Data Quality Control (QC) in Association Studies

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Introduction

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  - decrease in power
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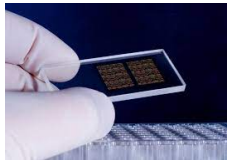
- Tutorials
  - Anderson et al. Nature Protocols 2010, doi:10.1038/nprot.2010.116
  - Turner et al. Curr Protoc Hum Genet. 2011. doi:10.1002/0471142905.hg0119s68
  - Marees et al. Int J Methods Psychiatr Res. 2018. doi: 10.1002/mpr.1608

Genotype Calling

illumina



affymetrix

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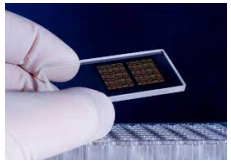

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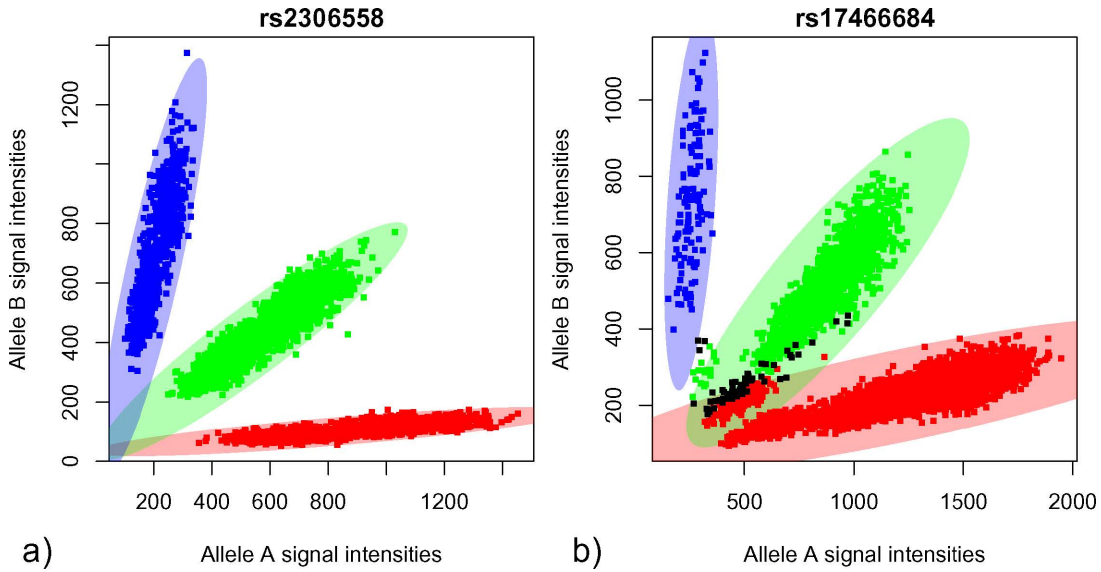

- Examples of cluster plots for two SNPs. One spot corresponds to one sample.
- Samples with genotypes AA and BB are red and blue, respectively. Heterozygous samples are shown in green; samples with **missing genotypes are black**. The ellipses represent the cluster boundaries as computed by ACPA.
- **a)** No samples in overlapping ellipses; **b)** Red samples lie in the green ellipse. At the bottom of the green ellipse, samples have been erroneously classified as red samples.

Schillert et al. BMC Proceedings 2009, 3(Suppl 7):S58 doi: 10.1186/1753-6561-3-S7-S58

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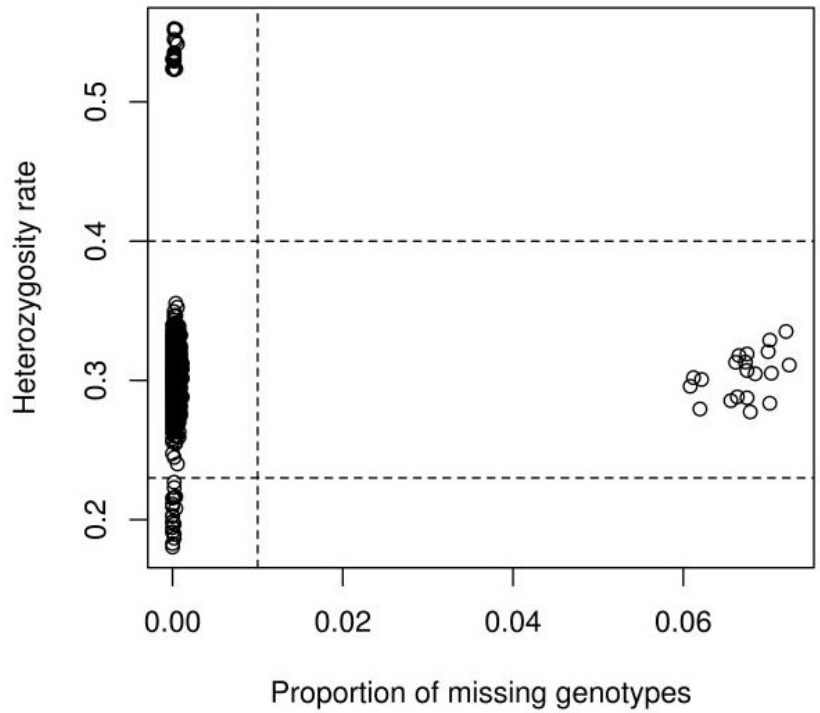
- Outlying population ancestry
  - confounding due to population structure

Call Rate and Heterozygosity

- There are samples with high levels of missing data and samples with unusually high and low heterozygosity



