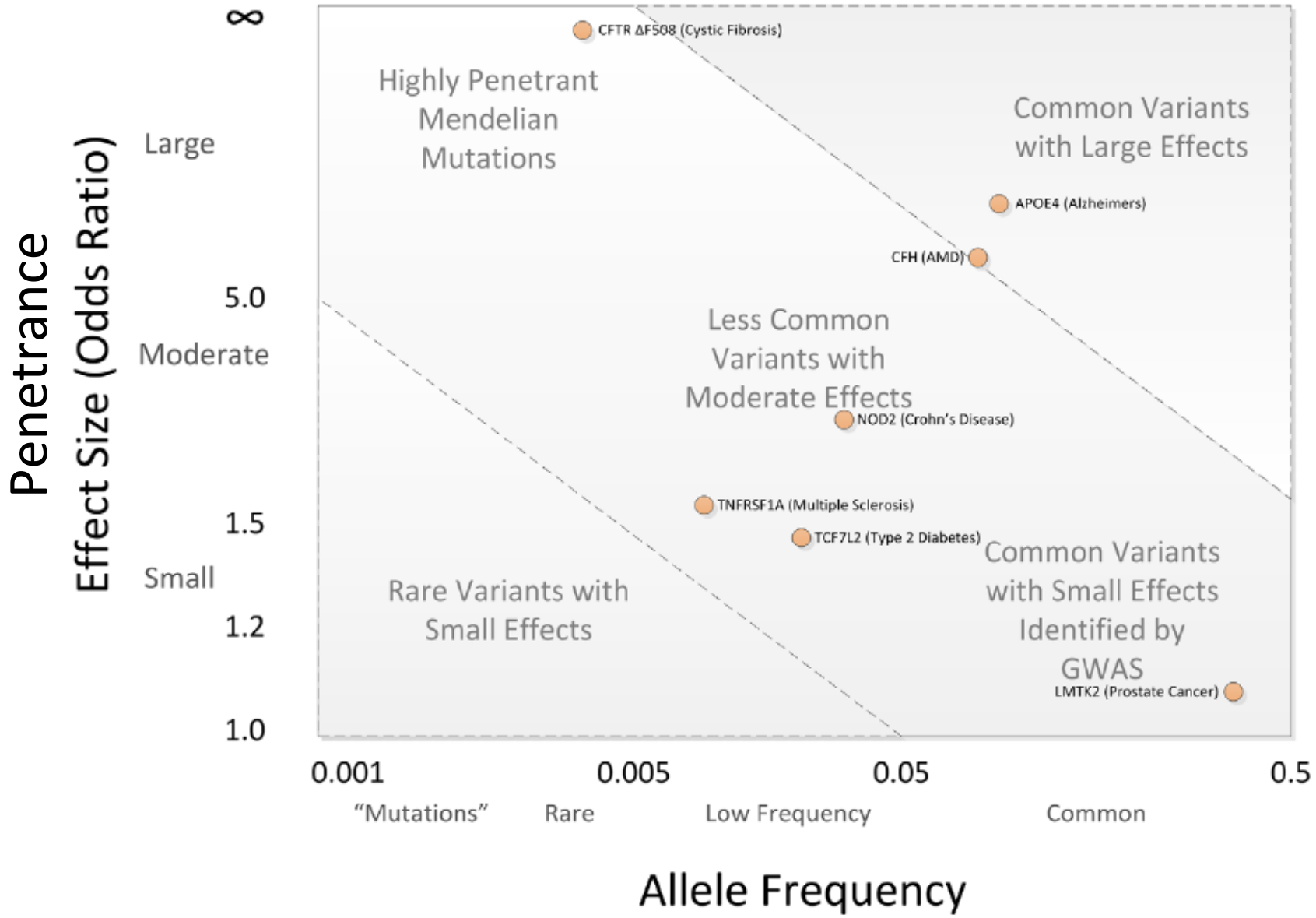
Genome Wide Association Studies

What are we trying to do?

- Correlate changes in genome with phenotype of interest.

GWAS!


