ACMG-ClinVar Penetrance RMarkdown

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Contents

Working Directory: /Users/jamesdiao/Documents/Kohane_Lab/2016-paper-ACMG-penetrance

1 Download, Transform, and Load Data

1.1 Collect ACMG Gene Panel

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

```
## Processed Table from ACMG Website 64 x 4 (selected rows):
```

	Disease_Name	Disease_MIM	Gene_Name	Gene_MIM
A1	Adenomatous polyposis coli	175100	APC	611731
A2	Aortic aneurysm, familial thoracic 4	132900	MYH11	160745
A5	Arrhythmogenic right ventricular cardiomyopathy, type 5	604400	TMEM43	612048
A10	Breast-ovarian cancer, familial 1	604370	BRCA1	113705
A11	Breast-ovarian cancer, familial 2	612555	BRCA2	600185
A12	Brugada syndrome 1	601144	SCN5A	600163
A13	Catecholaminergic polymorphic ventricular tachycardia	604772	RYR2	180902
A14	Dilated cardiomyopathy 1A	115200	LMNA	150330
A16	Ehlers-Danlos syndrome, type 4	130050	COL3A1	120180
A17	Fabry's disease	301500	GLA	300644
A18	Familial hypercholesterolemia	143890	APOB	107730
A20	Familial hypertrophic cardiomyopathy 1	192600	MYH7	160760
A28	Familial medullary thyroid carcinoma	155240	RET	164761
A30	Left ventricular noncompaction 6	601494	TNNT2	191045
A31	Li-Fraumeni syndrome 1	151623	TP53	191170
A32	Loeys-Dietz syndrome type 1A	609192	TGFBR1	190181
A37	Long QT syndrome 1	192500	KCNQ1	607542
A40	Lynch syndrome	120435	MLH1	120436
A44	Malignant hyperthermia	145600	RYR1	180901
A46	Marfan's syndrome	154700	FBN1	134797
A48	Multiple endocrine neoplasia, type 1	131100	MEN1	613733
A51	MYH-associated polyposis	608456	MUTYH	604933
A52	Neurofibromatosis, type 2	101000	NF2	607379
A53	Paragangliomas 1	168000	SDHD	602690
A57	Peutz-Jeghers syndrome	175200	STK11	602216
A58	Pilomatrixoma	132600	MUTYH	604933
A59	PTEN hamartoma tumor syndrome	153480	PTEN	601728
A60	Retinoblastoma	180200	RB1	614041
A61	Tuberous sclerosis 1	191100	TSC1	605284
A63	Von Hippel-Lindau syndrome	193300	VHL	608537
A64	Wilms' tumor	194070	WT1	607102

```
## ACMG-56 Genes:
```

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

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 126349 x 14 (selected rows/columns):
```

VAR_ID	CHROM	POS	ID	REF	ALT	CLNSIG	CLNDBN	CLNDSDBID	INTERP
1_955597_G_T	1	955597	rs115173026	G	T	2	not_specified	CN169374	FALSE
1_955619_G_C	1	955619	rs201073369	G	C	255	not_specified	CN169374	FALSE
1_957568_A_G	1	957568	rs115704555	A	G	2	not_specified	CN169374	FALSE

Table continues below

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: 56 x 6 (selected rows):
```

gene	name	chrom	start	end	downloaded
APC	NM_001127511	5	1.12e+08	112181936	TRUE
MYH11	NM_001040113	16	15796991	15950887	TRUE
ACTA2	NM_001141945	10	90694830	90751154	TRUE
MYLK	NM_001321309	3	123331142	123603149	TRUE
TMEM43	NM_024334	3	14166439	14185180	TRUE

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

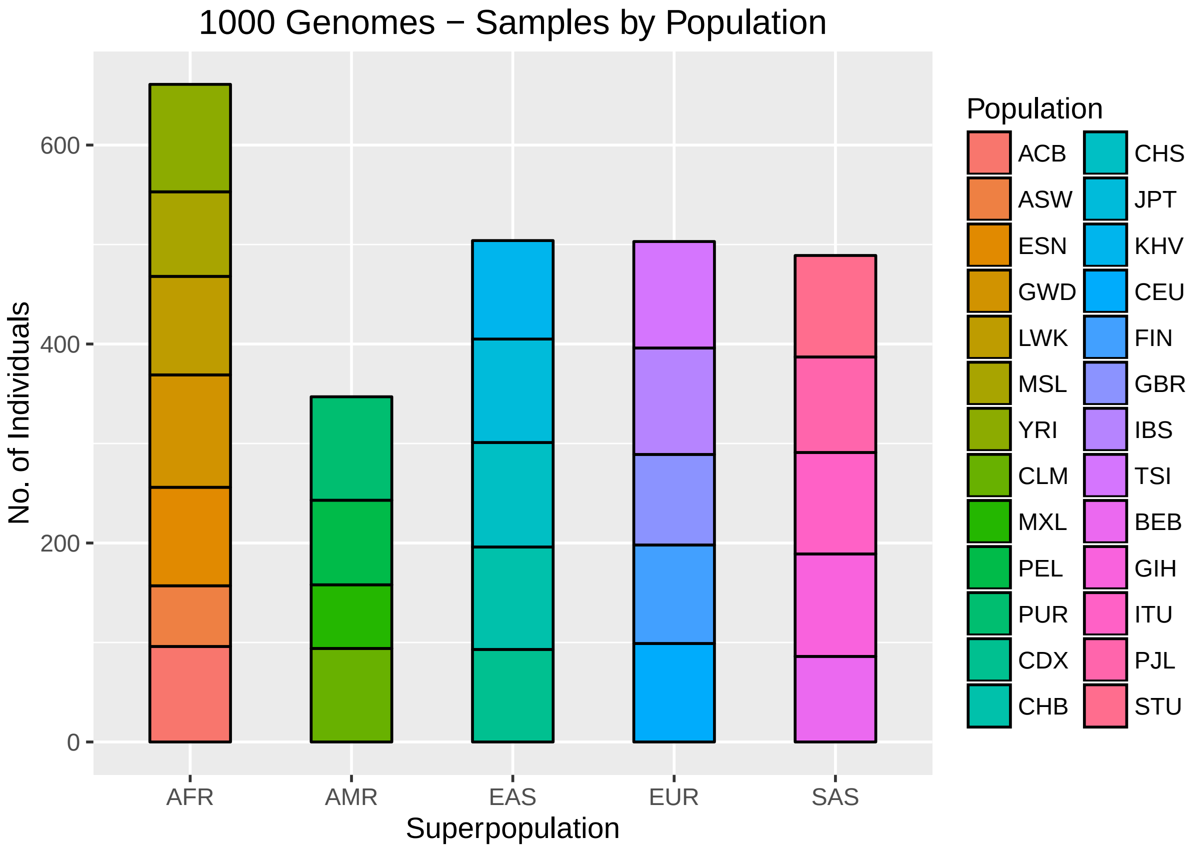
1.4 Collect 1000 Genomes Phase 3 Populations Map

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
NA18908	YRI	AFR	male
NA20296	ASW	AFR	female
HG02144	ACB	AFR	female
NA19789	MXL	AMR	male
HG00419	CHS	EAS	female
HG02373	CDX	EAS	male
HG01607	IBS	EUR	female
HG01776	IBS	EUR	female
NA20587	TSI	EUR	female
HG03727	ITU	SAS	male

```
## Population Distribution
```



1.5 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: 139335 x 2516 (selected rows/columns):
```

GENE	AF_1000G	VAR_ID	CHROM	POS	ID	REF	ALT	HG00096	HG00097	HG00099	HG00100	HG00101	HG00102
APC	0.0001997	5_112043211_A_G	5	1.12e+08	rs554351451	A	G	0	0	0	0	0	0
APC	0.0001997	5_112043231_G_A	5	1.12e+08	rs575784409	G	A	0	0	0	0	0	0
APC	0.005391	5_112043234_C_T	5	1.12e+08	rs115658307	C	T	0	0	0	0	0	0
APC	0.0001997	5_112043252_G_A	5	1.12e+08	rs558562104	G	A	0	0	0	0	0	0
APC	0.008786	5_112043263_C_T	5	1.12e+08	rs138386816	C	T	0	0	0	0	0	0

Table continues below

1.6 Import and Process 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 ExAC VCFs: 58873 x 45 (selected rows/columns):
```

GENE	AF_EXAC	AF_EXAC_AFR	AF_EXAC_AMR	AF_EXAC_EAS	AF_EXAC_EUR	AF_EXAC_SAS	VAR_ID	CHROM	POS	ID	REF	ALT
APC	8.13e-05	0	0	0	0	0.0001313	5_112043365_G_C	5	1.12e+08	.	G	C
APC	8.131e-05	0	0	0	0	0.0001313	5_112043382_A_G	5	1.12e+08	.	A	G
APC	0.1112	0.07979	0.1022	0	0.1063	0.1185	5_112043384_T_G	5	1.12e+08	rs78429131	T	G
APC	8.131e-05	0	0	0	0	0.0001313	5_112043392_C_T	5	1.12e+08	.	C	T
APC	8.134e-05	0	0	0	0	0.0001313	5_112043412_C_G	5	1.12e+08	.	C	G

Table continues below

1.7 Merge ClinVar with 1000 Genomes and ExAC

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

Subset_ClinVar	Number_of_Variants
Total ClinVar	126349
LP/P-ClinVar	33033
LP/P-ClinVar & ACMG	6252
LP/P-ClinVar & ACMG & ExAC	826
LP/P-ClinVar & ACMG & 1000 Genomes	122

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

Subset_1000_Genomes	Number_of_Variants
Total 1000_Genomes & ACMG	139335
1000_Genomes & ACMG & ClinVar	4891
1000_Genomes & ACMG & LP/P-ClinVar	122

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

Subset_ExAC	Number_of_Variants
Total ExAC & ACMG	58873
ExAC & ACMG & ClinVar	10043
ExAC & ACMG & LP/P-ClinVar	826