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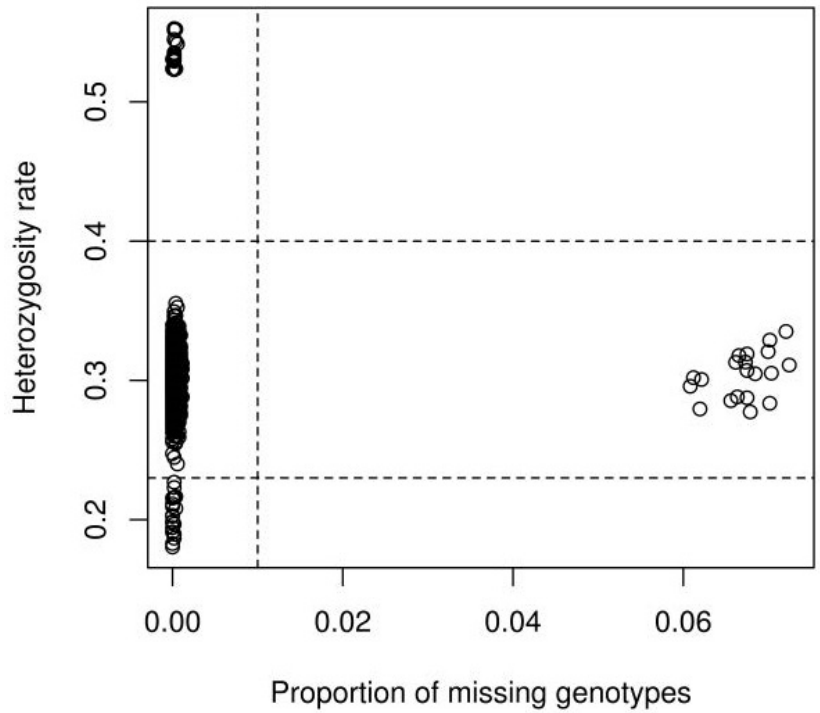

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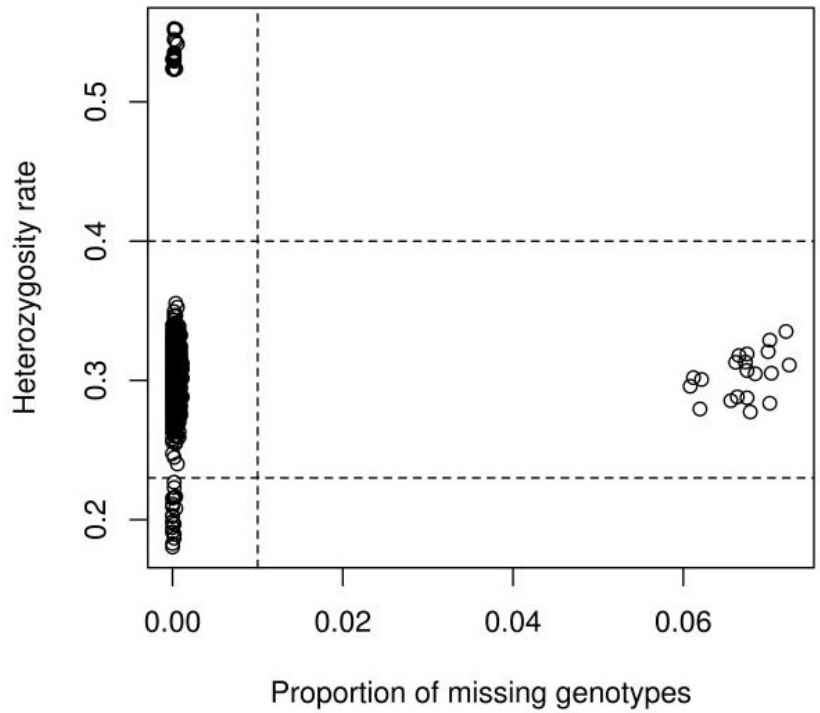

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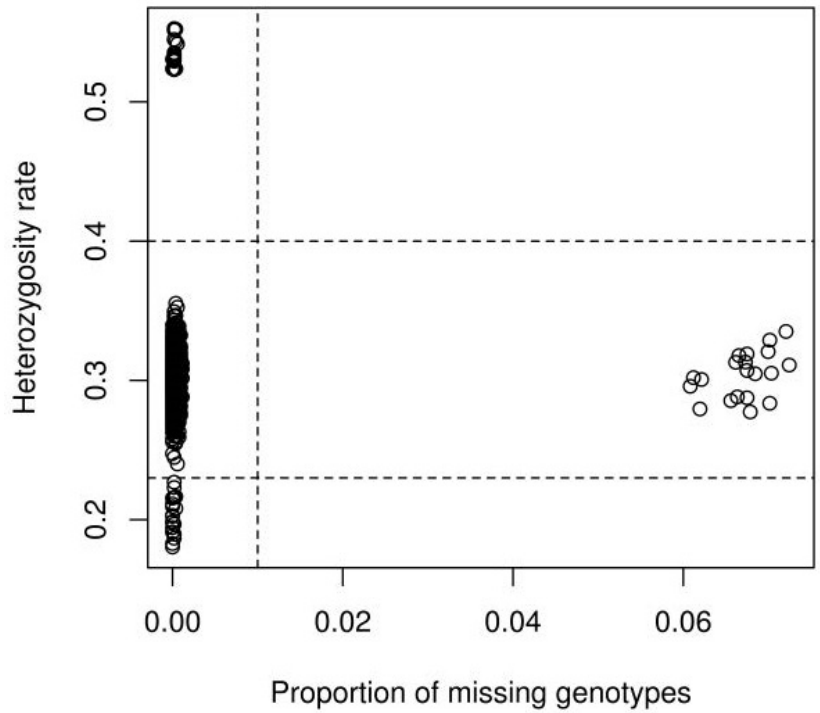

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- Dashed lines denote QC thresholds (exclude samples with missing call rate > 0.1, and samples with heterozygosity rate < 0.23 and > 0.4)
- Rule of thumb: remove individuals who deviate $\pm$ 3 SD from the samples' heterozygosity rate mean

