GWAS 1: What is a GWAS?

Matti Pirinen, University of Helsinki

Last updated: 2-March-2023

The slide set referred to in this document is "GWAS 1".

This course is about **statistical/computational ideas and methods** that are used in **genome-wide association studies (GWAS)**. (Note that on these notes 'GWAS' is both singular and plural while other texts may use 'GWASs' or 'GWASes' for plural.) More generally, these same methods are useful for analyzing large data sets of many other types, but, on this course, we will approach these from the angle of GWAS.

A GWAS quantifies statistical association between genetic variation and phenotypes. A **phenotype**, also called a **trait**, can be any measured or observed property of an individual. Examples of phenotypes are quantitative traits like standing height or concentration of cholesterol particles in circulation, or binary traits like diagnoses of multiple sclerosis or schizophrenia.

Why do we do GWAS? (slides 2-7) We do GWAS because a statistical association between a particular physical region of the genome and the phenotype

- can point to **biological mechanisms** affecting the phenotype,
- can allow **prediction** of the phenotype from genomic information.

These results may further benefit

- **medicine** by leading to molecular or environmental interventions against harmful phenotypes,
- **biotechnology** by improving the ways we utilize microbes, plants or animals,
- **forensics** by more accurate identification of an individual from a DNA sample,
- biogeographic **ancestry inference** of individuals, populations and species,
- our understanding of the role of **natural selection** and other **evolutionary forces** in the living world.

The genome of an individual remain (nearly) constant throughout the individual's lifetime. This is a truly remarkable property compared to, e.g., other molecular sources of information (such as metabolomics, metagenomics, transcriptomics, proteomics or epigenomics) or environmental factors that may vary widely across time. Therefore, the genome seems an ideal starting point for scientific research: it needs to be measured only once for an individual and there is no *reverse causation* from the phenotype to genome (with cancer as an important exception).

Ethical aspects As with any powerful technique, the utilization of results from GWAS also raises many new and difficult ethical questions and the legislation of utilization of genome information is under active development around the world. For example, we need concrete answers to

- who can access an individual's genetic information and for which purpose: individual himself/herself?, researchers?, medical professionals?, insurance companies?, employers?, school system?, everyone?

- what kind of information should be returned back to an individual: genetic risk for a disease for which some preventive measures exist vs. a disease with no actionable measures known?, genetic prediction of sensitive traits such as IQ?, genetic ancestry or family information that does not match prior expectations of the individual, for example, due to false paternity?
- what kind of information, if any, should be returned to a relative of a tested individual given that the relative may also have some of the same genetic variants?
- when is gene editing allowed: to cure severe disease?, to prevent a severe mutation to be passed on to next generation?, to design the next generation to have some favorable genetic variants?

When working with human genome data, we should always keep it clear in mind that there are such profound questions related to these data, and that the data we handle will likely turn out to be more powerful than most of us can imagine today. Human genome data are never 'just data' but include highly personal information, and they need to be handled with high respect and care. Access to genetic data requires a written agreement between the researcher and the data provider about how and for which purpose the data can be used.

Contents of this course (slides 30-31) The plan is to discuss the following topics (in varying level of detail):

1. What is a GWAS?
2. Statistics of GWAS (regression coefficients, P-values, statistical power, Bayes factors)
3. Genetic relatedness and population structure
4. Confounding and covariates in GWAS
5. Haplotypes, linkage disequilibrum, imputation, fine-mapping
6. Linear mixed models and heritability
7. Summary statistics and meta-analysis
8. Polygenic scores
9. Success and critisism of GWAS
10. Human genetics research at FIMM

1.1 Genetic variation (slides 8-13)

We all carry two nuclear genomes (i.e. genomes located in cell nucleus), one inherited from each of our two parents. Additionally, we have a small mitochondrial genome, assumed to be inherited exclusively from the mother, but on this course the term 'genome' refers to the nuclear genome.

Human genome is 3.2 billion **nucleotides** (or **base pairs** or **DNA letters** A,C,G,T) long sequence (see yourgenome.org), that is divided into separate physical pieces called **chromosomes** (see yourgenome.org). There are 22 **autosomal** (non-sex related) chromosomes and two **sex chromosomes** (X chromosome and Y chromosome). Normally, humans have two copies of each autosome and individuals with one copy of X and one of Y are males whereas individuals who have two copies of X are females. Abnormal number of autosomal chromosomes (called autosomal **aneuploidies**) typically cause severe consequences or an early death if present in all cells of an individual. The most common non-lethal exception is the Down syndrome (3 copies of chr 21). While autosomal aneuploidies are often lethal, there are several non-lethal sex chromosomal aneuploidies. **Mosaicism**, where some cells have abnormal chromosome numbers are also possible and are often present in cancer cells.

There are three types of pairings that come up when we analyse genomes.

- First, the DNA is most of the time a double-stranded molecule whose two strands (i.e. the two DNA molecules) are glued together by the chemical base pairings A-T and C-G. This base pairing is a key to the copying mechanism of the DNA that is needed before any cell division (see yourgenome.org) and the DNA molecules that are linked through base pairing carry exactly the same information, just written in the alternative language where we habe swapped A with T and C with G. To make a distinction between the two DNA molecules, it has been agreed that, based on chemical properties, one of the two DNA strands is called the **forward strand** (or **positive (+) strand**) and the other the **reverse strand** (or **negative (-) strand**). Thus, for example, when the + strand contains base A, the corresponding base on the - strand is T and vice versa.

- Second, the two **homologous** choromosomes of an individual (e.g. paternal chr 13 with maternal chr 13, or in a male, maternal X and paternal Y) can be thought of as a pair. Thus, we say that the human genome consists of 22 autosomes + X / Y, but each individual has two copies of each homologous chromosome, which sums to 46 unique chromosomes that are divided into 23 pairs of homologous chromosomes.

- Third, before any **cell division** each of the 46 unique chromosomes of an individual copies itself and the two copies (called sister chromatids) are paired with each other physically to make an X-like shape that is often used to illustrate chromosomes in pictures. Such pictures actually contain 92 chromosomes since each unique chromosome is duplicated in it, but we typically say that there are 46 replicated chromosomes rather than that there are 92 chromosomes. This pairing of sister chromatids after the copying mechanism is important in cell division so that the resulting two offspring cells will receive the correct set of choromosomes. In mitosis (ordinary cell division), each of the two new cells has one set of the 46 unique chromosomes. In meiosis (cell division creating sex cells), the gametes (sperm and eggs) are formed to have only one copy of each homologous chromosome and thus have 23 unique chromosomes. During meiosis, the process of **recombination** shuffles the homologous copies of the paternal and maternal chromosomes in such a way that each of the offspring's chromosomes will be a mixture of its grandparental chromosome segments.

Terms

- **Gene.** The most obvious way how genetic variation can affect phenotypes is through variation in how **genes** function. Genes are segements of DNA that code for proteins (see yourgenome.org) and variation in the physical structure of the protein or in the time and place where the protein is made can have phenotypic consequences. Therefore, we are very interested in how genetic variation can affect the function of genes, and a lot of this is still unknown. Protein coding genes cover less than 2% of the whole human genome, but the remaining 98% can affect the regulation of genes in many ways.

- **Locus (pl. loci).** A continuous region of the genome is called a locus (plural loci). It can be of any size (e.g. a single nucleotide site of length 1 bp or a region of 10 milion base pairs, 10 Mbp). **GWAS loci** are regions that include a clear statistical association with the phenotype of interest.

1.1.1 Genetic variants

At any one position of the genome (e.g. nucleotide site at position 13,475,383 of chromosome 1, denoted by chr1:13,475,383) variation can exist between the genomes in the population. For example, my paternal chromosome can have a base A and maternal chromosome can have a base G (on the +strand of the DNA) at that position. (slide 11). Such a one-nucleotide variation is called a **single-nucleotide variant (SNV)** and the different versions are called **alleles**. So in the example case, I would be carrying both an allele A and an allele G at that SNV, whereas you might be carrying two copies of allele A at the same SNV. My **genotype** would be AG and yours AA. An individual having different alleles on his/her two genomes is **heterozygous** at that locus, and an individual having two copies of the same allele is **homozygous** at that locus. If neither of the alleles is very rare in the population, say, the **minor allele frequency (MAF)** is $> 1\%$ in the population, the variant is called a polymorphism, **single-nucleotide polymorphism (SNP)**.