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): 6714 x 13 (selected rows/columns)
```

VAR_ID	Gene(s)	Condition(s)	Frequency
X_100652891_C_G	GLA	Fabry disease	GMAF:0.00050(G)
11_47374186_C_G	MYBPC3	Primary familial hypertrophic cardiomyopathy	GMAF:0.00020(G)
11_47355233_C_G	MYBPC3	Familial hypertrophic cardiomyopathy 4	GMAF:0.00020(G)
11_47364162_C_G	MYBPC3	Familial hypertrophic cardiomyopathy 4	GMAF:0.00020(G)
14_23886482_G_C	MYH7	not specified	GMAF:0.00020(C)
14_23893148_C_G	MYH7	Primary dilated cardiomyopathy	GO-ESP:0.00046(G)
1_17355075_A_T	SDHB	Gastrointestinal stromal tumor	GMAF:0.00120(T)
1_17380507_G_C	SDHB	Cowden syndrome 2	GO-ESP:0.01323(C)

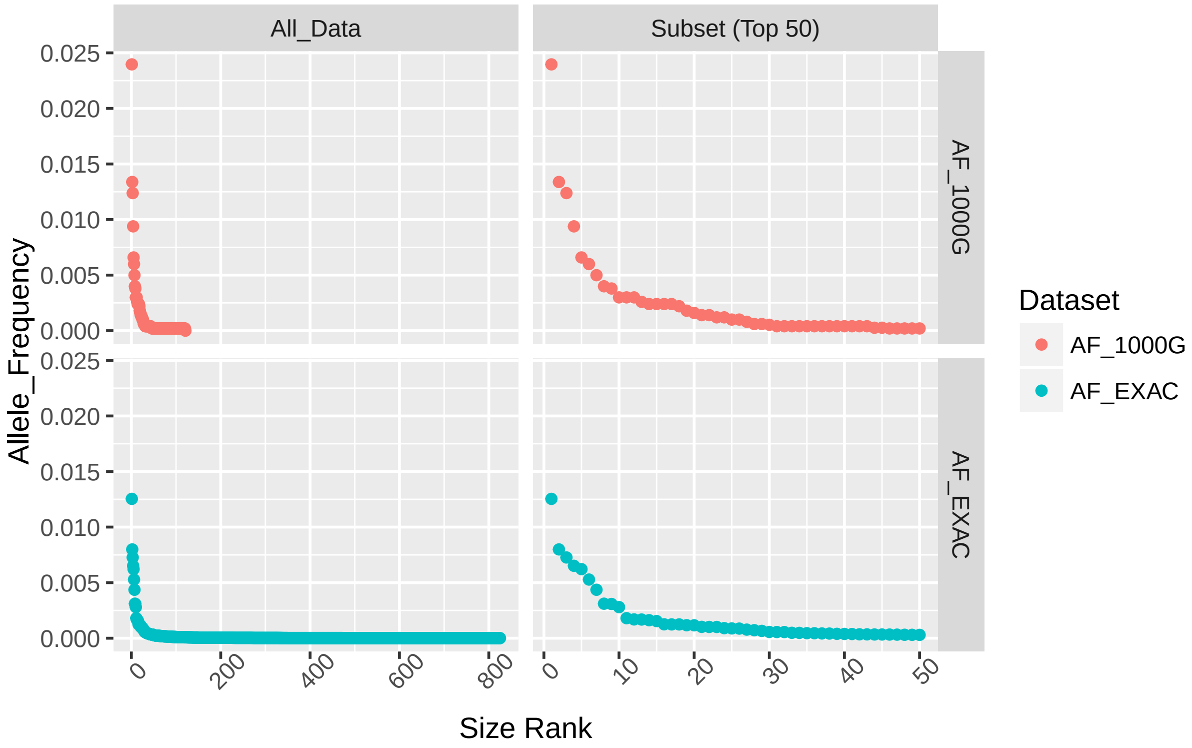
```
## Breakdown of ClinVar Query Results Table:
```

Subset	Number_of_Variants
Initial Count	12525
Filter Substitutions (N>N')	6732
Filter Coupling/Bad-Locations	6714
In ClinVar VCF	509
In LP/P-ClinVar VCF	503
ˆ & ACMG & ExAC	49
ˆ & ACMG & 1000 Genomes	9
ˆ & ACMG & ExAC & 1000 Genomes	8

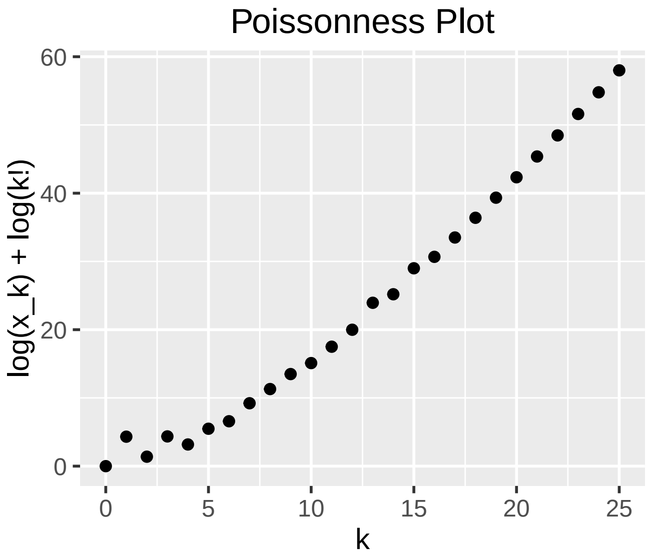
```
## Note the 12-fold reduction after merging the online query results with the VCF.
```

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.

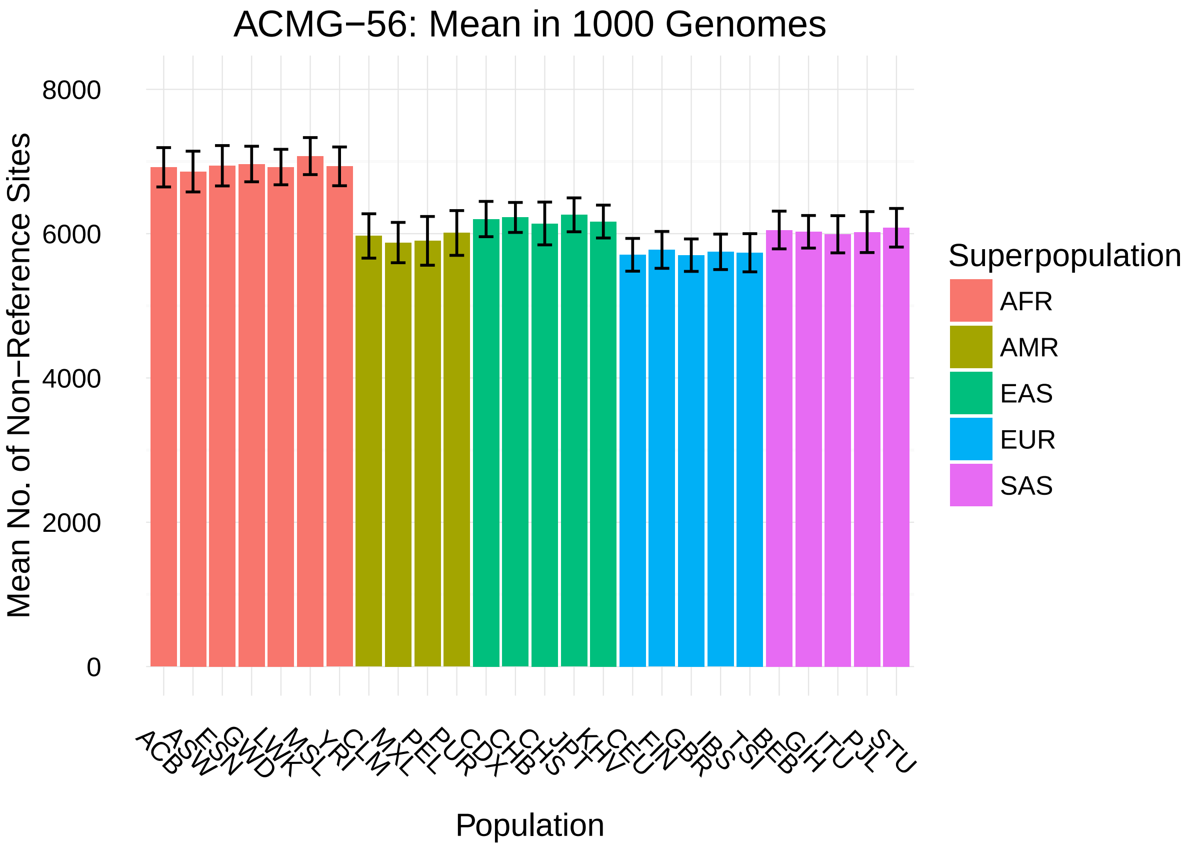
Ex: the genotype of 3 variants in 3 people looks like this:

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

Count the number of non-reference sites per individual:

HG00097	HG00099	HG00100
0	1	3