Penetrance
Effect Size (Odds Ratio)
∞
Large
5.0
Moderate
1.5
Small
1.2
1.0
CFTR ΔF508 (Cystic Fibrosis)
Highly Penetrant Mendelian Mutations
Common Variants with Large Effects
APOE4 (Alzheimers)
CFH (AMD)
Less Common Variants with Moderate Effects
NOD2 (Crohn's Disease)
TNFRSF1A (Multiple Sclerosis)
TCF7L2 (Type 2 Diabetes)
Common Variants with Small Effects Identified by GWAS
LMTK2 (Prostate Cancer)
Rare Variants with Small Effects
0.001
0.005
0.05
0.5
"Mutations"
Rare
Low Frequency
Common
Allele Frequency

How tell if a variant is correlated to a phenotype?

- Correlation
- Probability
- Odds ratio/Relative Risk

Different kinds of Phenotypes

- Categorical
  - Have diabetes or don't
  - Have phenylketonuria or don't
- Quantitative
  - Gradient of disease or biomarker of interest
    - Age of onset of Alzheimer's
    - Blood Pressure
    - HDL concentrations

Covariance and Correlation

Want:

- Degree of association between 2 variables, X &Y
- Given observations $x_1,\ldots,x_n$ and $y_1,\ldots,y_n$
  - Covariance (sum of O-E divided by number -1)

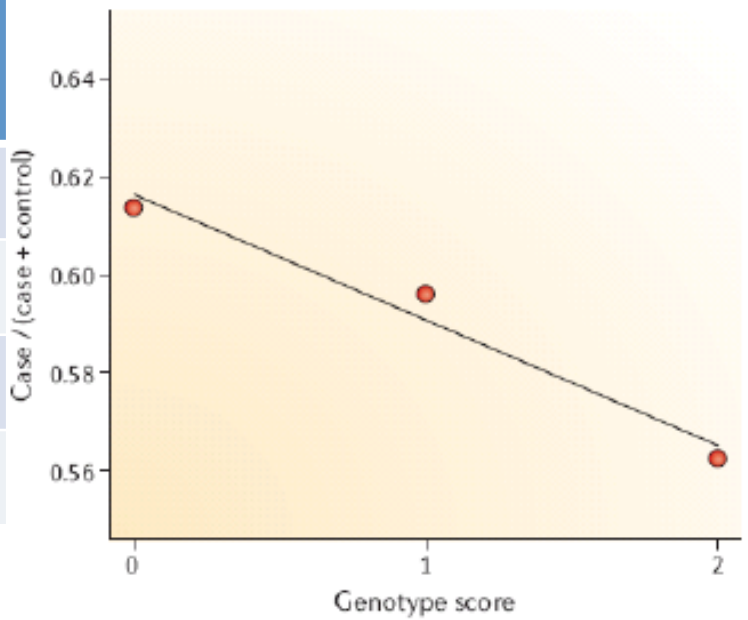
$$\frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{n-1}$$

  - Correlation coefficient

$$r=\frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2\sum_{i=1}^{n}(y_i-\bar{y})^2}}$$

Case/Control Study (Categorical)

Genotype	Case	Control	Total
AA	$N_{case,AA}$	$N_{control,AA}$	N_{AA}
Aa	$N_{case,Aa}$	$N_{control,Aa}$	N_{Aa}
aa	$N_{case,aa}$	$N_{control,aa}$	N_{aa}
Total	N_{case}	$N_{control}$	N

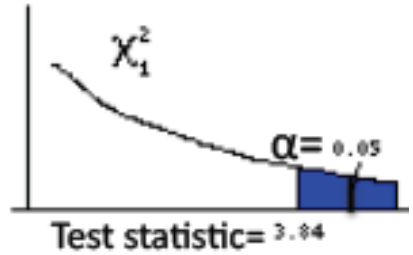


X^2 test with 1 or 2 df (1 if single allele, 2 if genotype)