There are over 10 million SNPs in the human genome. More complex genetic variation (slide 10) include structural variation (SV) such as copy number variants (CNVs), that include duplications or deletions of genomic regions, or rearrangements of the genome, such as inversions or translocations of DNA segments (see yourgenome.org).

A predefined set of 500,000 - 1,000,000 SNPs can be measured reliably and fairly cheaply (< 50 euros/sample) by DNA microarrays, which has been the single most important factor making GWAS possible (slide 12; Illustration). On this course, we consider SNPs as the canonical type of genetic variation. Typically, the SNPs are **biallelic**, i.e., there are only two alleles present in the population and this is what we assume in the following. In principle, however, all four possible alleles of a SNP could be present in the population.

Ambiguous SNPs. If the two alleles of a SNP are either C,G or A,T we call the SNP *ambiguous* because the strand information must be available and correct in order to make sense of the genotypes at this SNP. This is because allele C on +strand would be called allele G on -strand and if this SNP is reported with respect to different strands in different studies, the results get mixed up. The same problem does not happen with the other SNPs, e.g., a SNP with alleles A,C, because this SNP contains alleles T,G on the opposite strand and we could unambiguously match A to T and C to G between the studies. Note that we can resolve most ambiguous SNPs reliably based on the allele frequencies as long as the minor allele frequency is not close to 50%. If we are combining several studies, we should always start by plotting the allele frequencies between the studies after the alleles should be matching each other in order to see that the frequencies indeed approximately match across the studies.

Genome builds. Our map of the human genome is constantly improving as gaps in the reference genome are being closed, sequencing errors corrected and more structural variation detected. A consequence is that the coordinates of certain SNV/SNP will get updated whenever a new build of the human genome is published. The current build is called **GRCh38** for Genome Reference Consortium human build 38 and also nicknamed as hg38 for human genome build 38. It was published in 2013 and its predecessor, GRCh37, confusingly also called hg19, is still in use in some of the data sets. Thus, when communicating genomic coordinates with others, you should always specify which build is to be considered. For example, a SNV rs121964904 causing aspartylglucosaminuria is located at position 4:177,438,764 in build 38 whereas its position in build 37 was 4:178,359,918. The mapping between builds can be attempted via LiftOver tools

Some catalogues of genetic variation A large part of the genetics research over the last 30 years have been driven by international projects aiming to catalogue genetic variation in public domain.

- The Human Genome Project 1990-2003 established a first draft of a human genome sequence.
- The HapMap project 2002-2009 studied the correlation structure of the common SNPs.
- The 1000 Genomes project 2008-2015, expanded HapMap to genome sequence information across the globe and currently remains a widely-used reference for global allele frequency information. 1000G project was able to characterize well common variation in different *populations*, but missed many rare variants of single individuals because the costs of very accurate sequencing were too high. The tremendous impact of the 1000G project stems from the fact that everyone can download the individual level genome data of the 1000G samples from the project's website and use it in their own research.
- Exome Aggregation Consortium (ExAC) 2014-2016 concentrated only on the protein coding parts of the genome, so called *exons*, that make up less than 2% of the genome and was able to provide accurate sequence data for the exomes of over 60,000 individuals. This effort has been particularly important for medical interpretation of rare variants seen in clinics that diagnose patients with severe disease. ExAC provides summary level information through browser and downloads but individual level data cannot be downloaded.
- Genome Aggregation Database (gnomAD) is expanding the ExAC database and also includes additional whole genome sequencing information. It is the current state-of-the-art among the public genome variation databases. Note that gnomAD v2 uses human genome build 37 but v3 uses build 38.

1.1.2 Genotypes and Hardy-Weinberg equilibrium

Let's consider one SNP in the population. The SNP has two alleles that could be called by their nucleotides, but with quantitative analyses in mind, we name the alleles in such a way that the **minor allele** (the one that is less common in the population) is called allele 1 and the **major allele** (the one that is more common in the population) is called allele 0. (There is no general rule that GWAS results are reported as allele 1 corresponding to the minor allele, and even if there was, the minor allele could differ between two data sets/populations, so consistency across studies needs always to be checked.) Let's denote the **minor allele frequency (MAF)** by f. Since each individual has two copies of the genome, there are individuals with three possible genetic types (called **genotypes**) at this SNP. We denote each genotype by the number of copies of allele 1 that the genotype contains (i.e. genotype can be 0, 1 or 2). If we assume that, at this SNP, the two alleles in one individual are sampled at random from the population, then the relative genotype frequencies in the population follow the binomial distribution $\mathrm{Bin}(2, f)$:

genotype	expected frequency from Bin(2,f)
0	$(1-f)^2$
1	$2\,f(1-f)$
2	f^2

These frequencies are called the **Hardy-Weinberg equilibrium** (HWE) genotype frequencies (or HW proportions) and they define the theoretical equilibrium genotype frequencies given the value of f in an ideal *randomly mating* population without *selection*, *migration*, or *genetic drift* (=statistical fluctuations due to finite population size). In practice, most variants in human populations do approximately follow HWE. Clear deviations from HWE could point to, for example, recent *population structure* (e.g. two populations have admixed), *assortative mating* (individuals tend to mate with partners of their kind) or natural selection (e.g. genotype 1 is very advantageous whereas genotype 2 is lethal and hence completely absent from the population). On the other hand, technical problems in *genotype calling* (i.e. in determining genotypes from the intensity measures from a genotyping chip) can also cause deviations from HWE, either because of bad quality data or because the variation is not a biallelic SNP and has more than two alleles (slide 13). Therefore, often variants which do not follow HW frequencies are excluded from many GWAS analyses as part of the *quality control* (QC) procedure.

Testing HWE. To test for (deviations from) HWE a one degree of freedom chi-square test can be used where the expected counts are derived assuming HWE given the allele frequencies.

Suppose that among N individuals from a population we have observed genotype counts n_0, n_1, n_2 for genotypes 0,1 and 2, respectively, with $N = n_0 + n_1 + n_2$. Our estimate for population frequency of allele 1 is $\widehat{f} = (n_1 + 2\,n_2)/(2\,N)$, and the expected genotype counts under HWE are $h_0 = N(1-\widehat{f})^2$, $h_1 = 2N\widehat{f}(1-\widehat{f})$ and $h_2 = N\widehat{f}^2$. The test statistic measures the deviation between the observed counts and the expected counts:

$$t_{HWE} = \sum_{i=0}^{2} \frac{(n_i - h_i)^2}{h_i}.$$

If HWE holds, then t_{HWE} follows approximately a chi-square distribution with 1 degree of freedom, which is used for deriving a P-value. If the theoretical chi-square distribution is used, the test is asymptotic and hence not necessarily valid for a small sample size or very rare variants, and a test statistic distribution based on permutations should be used, e.g., by using the R package HardyWeinberg.

Example 1.1. Look up the SNP rs429358 from the Ensmbl browser https://www.ensembl.org/. Choose 'Human' and type 'rs429358'; you'll see the variant's chromosome (19), position (44,908,684 in the genome version GRCh38 mentioned at top left) and the two alleles, T and C, of which C is predicted to be the 'ancestral' that is, the older allele, and C is also the minor allele with an average MAF of 15% across human populations. Next click 'Population Genetics' to see allele and genotype frequencies in different human populations. (Familiarize with given populations by hovering mouse above them.) Scrolling down, in the

1000 Genomes project Phase 3 (1000G) Finnish data, the minor allele C has frequency $37/198 \approx 18.7\%$ and the observed genotype counts are 66 (TT), 29 (TC) and 4 (CC) individuals. Let's use these values to visualize the genotype distribution and apply the standard test for HWE.