```
## Mean = 1.33
```



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

2.2.0.2 For 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:

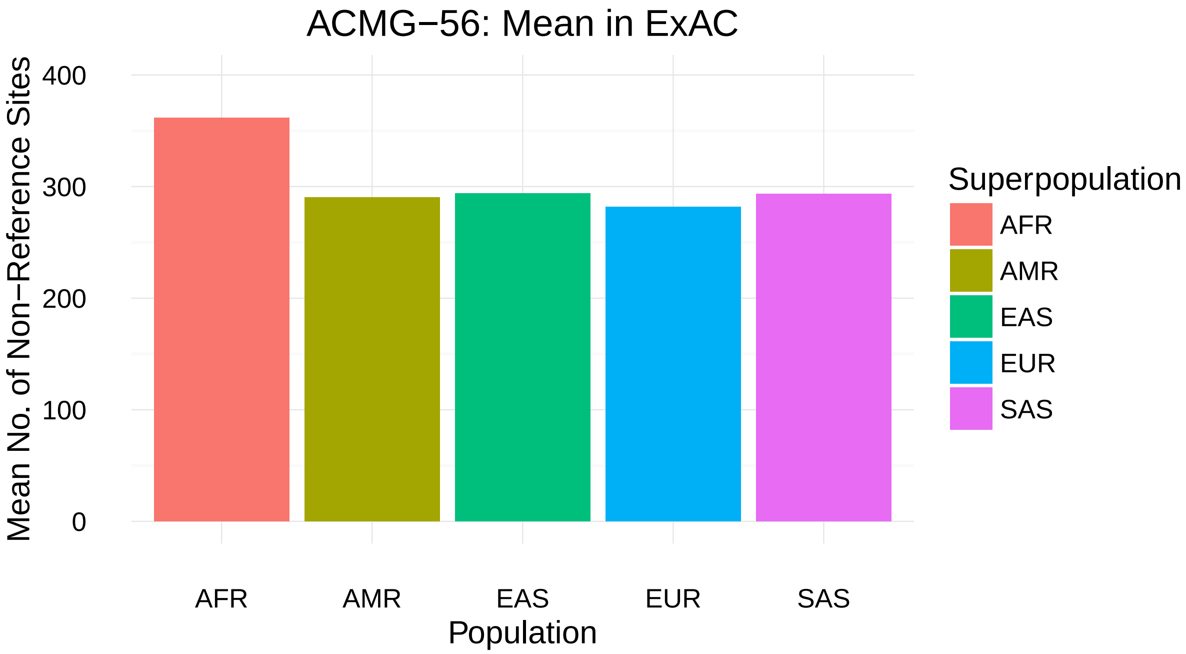
	AFR	AMR	EAS	EUR	SAS
Variant 1	0.1	0.2	0	0	0.3
Variant 2	0.2	0	0.3	0	0.1

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 $\sum E(V_i) = \sum(columns)$.

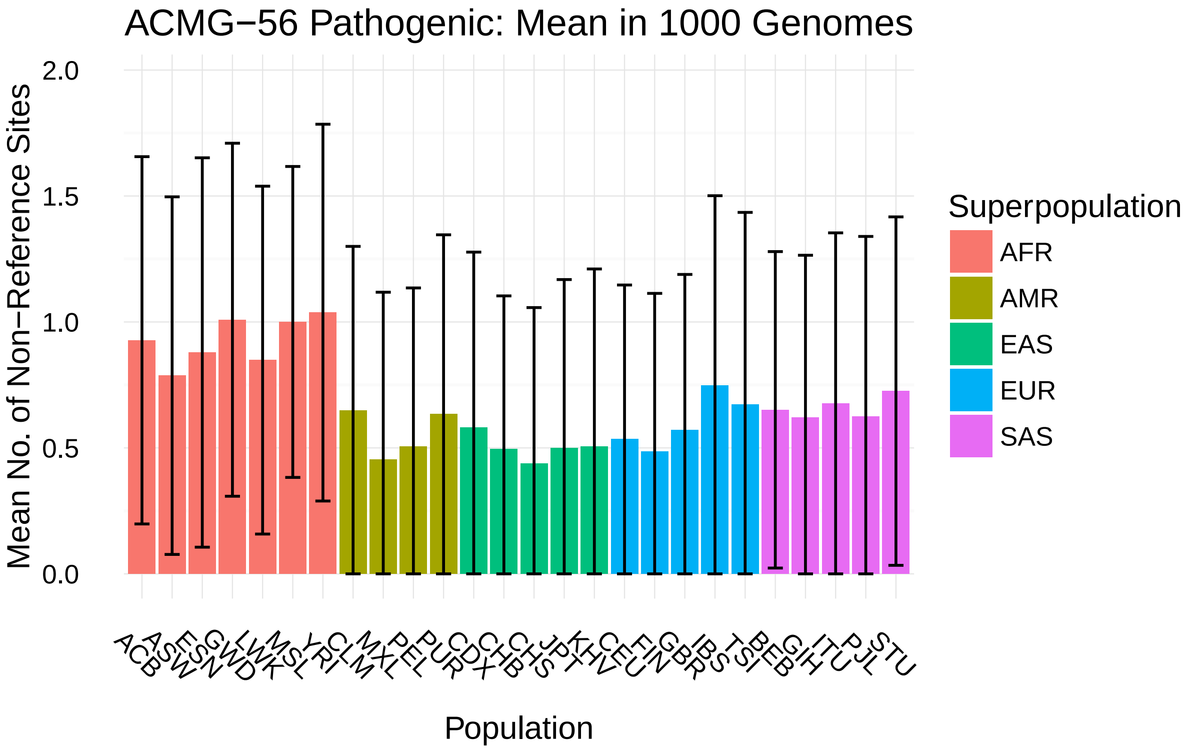
AFR	AMR	EAS	EUR	SAS
0.55	0.36	0.51	0	0.7

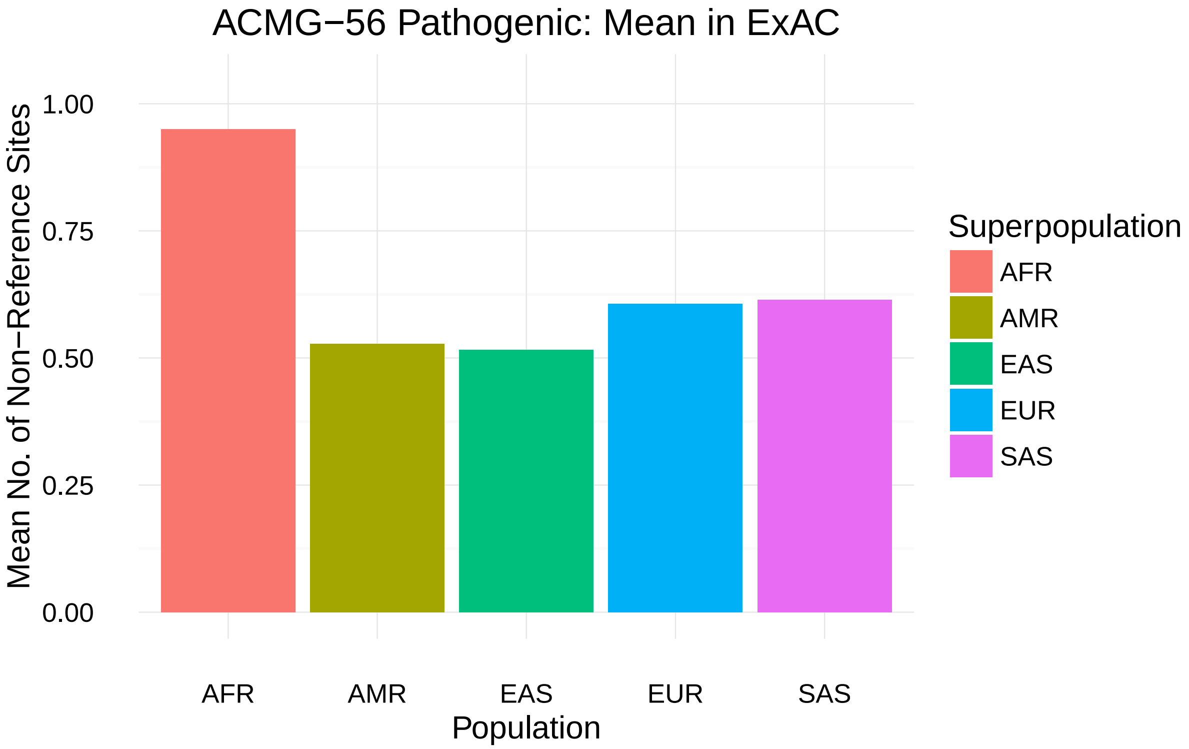


2.3 Pathogenic Non-Reference Sites

2.3.0.1 For 1000 Genomes and ExAC

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





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.

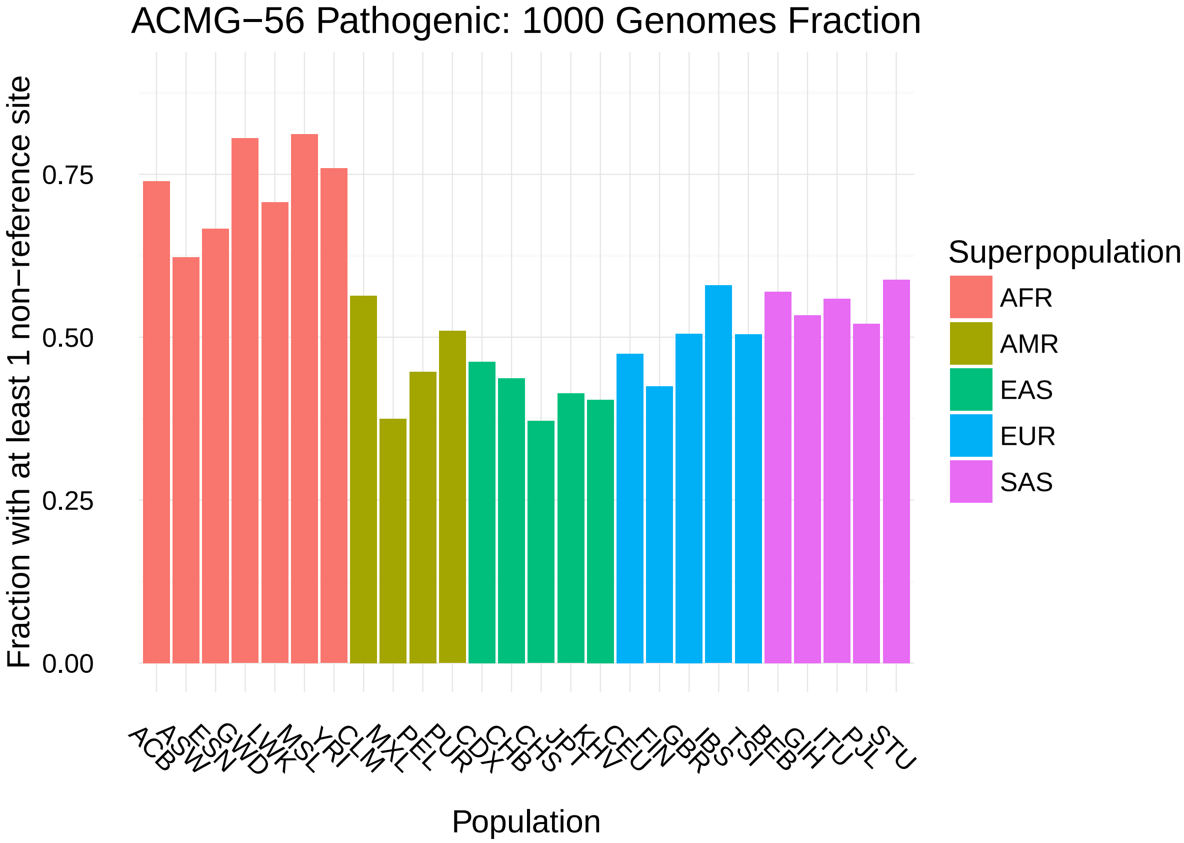
Ex: the genotype of 3 variants in 3 people looks like this:

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

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

HG00097	HG00099	HG00100
0	1	1

