ACMG-ClinVar Penetrance RMarkdown

James Diao, under the supervision of Arjun Manrai

December 6, 2016

Contents

Working Directory: /Users/jamesdiao/Documents/Kohane_Lab/2016-paper-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) INTERP = Pathogenicity (likely pathogenic or pathogenic; CLNSIG = 4 or 5)

```
## Processed ClinVar data frame 128262 x 14 (selected rows/columns):
```

VAR_ID	CHROM	POS	ID	REF	ALT	CLNSIG
1_949523_C_T	1	949523	rs786201005	C	T	5
1_949739_G_T	1	949739	rs672601312	G	T	5
1_955597_G_T	1	955597	rs115173026	G	T	2
1_955619_G_C	1	955619	rs201073369	G	C	255
1_957568_A_G	1	957568	rs115704555	A	G	2
1_957605_G_A	1	957605	rs756623659	G	A	5

Table continues below

CLNDBN	CLNDSDBID	INTERP
Immunodeficiency_38_with_basal_ganglia_calcification	CN221808:616126	TRUE
Immunodeficiency_38_with_basal_ganglia_calcification	CN221808:616126	TRUE
not_specified	CN169374	FALSE
not_specified	CN169374	FALSE
not_specified	CN169374	FALSE
Congenital_myasthenic_syndrome	C0751882:ORPHA590	TRUE

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/2016-paper-ACMG-penetrance/1000G/

```
## Download report: region and successes: 59 x 6 (selected rows):
```

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

```
## File saved as download_output.txt in Supplementary_Files
```

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: 96742 x 48 (selected rows/columns):
```

	GENE	AF_GNOMAD	VAR_ID
37501	FBN1	0.00003180	15_48738899_G_A
279211	RYR1	0.00001580	19_38981366_C_T
42828	ACTA2	0.00001200	10_90699222_A_G
25762	SDHC	0.00000400	1_161326623_G_A
11215	MSH6	0.00000798	2_48027603_T_C

```
## Processed ExAC VCFs: 59883 x 45 (selected rows/columns):
```

	GENE	AF_EXAC	VAR_ID
269	BRCA1	0.000008237	17_41223051_G_A
9753	APC	0.000016480	5_112175686_T_A
16371	SDHC	0.000024710	1_161310401_C_T
21853	WT1	0.000248100	11_32461657_A_G
67215	CACNA1S	0.005326000	1_201028268_C_A

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
HG03121	ESN	AFR	female
HG02031	KHV	EAS	female
NA18582	CHB	EAS	female
NA18977	JPT	EAS	male
NA20761	TSI	EUR	female
NA21097	GIH	SAS	female

1.7 Merge ClinVar with gnomAD, ExAC, and 1000 Genomes

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

Subset_ClinVar	Number_of_Variants
Total ClinVar	128262
LP/P	33914
ACMG LP/P	6951
ACMG LP/P in gnomAD	1220
ACMG LP/P in ExAC	868
ACMG LP/P in 1000 Genomes	133

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

Subset_gnomAD	Number_of_Variants
ACMG in gnomAD	96742
ClinVar-ACMG in gnomAD	12547
LP/P-ACMG in gnomAD	1220

```
## Breakdown of ACMG-ExAC Variants
```

Subset_gnomAD	Number_of_Variants
ACMG in ExAC	59883
ClinVar-ACMG in ExAC	10193
LP/P-ACMG in ExAC	868

```
## Breakdown of ACMG-1000G Variants
```

Subset_gnomAD	Number_of_Variants
ACMG in 1000G	141466
ClinVar-ACMG in 1000G	4965
LP/P-ACMG in 1000G	133

1.8 Comparison with ClinVar Browser Query Results

`clinvar_query.txt` contains all results matched by the search query: "(APC[GENE] OR MYH11[GENE]... OR WT1[GENE]) AND (clinsig_pathogenic[prop] OR clinsig_likely_pathogenic[prop])" from the ClinVar website. The exact query is saved in /Supplementary_Files/query_input.txt
This presents another way of collecting data from ClinVar.

Intermediate step: convert hg38 locations to hg19 using the Batch Coordinate Conversion tool (liftOver) from UCSC Genome Browser Utilities.

```
## ClinVar Query Results Table (substitutions only): 6445 x 13 (selected rows/columns)
```

VAR_ID	Gene(s)	Condition(s)	Frequency
1_17350520_G_C	SDHB	Paragangliomas 4	NA
1_45798631_T_A	MUTYH	Hereditary cancer-predisposing syndrome	NA
1_201334766_A_T	TNNT2	Familial hypertrophic cardiomyopathy 2	NA
3_38646328_G_C	SCN5A	Atrial fibrillation, familial, 10	NA
3_38647447_G_C	SCN5A	Atrial fibrillation, familial, 10	NA

Table continues below

Clinical significance (Last reviewed)	Review status
Pathogenic/Likely pathogenic, not provided(Last reviewed: Feb 2, 2015)	criteria provided, single submitter
Pathogenic(Last reviewed: Sep 17, 2015)	criteria provided, single submitter
Pathogenic/Likely pathogenic(Last reviewed: Feb 27, 2016)	criteria provided, multiple submitters, no conflicts
Pathogenic(Last reviewed: Apr 15, 2008)	no assertion criteria provided
Pathogenic(Last reviewed: Apr 15, 2008)	no assertion criteria provided

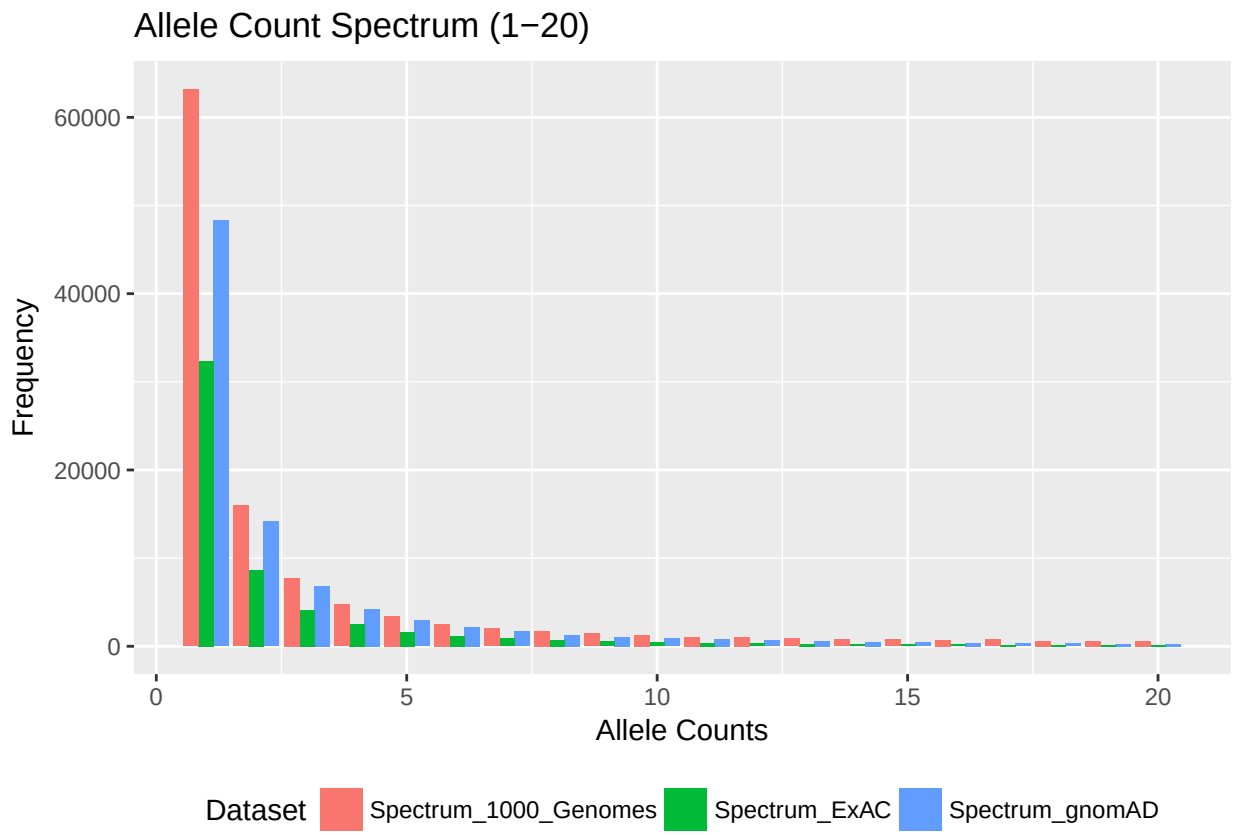
```
## Breakdown of ClinVar Query Results Table:
```

Subset	Number_of_Variants
Initial Count	14097
Filter Substitutions (N>N')	7039
Filter Coupling/Bad-Locations	6445
In ClinVar VCF	522
In LP/P-ClinVar	520
In LP/P-ACMG & gnomAD	49
In LP/P-ACMG & ExAC	37
In LP/P-ACMG & 1000G	1

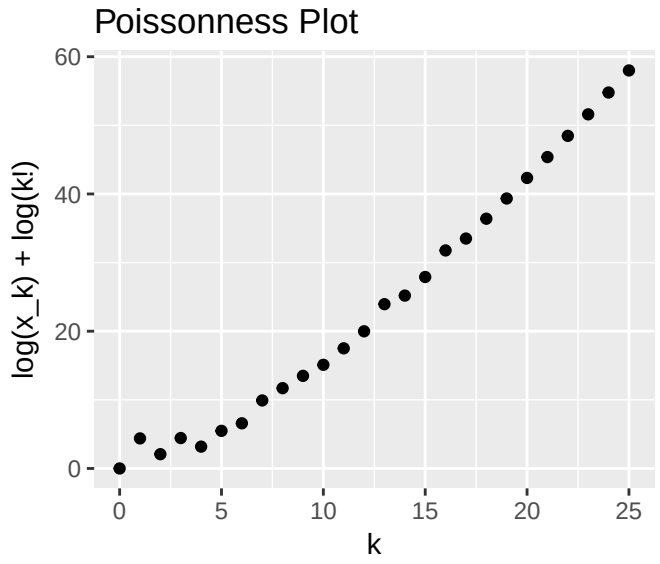
```
## Note the large reduction after merging the online query results with the VCF.
```

2 Plot Summary Statistics Across Populations

2.1 Distribution of Allele Frequencies



The distribution of allele frequencies is approximately Poisson, with "Poissonness plot" correlation = 0.99. The Poissonness plot (Hoaglin 1980) is defined as the plot of $log(x_k) + log(k!)$ vs. k, as shown below:



2.2 Overall Non-Reference Sites

2.2.0.1 For 1000 Genomes

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

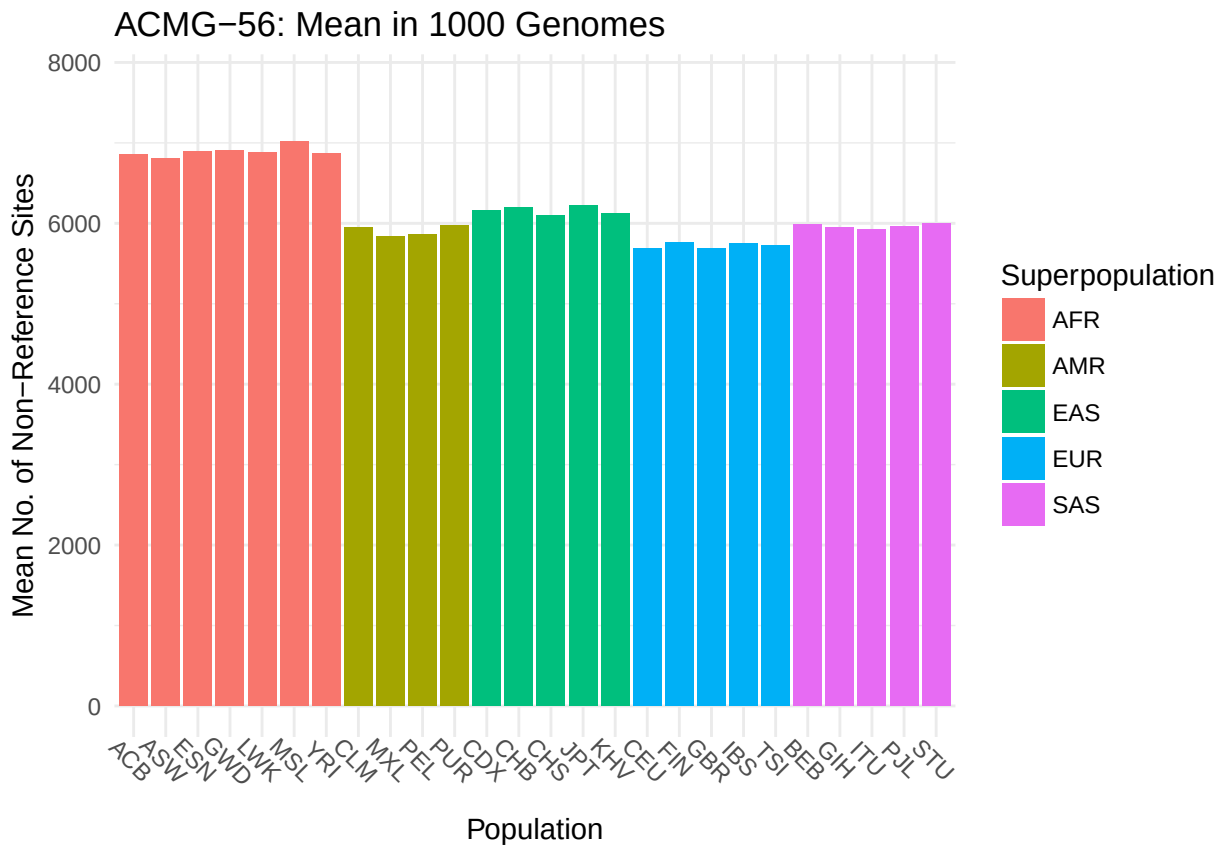
Ex: the genotype of 3 variants in 3 people looks like this:

	HG00097	HG00099	HG00100
Variant 1	0	0	0
Variant 2	0	0	0
Variant 3	0	0	0

Count the number of non-reference sites per individual:

HG00097	HG00099	HG00100
0	0	0