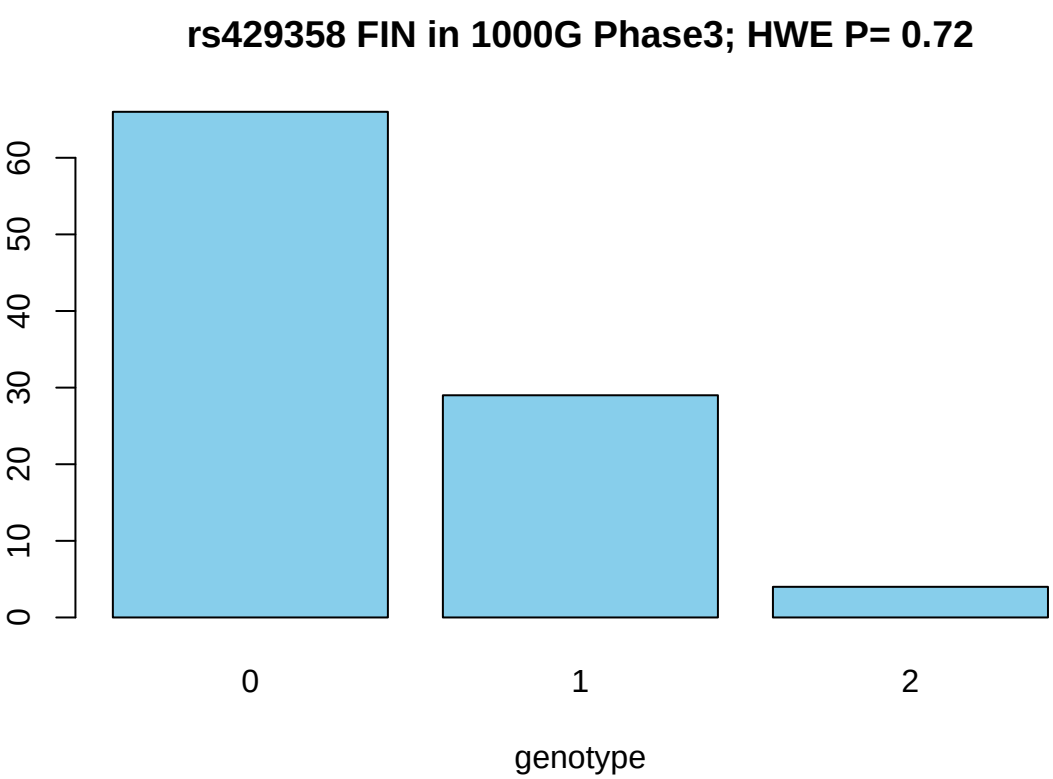
```
geno = c(66, 29, 4)
N = sum(geno) # number of individuals
f = sum(geno * c(0,1,2)) / (2*N) #(66*0 + 29*1 + 4*2) / (2*(66+29+4))
f # MAF
```

```
## [1] 0.1868687
```

```
hwe.prop = c( (1-f)^2, 2*f*(1-f), f^2) # these would be the genotype freqs under HWE
rbind(obs = geno/N, hwe = hwe.prop) # print the observed genotype freqs and HWE expectations
```

```
##          [,1]      [,2]       [,3]
## obs 0.6666667 0.2929293 0.04040404
## hwe 0.6611825 0.3038976 0.03491991
```

```
# For testing HWE we use chi-square test even though counts are quite small in last cell:
hwe.test = sum( (geno - N*hwe.prop)^2 / (N * hwe.prop)) # HWE test statistic
hwe.p = pchisq(hwe.test, df = 1, lower = FALSE) # P-value from the test
barplot(geno, main = paste("rs429358 FIN in 1000G Phase3; HWE P=", signif(hwe.p, 3)),
        names = c(0, 1, 2), xlab = "genotype", col = "skyblue")
```



To get familiar with how to generate realistic genotype data, let's also make example genotype data for $n = 1000$ additional Finns at this variant, both by sampling from the genotype frequencies and by sampling from the allele frequencies (assuming HWE). Since this variant seems to follow HWE, we do not expect qualitative differences between the two simulation approaches.

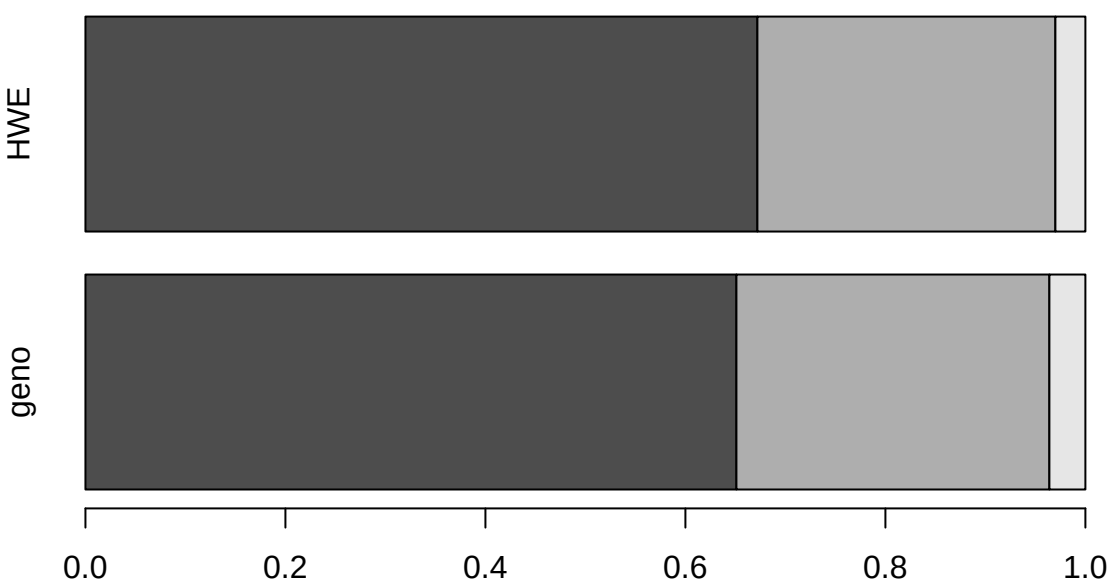
```r
set.seed(19) #setting seed guarantees the same simulation results every time this code is run
n = 1000
sample.from.geno = sample(c(0,1,2), prob = geno, size = n, replace = T) #sample from genotype frequenci
# replace = TRUE means sampling with replacement, that is,
# each genotype can be sampled many times, always with the same probabilities given in 'prob'
tab = table(sample.from.geno) # table() counts how many times each value is present
counts.from.geno = rep(0, 3) # How many carriers of each genotype?
counts.from.geno[ 1 + as.numeric( names(tab) )] = as.numeric(tab) #works even if some count is 0

# To sample from HWE frequencies, we could use:
# sample.from.hwe = sample(c(0, 1, 2), prob = c( (1-f)^2, 2*f*(1-f), f^2), size = n, replace = T)
# but a simpler way is to sample n genotypes directly from Bin(2,f) distribution:
sample.from.hwe = rbinom(n, size = 2, p = f)
counts.from.hwe = rep(0, 3) #Let's count how many carriers of each genotype
for(ii in 0:2){ #this is another way to do the counting compared to table() above
  counts.from.hwe[ii+1] = sum(sample.from.hwe == ii)}

rbind(geno = counts.from.geno / n, hwe = counts.from.hwe / n)
```

```
##       [,1]  [,2]  [,3]
## geno 0.651 0.313 0.036
## hwe  0.672 0.298 0.030
```

```r
barplot(cbind(counts.from.geno / n, counts.from.hwe / n),
        names = c("geno","HWE"), beside = F, horiz = T)
```



They *look* pretty similar but to do statistical inference we should also quantify the uncertainty of the estimates (e.g. by 95% intervals). For small counts, a Bayesian credible interval called *Jeffreys interval* behaves more consistently than the standard 95% confidence interval, whereas for larger counts the two approaches agree. Details of the two approaches are here.

Let's make Jeffreys intervals for the estimates of each genotype frequency in both of the data sets.

```
interval.from.geno = matrix(NA, ncol = 2, nrow = 3) #empty matrix
interval.from.hwe = matrix(NA, ncol = 2, nrow = 3)
for(ii in 1:3){ #find intervals while looping over 3 genotypes
  interval.from.geno[ii,] = qbeta(c(0.025, 0.975), counts.from.geno[ii] + 0.5, n-counts.from.geno[ii] +
  interval.from.hwe[ii,] = qbeta(c(0.025, 0.975), counts.from.hwe[ii] + 0.5, n-counts.from.hwe[ii] + 0.5
}
```

Now we can print out the observed genotype frequency (1st col) and its 95% interval (2nd and 3rd cols) for both data sets and compare whether the estimates seem similar given the uncertainty described by the intervals:

```
cbind(geno.est = counts.from.geno / n, interval.from.geno,
      hwe.est = counts.from.hwe / n, interval.from.hwe )
```

```
##      geno.est                        hwe.est
## [1,]    0.651 0.62105469 0.68007266   0.672 0.64243720 0.70056879
## [2,]    0.313 0.28483127 0.34224942   0.298 0.27026622 0.32690115
## [3,]    0.036 0.02576052 0.04891794   0.030 0.02074395 0.04196844
```

All estimates are within other data set's 95% credible interval and we have no reason to suspect frequency differences between the two data sets.

