0 | 0.1 |

The probability of having at least 1 non-reference site at each variant - (0|1) (1|0) or (1|1) is given by $1 - (1 - AF)^2$. Note that this is approximately $2 * AF$ when $AF$ is small:

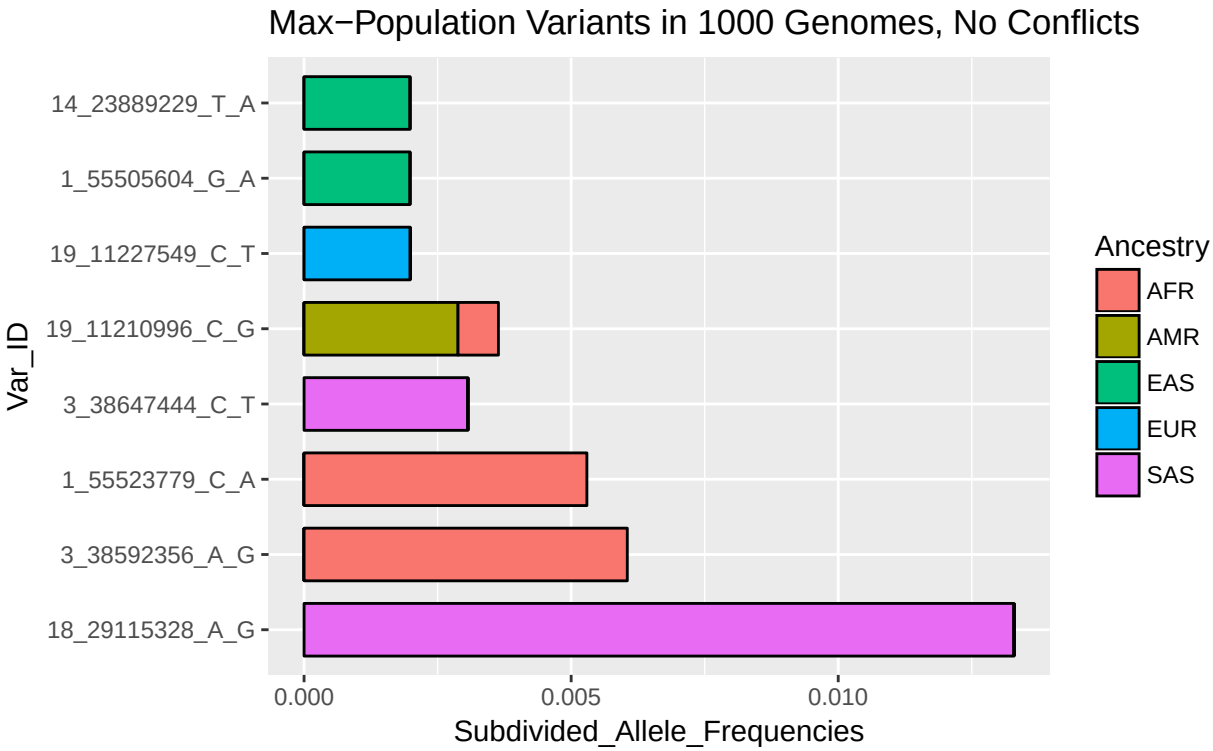
| | AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|---|
| **Variant 1** | 0.19 | 0.36 | 0 | 0 | 0.51 |
| **Variant 2** | 0.36 | 0 | 0.51 | 0 | 0.19 |