```
## Mean = 0.667
```



2.4.0.2 For 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:

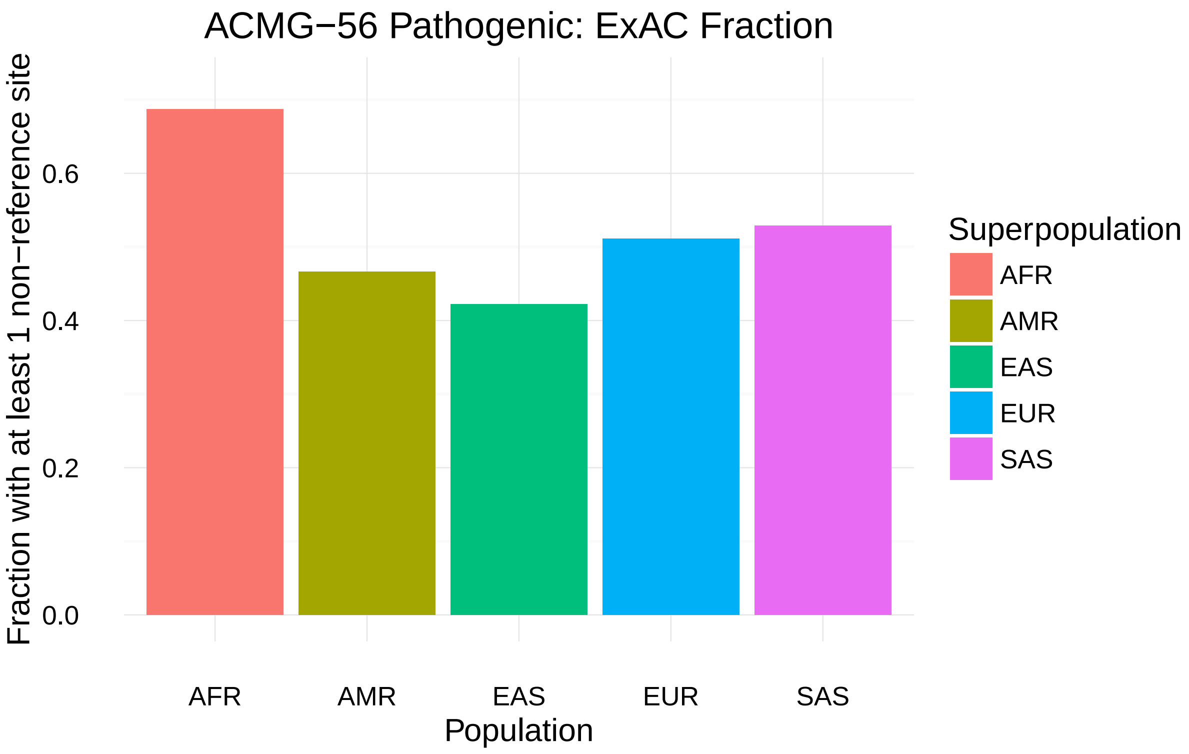
	AFR	AMR	EAS	EUR	SAS
Variant 1	0.1	0.2	0	0	0.3
Variant 2	0.2	0	0.3	0	0.1

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

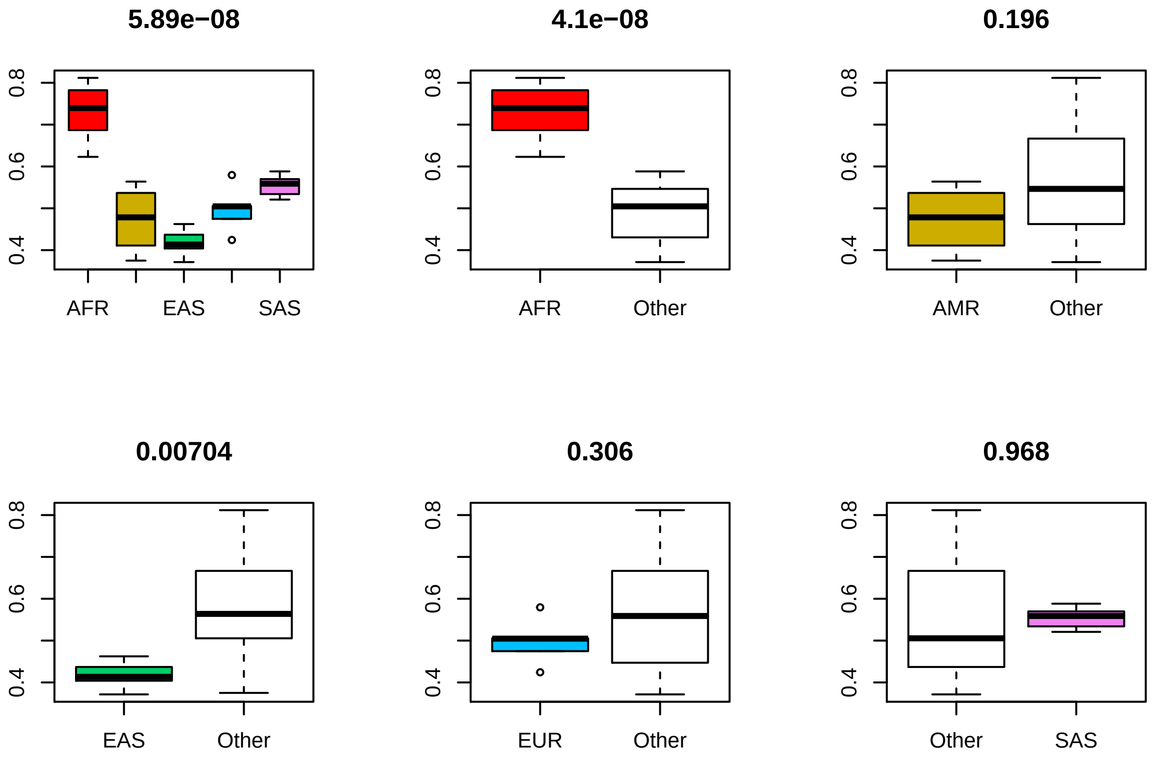
The expected (mean) number of non-reference sites is given by $1 - \prod(1 - AF)^2$.

AFR	AMR	EAS	EUR	SAS
0.4816	0.36	0.51	0	0.6031

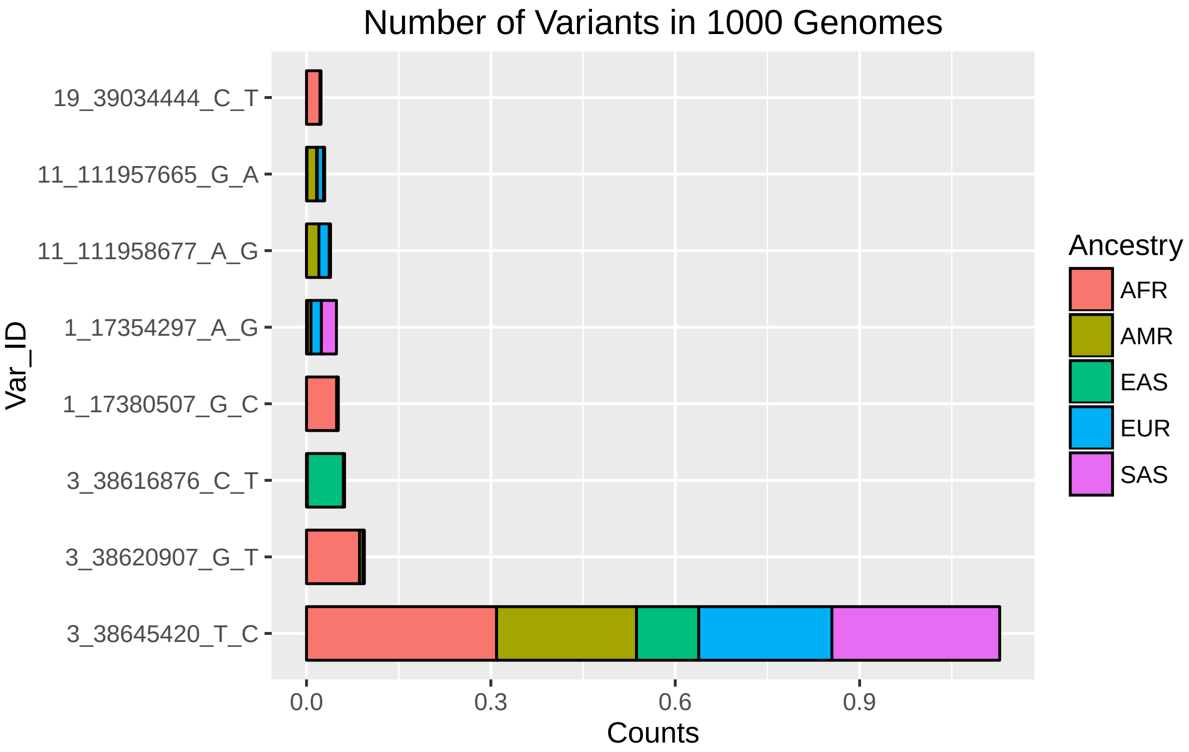


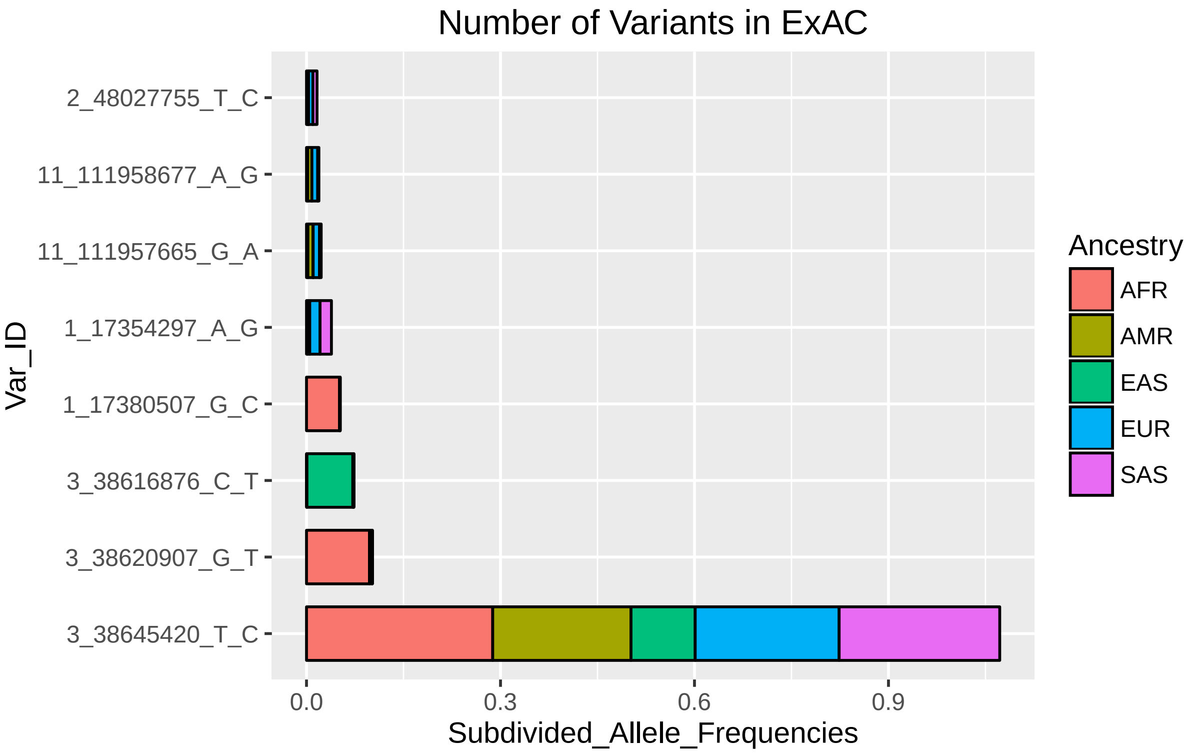
2.5 Test Statistics for Ancestral Differences

F-statistic/T-statistic: probability that the different groups are sampled from distributions with the same mean. These plots are from 4(a) - 1000 Genomes Fraction with 1+ Non-Reference Site, but can be replicated for plots 2(ab) and 3(ab) as well.



2.6 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 of Disease Prevalence 30 x 16 (selected rows/columns):
```