A standard two-sample chi-square test can also be carried out to quantify the frequency difference using a P-value:

```
chisq.test(rbind(counts.from.geno, counts.from.hwe)) # tests whether rows have same distribution
```

```
##
##  Pearson's Chi-squared test
##
## data:  rbind(counts.from.geno, counts.from.hwe)
## X-squared = 1.247, df = 2, p-value = 0.5361
```

Unsurprisingly, this does not indicate any frequency difference between the two data sets as P-value is large (0.5361). We'll come back to interpretation of P-values later.

1.2 What is a genome-wide association study?

Let's look at some recent examples of GWAS (slide 14-15). Two main types of GWAS are studying quantitative traits or disease phenotypes.

Example 1.2. QT-GWAS (slides 16-20) GWAS on body-mass index (BMI) by Locke et al. (2015) combined data of 339,000 individuals from 125 studies around the world to study the association of SNPs and BMI. It highlighted 97 regions of the genome with convincing statistical association with BMI. Pathway analyses provided support for a role of the central nervous system in obesity susceptibility and implicated new genes and pathways related to synaptic function, glutamate signalling, insulin secretion/action, energy metabolism, lipid biology and adipogenesis.

Example 1.3. Disease GWAS (slides 22-24) GWAS on migraine by Hautakangas et al. (2022) combined genetic data on 102,000 cases (individuals with migraine) and 771,000 controls (individuals with no known migraine) originating from 25 studies. Genetic data was available on millions of genetic variants. At each variant, the genotype distribution between cases and controls were compared. 123 regions of the

genome showed a convincing statistical association with migraine. Two of the 123 regions contained genes (namely *CALCA/CALCB* and *HTR1F*) that are targets of recent molecular therapies for migraine (namely CGRP-antibodies and ditans), which raises hopes that the remaining 121 could provide other clues for drug development. Downstream analyses combined the genes into pathways and cell types and highlighted enrichment of signals near genes that are active in vascular system but also those in central nervous system. This gives biological evidence that migraine is a neuro-vascular disorder, rather than only vascular or only neuronal.

Terms:

- **Monogenic** phenotype is determined by a single gene/locus.
- **Oligogenic** phenotype is influenced by a handful of genes/loci.
- **Polygenic** phenotype is influenced by many genes/loci.
- **Complex trait** is a (quantitative) phenotype that is not monogenic. Typically polygenic and also influenced by many environmental factors.
- **Common disease** is a disease/condition that is common in the population (say, prevalence of 0.1% or more). Examples: MS-disease (prevalence in the order of 0.1%), schizophrenia (~1%) or Type 2 diabetes (~10%).
- **Common variant** has frequency of at least 1%.
- **Low-frequency variant** has frequency of at least 0.1% and lower than a common variant.
- **Rare variant** has frequency lower than a low-frequency variant.

GWAS have shown us that, very generally, complex traits and common diseases are highly polygenic, and many common variants with each showing only a small effect size influence these phenotypes. We don't yet know which are the exact causal variants for each phenotype because of the correlation structure among genetic variants (this is the fine-mapping problem we'll look later). We also don't yet know very accurately how rare variants affect each phenotype because that would require very large sample sizes being studied by *genome sequencing* techniques, not only by SNP arrays.

1.2.1 Quantitative traits

Let's mimick the data we see on slide 4. The phenotype is LDL-cholesterol level and we assume that the trait distributions of individuals with 0, 1 or 2 copies of allele T at SNP rs11591147 are Normal distributions with SD=1 and with means of 0.02, -0.40 and -2.00, respectively. Allele T frequency is 4% in Finland. Let's simulate $n = 10,000$ individuals and boxplot them by genotype.

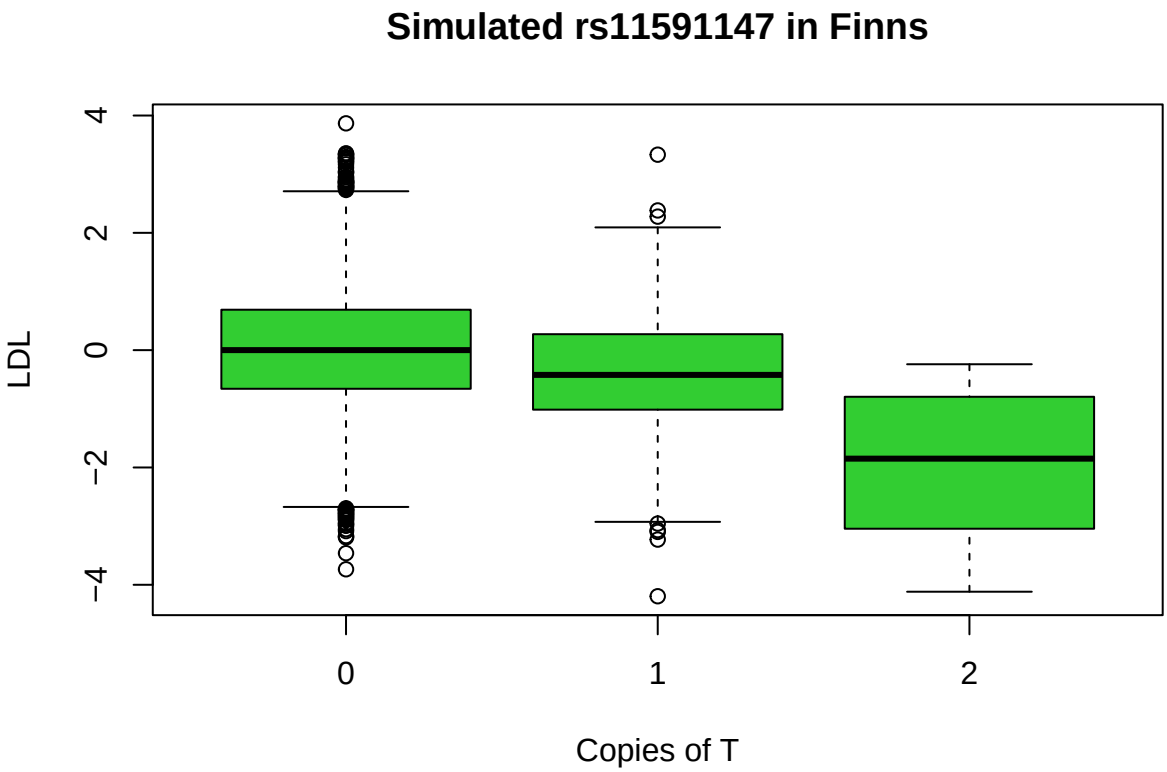
```
n = 10000
f = 0.04
mu = c(0.02, -0.40, -2.00) #mean of each genotype
sigma = c(1, 1, 1) #SD of each genotype

x = rbinom(n, size = 2, p = f) #genotypes for 'n' individuals assuming HWE
table(x)/n #(always check that simulated data is ok before starting to work with it!)
```

```
## x
##      0      1      2
## 0.9212 0.0772 0.0016
```

```
y = rep(NA,n) #make empty phenotype vector
for(ii in 0:2){ #go through each genotype group: 0, 1, 2.
  y[x == ii] = rnorm(sum(x == ii), mu[1+ii], sigma[1+ii]) } #generate trait for group ii

boxplot(y ~ x, main = "Simulated rs11591147 in Finns", ylab = "LDL",
        xlab = "Copies of T", col = "limegreen")
```



We see that the phenotype varies with genotype in such a way that each additional copy of allele T decreases the level of LDL.

Additive model The simplest way to analyze these data statistically is to use an **additive model**, that makes the assumption that the means of the groups depend additively on the number of allele 1 in the genotype, and that the SDs of the genotype groups are constant. Thus, we fit a linear model $y = \mu + x\beta + \varepsilon$, where y is the phenotype, x is the genotype (0,1 or 2) and parameters to be estimated are

- μ, the mean of genotype 0 and
- β, the effect of each copy of allele 1 on the mean phenotype.

The error terms ε are assumed to have an identical Normal distribution $N(0, \sigma^2)$ where σ^2 is not known and will be estimated from the data. Let's fit this linear model in R using `lm()`.

```
lm.fit = lm(y ~ x)
summary(lm.fit)
```

```
##
## Call:
## lm(formula = y ~ x)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.7634 -0.6652 -0.0119  0.6759  3.8529
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.01358    0.01032   1.316    0.188
```

```
## x           -0.44553    0.03570 -12.480   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9915 on 9998 degrees of freedom
## Multiple R-squared:  0.01534,    Adjusted R-squared:  0.01524
## F-statistic: 155.7 on 1 and 9998 DF,  p-value: < 2.2e-16
```

The `summary(lm.fit)` command produced

- parameter estimates (or Coefficients) $\widehat{\mu}$ and $\widehat{\beta}$,
- their standard errors (SE) (estimates for square root of the sampling variance of the parameter estimators),
- t-statistic (estimate/SE) and
- P-value under the null hypothesis that the parameter is 0 and the errors are uncorrelated and follow the distribution $N(0, \sigma^2)$.