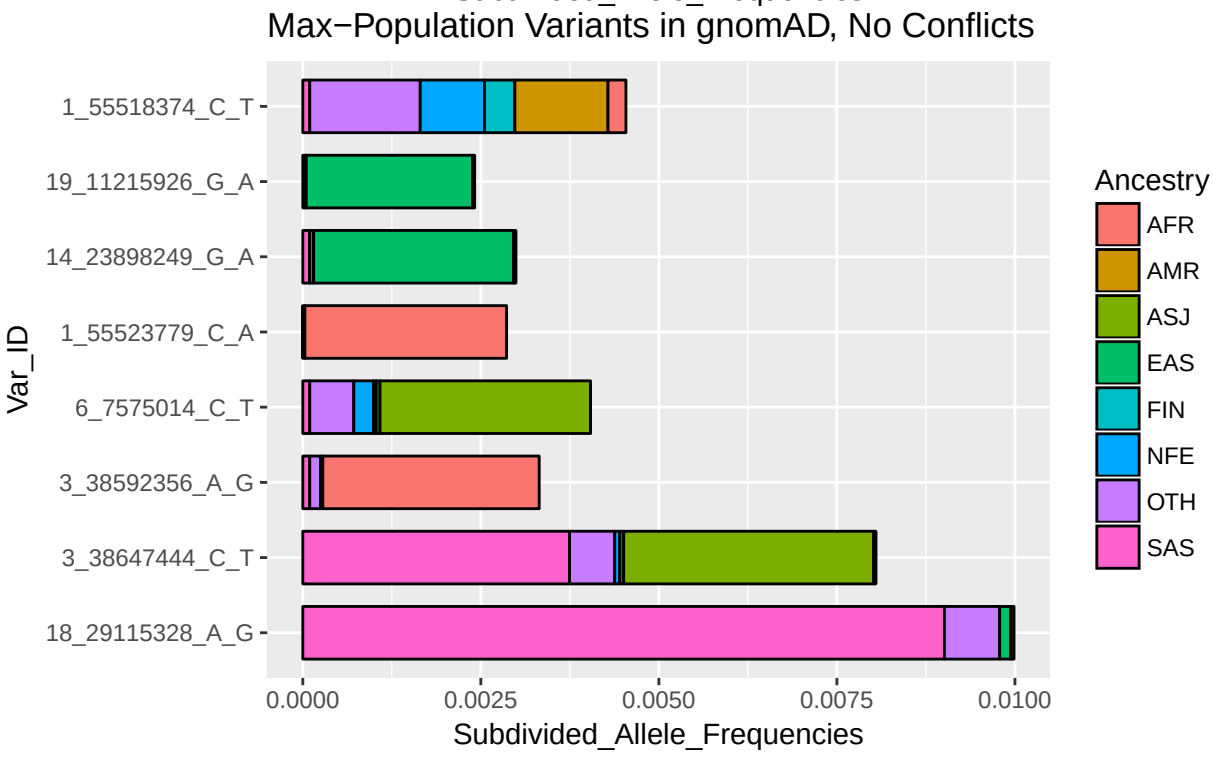
The expected (mean) number of non-reference sites is given by $1 - \prod(1 - AF)^2$.

| AFR | AMR | EAS | EUR | SAS |
|---|---|---|---|---|
| 0.4816 | 0.36 | 0.51 | 0 | 0.6031 |



### 1.10 Common Pathogenic Variants by Ancestry



# 2 Penetrance Estimates

## 2.1 Bayes' Rule as a Model for Estimating Penetrance

Let $V_x$ be the event that an individual has 1 or more variant related to disease $x$,
and $D_x$ be the event that the individual is later diagnosed with disease $x$.

In this case, we can define the following probabilities:
1. Prevalence = $P(D_x)$
2. Population Allele Frequency (PAF) = $P(V_x)$
3. Case Allele Frequency (CAF) = $P(V_x|D_x)$
4. Penetrance = $P(D_x|V_x)$