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

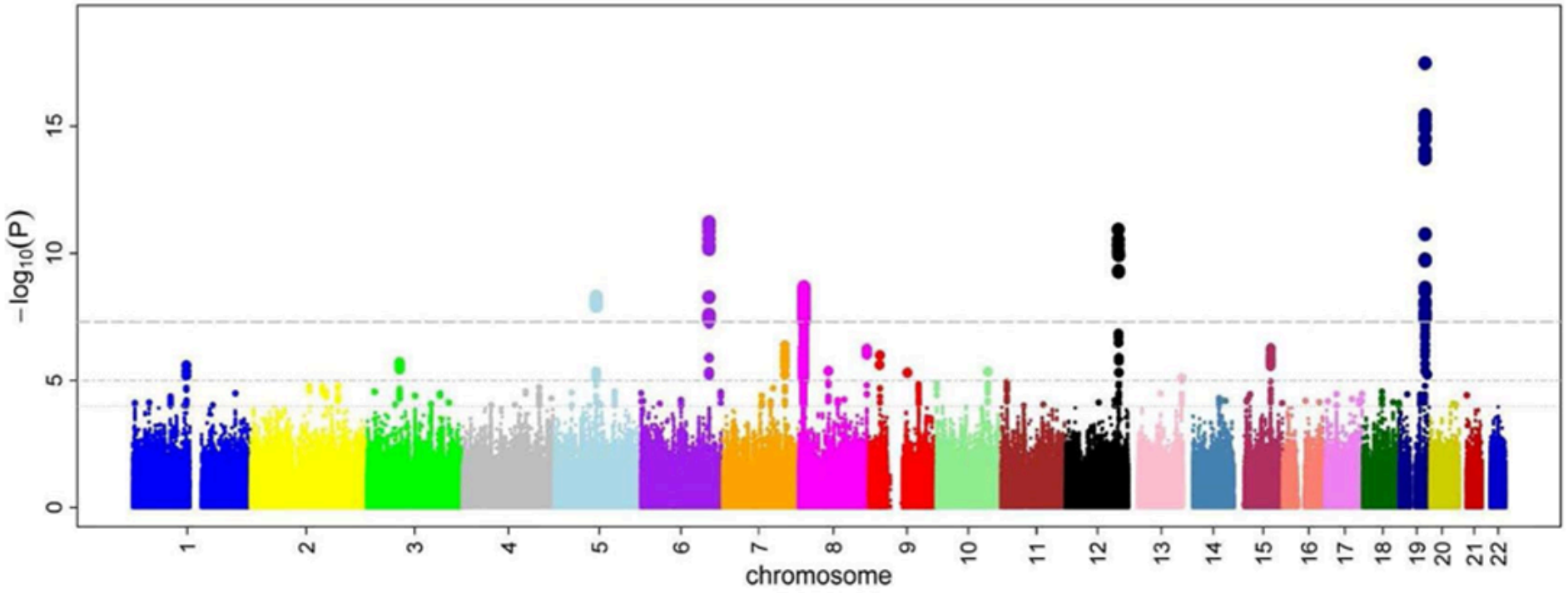
O_i = observed frequency for i^{th} outcome
(the value can be read off of the contingency table)
E_i = expected frequency for i^{th} outcome
(the value can be obtained as described in the previous slides)
n = total number of outcomes

The probability distribution of this statistic is given by the chi-square distribution.



Using chi-square test, we can test how well observed values fit expected values computed under the independence hypothesis

Manhattan Plot



GWAS for microcirculation

M. Kamran Ikram et al - Ikram MK et al (2010) Four Novel Loci (19q13, 6q24, 12q24, and 5q14) Influence the Microcirculation In Vivo. PLoS Genet. 2010 Oct 28;6(10):e1001184. doi:10.1371/journal.pgen.1001184.g001

Let’s do a GWAS

Allele	Short Hair	Long Hair	Totals	Expected
T	3	12	15	7.5
G	9	0	9	4.5
Totals	12	12	24	

Allele	Short Hair	Long Hair	Expected
T	3	12	7.5
G	9	0	4.5

$$\chi^2 = \sum_{i=o}^{\infty} \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2 = \left(\frac{(3-7.5)^2}{7.5}\right) + \left(\frac{(9-4.5)^2}{4.5}\right) + \left(\frac{(12-7.5)^2}{7.5}\right) + \left(\frac{(0-4.5)^2}{4.5}\right)$$

$$\chi^2 = 14.4$$

Significance

	P										
DF	**0.995**	**0.975**	**0.20**	**0.10**	**0.05**	**0.025**	**0.02**	**0.01**	**0.005**	**0.002**	**0.001**
1	0.0000393	0.000982	1.642	2.706	3.841	5.024	5.412	6.635	7.879	9.550	10.828
2	0.0100	0.0506	3.219	4.605	5.991	7.378	7.824	9.210	10.597	12.429	13.816
3	0.0717	0.216	4.642	6.251	7.815	9.348	9.837	11.345	12.838	14.796	16.266
4	0.207	0.484	5.989	7.779	9.488	11.143	11.668	13.277	14.860	16.924	18.467
5	0.412	0.831	7.289	9.236	11.070	12.833	13.388	15.086	16.750	18.907	20.515