Under the assumptions of linear model, the sampling distribution of the t-statistic is t-distribution and hence level q confidence intervals are determined as $\widehat{\beta} \pm a \times \text{SE}$, where a is the $(1-q)/2$ quantile of the t-distribution with $n-2$ degrees of freedom. When σ^2 is known, the t-distribution is replaced by a Normal distribution, and same is approximately true when n becomes large, even if the estimate $\widehat{\sigma^2}$ is used for computing SE. In these cases, we often talk about z-statistic instead of t-statistic. In GWAS analyses, we typically have thousands of samples and use z-scores and the Normal approximation by default.

The last paragraph in the output tells about the full model fit. We can measure how much variation in y is left unexplained by the model by computing **residual sum of squares** (RSS):

$$RSS = \sum_{i=1}^{n} \left(y_i - \widehat{\mu} - x_i \widehat{\beta} \right)^2 .$$

R^2 is the proportion of variance explained by the linear model, that is, one minus the proportion left unexplained:

$$R^2 = 1 - \frac{\frac{RSS}{n-1}}{\widehat{\text{Var}}(y)}.$$

An adjusted version of R^2 penalizes for additional predictors and is defined here as

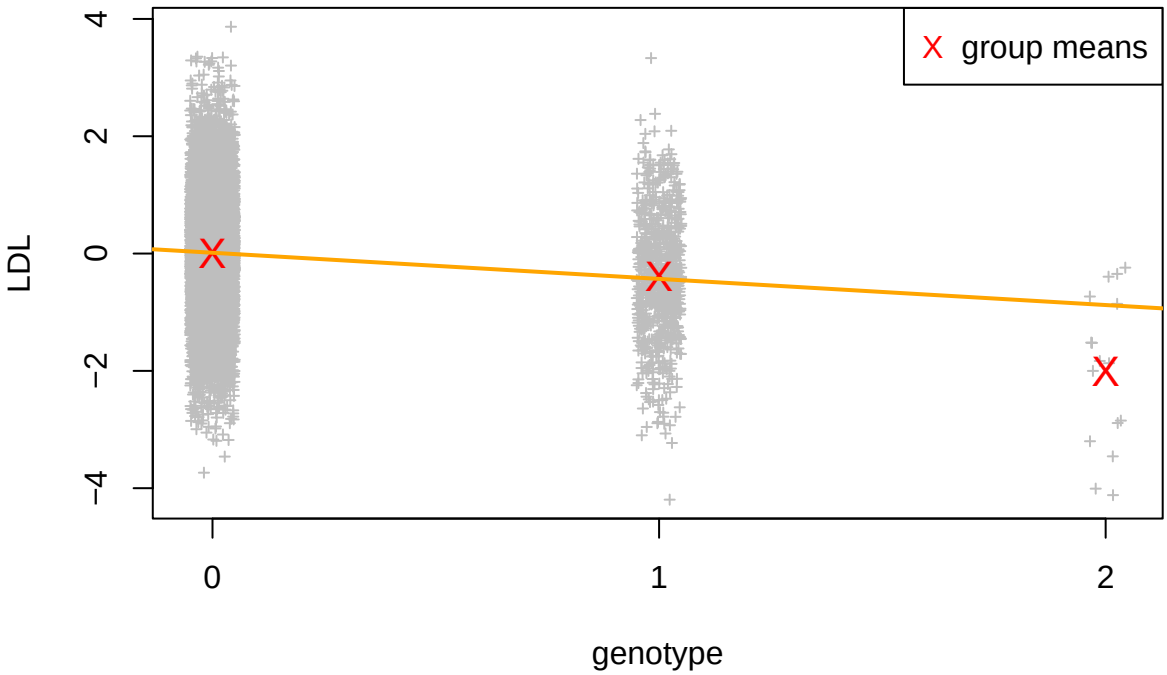
$$R^2_{adj} = 1 - \frac{\frac{RSS}{n-2}}{\widehat{\text{Var}}(y)}.$$

Note that if there is only the intercept parameter μ in the model, then $R^2 = R^2_{adj} = 0$, and if the model explains data perfectly ($RSS = 0$), then $R^2 = R^2_{adj} = 1$. In other cases, R^2 values are between 0 and 1 and larger values mean more variance explained by the model.

R^2 should not be the only measure used to judge how suitable the model is for the data. One should also plot the data and the model fit in different ways to assess this question. (Of course not for all variants in GWAS, but for the most interesting ones.) For this simple linear model, a scatter plot and a regression line is a good way to assess whether we observe any deviations from the assumption of additivity. Additionally, the differences in residual variation between the genotype groups could indicate interaction effects between the genetic variant and some other genetic or environmental variable.

```r
plot( x + runif(n, -0.05, 0.05), y, xlab = "genotype", ylab = "LDL", xaxt = "n",
      pch = 3, cex = 0.5, col = "gray")
#runif() adds some jitter to x so that all points are not on top of each other
```

```
axis(1, at = 0:2, labels = 0:2)
points(0:2, c(mean(y[x==0]), mean(y[x==1]), mean(y[x==2])), col = "red", pch = "X", cex = 1.3)
abline(lm.fit, col = "orange", lwd = 2)
legend("topright", pch = "X", legend ="group means", col = "red")
```



Conclusion: We see a statistically highly significant association between the genotype and phenotype where a copy of allele T decreases LDL levels by 0.45 units. This variant explains about 1.5% of the variation in LDL-cholesterol levels. We also see that individuals homozygous for allele T (genotype 2) have on average lower levels of LDL than the model predicts, which indicates a deviation from the additivity assumption. Let's next fit a full 2-parameter model to quantify this deviation.

Full model Let's add a new parameter γ to the model to describe the residual effect for group 2 after the additive effect β has been accounted for. The model is $y = \mu + x\beta + z\gamma + \varepsilon$, where z is the indicator of genotype 2, i.e., $z_i = 1$ if individual i has genotype 2 and otherwise $z_i = 0$. This is the full model, where the means of each of the three genotype groups can be determined freely as we have 3 free parameters (genotype 0: μ; genotype 1: $\mu + \beta$ and genotype 2: $\mu + 2\beta + \gamma$).

```
z = as.numeric( x == 2 ) #z is indicator for genotype group 2
lm.full = lm( y ~ x + z )
summary(lm.full)
```

```
##
## Call:
## lm(formula = y ~ x + z)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.8095 -0.6675 -0.0128  0.6760  3.8548
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)  0.01165    0.01032   1.129    0.259
## x           -0.39750    0.03711 -10.711  < 2e-16 ***
## z           -1.20614    0.25789  -4.677 2.95e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9905 on 9997 degrees of freedom
## Multiple R-squared:  0.01749,    Adjusted R-squared:  0.01729
## F-statistic: 88.97 on 2 and 9997 DF,  p-value: < 2.2e-16
```

It seems that also the new variable is useful (large effect compared to SE and small P-value). Now the interpretation of coefficients is that genotype 1 has average phenotype of -0.38 and genotype 2 has average phenotype $0.01 - 0.398 \cdot 2 - 1.206 = -1.99$.

Note also that the full model gives the same model fit and is simply a different parameterization of the linear regression model that treats the genotype as a factor with three levels.

```r
lm.full2 = lm( y ~ as.factor(x) )
summary(lm.full2)
```

```
##
## Call:
## lm(formula = y ~ as.factor(x))
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.8095 -0.6675 -0.0128  0.6760  3.8548
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.01165    0.01032   1.129    0.259
## as.factor(x)1 -0.39750    0.03711 -10.711  < 2e-16 ***
## as.factor(x)2 -2.00115    0.24784  -8.074 7.56e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9905 on 9997 degrees of freedom
## Multiple R-squared:  0.01749,    Adjusted R-squared:  0.01729
## F-statistic: 88.97 on 2 and 9997 DF,  p-value: < 2.2e-16
```

This second parameterization treats group 0 as the baseline and reports the deviations between the other two groups and the baseline as effect estimates. If we, instead, are interested to quantify how much deviation the data show from additivity, then the coefficient γ from the first parameterization is the most suitable.