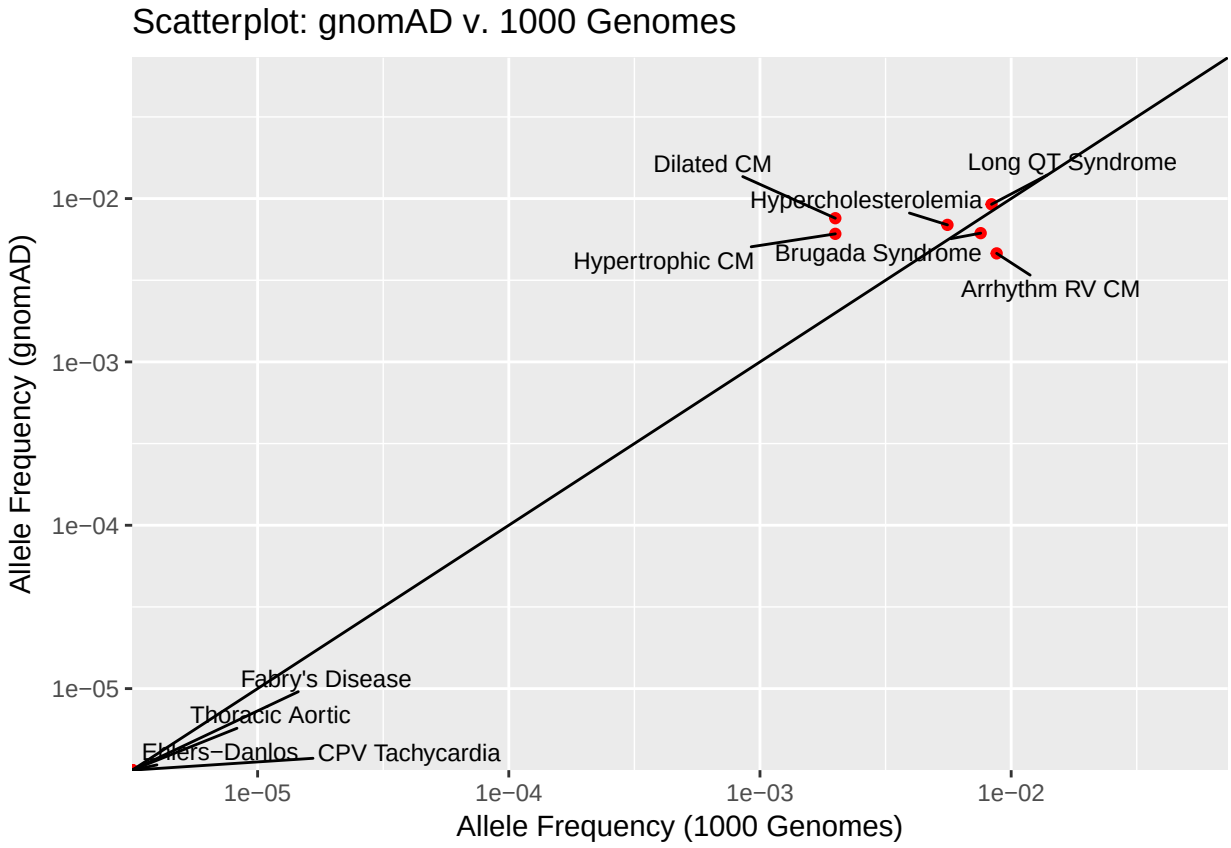
By Bayes' Rule, the penetrance of a variant related to disease $x$ may be defined as:

$$P(D_x|V_x) = \frac{P(D_x) * P(V_x|D_x)}{P(V_x)} = \frac{(Prevalence)(Population\ Allele\ Frequency)}{(Case\ Allele\ Frequency)}$$