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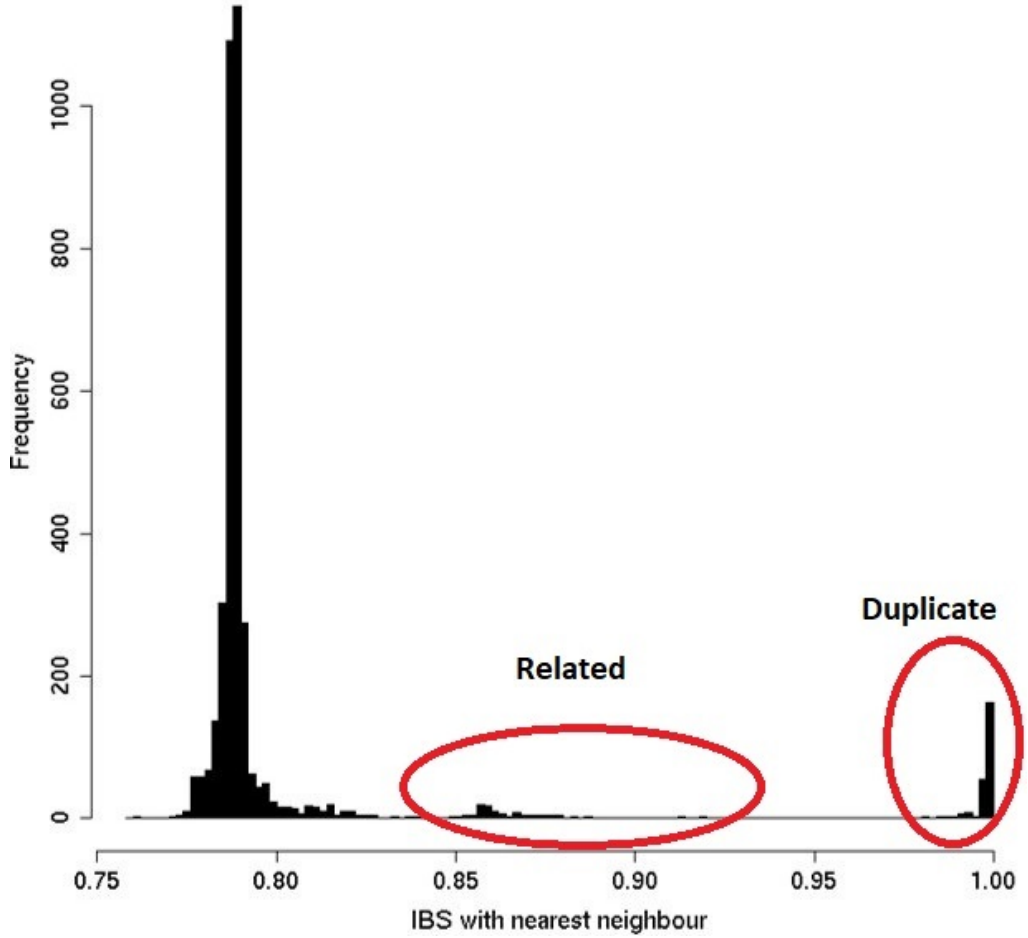
- Common to plot histogram of IBS of each individual with "nearest neighbour"

IBS Distribution



- For each individual, the distance to its nearest neighbour is calculated
- Remove one sample from each duplicate or related pair (usually one with lowest call rate)
- Alternative: take account of relatedness in analysis
- The absolute amount of IBS sharing depends on allele frequencies in the population
- Methods that estimate kinship or relatedness coefficients typically aim for estimating identity-by-descent (IBD)

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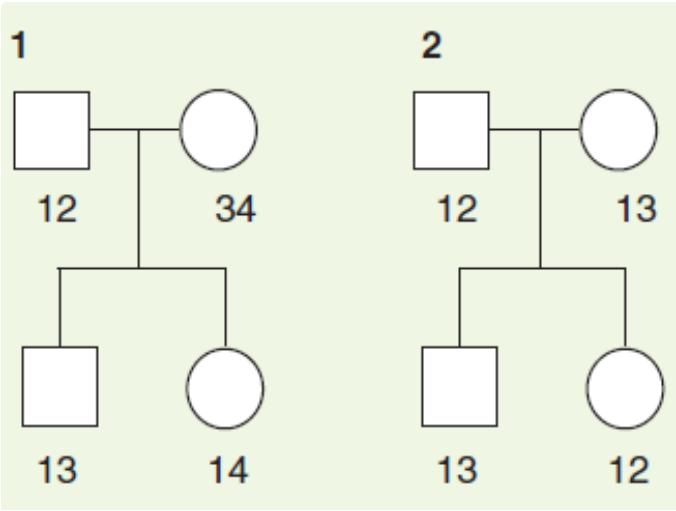

- Pedigree 1: Siblings share allele 1 IBD (inherited from the father)
- Pedigree 2: Siblings share allele 1 IBS (inherited from different parents)

Forabosco et al. Expert Rev. Mol. Diagn. 5(5), (2005). doi: 10.1586/14737159.5.5.781