```
## Mean = 0
```



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

2.2.0.2 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:

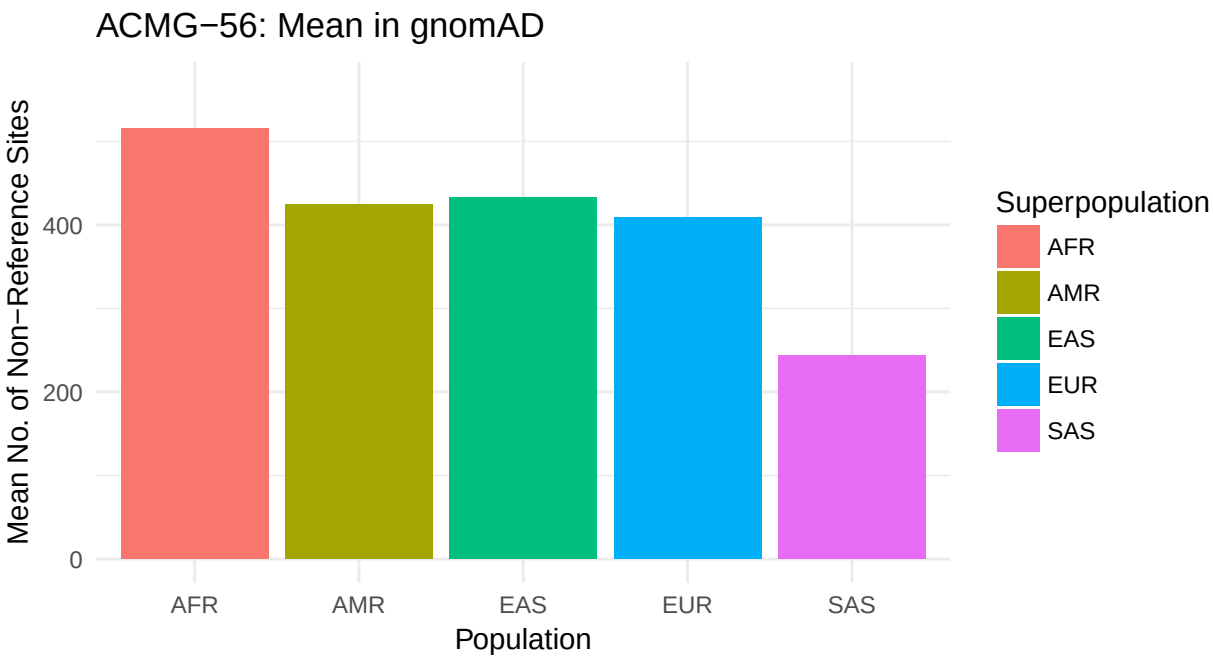
	AFR	AMR	EAS	EUR	SAS
Variant 1	0.1	0.2	0	0	0.3
Variant 2	0.2	0	0.3	0	0.1

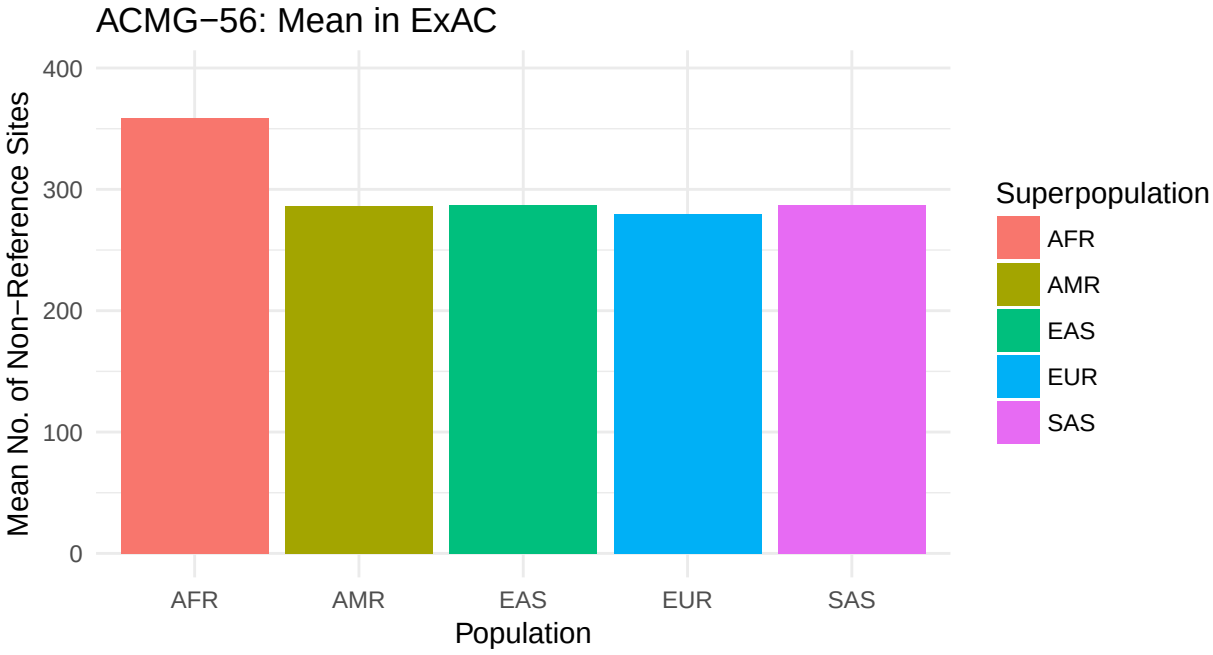
The probability of having at least 1 non-reference site at each variant - (0|1) (1|0) or (1|1) is given by $1 - (1 - AF)^2$. Note that this is approximately $2 * AF$ when AF is small:

	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

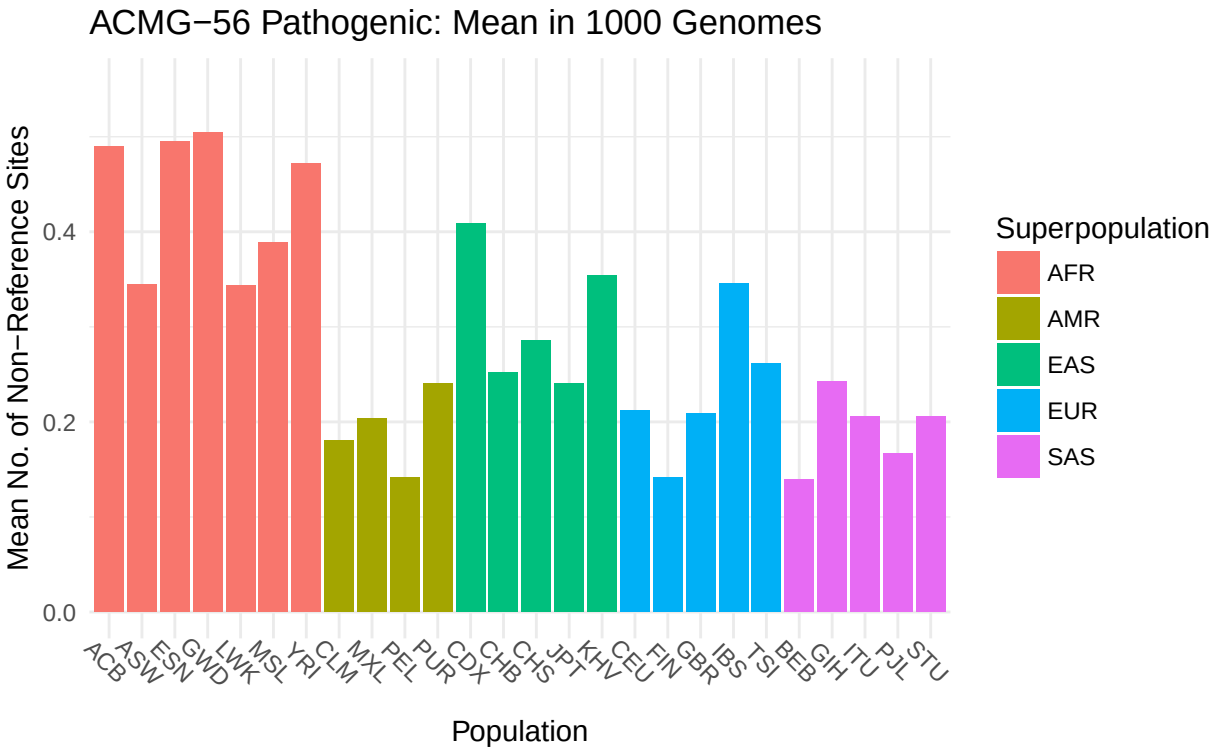

ACMG−56: Mean in gnomAD
Mean No. of Non−Reference Sites
0
200
400
AFR
AMR
EAS
EUR
SAS
Population
Superpopulation
AFR
AMR
EAS
EUR
SAS

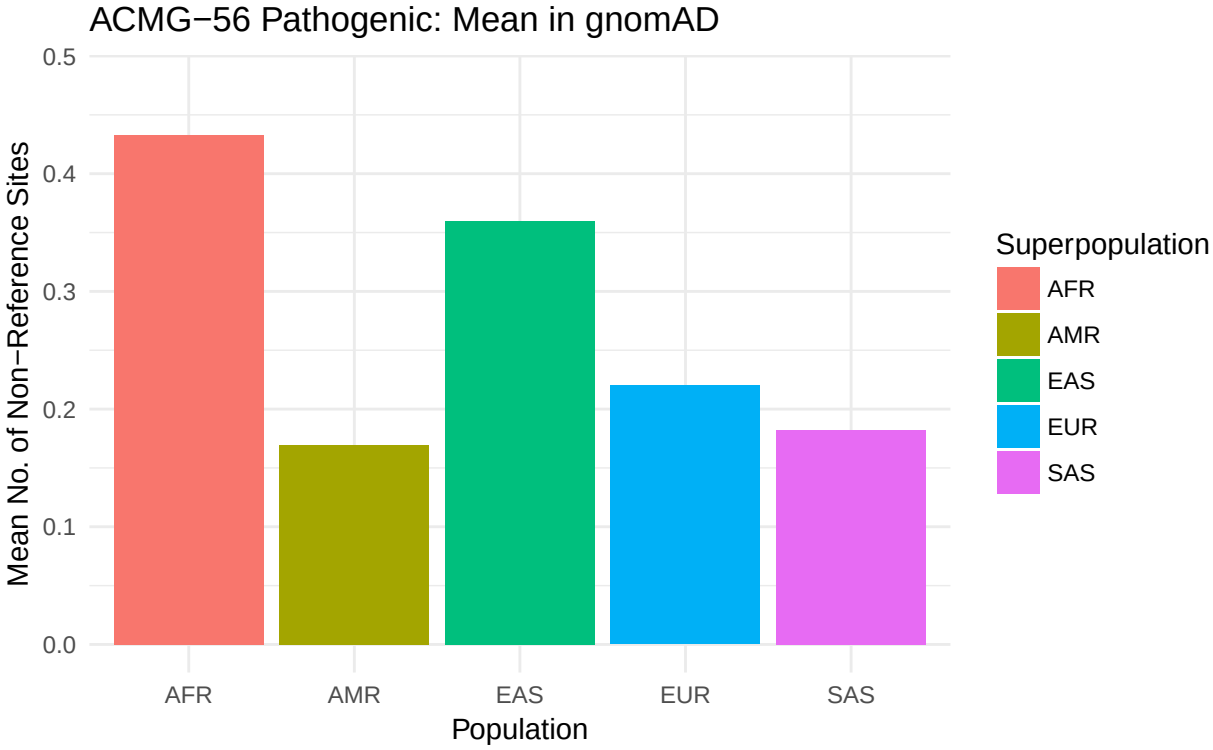

ACMG−56: Mean in ExAC
Mean No. of Non−Reference Sites
0
100
200
300
400
AFR