The second parameterization is also the same model as the traditional Analysis of Variance (ANOVA). But here we want to work within the regression model framework rather than ANOVA framework for reasons that become clear once we start considering covariates and confounders.

Note that all these models make the same assumption that SD is constant across the genotype groups.

Current approach to quantitative phenotypes in GWAS Almost all GWAS analyze quantitative traits using the additive model, i.e., a linear regression model with a single parameter for genetic effect. The full model is typically used only for a small group of interesting variants identified by the additive model to check if they show deviations from additivity. The main reason for this is that the additive model is usually

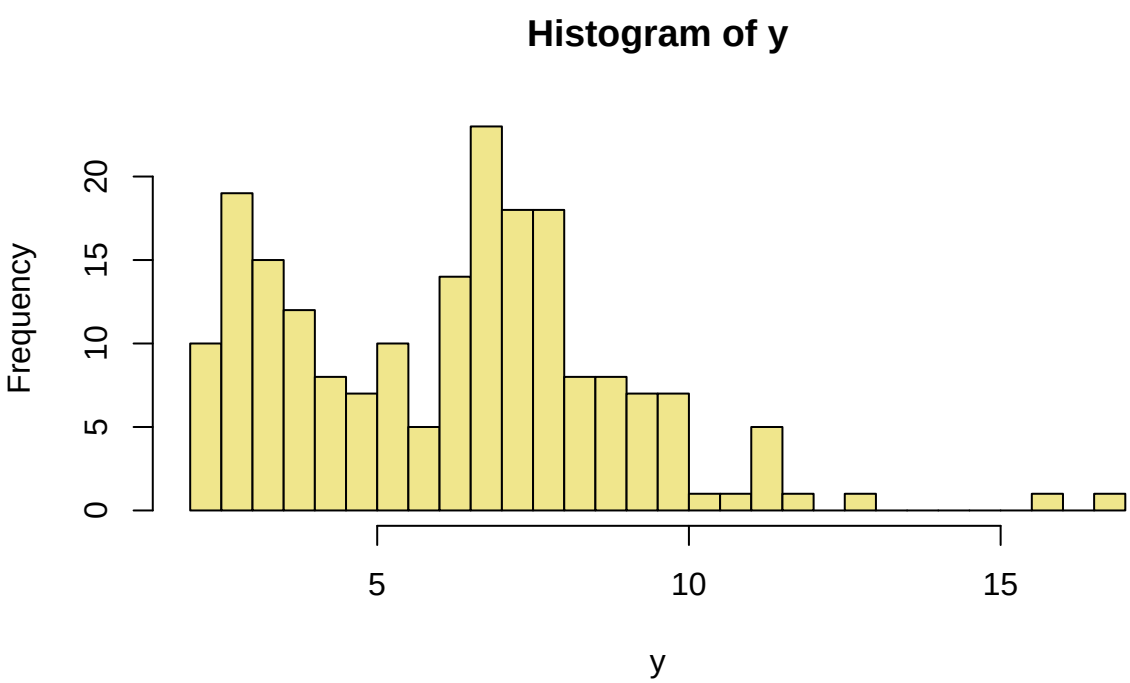
almost as powerful to find associations as the full model even when deviations from additivity are present in the data, since typically one of the genotype groups is much smaller than the other two and hence does not affect much the statistical model fit. Additionally, our current understanding is that most associations follow well the additive model and the additive model has more power than the full model when the additivity assumption is approximately true. (But note that our current understanding may be biased in favor of the additive model since we do not usually look very carefully for non-additive effects.) It would seem useful to run both the additive and full model in GWAS, but this is often not done because with millions of variants to be analyzed, there are a lot of results to handle already when only the simplest model is applied.

Quantile normalization (QN). Often in large GWAS the quantitative phenotype is forced to follow a Normal distribution by a procedure called *quantile normalization* or *inverse-Normal transformation*. This adds robustness to the analysis since possible phenotypic outliers have a smaller effect on the coefficients while we still keep in the data all the information provided by the ordering of the original trait values. This also harmonizes the trait distributions across multiple cohorts by forcing them to look similar.

To apply QN to a set of n trait values, we first regress out from the trait values the central covariates (such as sex and age) using a linear model. "Regressing out" means that we fit a linear model predicting the trait value using the covariates and collect the residuals from that model; the interpretation of the residuals is what remains from the trait values once the covariate effects have been removed. Then we order the residuals of the regression in ascending order $\widehat{r}_{(1)} \leq \ldots \leq \widehat{r}_{(n)}$. Now the QN'ed trait values for the sample is taken from the inverse of the cumulative distribution function of the Normal distribution at n equally spaced values between 0 and 1, and the resulting values $q_1 \leq \ldots \leq q_n$ are matched to the individuals so that the value q_i becomes the trait value for individual who corresponds to residual $\widehat{r}_{(i)}$. Advantage of QN is robustness to outliers and to systematic differences in measurements between studies. A disadvantage is that we lose some information of the phenotype distribution, which could have been useful if it were modeled properly.

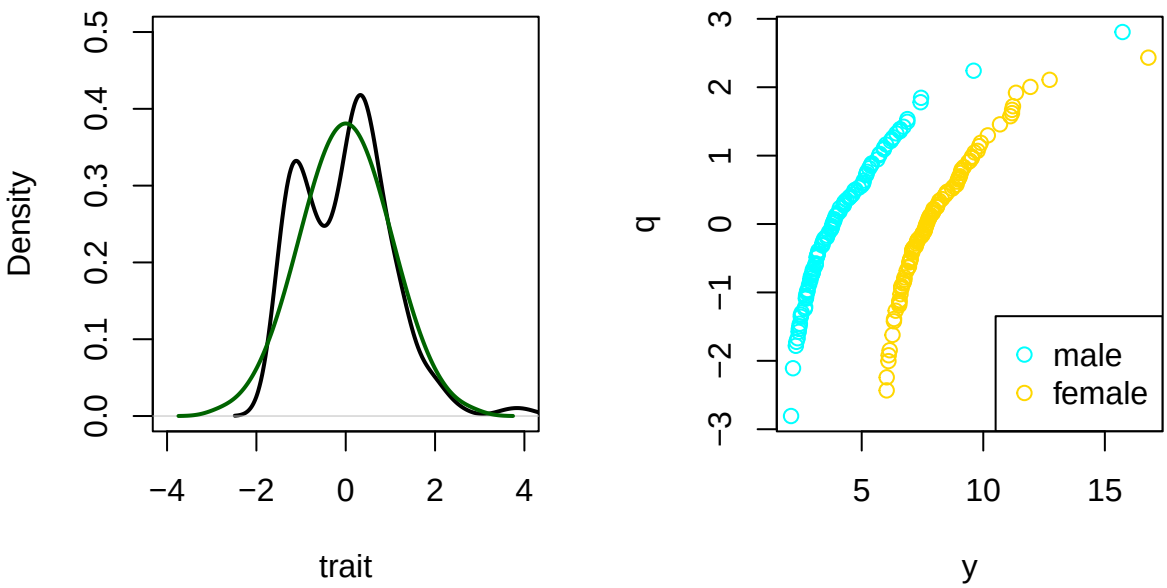
For an example, let's do QN for 100 males and 100 females where the phenotype in males follows $2 + \text{Gamma}(\text{shape} = 1.5, \text{scale} = 1.5)$ and in females $6 + \text{Gamma}(\text{shape} = 1.5, \text{scale} = 1.5)$.

```
n = 200 #males + females
fem = rep( c(0,1), each = n/2) #who is female
y = 2 + rgamma(n, shape = 1.5, scale = 1.5) #males have shift of 2
y[fem == 1] = 4 + y[fem == 1] #females have shift of 6 = 2 + 4
hist(y, breaks = 30, col = "khaki") #shows some outliers compared to mixture of 2 Normals
```



```r
#regress out sex and take residuals
lm.fit = lm(y ~ fem)
r = residuals(lm.fit)

#find QN'ed trait values from qnorm = inverse of cumulative distribution of Normal
inv.normalise <- function(x) { #this would also tolerate NAs
  return( qnorm( (rank(x, na.last = "keep") - 0.5) / sum(!is.na(x))))}
q = inv.normalise(r)
#Let's plot y and q (after scaling to mean=0, var=1)
par(mfrow = c(1,2))
plot(density(scale(y)), col = "black", lwd = 2, xlim = c(-4,4), ylim = c(0,0.5),
     xlab = "trait", main = "" )
lines(density(scale(q)), col = "darkgreen", lwd = 2)
plot(y, q, col = c("cyan","gold")[1+fem])
legend("bottomright", col = c("cyan","gold"), pch = 1, leg = c("male","female") )
```



We see that (right plot) the outliers on the original scale (x-axis) are still the most extreme values in QN-scale on y-axis, but they are now close to the other values and not anymore outliers. The QN'ed trait, obviously, looks perfectly Normal (green curve on left plot) while in the original trait we see outliers on right tail. We also see that sex caused original y to have a two modal distribution with peaks roughly at -1 and +1 on standardized scale, but that the effect of sex was removed by the regression step **before** QN was applied.