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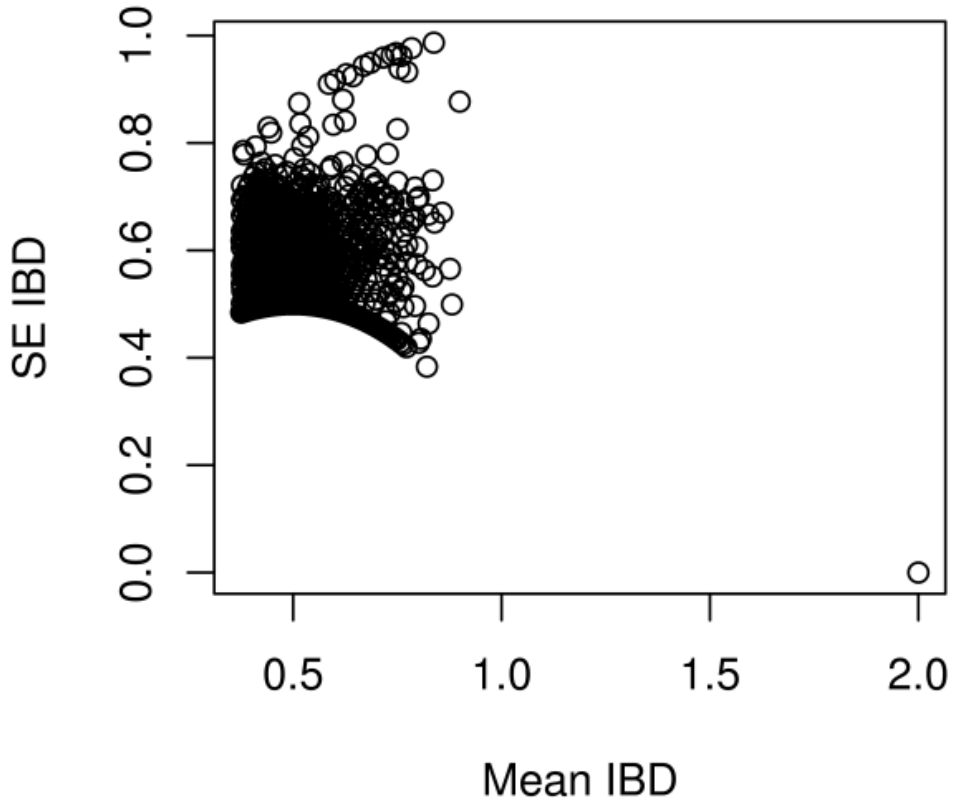
- Prune the data for LD before assessing IBD
  - shared region of high LD results in more shared variants than one of low LD, even if the two regions are the same size

IBD Plot



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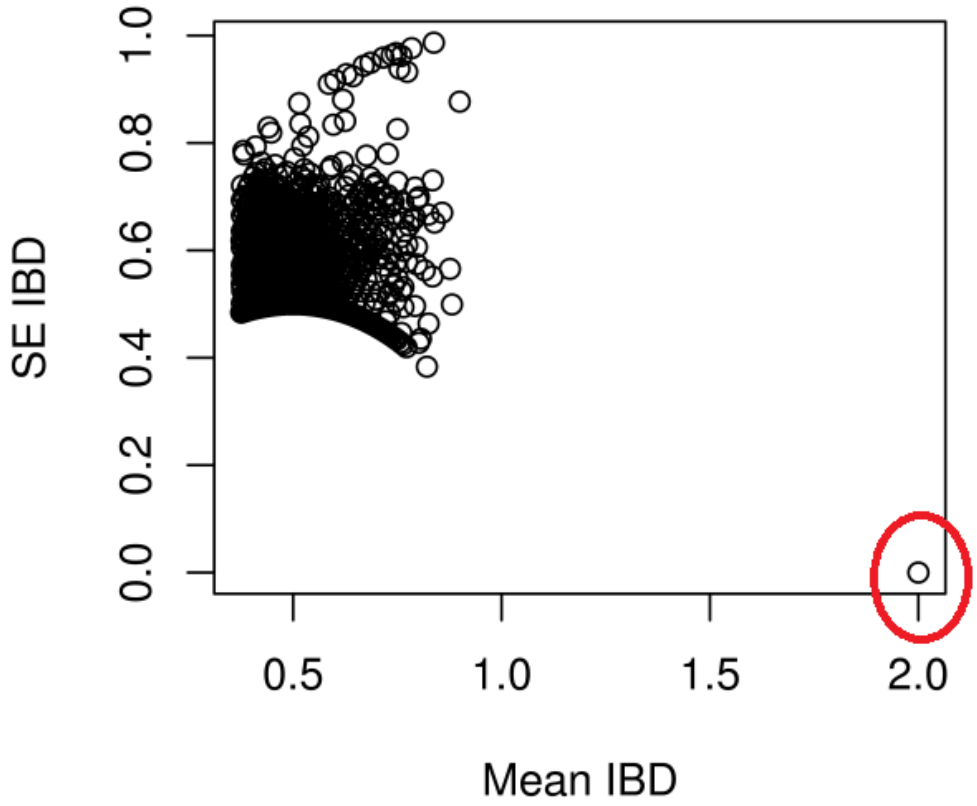

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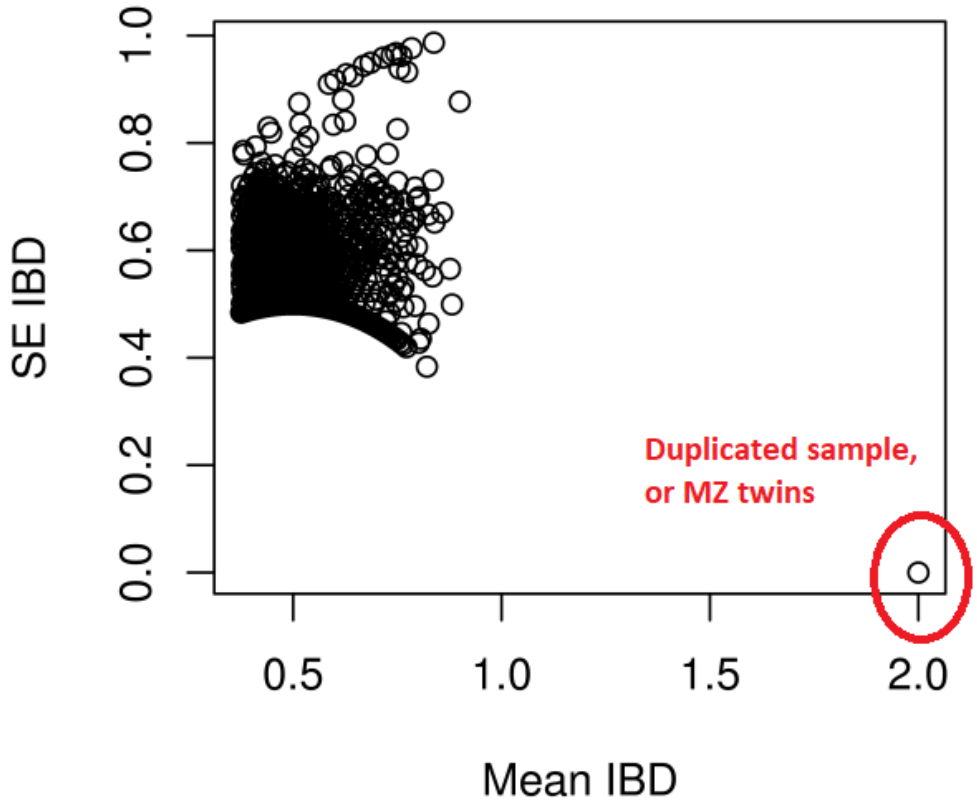