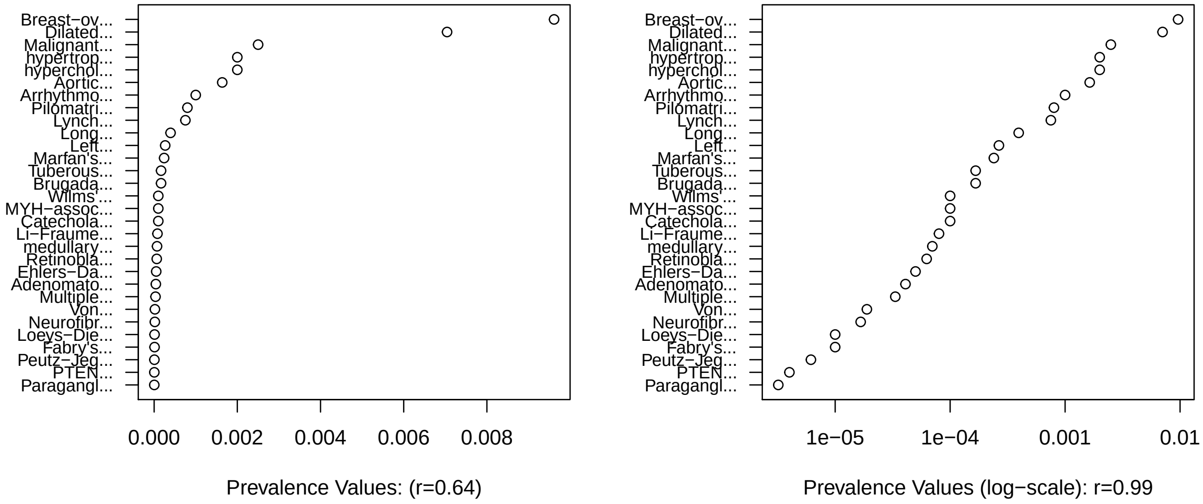
Gene	Disease	Disease_MIM	Tags
BRCA1;BRCA2	Breast-ovarian cancer familial	604370;612555	breast;ovarian
SCN5A	Brugada syndrome	601144	brugada
COL3A1	Ehlers-Danlos syndrome	130050	ehler;danlos
TP53	Li-Fraumeni syndrome	151623	fraumeni

Table continues below

Inverse.Prevalence.1	Inverse.Prevalence.2	year	first.author	citations
104	NA	2013	NA	NA
10000	2000	2006	Antzelevitch	11
20000	NA	2010	Malfait	116
20000	5000	1999	Schneider	47

3.3 Distribution of Prevalences

Later, we face the question of how to compute point estimates for penetrance, which requires a point estimate of prevalence. We decided to combine the upper and lower bounds of prevalence ranges using the geometric-mean, or log-average, because the prevalences seem to be distributed most uniformly on a logarithmic-scale.

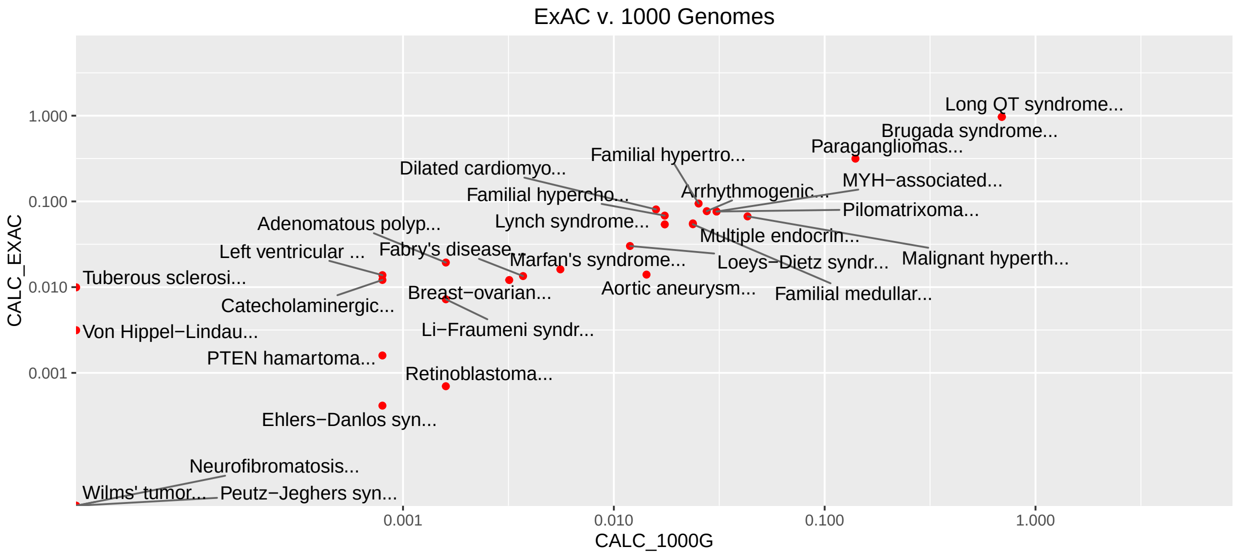


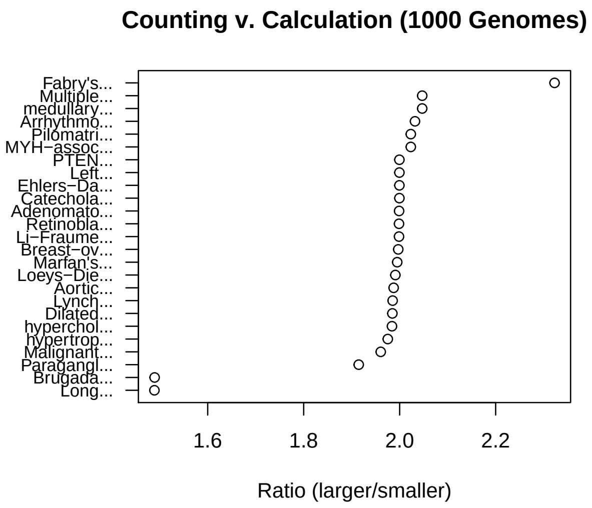
3.4 Collect and Aggregate Allele Frequencies at the Disease-Level

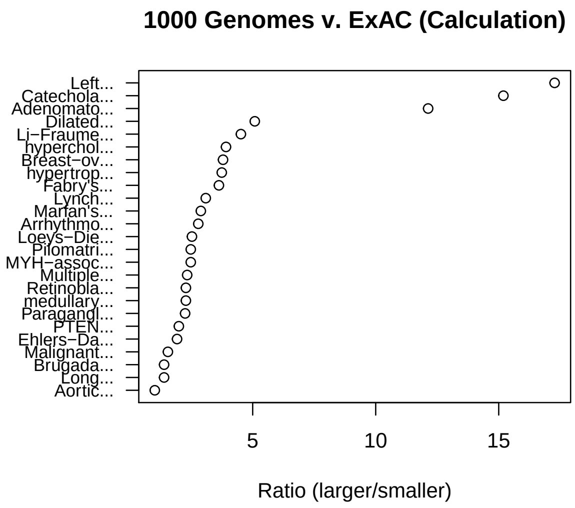
We define AF(disease) as the probability of having at least 1 variant associated with the disease.
The frequencies across the relevant variants can be aggregated in two ways:
(1) By direct counting, from genotype data in 1000 Genomes.
(2) AF(disease) $= 1 - \prod_{variant}(1 - AF_{variant})$, from population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

Correlation Table:

	COUNT_1000G	CALC_1000G	CALC_EXAC
COUNT_1000G	1	0.9994	0.9905
CALC_1000G	0.9994	1	0.9943
CALC_EXAC	0.9905	0.9943	1

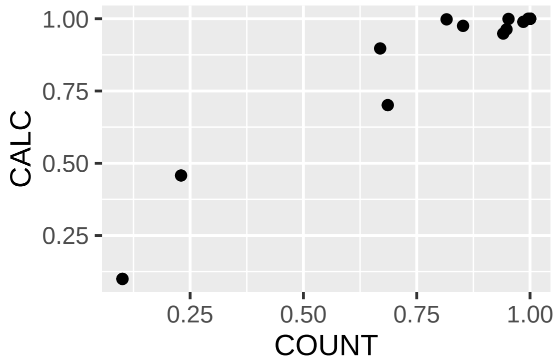






The median AF(disease) ratio between counting and calculation is: 1.999.
The median AF(disease) ratio between ExAC and 1000 Genomes is: 2.531.