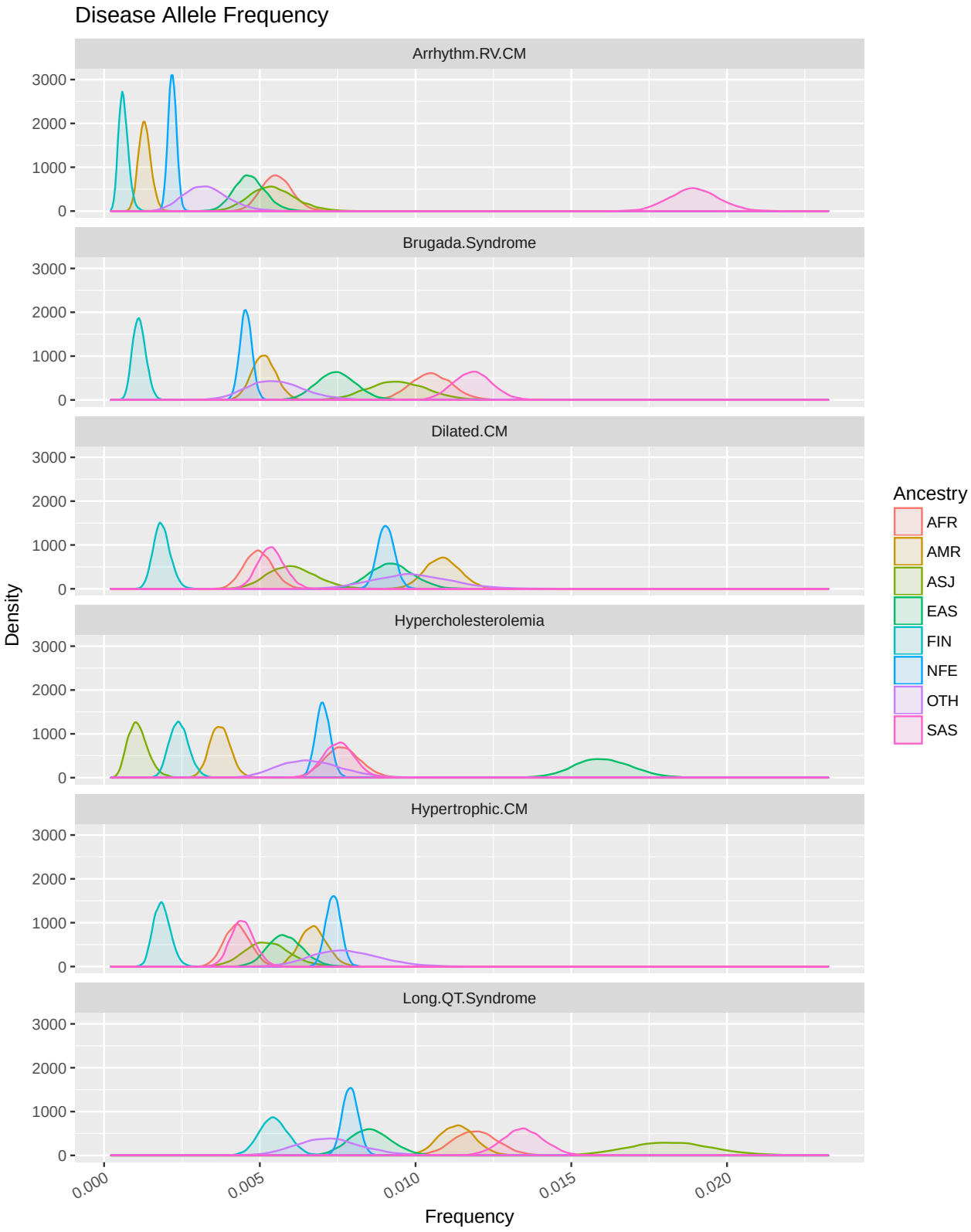
To compute penetrance estimates for each of the diseases related to the ACMG-59 genes, we will use the prevalence data we collected into `Literature_Prevalence_Estimates.csv`, allele frequency data from 1000 Genomes/ExAC/gnomAD, and a broad range of values for case allele frequency.

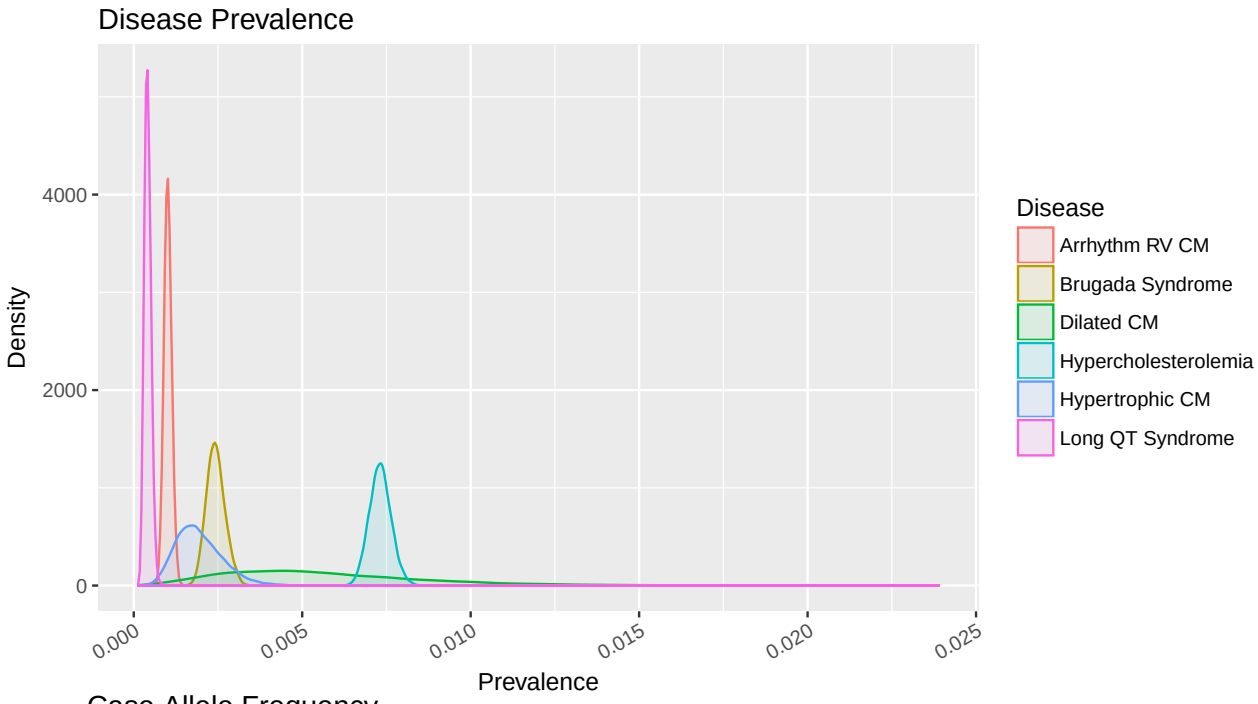
## 2.2 Collect and Aggregate Allele Frequencies at the Disease-Level