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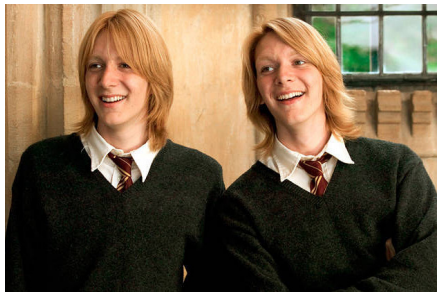


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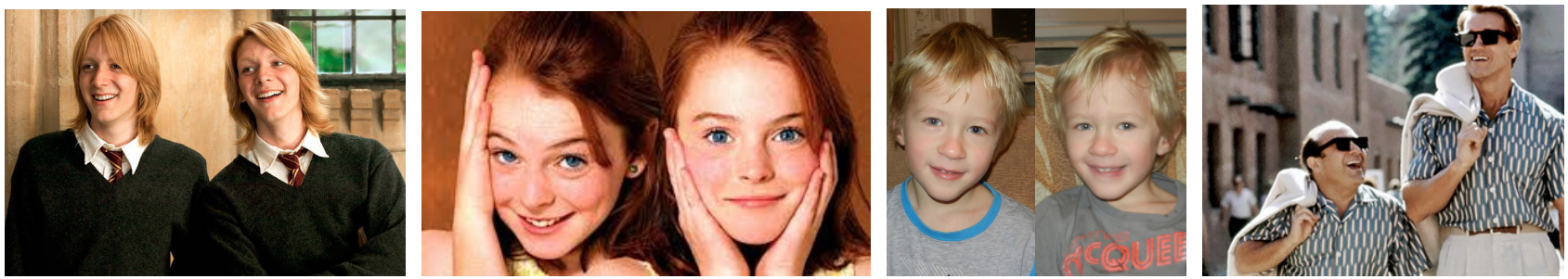

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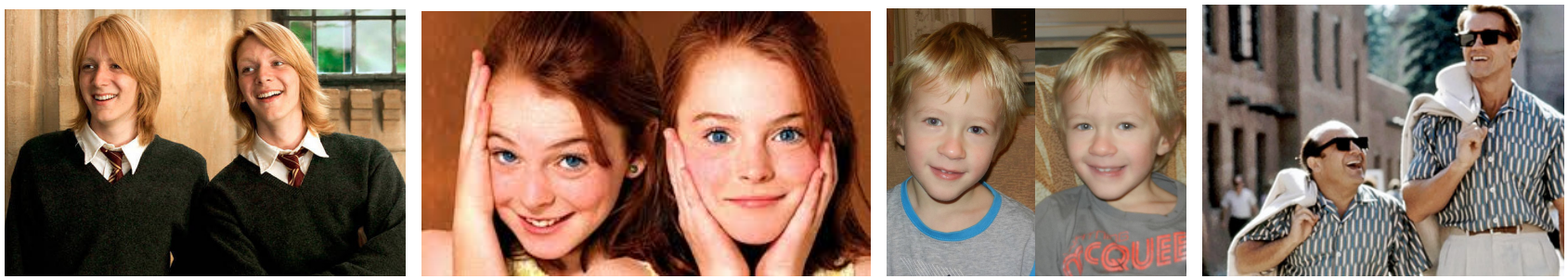

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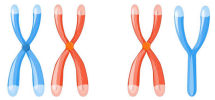
- Mixed modelling approaches account for "relatedness" between individuals (families, cryptic relatedness, population structure) by allowing for kinship

Gender Check - X Chromosome



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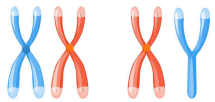

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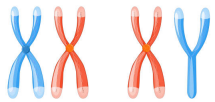