AMR
EAS
EUR
SAS
Population
Superpopulation
AFR
AMR
EAS
EUR
SAS

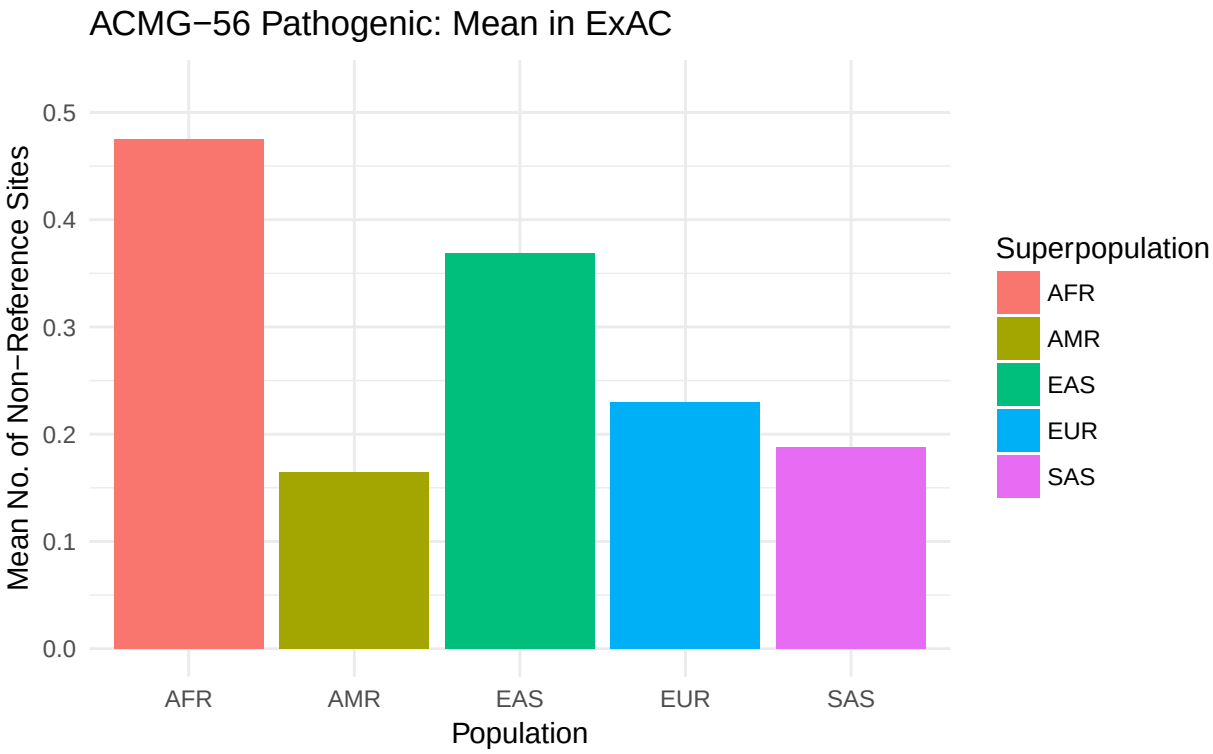
2.3 Pathogenic Non-Reference Sites

2.3.0.1 For 1000 Genomes and gnomAD/ExAC

This is the same procedure as above, but performed only on the subset of variants that are pathogenic.






ACMG−56 Pathogenic: Mean in ExAC
Mean No. of Non−Reference Sites
0.5
0.4
0.3
0.2
0.1
0.0
AFR
AMR
EAS
EUR
SAS
Population
Superpopulation
AFR
AMR
EAS
EUR
SAS

2.4 Fraction of Individuals with Pathogenic Sites

2.4.0.1 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-56 genes.

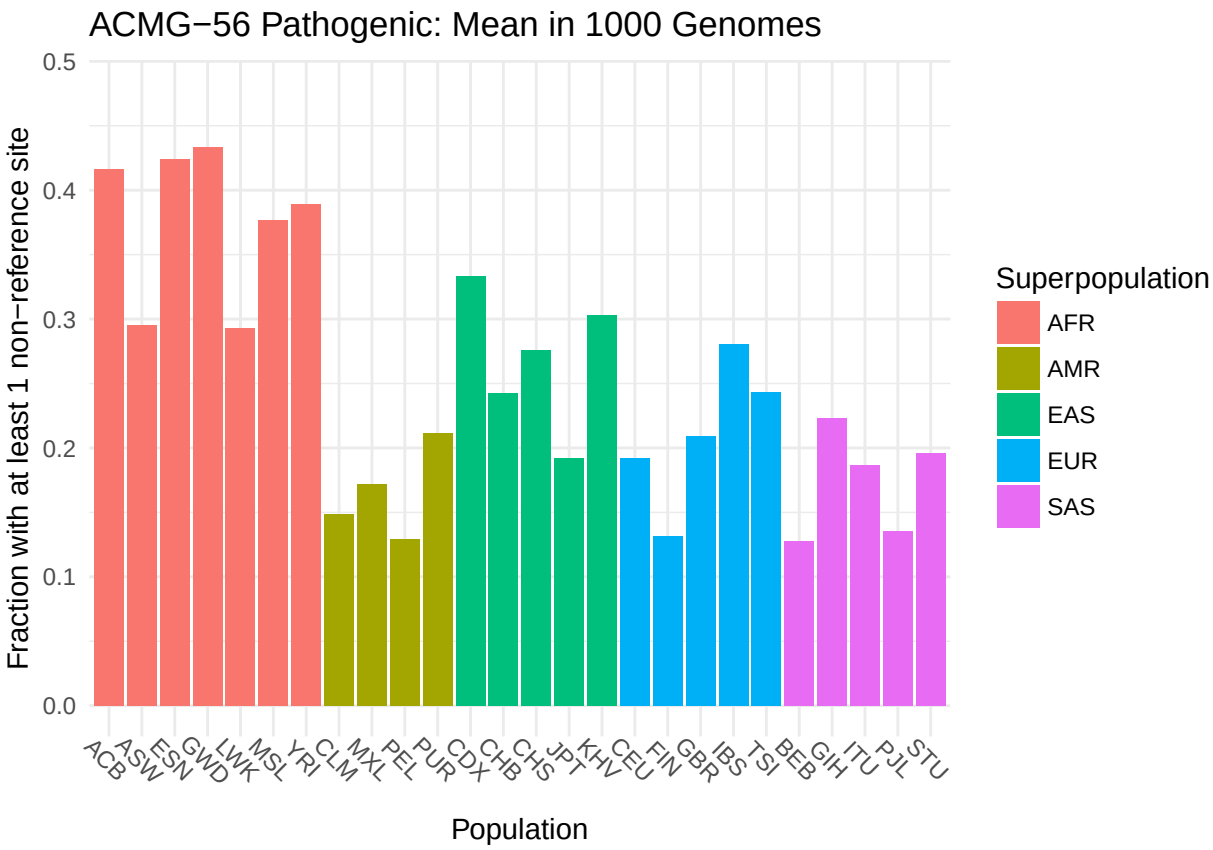
Ex: the genotype of 3 variants in 3 people looks like this:

	HG00097	HG00099	HG00100
Variant 1	0	0	0
Variant 2	0	0	0
Variant 3	0	0	0

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

HG00097	HG00099	HG00100
0	0	0