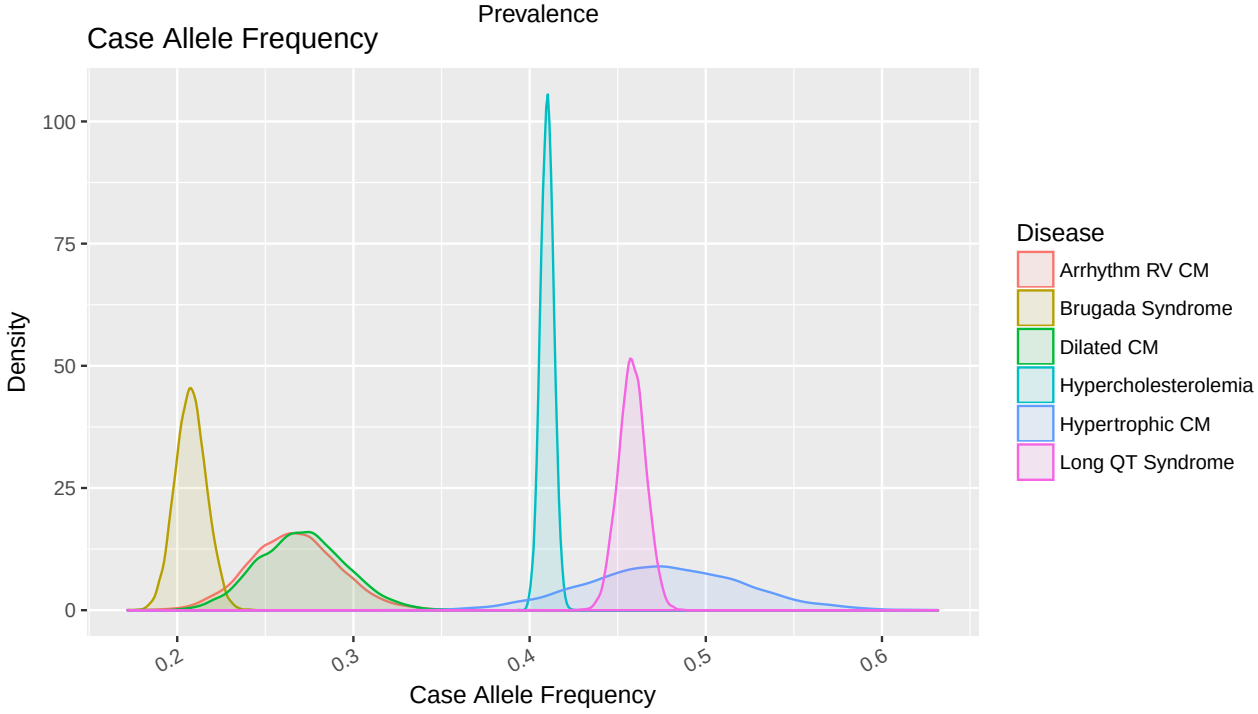
We define AF(disease) as the probability of having at least 1 variant associated with the disease. The variants can be assigned to diseases in two ways:
(1) By associating it by MIM. An MIM code is assigned for around 31% of assertions in each dataset.
(1) By associating it by MedGen. An MIM code is assigned for around 22% of assertions in each dataset.
(2) By associating it by gene. All variants are associated with genes, but some variants may be designated as pathogenic for non-ACMG conditions.
The frequencies across the relevant variants can be aggregated in two ways:
(1) By direct counting, from genotype data in 1000 Genomes.
(2) AF(disease) = $1 - \prod_{variant}(1 - AF_{variant})$, from population data in 1000 Genomes, ExAC, or gnomAD (assumes independence).