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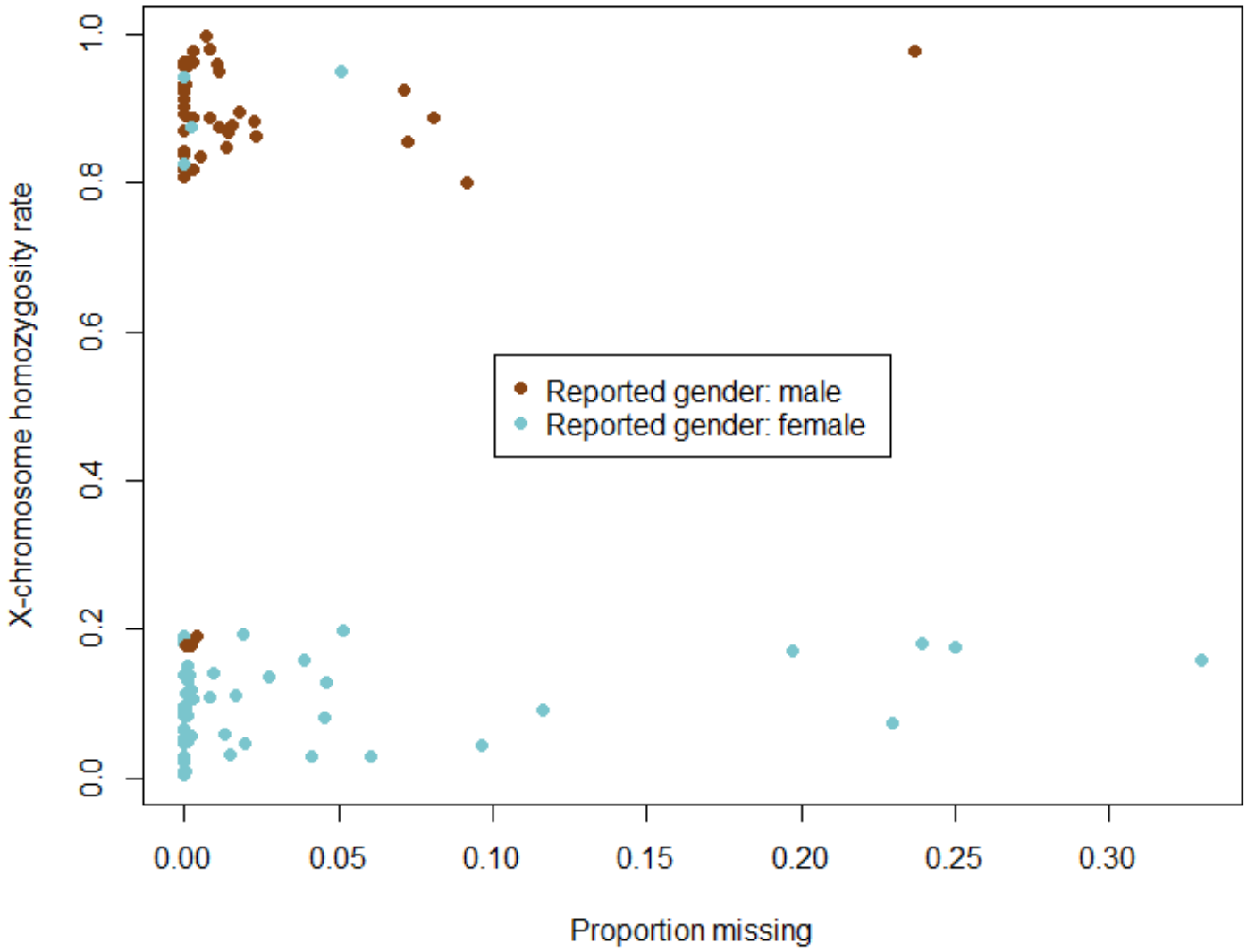
- Gender error reported for mismatch in reported and genetic sex
- Discrepancies with external gender information may reflect:
  - errors in external data
  - sample mix-up

Gender Check - Plot



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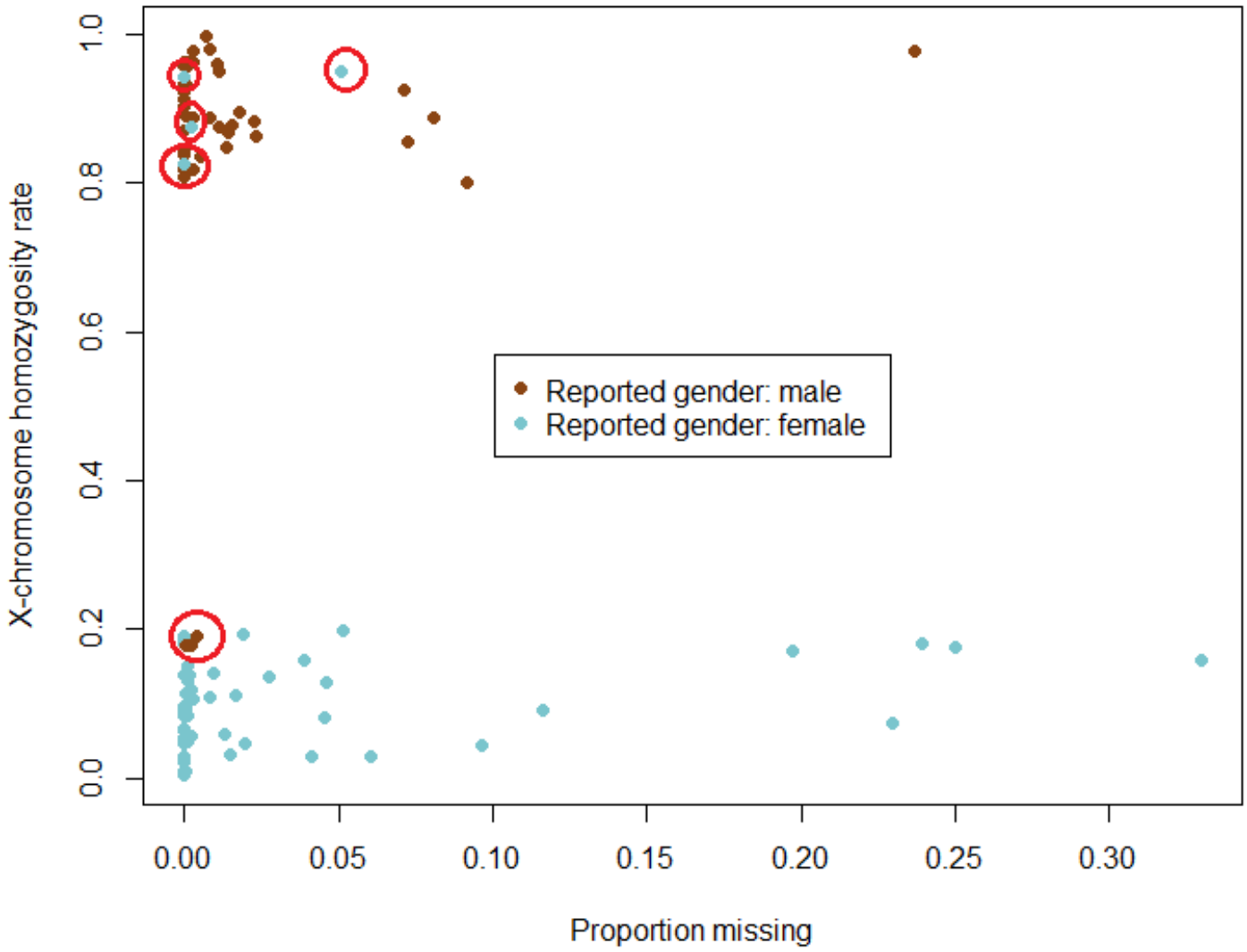