```
## Mean = 0
```



2.4.0.2 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:

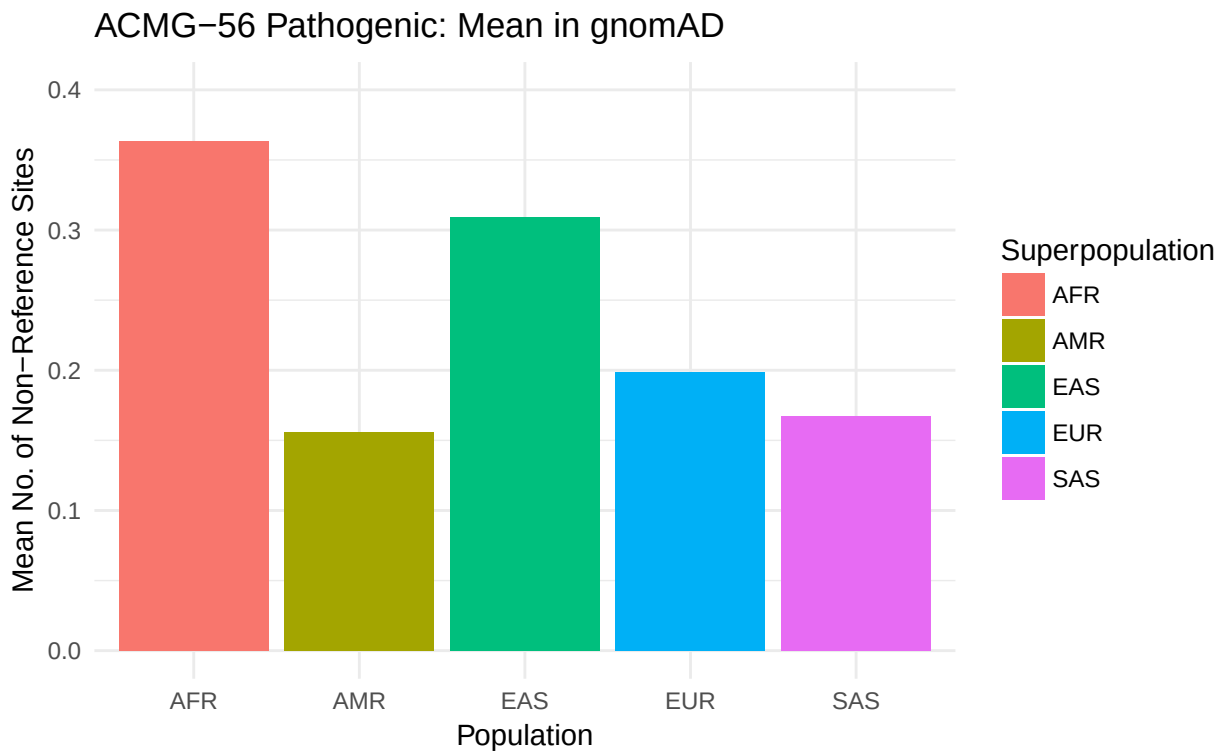
	AFR	AMR	EAS	EUR	SAS
Variant 1	0.1	0.2	0	0	0.3
Variant 2	0.2	0	0.3	0	0.1

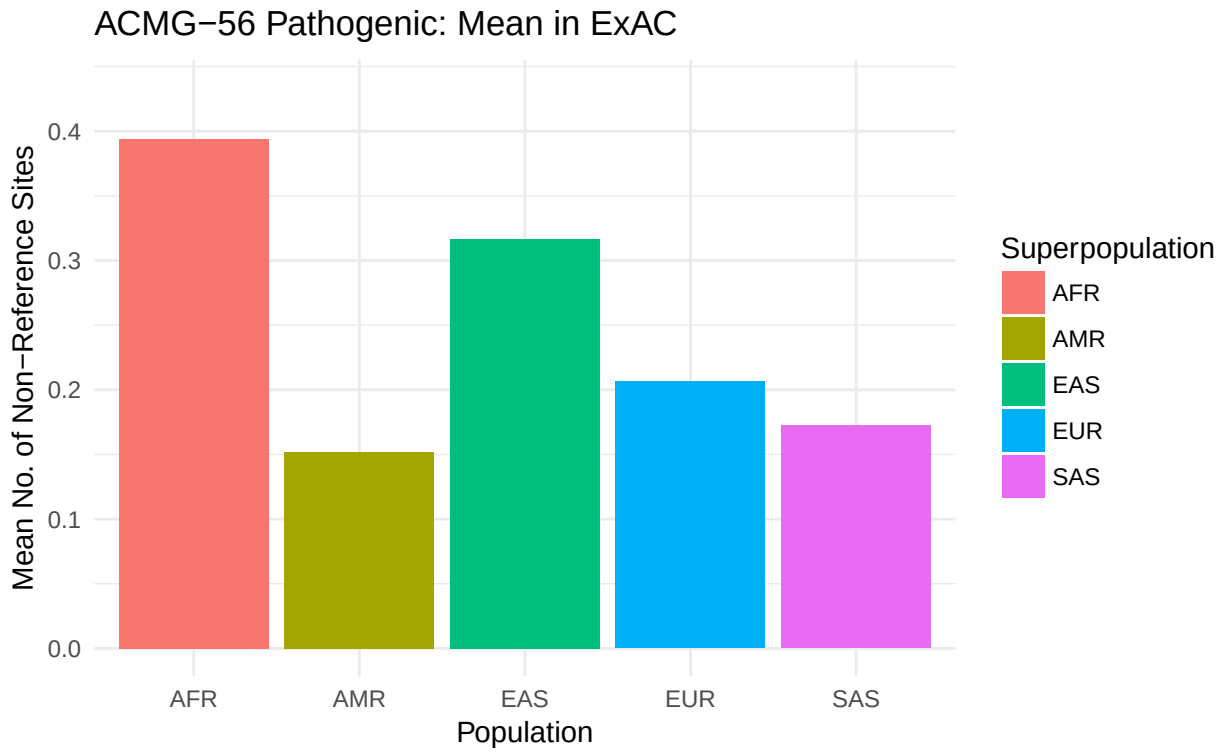
The probability of having at least 1 non-reference site at each variant - (0|1) (1|0) or (1|1) is given by $1 - (1 - AF)^2$. Note that this is approximately $2 * AF$ when AF is small:

	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

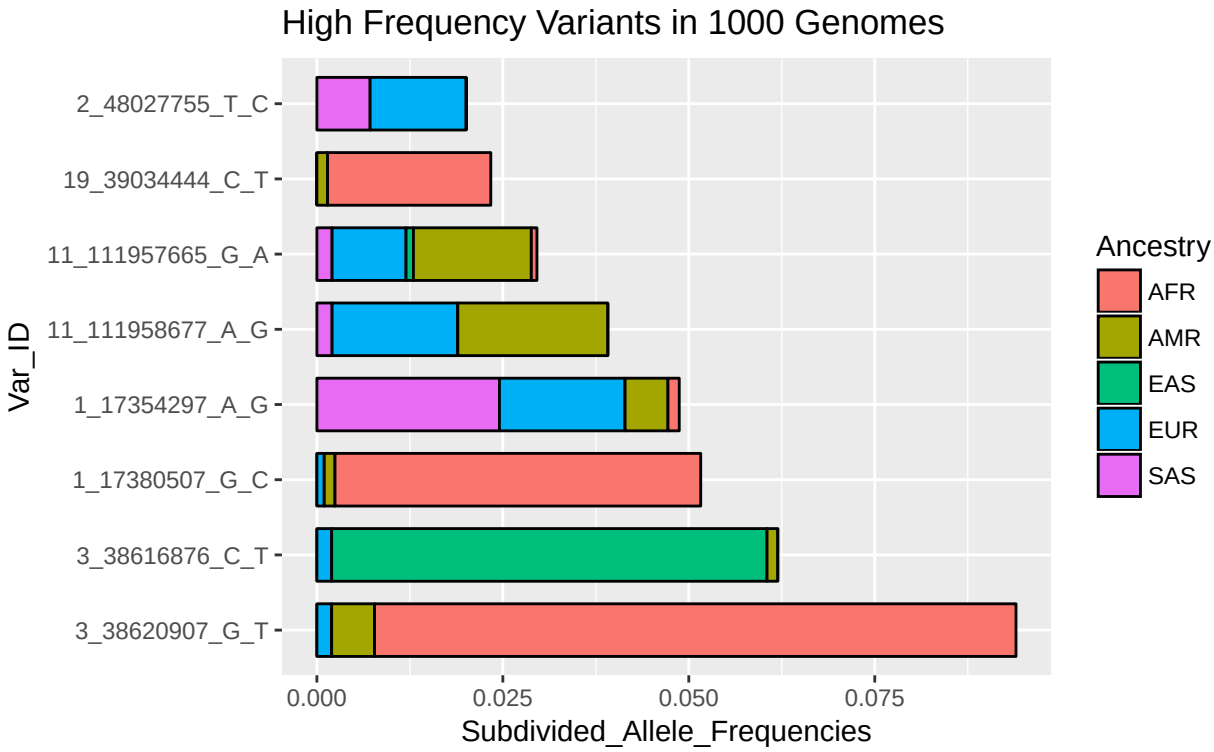
The expected (mean) number of non-reference sites is given by $1 - \prod(1 - AF)^2$.

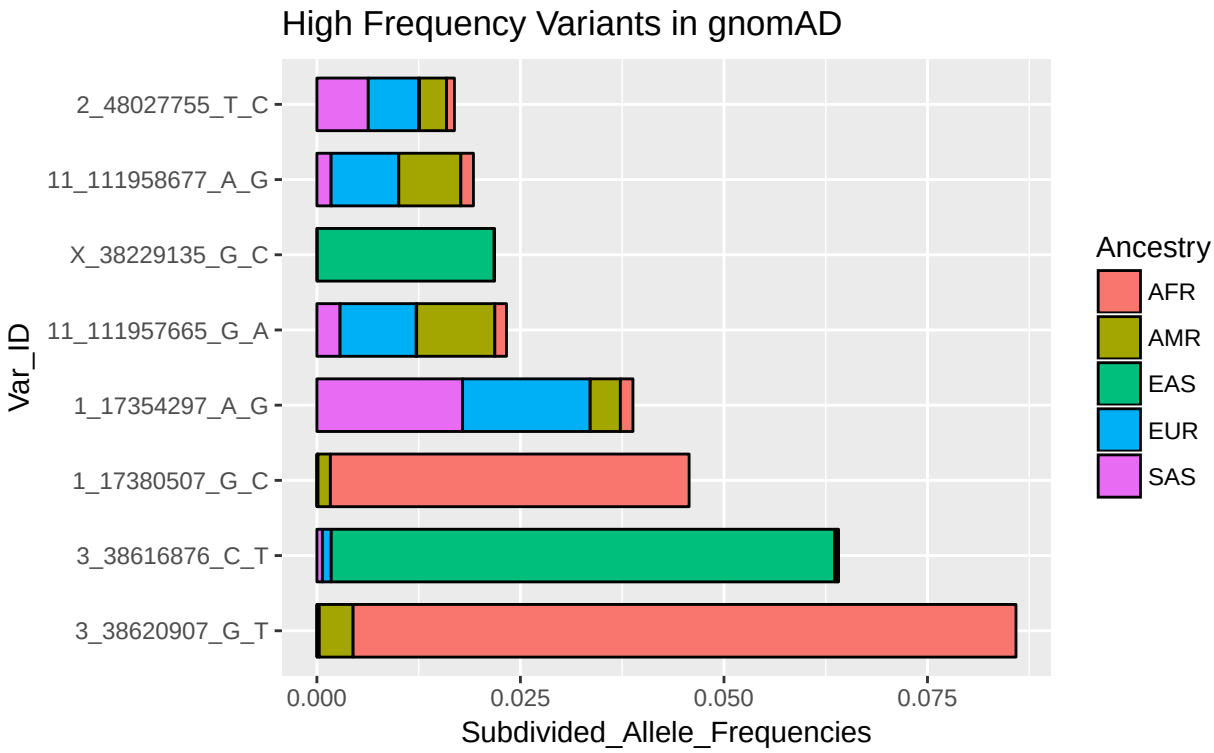
AFR	AMR	EAS	EUR	SAS
0.4816	0.36	0.51	0	0.6031


ACMG−56 Pathogenic: Mean in gnomAD
Mean No. of Non−Reference Sites
0.4
0.3
0.2
0.1
0.0
AFR
AMR
EAS
EUR
SAS
Population
Superpopulation
AFR
AMR
EAS
EUR
SAS


ACMG−56 Pathogenic: Mean in ExAC
Mean No. of Non−Reference Sites
0.4
0.3
0.2
0.1
0.0
AFR
AMR
EAS
EUR
SAS
Population
Superpopulation
AFR
AMR
EAS
EUR
SAS

2.5 Common Pathogenic Variants by Ancestry





3 Penetrance Estimates

3.1 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. Allele Frequency = $P(V_x)$
3. Allelic Heterogeneity = $P(V_x|D_x)$
4. Penetrance = $P(D_x|V_x)$

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

$$P(D_x|V_x) = \frac{P(D_x) * P(V_x|D_x)}{P(V_x)} = \frac{Prevalence * Allelic.Heteogeneity}{Allele.Frequency}$$