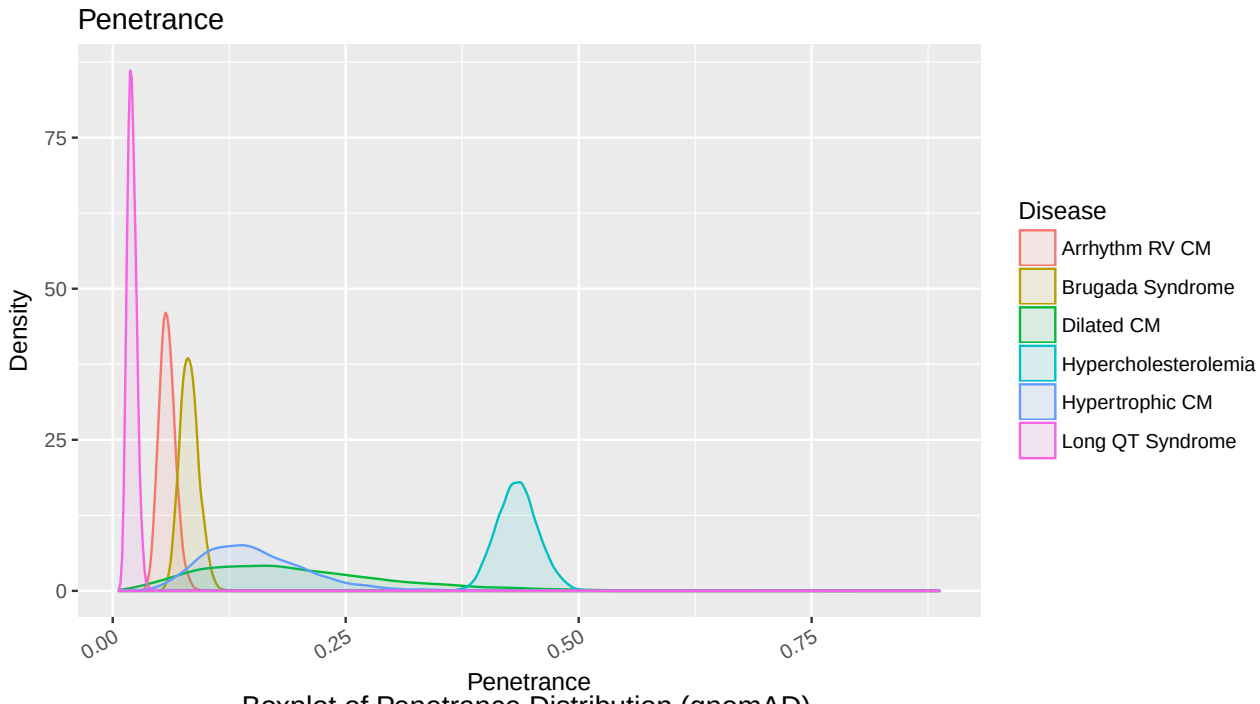

Scatterplot: gnomAD v. 1000 Genomes
Allele Frequency (gnomAD)
1e−02
1e−03
1e−04
1e−05
Dilated CM
Long QT Syndrome
Hypercholesterolemia
Hypertrophic CM
Brugada Syndrome
Arrhythm RV CM
Fabry's Disease
Thoracic Aortic
Ehlers−Danlos
CPV Tachycardia
1e−05
1e−04
1e−03
1e−02
Allele Frequency (1000 Genomes)