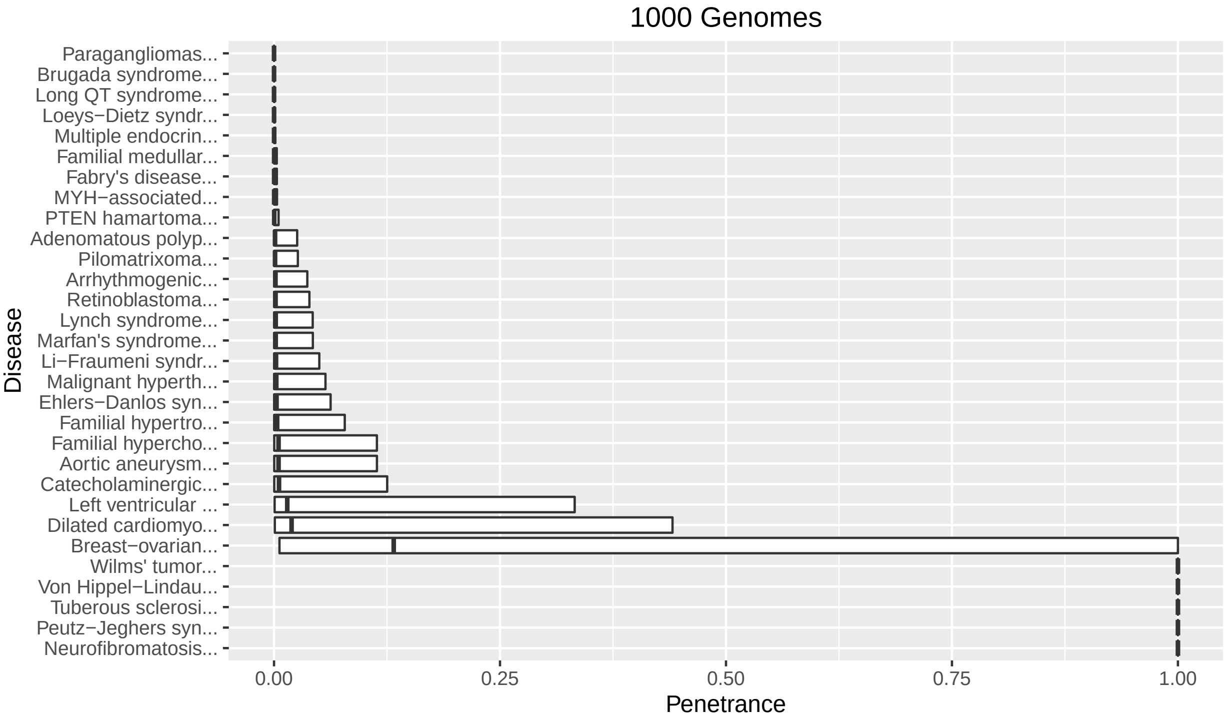
Sampling 10000 variants from 1000 Genomes to test deviations from assuming independence.

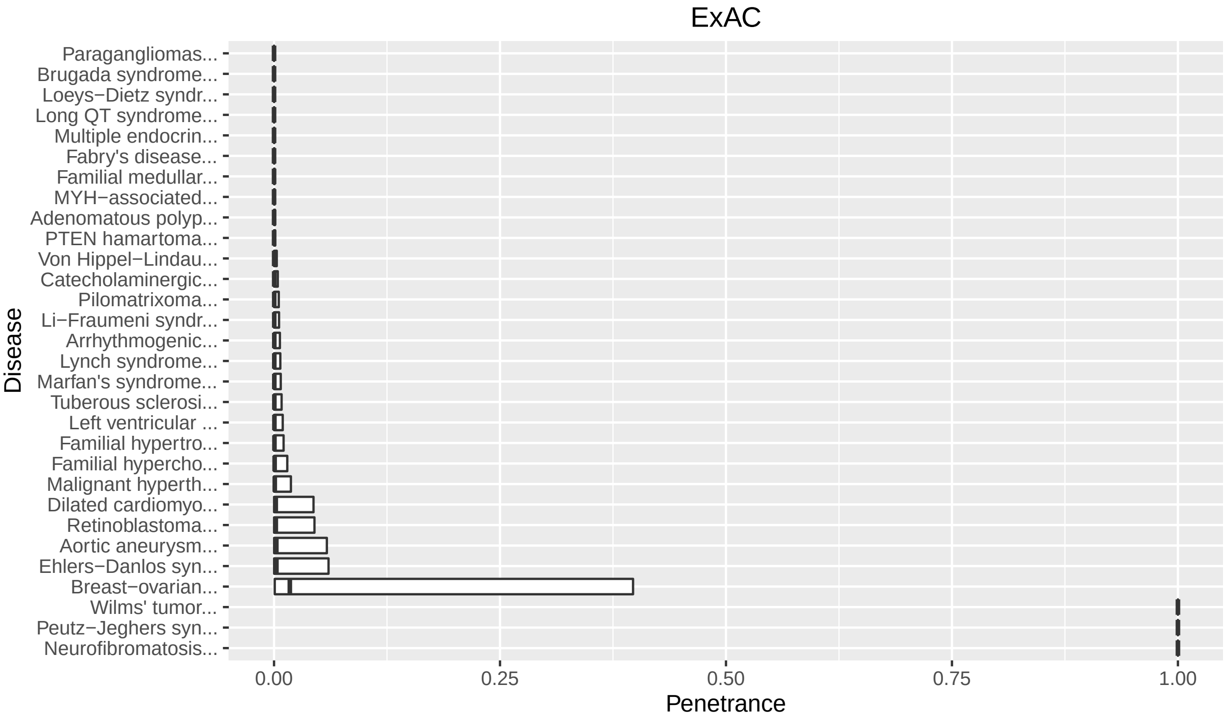


Pearson correlation: 0.97.

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

The left end of the boxplot indicates P(V|D) = 0.001,
the bold line in the middle indicates P(V|D) = 0.022,
the right end of the boxplot indicates P(V|D) = 0.5.



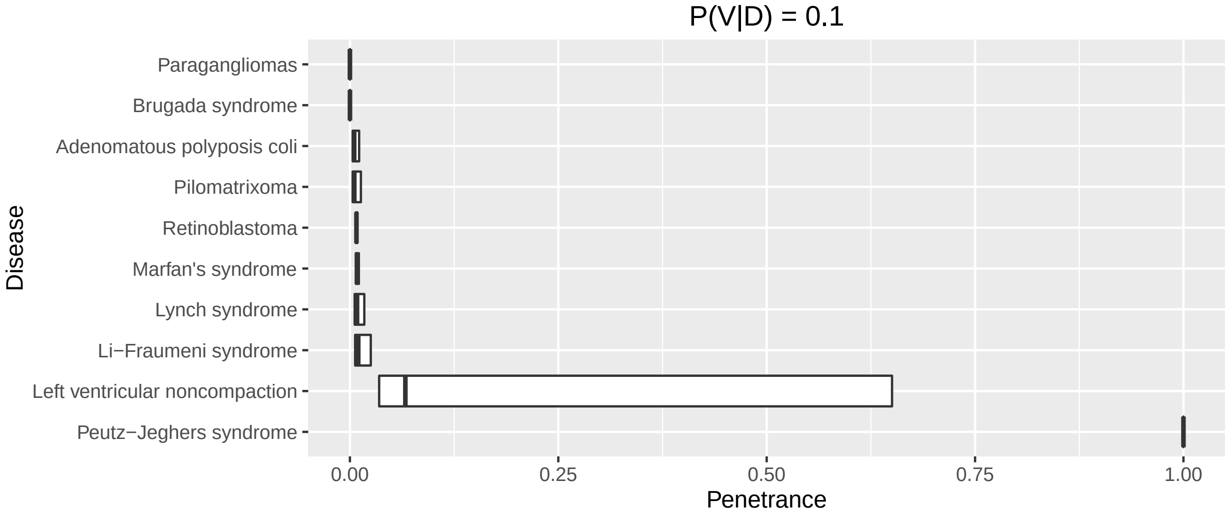


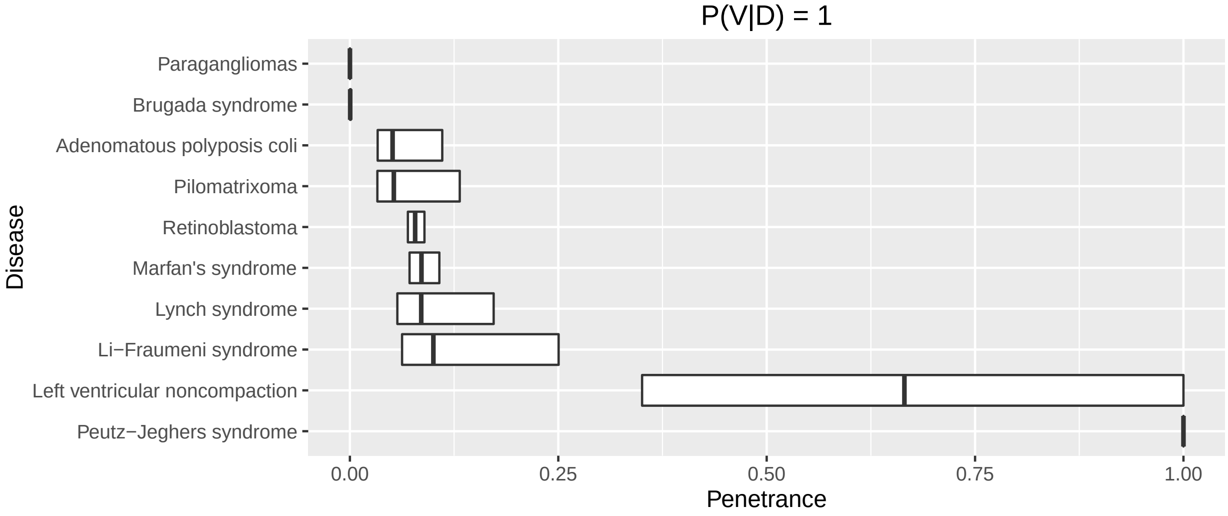
Note: the bold black lines at 1.0 all indicate no allele frequency (disease_AF) data. (Disease_AF = 0 returns "infinite penetrance", which is capped at 1).

3.6 Penetrance as a Function of P(D)

The left end of the boxplot indicates P(D) = upper value,
the bold line in the middle indicates P(D) = geometric_mean(values),
the right end of the boxplot indicates P(D) = lower value.