To compute penetrance estimates for each of the diseases related to the ACMG-56 genes, we will use the prevalence data we collected into `Literature_Prevalence_Estimates.csv`, allele frequency data from 1000 Genomes and ExAC, and a broad range of values for allelic heterogeneity.

3.2 Import Literature-Based Disease Prevalence Data

Data Collection: 1. Similar disease subtypes were grouped together (e.g., the 8 different types of familial hypertrophic cardiomyopathy), resulting in 30 disease categories across 56 genes.
2. The search query "[disease name] prevalence" was used to find articles using Google Scholar.
3. Prevalence estimates were recorded along with URL, journal, region, publication year, sample size, first author, population subset (if applicable), date accessed, and potential issues. Preference was given to studies with PubMed IDs, more citations, and larger sample sizes.

Prevalence was recorded as reported: either a point estimate or a range. Values of varying quality were collected across all diseases.

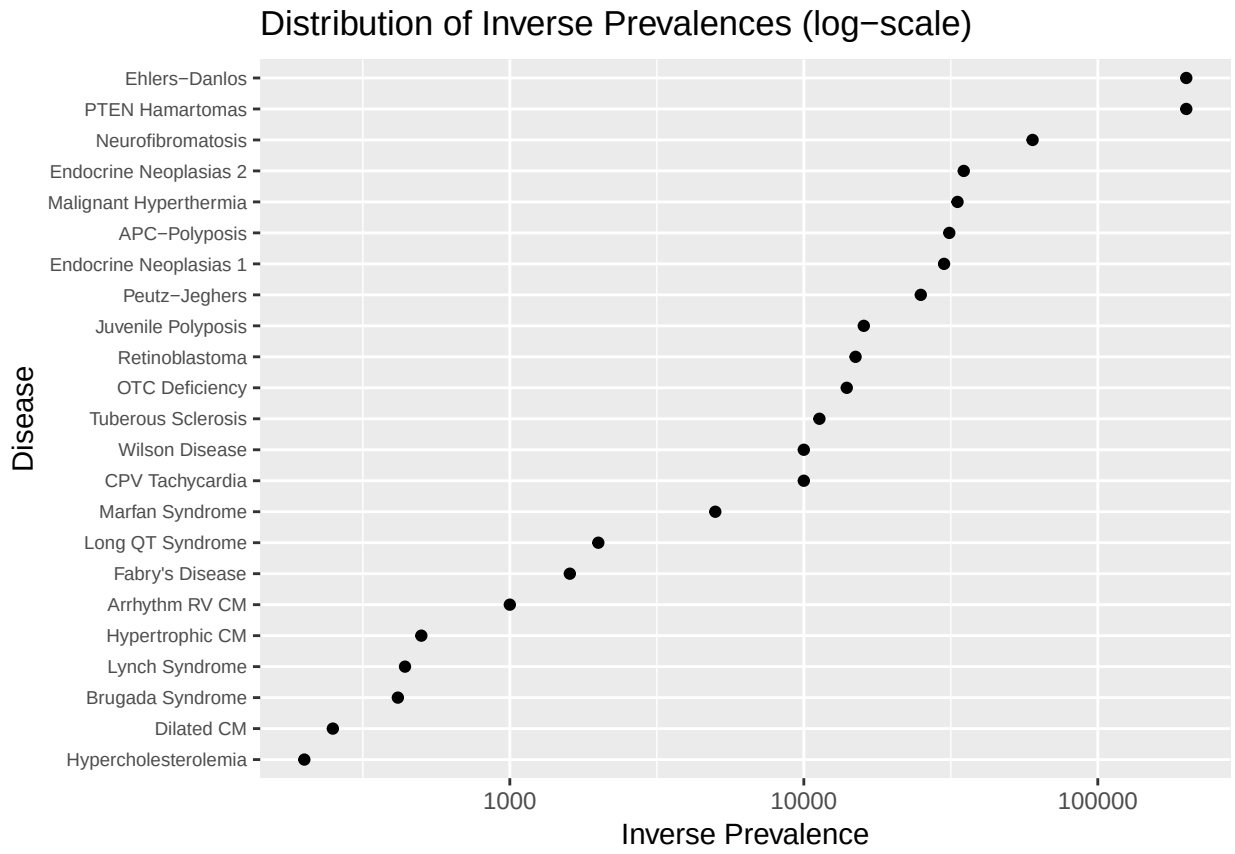
```
## Table of Literature-Based Estimates 23 x 15 (selected rows/columns):
```

Gene	Phenotype
BMPR1A|SMAD4	Juvenile polyposis
MEN1	Multiple endocrine neoplasia type 1
RB1	Retinoblastoma
MYBPC3|MYH7|TNNI3|TPM1|MYL3|ACTC1|PRKAG2|GLA|MYLH	Hypertrophic cardiomyopathy