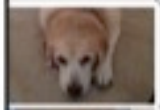
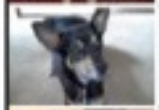
By alleles – 1 df

$X^2 = 14.4$

Therefore $p<0.001$

What we actually care about

- How much does this allele/genotype increase your risk of a disease/phenotype
- Odds ratio (OR) or Risk ratio (RR)
  - Risk ratio depends on penetrance

Dog	Coat Length	chr32 7420804	chr32 7472206	chr32 7473337	chr32 7479580	chr32 7482967	chr32 7490570	chr32 7492364
	Short Coat	TC	AA	GT	TT	AG	TT	CG
	Short Coat	TC	AA	GG	TT	GG	CC	GG
	Short Coat	CC	GA	GT	CT	AG	CT	
	Short Coat	TC	GA	GG	CT	AA	TT	CG
	Short Coat	CC	GA	GT	CT	AG	CT	GG
	Short Coat	TC	AA	GG	TT	GG	CC	CG
	Long Coat	CC	AA	TT	TT	GG	TT	GG
	Long Coat	TC	AA	TT	TT	GG	TT	GG
	Long Coat	TC	AA	TT	TT	GG	TT	GG
	Long Coat	CC	AA	TT	TT	GG	CT	CG
	Long Coat	TT	AA	TT	TT	GG	TT	GG
	Long Coat	CC	AA	TT	TT	GG	TT	GG

Chr32 7490570

Allele	Short	Long	Totals	Expected
T	6	11	17	8.5
C	6	1	7	3.5
Totals	12	12	24	

$$\chi^2 = \sum_{i=o}^{\infty} \frac{(O_i - E_i)^2}{E_i}$$

$$\chi^2 = \left(\frac{(6-8.5)^2}{8.5}\right) + \left(\frac{(11-8.5)^2}{8.5}\right) + \left(\frac{(6-3.5)^2}{3.5}\right) + \left(\frac{(1-3.5)^2}{3.5}\right)$$