Disease	Prevalence_Ratio
Retinoblastoma	1.3
Marfan's syndrome	1.5
Lynch syndrome	3.0
Adenomatous polyposis coli	3.3
Li-Fraumeni syndrome	4.0
Paragangliomas	4.0
Pilomatrixoma	4.0
Brugada syndrome	5.0
Peutz-Jeghers syndrome	12.0
Left ventricular noncompaction	18.6

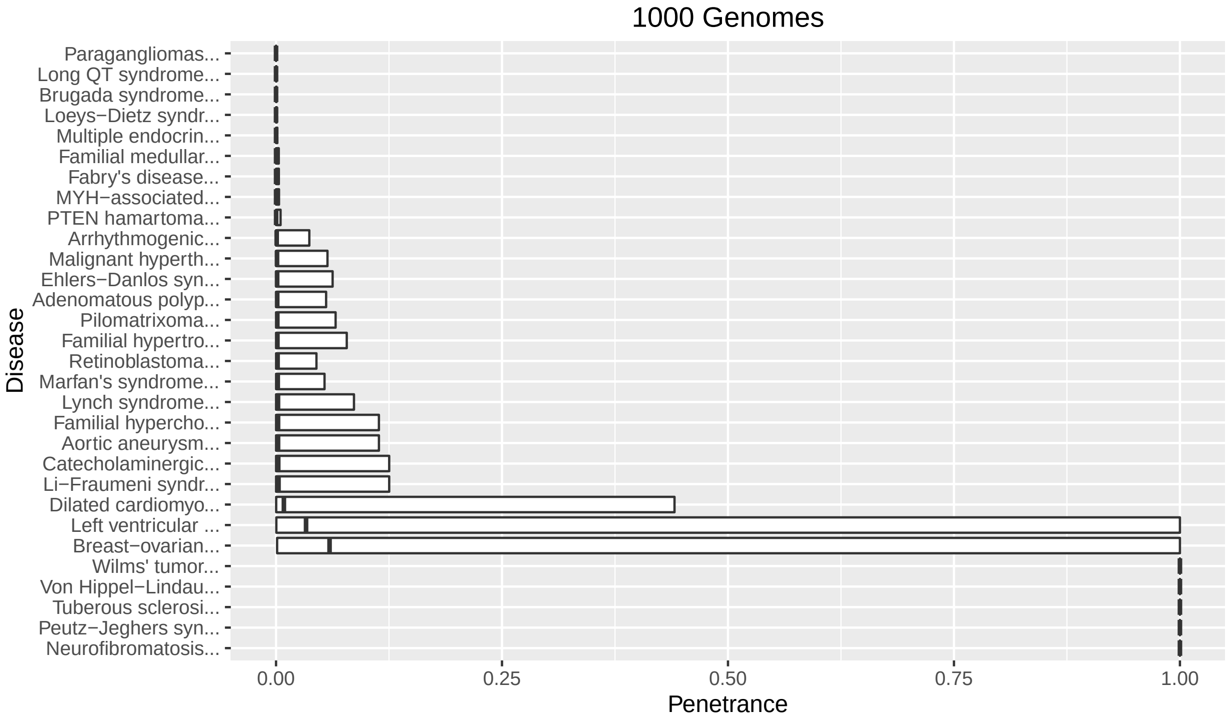


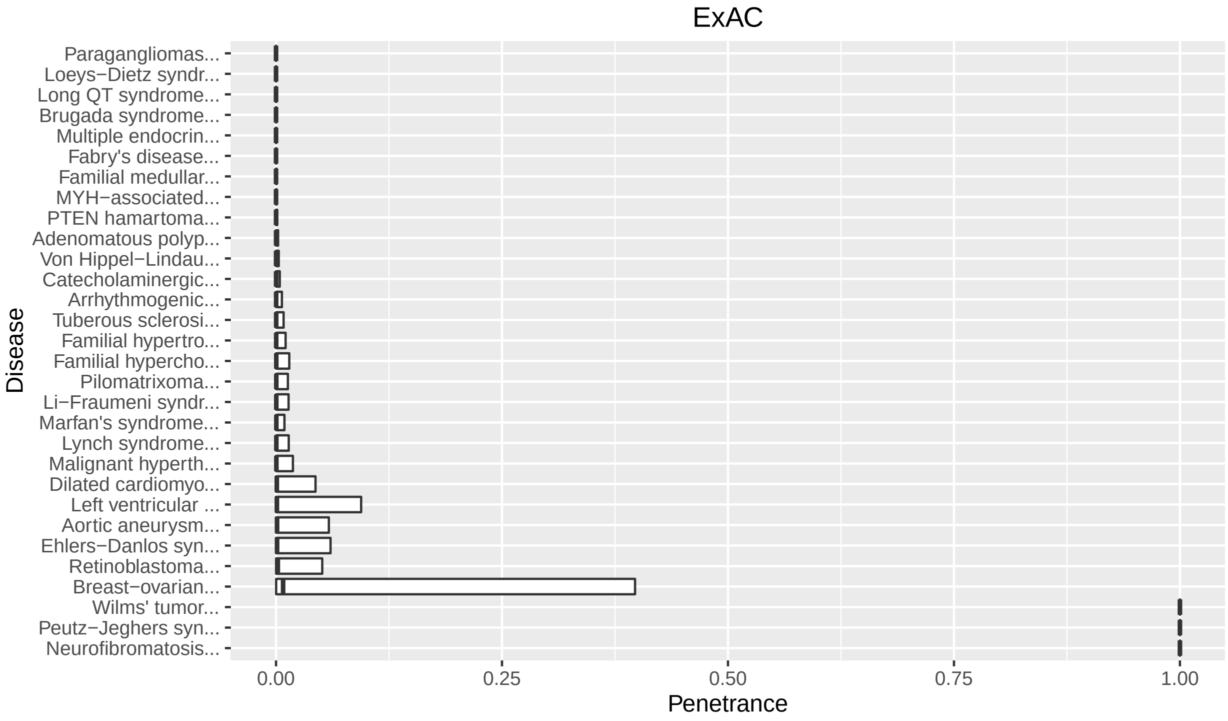


This can only be computed in 10 cases where a prevalence range was given, rather than a point estimate.

3.7 Max/Min Penetrance as a Function of P(D) and P(V|D)

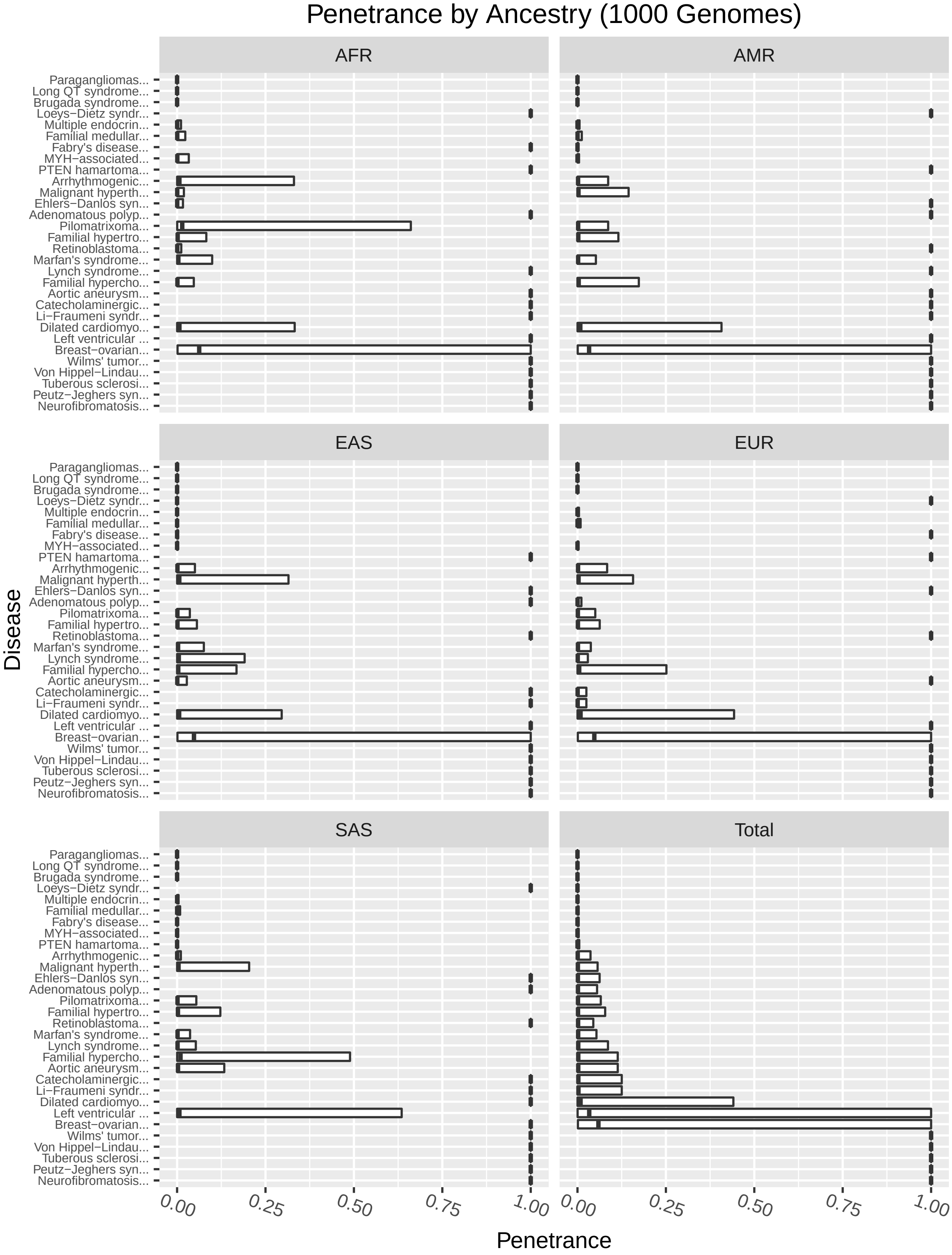
The left end of the boxplot indicates P(D) AND P(V|D) = lower value,
the bold line in the middle indicates P(D) AND P(V|D) = geometric_mean(values),
the right end of the boxplot indicates P(D) AND P(V|D) = upper value.