Table continues below

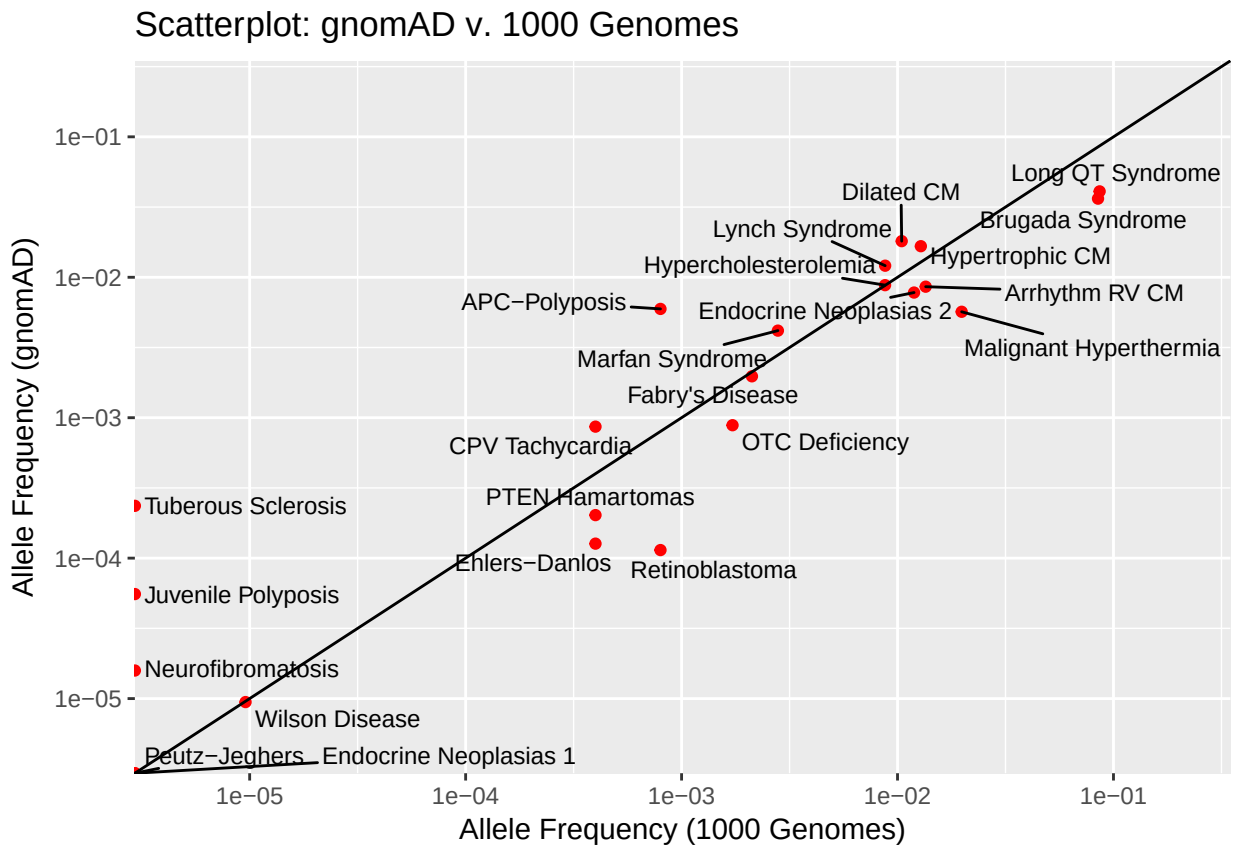
Abbreviation	Inverse_Prevalence	Allelic_Heterogeneity
JPS	16000	0.4
MEN1	30000	0.9
RB	15000	0.97
HCM	500	0.6

3.3 Distribution of Prevalences



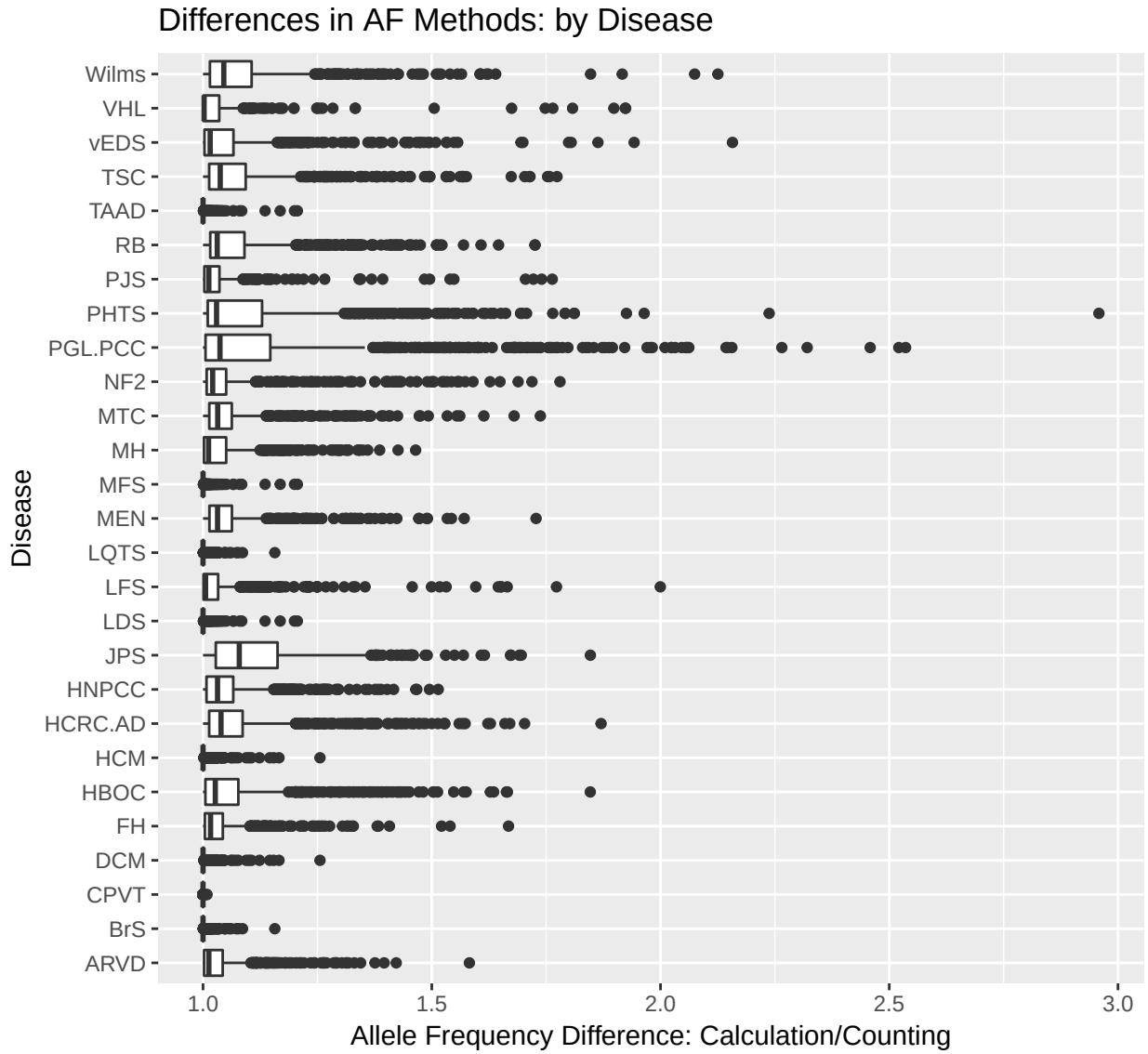
3.4 Collect and Aggregate Allele Frequencies at the Disease-Level

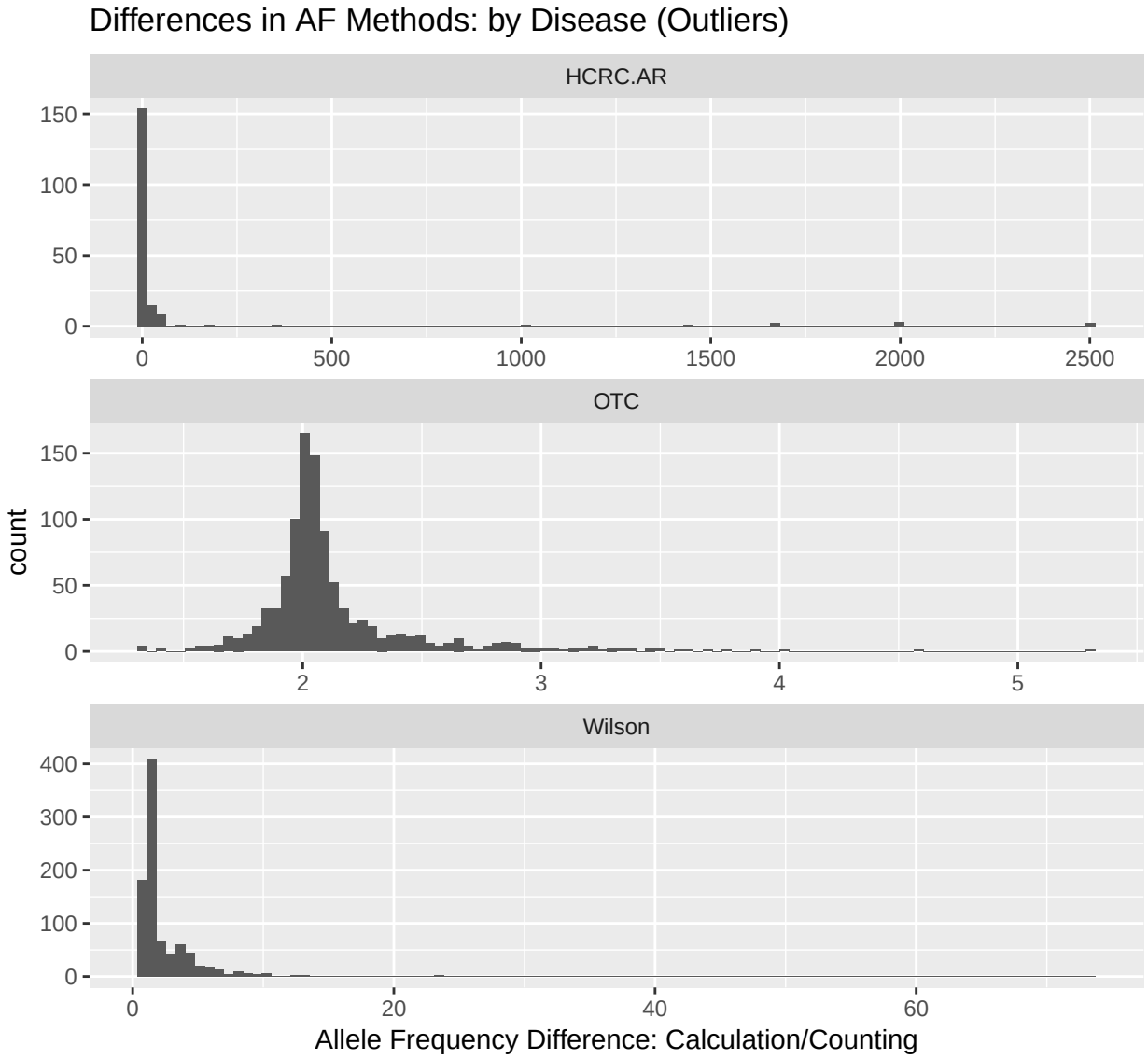
We define AF(disease) as the probability of having at least 1 variant associated with the disease.
The frequencies across the relevant variants can be aggregated in two ways:
(1) By direct counting, from genotype data in 1000 Genomes.
(2) AF(disease) $= 1 - \prod_{variant}(1 - AF_{variant})$, from population data in ExAC (assumes independence).

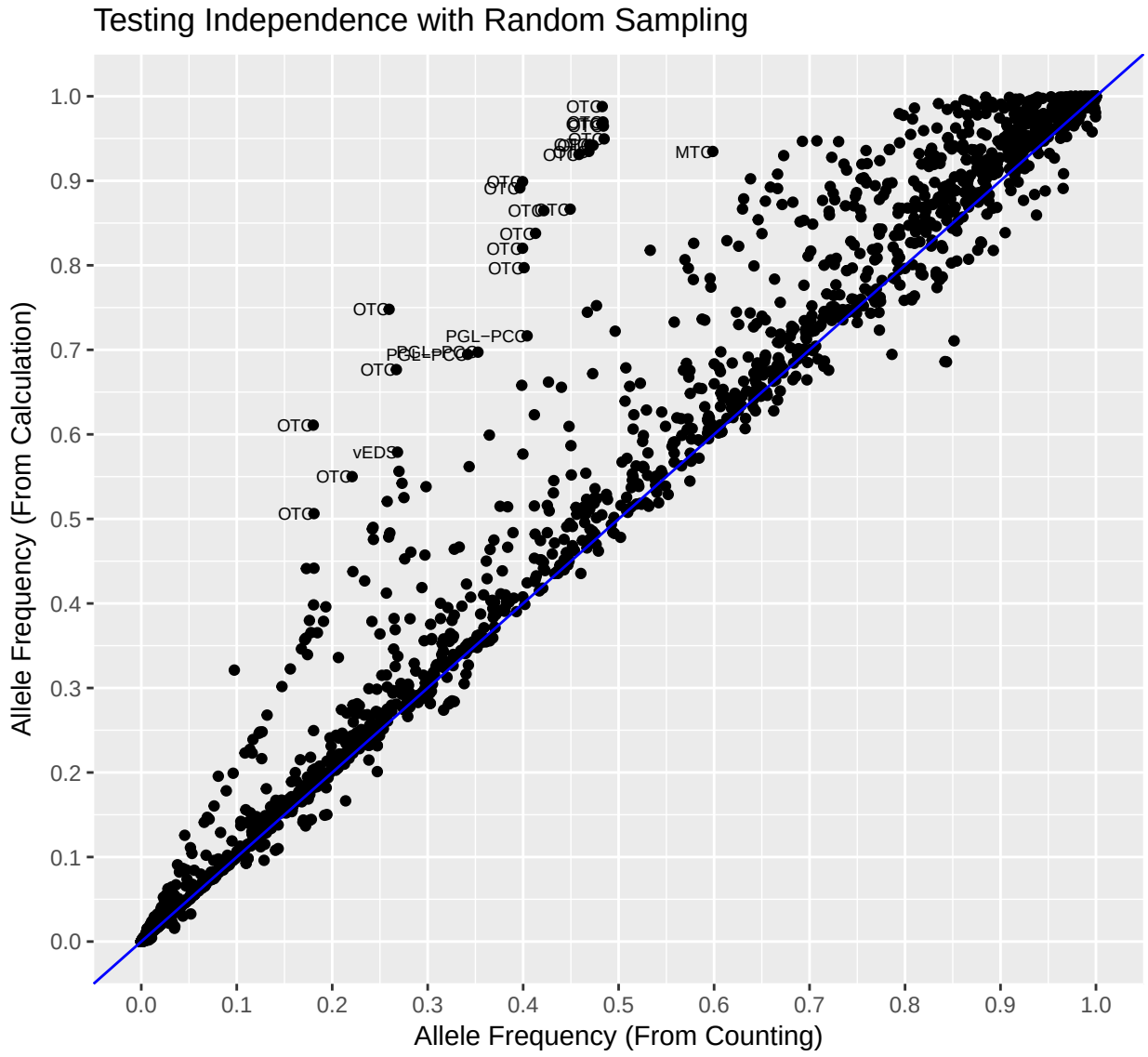


Ratio_1000G (red, top) computes AF(calculation in 1000 Genomes) / AF(counting in 1000 Genomes).
Ratio_gnomAD (blue, bottom) computes AF(calculation in gnomAD) / AF(calculation in 1000 Genomes).

Sampling 1000 variants from all variants in 1000 Genomes to test deviations from independence assumptions. Repeat for 1000 trials and plot the distribution of disease-level allele frequencies (1000 points per disease).




Differences in AF Methods: by Disease (Outliers)
HCRC.AR
OTC
Wilson
count
Allele Frequency Difference: Calculation/Counting

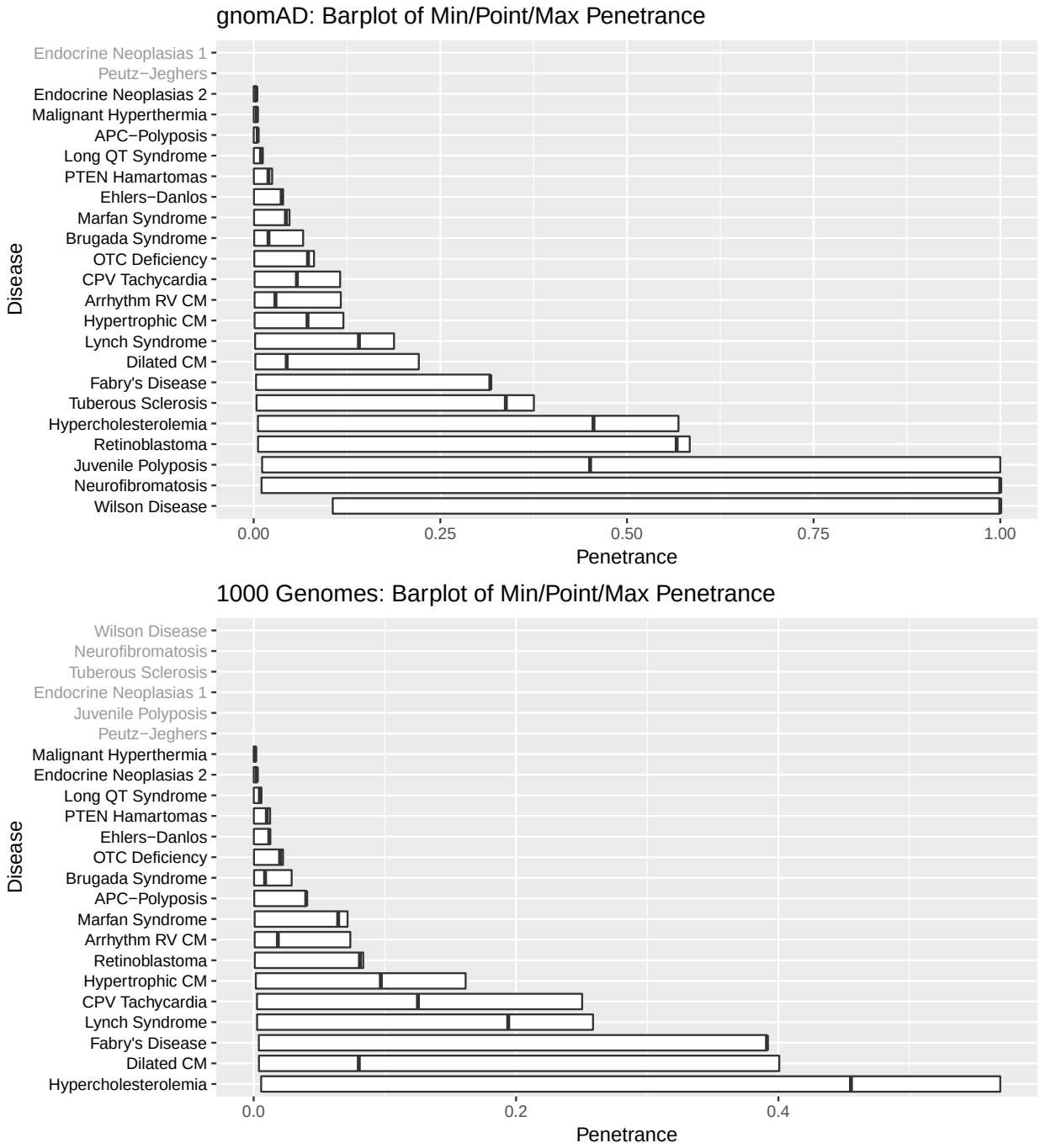