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Gender Check - Examples

Reported gender	Homozygosity rate	Gender according to SNPs
Male	0.98	Male
Female	0.03	Female
Female	0.99	Male
Female	0.28	Unknown*
Female	0.35	Unknown**

* Likely a female with sex chromosome anomaly (e.g. XX/XO mosaic, loss-of-heterozygosity on X)
** Likely a male with sex chromosome anomaly (e.g. XXY or XX/XY mosaic)

Adapted from Turner et al. Curr Protoc Hum Genet, (2011). doi:10.1002/0471142905.hg0119s68

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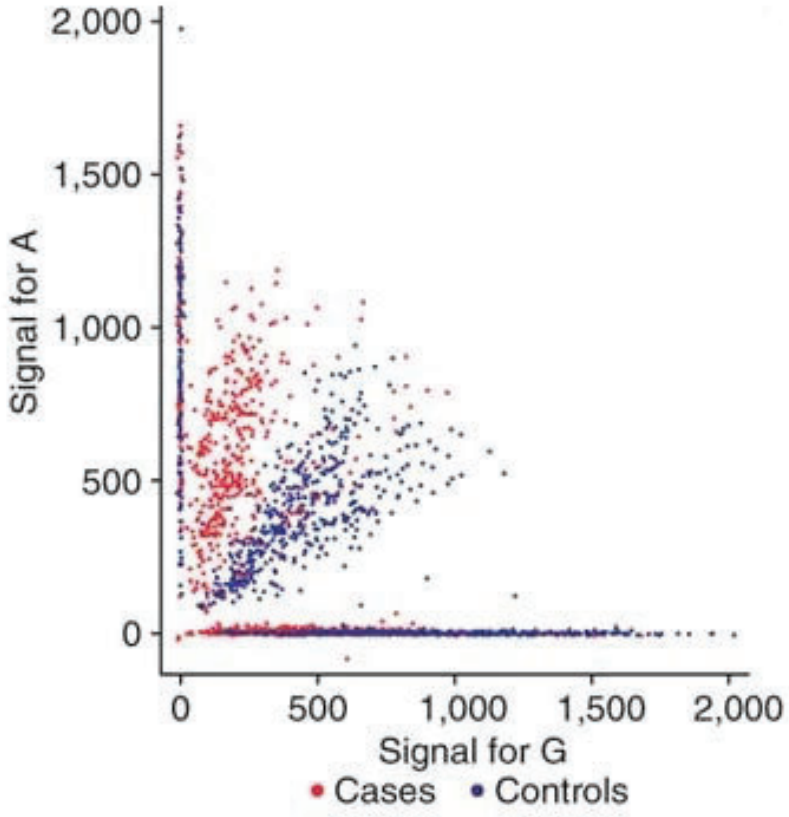
- Extreme differential call rates between cases and controls

Effect of Differential Call Rate

Clayton et al. Nature Genetics, (2005). doi:10.1038/ng1653



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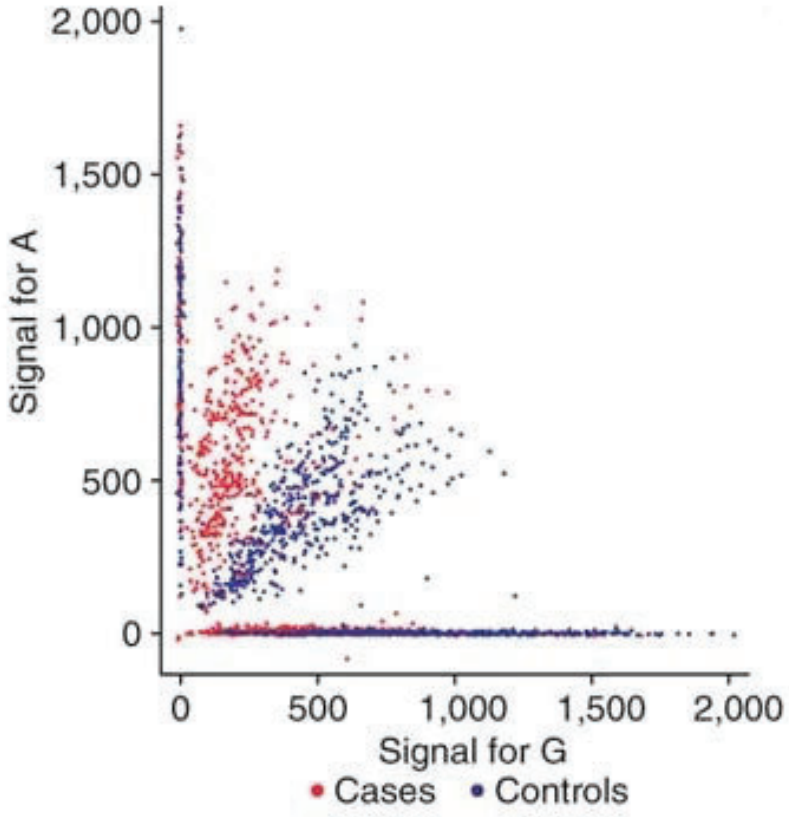