1.2.2 Binary phenotypes

How do we look for the association between a genotype and a binary disease status? Here the phenotype is 0-1 indicator whether an individual is a **case** (y=1; has the disease) or a **control** (y=0; does not have a disease). If we take a practical approach, we could just apply the linear regression model to binary outcome variable but, conceptually, this does not feel right because the error terms cannot be assumed to be normally distributed. Instead, we will use a generalized linear model called **logistic regression**. To derive the model, let's first define the effect size parameter for binary outcomes.

Relative risk and odds ratio To measure whether a genotype associates with disease status, we consider a **relative risk** (RR) parameter for genotype 1

$$\lambda_1 = \frac{\Pr(Y = 1 \,|\, X = 1)}{\Pr(Y = 1 \,|\, X = 0)}.$$

Thus λ_1 tells how many times larger the risk of getting the disease is for individuals with genotype 1 compared to individuals with genotype 0. If

- $\lambda_1 = 1$, there is no association with disease,
- $\lambda_1 > 1$, genotype 1 confers risk for disease,
- $\lambda_1 < 1$, genotype 1 confers protection from disease.

Similarly we define relative risk λ_2 as the factor by which genotype 2 multiplies the disease risk of genotype 0.

Relative risk is an intuitive measure but it turns out not to be very easy to estimate in the regression setting, particularly in the typical GWAS setting. However, if we modify the parameter slightly from comparing risks to comparing odds, we will get a measure that can be estimated in practice. The odds corresponding to a probability value p are defined as $p/(1-p)$, i.e., the odds tell how many times as probable the event is to occur than it is to not occur. If probability of a disease is $p = 50\%$, then the odds of disease are 1, if $p = 1\%$ then odds are $1/99 = 0.0101$ and if $p = 99\%$ then odds are 99. Just like with RR we measured the *relative* increase in risk between two genotypes, with odds we use a relative measure called **odds ratio (OR)** to describe relative increase in odds between genotypes. Thus, the odds ratio for genotype 1 is

$$\mathrm{OR}_1 = \frac{\Pr(Y = 1 \,|\, X = 1)}{\Pr(Y = 0 \,|\, X = 1)} : \frac{\Pr(Y = 1 \,|\, X = 0)}{\Pr(Y = 0 \,|\, X = 0)} = \frac{\Pr(Y = 1 \,|\, X = 1)\Pr(Y = 0 \,|\, X = 0)}{\Pr(Y = 1 \,|\, X = 0)\Pr(Y = 0 \,|\, X = 1)}.$$

By Bayes formula we can swap the roles of Y and X in the above formula, e.g., by writing $\Pr(Y = 1 \,|\, X = 1) = \Pr(X = 1 \,|\, Y = 1)\Pr(Y = 1) \,/\, \Pr(X = 1)$. By applying this to all 4 terms, we get

$$\mathrm{OR}_1 = \frac{\Pr(X = 1 \,|\, Y = 1)\Pr(Y = 1)\Pr(X = 0)\Pr(X = 0 \,|\, Y = 0)\Pr(Y = 0)\Pr(X = 1)}{\Pr(X = 0 \,|\, Y = 1)\Pr(Y = 1)\Pr(X = 1)\Pr(X = 1 \,|\, Y = 0)\Pr(Y = 0)\Pr(X = 0)} = \frac{\Pr(X = 1 \,|\, Y = 1)\Pr(X = 0 \,|\, Y =}{\Pr(X = 0 \,|\, Y = 1)\Pr(X = 1 \,|\, Y =}$$

This shows that we can estimate our target odds ratio for the disease between different genotypes equally well by collecting individuals based on disease status and observing their genotype distributions. This is the important property why odds ratios are possible to measure in a typical disease GWAS that collects individuals by disease status and then measures the genotypes.

Example 1.4. OR and risk. Suppose that we know that there is a strong risk variant for Alzheimer's disease (AD) with OR=3.0 (for one copy of the risk allele). Suppose that non-carriers of the risk allele have a lifetime risk of 15% of AD. How large is the lifetime risk for carriers of one or two copies of the risk variant?

Let $p_0 = 0.15$ be the risk in non-carriers. We know that odds of carriers of one copy are $p_1/(1-p_1) = 3.0 \cdot p_0/(1-p_0) = 3.0 \cdot 0.15/0.85 = 0.5294$. Hence the risk is $p_1 = 0.5294/(1+0.5294) = 0.346$. That is, 35%.

For carriers of two copies, $p_2/(1-p_2) = 3.0 \cdot p_1/(1-p_1) = 3.0 \cdot 0.5294 = 1.5882$ and $p_2 = 1.5882/(1+1.5882) = 0.61363$. That is, 61%.

Inference for OR based on counts In practice, GWAS analyses for disease studies are done using regression models since they have a possibility to account for confounding covariates. However, sometimes it is also useful to be able to quickly estimate OR parameters and their uncertainty using count data without access to regression models. But remember that such estimates cannot be adjusted for covariates and therefore can be biased by confounding factors.

Suppose we have observed the following genotype counts:

group	genotype 0	genotype 1	genotype 2
cases	S_0	S_1	S_2
controls	R_0	R_1	R_2

Then the estimates for odds-ratio between genotype 1 and genotype 0, its logarithm and the standard error of the logOR are

$$\widehat{\mathrm{OR}}_1 = \frac{S_1 R_0}{S_0 R_1}, \qquad \log\left(\widehat{\mathrm{OR}}_1\right) = \log\left(\frac{S_1 R_0}{S_0 R_1}\right) \qquad \text{and} \qquad \mathrm{SE}\left(\log\left(\widehat{\mathrm{OR}}_1\right)\right) = \sqrt{\frac{1}{R_1} + \frac{1}{R_0} + \frac{1}{S_1} + \frac{1}{S_0}}.$$

In particular, standard error can only be calculated for logOR and not for OR because only the sampling distribution of logOR is approximately Normal.

The 95% confidence interval for logOR is, naturally, $(\mathrm{logOR} - 1.96 \cdot \mathrm{SE}, \mathrm{logOR} + 1.96 \cdot \mathrm{SE})$, and 95% CI for OR is $(\exp(\mathrm{logOR} - 1.96 \cdot \mathrm{SE}), \exp(\mathrm{logOR} + 1.96 \cdot \mathrm{SE}))$. Thus, the endpoints of the 95%CI must always be computed on the log-odds scale and then transformed to the OR scale.

Similar inference can be done for the OR_2 parameter measuring the odds-ratio between genotypes 2 and 0 by substituting the counts of genotype 1 with the counts of genotype 2 in the formulas above.

If any counts are very small, or even 0, the SE is not reliable. One can add a value of 0.5 to each of the observed counts to get an OR estimate even in these case, but one shouldn't rely on the SE estimate.

Logistic regression Logistic regression model takes the place of linear regression as the basic GWAS model when the phenotype is binary. It explains the logarithm of the odds of the disease by the genotype. The simplest model is the **additive** model:

$$\log\left(\frac{\Pr(Y = 1 \,|\, X = x)}{\Pr(Y = 0 \,|\, X = x)}\right) = \mu + x\beta.$$

Thus, μ is the logarithm of odds ('log-odds') for genotype 0 and β is the log of odds ratio (logOR) between genotype 1 and 0 (and $\exp(\beta)$ is the corresponding odds ratio). Similarly, 2β is the logOR between genotypes 2 and 0. This model is additive on the log-odds scale and hence multiplicative on the odds scale. Due to this duality, it is sometimes called additive model and sometimes called multiplicative model, which is a source of confusion. In these notes, it is called the additive model. In R, such a logistic regression model can be fitted by command `glm(y ~ x, family = "binomial")`.

To try out logistic regression, we should learn how to simulate some case-control data that follow the logistic regression model.