```
## 30 diseases x 1000 points = 30,000 points.This plot has been downsampled 10x and contains 3,000 poin
```

Pearson correlation: 0.99
Mean ratio (Calculation/Counting): 1.07

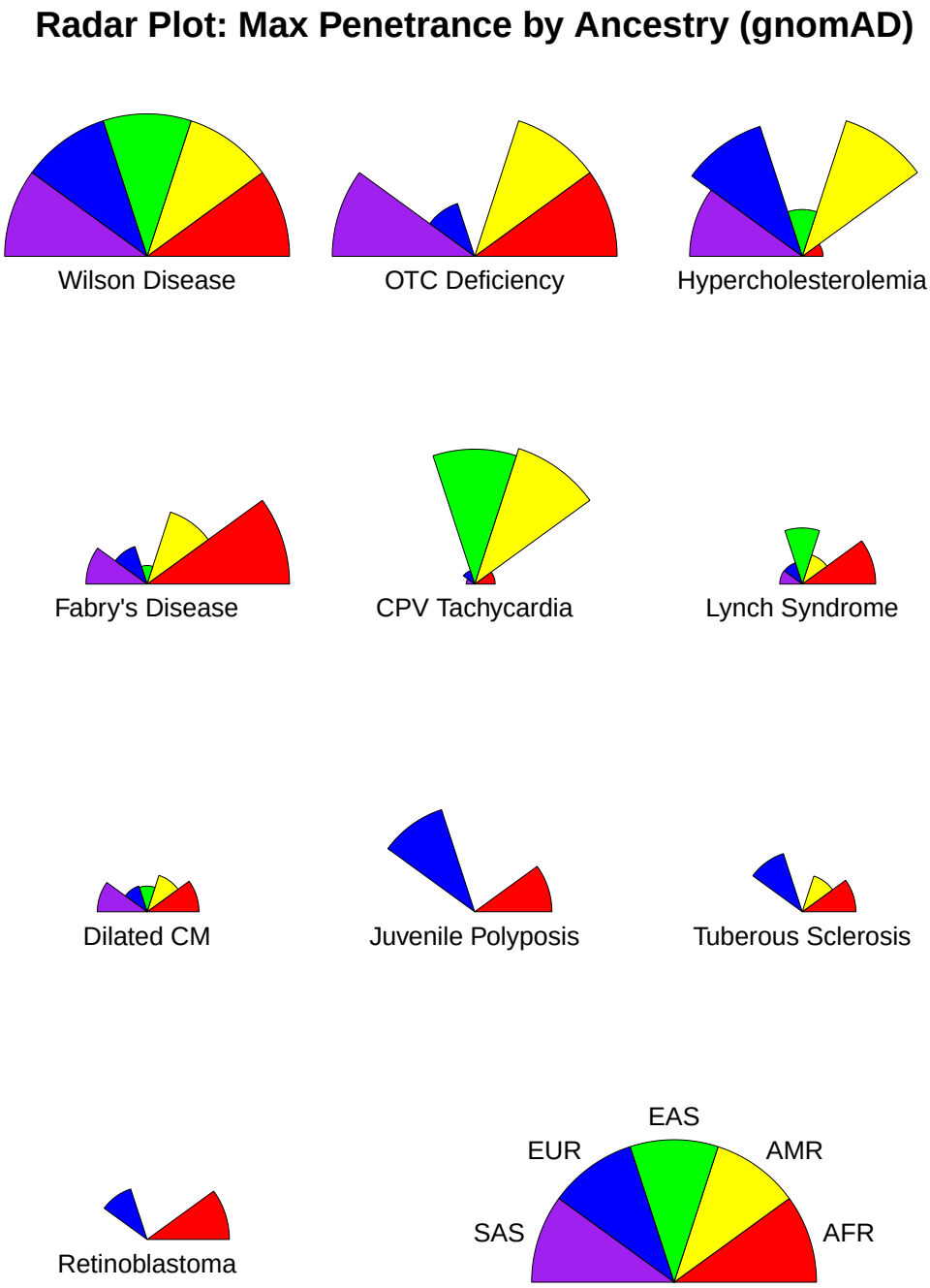
3.5 Penetrance as a Function of P(V|D)

The left end of the boxplot indicates P(V|D) = 0.01,
the bold line in the middle indicates P(V|D) = point value,
the right end of the boxplot indicates P(V|D) = 1.

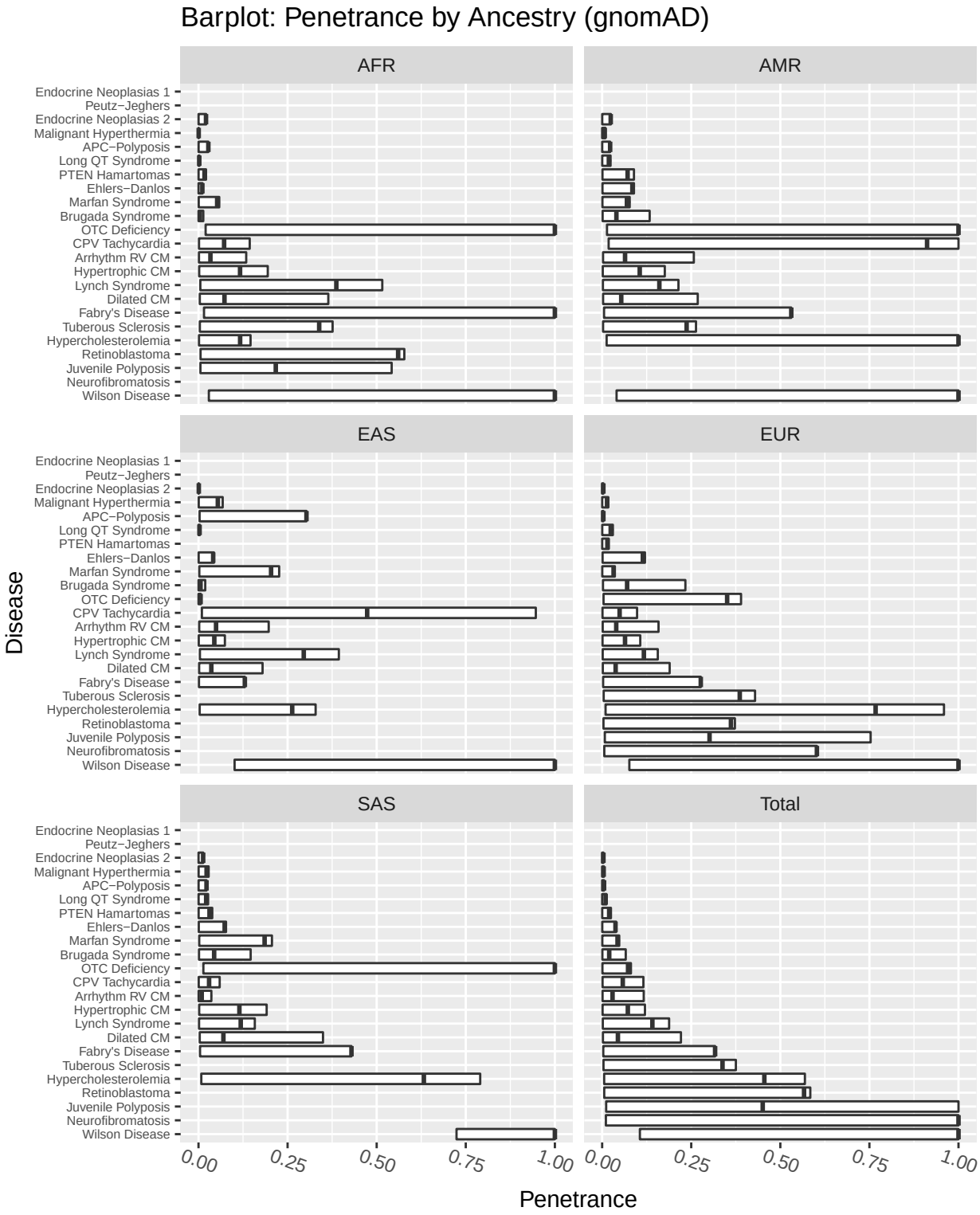


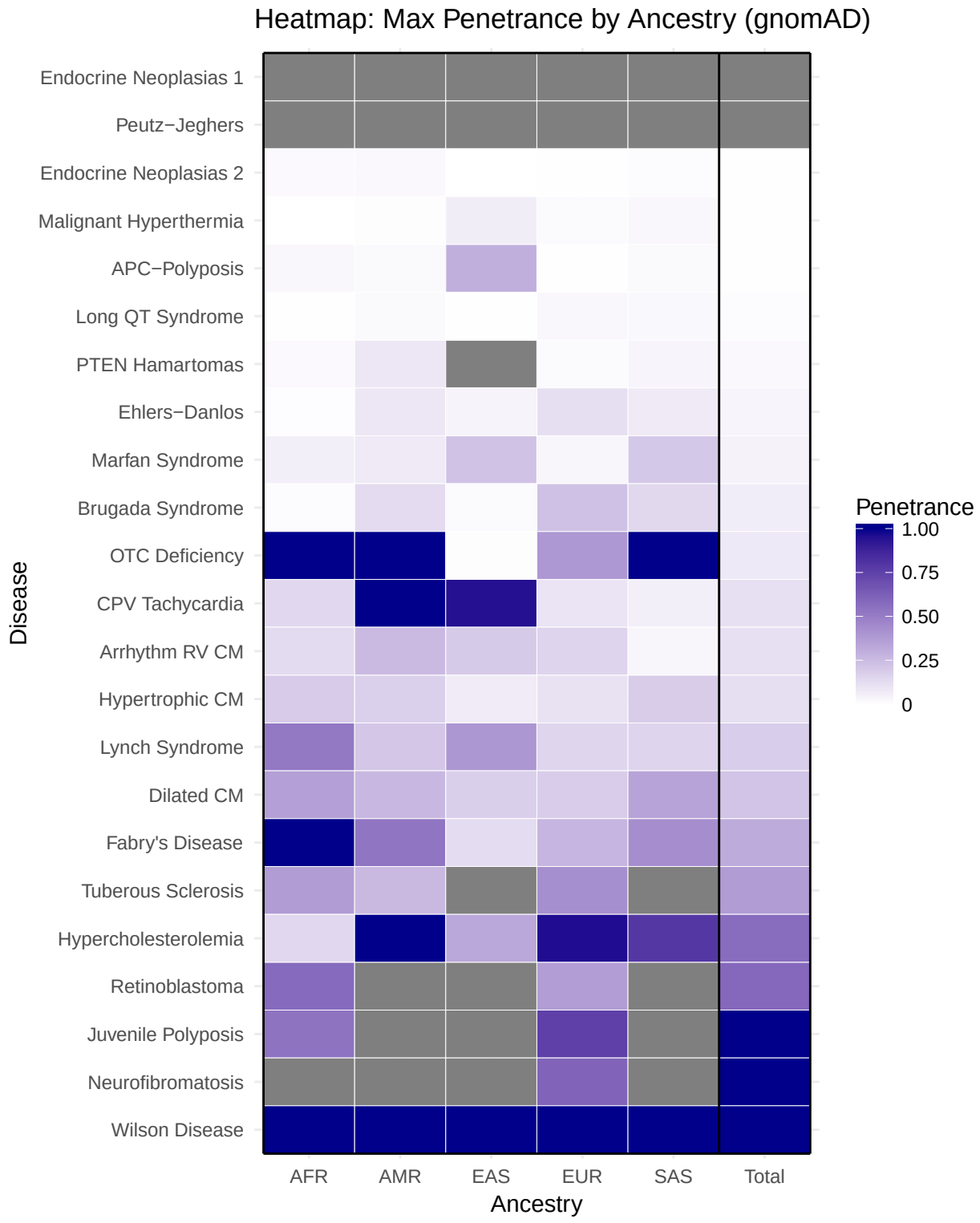
Note 1: the grayed-out empty lines at the top all indicate no allele frequency (disease_AF) data.
Note 2: Some diseases have mean theoretical penetrance = 1 because the assumed allelic heterogeneity is greater than is possible, given the observed prevalence and allele frequencies.

3.6 Penetrance Estimates by Ancestry



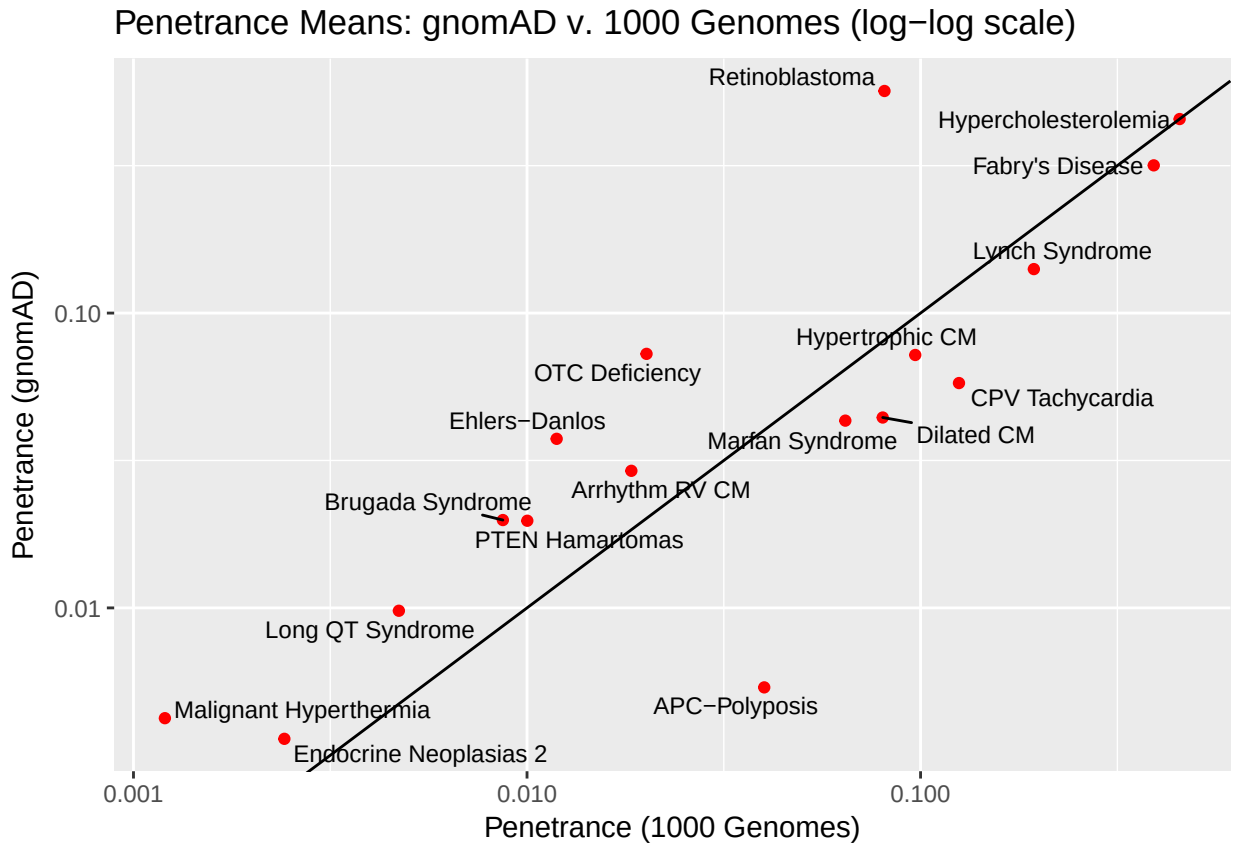