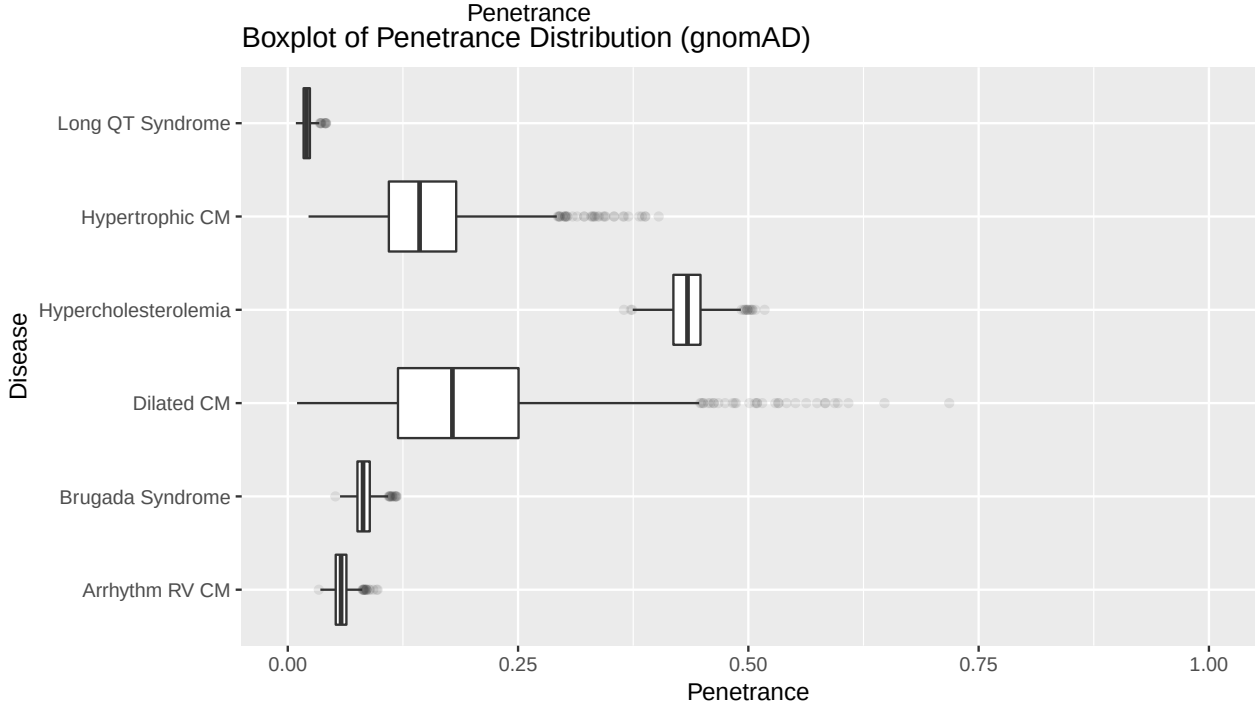
## 2.3 Bootstrapped Distribution of Penetrance