Example 1.5. Let's assume that our risk allele A has frequency 13% in controls, and that it is in HWE in controls. If the risk model is additive on log-odds scale with odds-ratio 1.43 per each copy of allele A, what are the genotype frequencies in cases?

Let's denote case frequencies by f_0, f_1, f_2 and control frequencies by q_0, q_1, q_2. From formulas above, we get that

$$f_1 = \Pr(A = 1 \,|\, Y = 1) = \mathrm{OR}_1 \frac{\Pr(A = 1 \,|\, Y = 0)}{\Pr(A = 0 \,|\, Y = 0)} \Pr(A = 0 \,|\, Y = 1) = \mathrm{OR}_1 \frac{q_1 f_0}{q_0},$$

$$f_2 = \Pr(A = 2 \,|\, Y = 1) = \mathrm{OR}_2 \frac{\Pr(A = 2 \,|\, Y = 0)}{\Pr(A = 0 \,|\, Y = 0)} \Pr(A = 0 \,|\, Y = 1) = \mathrm{OR}_2 \frac{q_2 f_0}{q_0}.$$

Since $f_0 + f_1 + f_2 = 1$, we get

$$f_0 = \left(1 + \mathrm{OR}_1 \frac{q_1}{q_0} + \mathrm{OR}_2 \frac{q_2}{q_0}\right)^{-1}.$$

Now we can compute the genotype frequencies in cases. (Note $\mathrm{OR}_2 = \mathrm{OR}_1^2$ under the additive model).

Let's write a function that computes the case and control frequencies given control allele frequencies and OR.

```r
case.control.freqs <- function(q, or){
  #if dimension of 'q' is 1 then 'q' is taken as allele 1 freq. in controls and HWE is assumed in contr
  #if dimension of 'q' is 3 then 'q' is taken as the genotype (0,1,2) frequencies in controls
  #if dimension of 'or' is 1, then 'or' is per each copy of allele 1
  #if dimension of 'or' is 2, then 'or[1]' is for genotype 1 vs.0 and 'or[2]' is geno 2 vs. 0

  if(length(q) == 1) q = c((1-q)^2, 2*q*(1-q), q^2) # assumes HWE in controls
  stopifnot(length(q) == 3)
  if(length(or) == 1) or = c(or, or^2)
  stopifnot(length(or) == 2)

  f = q / q[1] * c(1, or)
  f = f / sum(f)

  data.frame(cases = f, controls = q, row.names = c(0,1,2))
}
```

```r
or = 1.43
a.cntrl = 0.13
cc.f = case.control.freqs(a.cntrl, or)
cc.f
```

```
##        cases controls
## 0 0.67887986   0.7569
## 1 0.29012360   0.2262
## 2 0.03099654   0.0169
```

Let's generate 2000 cases and controls from these genotype frequencies and estimate the genetic effect using logistic regression.

```r
n = 2000
x.cases = sample(c(0, 1, 2), prob = cc.f$cases, size = n, replace = T)
x.controls = sample(c(0, 1, 2), prob = cc.f$controls, size = n, replace = T)
x = c(x.cases, x.controls) #genotypes of all samples
y = c(rep(1, n), rep(0, n)) #binary phenotype corresponding to genotypes: 1st cases, then controls
glm.fit = glm(y ~ x, family = "binomial")
summary(glm.fit)
```

```
##
## Call:
## glm(formula = y ~ x, family = "binomial")
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.4894  -1.1252  -0.1153   1.2305   1.2305
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.12409    0.03685  -3.368 0.000758 ***
## x            0.41655    0.06360   6.550 5.75e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 5545.2  on 3999  degrees of freedom
## Residual deviance: 5501.3  on 3998  degrees of freedom
## AIC: 5505.3
##
## Number of Fisher Scoring iterations: 4
```

What is the estimate and 95%CI on odds ratio scale?

```
b = summary(glm.fit)$coeff[2,1] #estimate, beta-hat
se = summary(glm.fit)$coeff[2,2] #standard error
#endpoints computed on logOR scale, then transformed to OR scale:
c(or.est = exp(b), low95 = exp(b - 1.96*se), up95 = exp(b + 1.96*se))
```

```
##   or.est    low95     up95
## 1.516720 1.338973 1.718063
```

Let's compare the result from the additive logistic regression model to the results that we get from the raw genotype counts between the genotypes 1 and 0 using the formulas above (see "Inference for OR based on counts"):

```
s1 = sum(x.cases == 1); s0 = sum(x.cases == 0); r1 = sum(x.controls == 1); r0 = sum(x.controls == 0)
or.1.counts = s1*r0 / (s0*r1)
se.1.counts = sqrt(sum( 1 / c(s1, s0, r1, r0) ) )
c(or.est = or.1.counts,
  low95 = exp(log(or.1.counts) - 1.96 * se.1.counts),
  up95 = exp(log(or.1.counts) + 1.96 * se.1.counts) )
```

```
##   or.est    low95     up95
## 1.580529 1.367507 1.826735
```

These are a bit different from the additive model results above. The reason is that the additive model above uses also the data from individuals with genotype 2 to estimate the OR parameter while the formulas do not. It turns out that if we applied the additive regression model only to the individuals having either genotype 0 or genotype 1, then we would get essentially the same results as we get from the raw counts:

```
glm.fit = glm(y[x != 2] ~ x[x != 2], family = "binomial")
b = summary(glm.fit)$coeff[2,1] #estimate, beta-hat
se = summary(glm.fit)$coeff[2,2] #standard error
exp(c( b, b - 1.96 * se, b + 1.96 * se ))
```

```
## [1] 1.580529 1.367512 1.826729
```

Remember that even though the count based inference and logistic regression inference based on additive model gives similar results here, they may give very different results when there are confounding covariates included in the regression model, and in such situations, we trust more the regression model results. We come back to this later on the material.

Similarly to the quantitative phenotypes, we can use the full model also for the binary data:

```r
z = as.numeric( x == 2 )
glm.full = glm( y ~ x + z, family = "binomial")
summary(glm.full)
```

```
##
## Call:
## glm(formula = y ~ x + z, family = "binomial")
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -1.40059  -1.12268  -0.07657   1.23314   1.23314
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.13011    0.03726  -3.492 0.000479 ***
## x            0.45776    0.07386   6.197 5.74e-10 ***
## z           -0.27459    0.24920  -1.102 0.270526
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 5545.2  on 3999  degrees of freedom
## Residual deviance: 5500.1  on 3997  degrees of freedom
## AIC: 5506.1
##
## Number of Fisher Scoring iterations: 4
```

In this example, we have no deviation from additivity as the data were simulated assuming the additive model.

Ascertained case-control studies Suppose we are studying MS disease whose prevalence is about 1/1000. Even if we collect 100,000 individuals from the population, we still would get only about 100 cases! In ascertained case-control studies we enrich cases by collecting a case sample directly from the diseased people and similarly we collect controls either from the general population or, even better, from the individuals we know are disease free. (For diseases with prevalence $< 1\%$ there is little difference between these two control ascertainment strategies.) Thus, by a phenotype-based ascertainment, we may have a GWAS of 10,000 individuals that is divided into sets of 5,000 cases and 5,000 controls. This approach gives much higher statistical power to detect associations than a population collection of 100 cases and ~100,000 controls. (We will do power analyses later on the course.)

Can we analyse such ascertained case-control samples using the same logistic regression model as we applied above or does the ascertainment cause some issues? The answer is yes we can. The parameter β is the logOR and we showed earlier that this parameter can be estimated also by ascertaining individuals based on their phenotypes and similar result also extends to the use of logistic regression. However, the parameter μ that determines the absolute odds of disease for genotype class 0 depends on the sampling strategy, i.e., on the proportion of cases in the data. Thus, in ascertained data, μ does NOT estimate the population prevalence parameter of the genotype group 0. However, we can still apply the logistic regression model to the ascertained case-control sample and estimate the three central association statistics: genetic effect β, its uncertainty (standard error), and P-value.

GWAS software As current GWAS consider 100,000s of individuals and millions of variants, those analyses are done with specialized software packages that read in specific file formats. The most popular software

is PLINK in its recent, efficient version 2.0. Another widely used software is SNPTEST.

On this course, we do not focus on the commands or input file formats of any particular GWAS software, since that alone would take already all our time and the software packages are in constant development, which means that data input formats and commands tend to change over time. Instead, the goal of this course is to understand why each analysis is done and how to interpret the output from analyses, especially from a statistical point of view. These skills are independent of any particular GWAS software.