```
## [1] These are the top 10 diseases by summed allele frequencies. NULL values are not plotted.
## [1] Each radius is proportional to the penetrance of the disease in the given population.
```


Barplot: Penetrance by Ancestry (gnomAD)
AFR
AMR
EAS
EUR
SAS
Total
Disease
Endocrine Neoplasias 1
Peutz−Jeghers
Endocrine Neoplasias 2
Malignant Hyperthermia
APC−Polyposis
Long QT Syndrome
PTEN Hamartomas
Ehlers−Danlos
Marfan Syndrome
Brugada Syndrome
OTC Deficiency
CPV Tachycardia
Arrhythm RV CM
Hypertrophic CM
Lynch Syndrome
Dilated CM
Fabry's Disease
Tuberous Sclerosis
Hypercholesterolemia
Retinoblastoma
Juvenile Polyposis
Neurofibromatosis
Wilson Disease
0.00
0.25
0.50
0.75
1.00
Penetrance



Dark gray boxes are NA: no associated variants discovered in that ancestral population.

3.7 Comparing Mean Penetrance between gnomAD and 1000 Genomes



The Pearson correlation is 0.68.
Max penetrance values computed using 1000 Genomes are 0.544-fold larger than those computed using gnomAD.

Note: Prevalence ranges of 5x were assumed for all point estimates of prevalence.
For example: a point estimate of 0.3 would be given the range [0.1, 0.5].

–>