$$\chi^2 = 5.0$$

Still significant 0.05>p>0.025

Allele	Short	Long	Totals
T	6	11	17
C	6	1	7
Totals	12	12	24

$$P(Short \mid T) = \frac{(Short \,\&\, T)}{(T)} = \frac{6}{17} = 0.35$$

$$P(Short \mid C) = \frac{(Short \,\&\, C)}{(C)} = \frac{6}{7} = 0.86$$

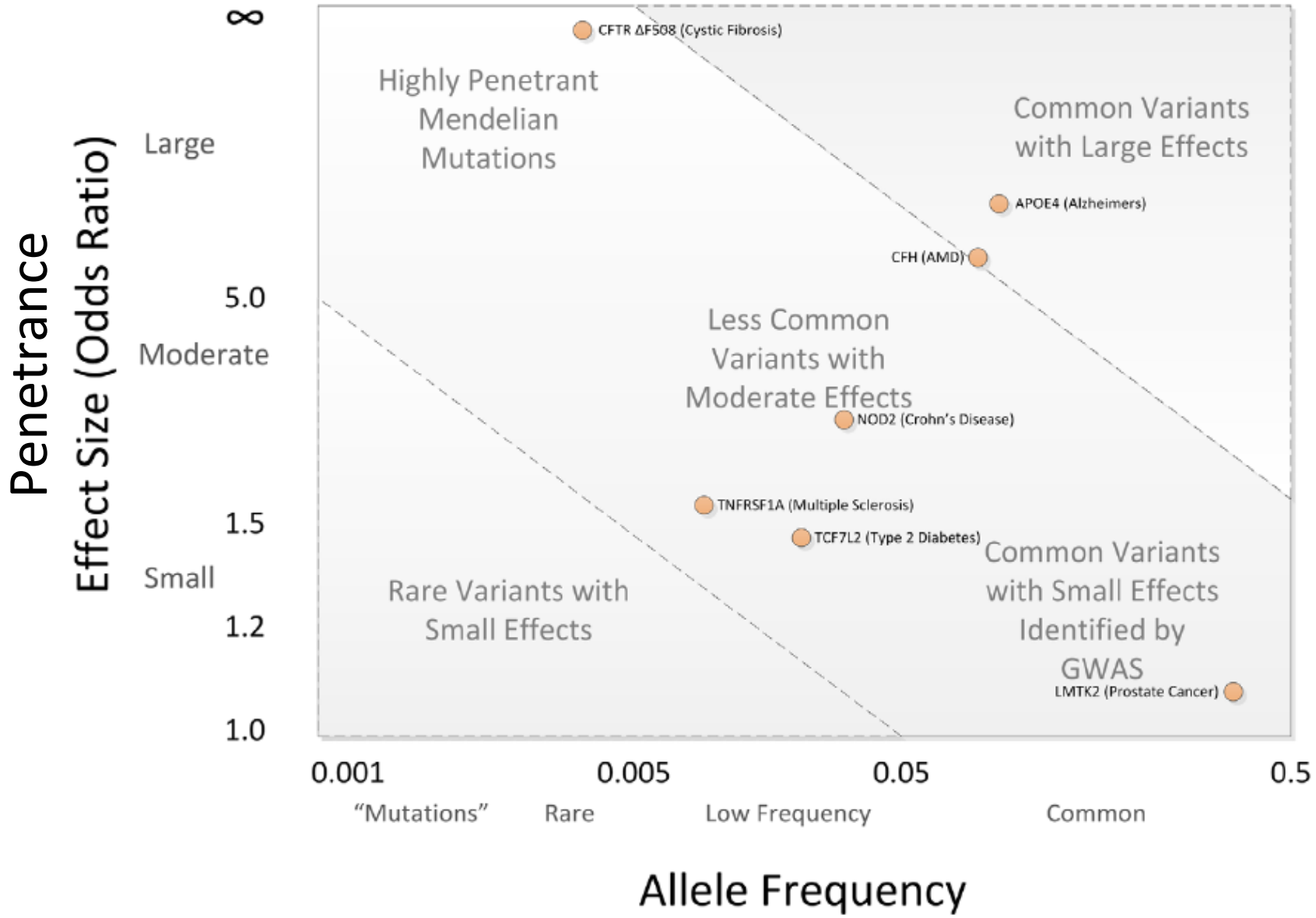
$$Odds(T) = \frac{P(Short \mid T)}{1 - P(Short \mid T)} = 0.55$$

$$Odds(C) = \frac{P(Short \mid C)}{1 - P(Short \mid C)} = 6.0$$

$$OR = \frac{Odds(C)}{Odds(T)} = 11$$

Quantitative Phenotypes

- Can't use χ^2
- Need to use a regression on the data
  - Linear is a good first step
- Lots of ongoing research on how to improve this


Penetrance
Effect Size (Odds Ratio)
∞
Large
5.0
Moderate
1.5
Small
1.2
1.0
CFTR ΔF508 (Cystic Fibrosis)
Highly Penetrant Mendelian Mutations
Common Variants with Large Effects
APOE4 (Alzheimers)
CFH (AMD)
Less Common Variants with Moderate Effects
NOD2 (Crohn's Disease)
TNFRSF1A (Multiple Sclerosis)
TCF7L2 (Type 2 Diabetes)
Common Variants with Small Effects Identified by GWAS
LMTK2 (Prostate Cancer)
Rare Variants with Small Effects
0.001
0.005
0.05
0.5
"Mutations"
Rare
Low Frequency
Common
Allele Frequency

Why do GWAS usually find common variants with moderate to large effects?

- Need large numbers of individuals to find rare variants (statistical power)
- Need moderate effect to reach statistical significance
- If very high effect, can still find, like Chr32 7473337

Some problems with GWAS

- Clinical phenotypes aren't always good enough
  - May be multiple pathophysiologies that have different causes
  - Clinical categories may be too broad to distinguish
- SNPs sometimes not clear how they relate
- Often find nothing
  - Moving toward pathway analysis instead of gene by gene

Questions?