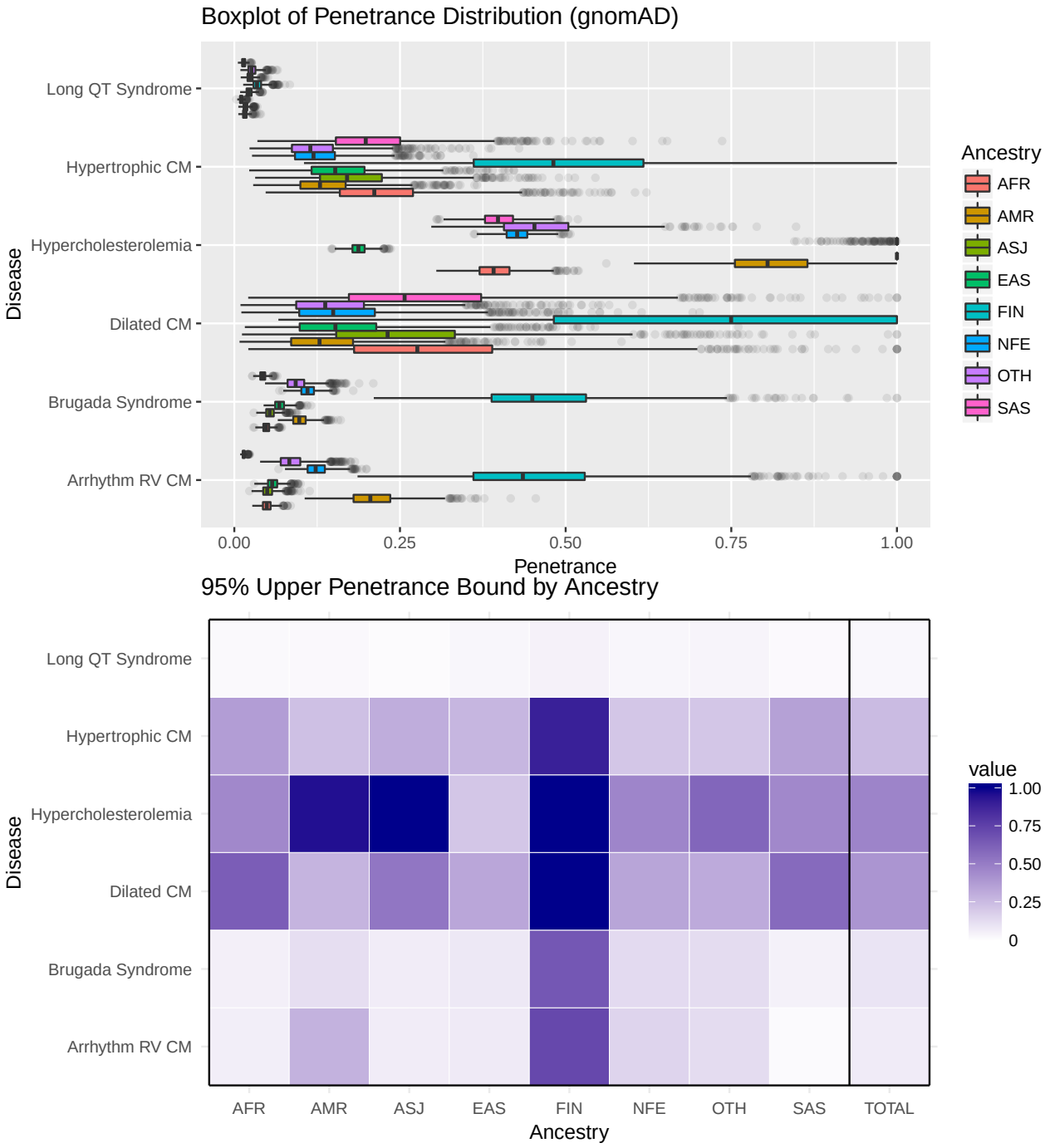



Disease Prevalence
Density
4000
2000
0
0.000
0.005
0.010
0.015
0.020
0.025
Prevalence
Disease
Arrhythm RV CM
Brugada Syndrome
Dilated CM
Hypercholesterolemia
Hypertrophic CM
Long QT Syndrome


Case Allele Frequency
Density
100
75
50
25
0
0.2
0.3
0.4
0.5
0.6
Case Allele Frequency
Disease
Arrhythm RV CM
Brugada Syndrome
Dilated CM
Hypercholesterolemia
Hypertrophic CM
Long QT Syndrome


Penetrance
Density
75
50
25
0
0.00
0.25
0.50
0.75
Penetrance
Disease
Arrhythm RV CM
Brugada Syndrome
Dilated CM
Hypercholesterolemia
Hypertrophic CM
Long QT Syndrome


Boxplot of Penetrance Distribution (gnomAD)
Disease
Long QT Syndrome
Hypertrophic CM
Hypercholesterolemia
Dilated CM
Brugada Syndrome
Arrhythm RV CM
0.00
0.25
0.50
0.75
1.00
Penetrance


Boxplot of Penetrance Distribution (gnomAD)
Long QT Syndrome
Hypertrophic CM
Hypercholesterolemia
Dilated CM
Brugada Syndrome
Arrhythm RV CM
Disease
0.00
0.25
0.50
0.75
1.00
Penetrance
Ancestry
AFR
AMR
ASJ
EAS
FIN
NFE
OTH
SAS
95% Upper Penetrance Bound by Ancestry
Long QT Syndrome
Hypertrophic CM
Hypercholesterolemia
Dilated CM
Brugada Syndrome
Arrhythm RV CM
Disease
AFR
AMR
ASJ
EAS
FIN
NFE
OTH
SAS
TOTAL
Ancestry
value
1.00
0.75
0.50
0.25
0