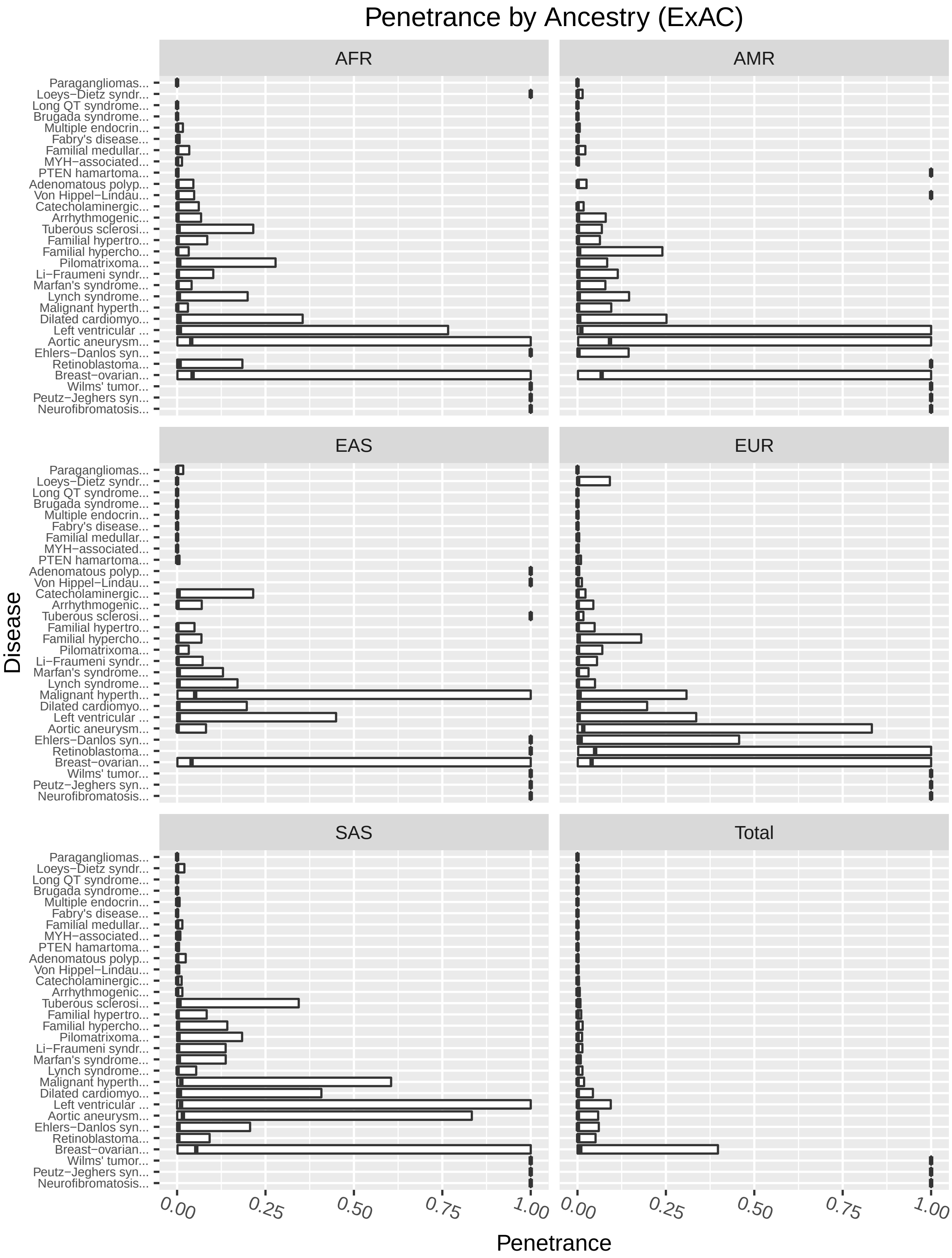




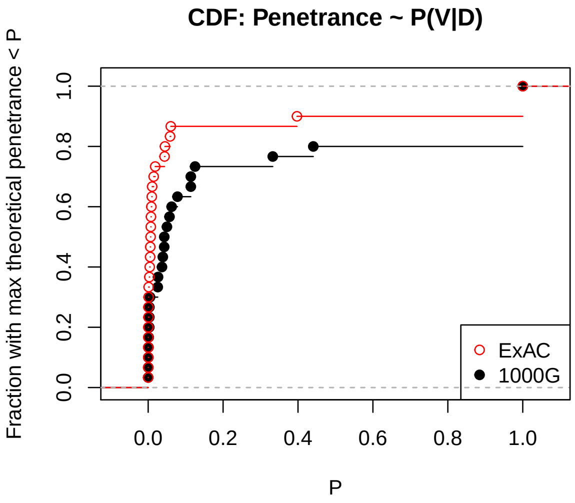
Note: Prevalence ranges of 5x were assumed for all point estimates of prevalence.
For example: a point estimate of 0.022 would be given the range 0.01-0.05.

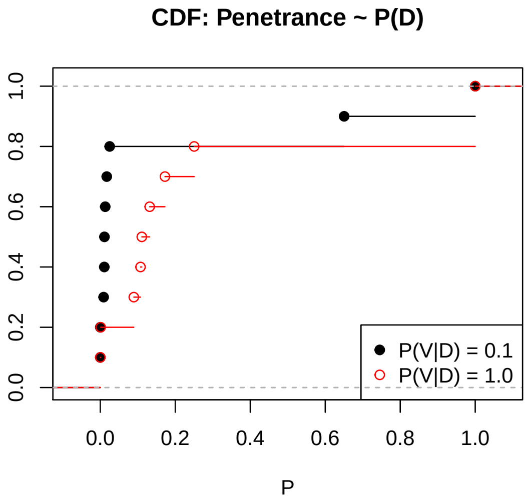
3.8 Penetrance Estimates by Ancestry

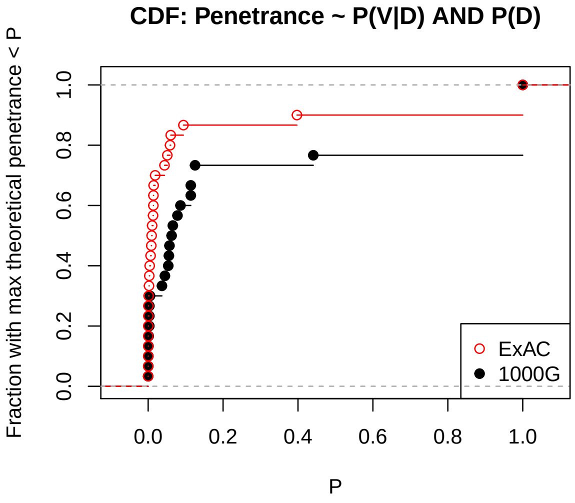



Penetrance by Ancestry (ExAC)
AFR
AMR
EAS
EUR
SAS
Total
Disease
Paragangliomas...
Loeys−Dietz syndr...
Long QT syndrome...
Brugada syndrome...
Multiple endocrin...
Fabry's disease...
Familial medullar...
MYH−associated...
PTEN hamartoma...
Adenomatous polyp...
Von Hippel−Lindau...
Catecholaminergic...
Arrhythmogenic...
Tuberous sclerosi...
Familial hypertro...
Familial hypercho...
Pilomatrixoma...
Li−Fraumeni syndr...
Marfan's syndrome...
Lynch syndrome...
Malignant hyperth...
Dilated cardiomyo...
Left ventricular ...
Aortic aneurysm...
Ehlers−Danlos syn...
Retinoblastoma...
Breast−ovarian...
Wilms' tumor...
Peutz−Jeghers syn...
Neurofibromatosis...
0.00
0.25
0.50
0.75
1.00
Penetrance

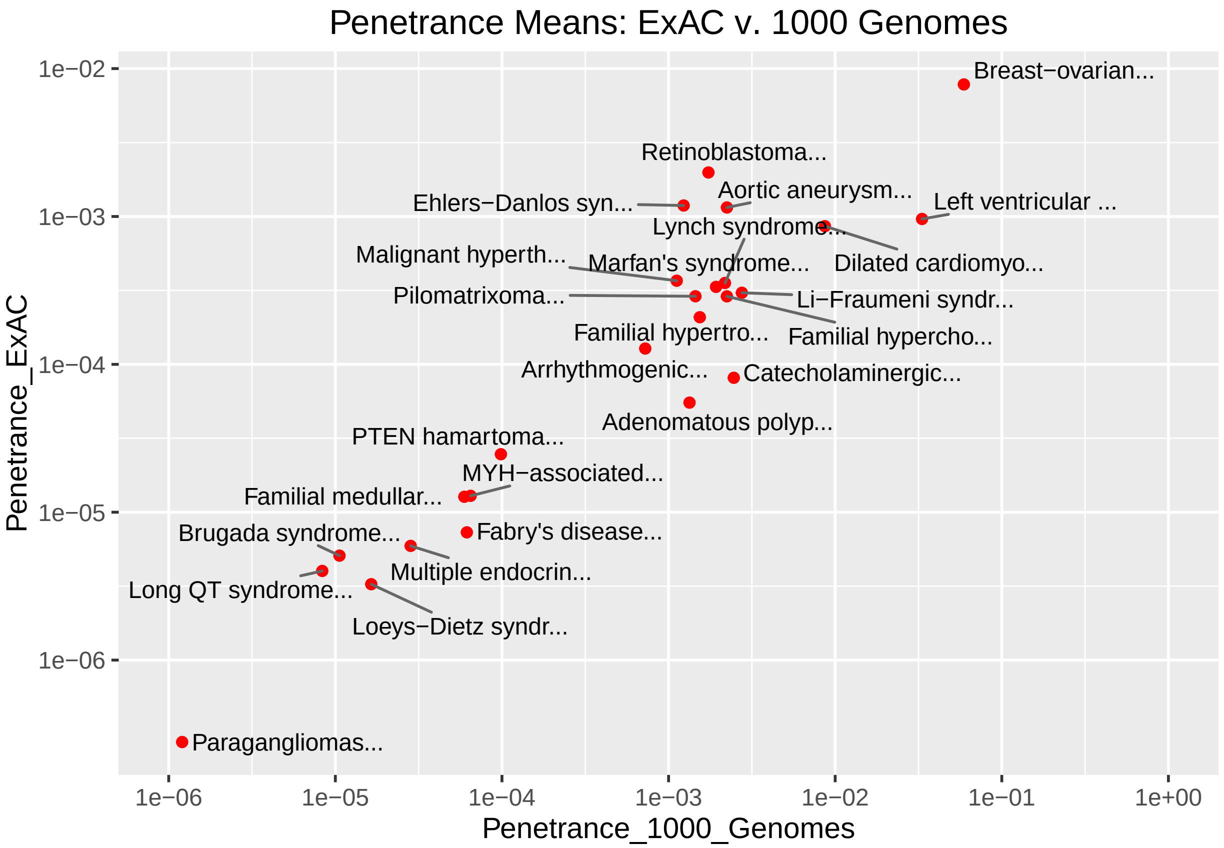
3.9 Empirical CDFs for All Penetrance Plots







3.10 Comparing Mean Penetrance between ExAC and 1000 Genomes



The Pearson correlation is 0.88.
Max penetrance values computed using 1000 Genomes are 7.3-fold larger than those computed using ExAC.