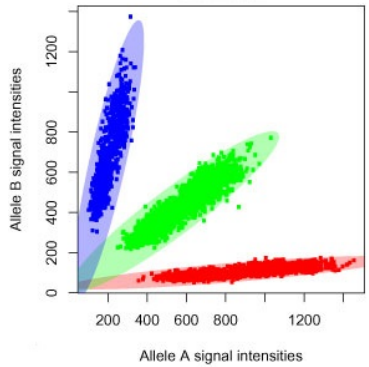

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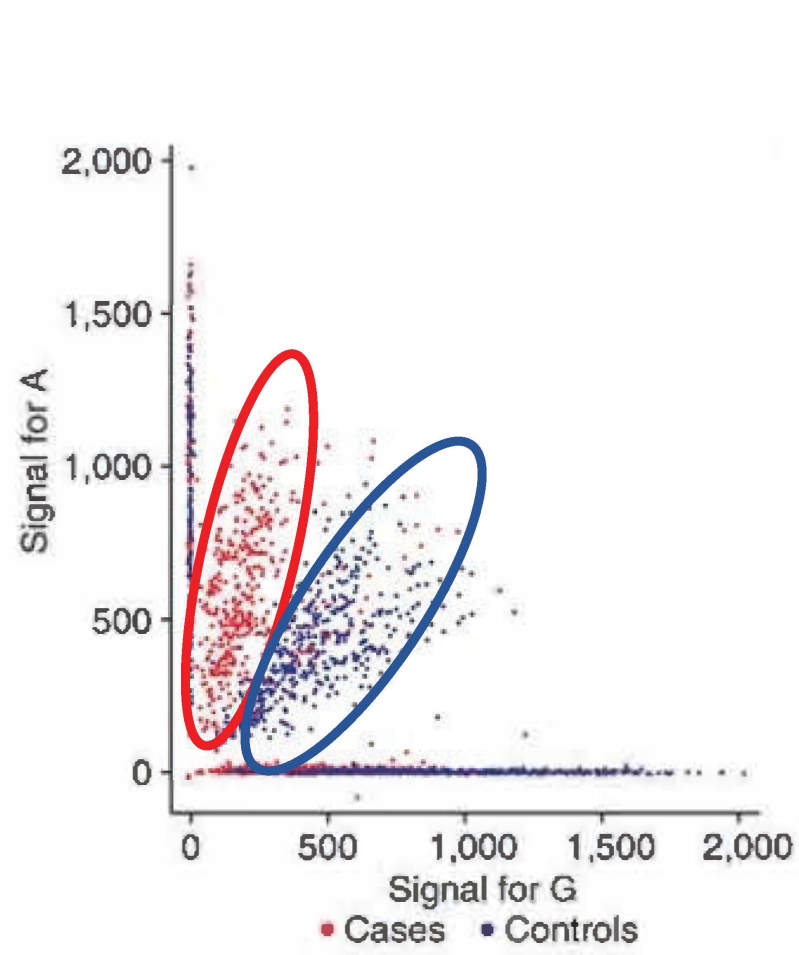

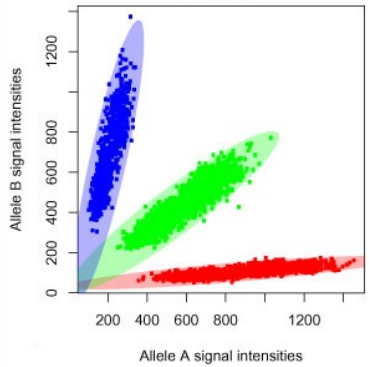

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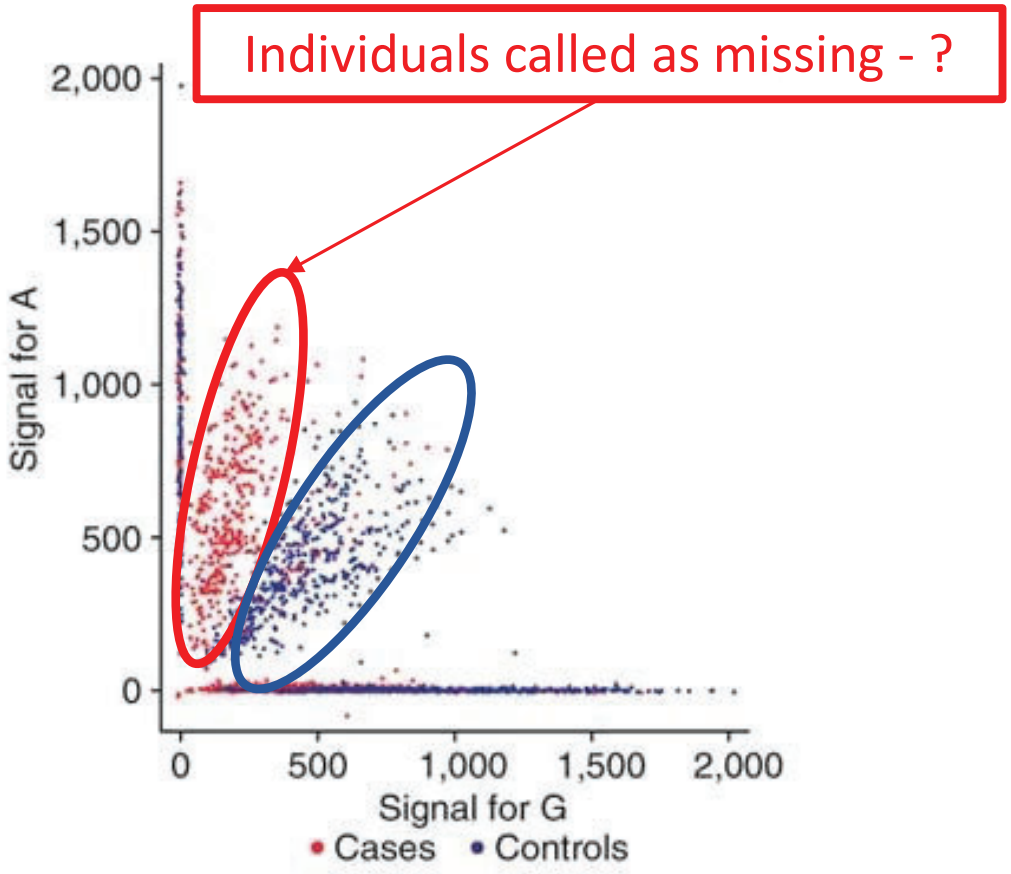

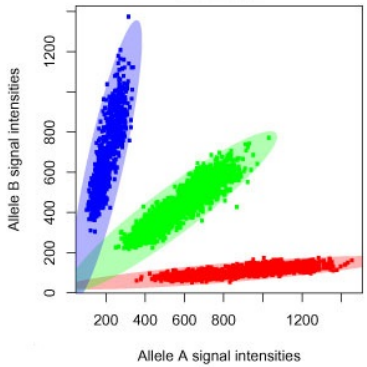

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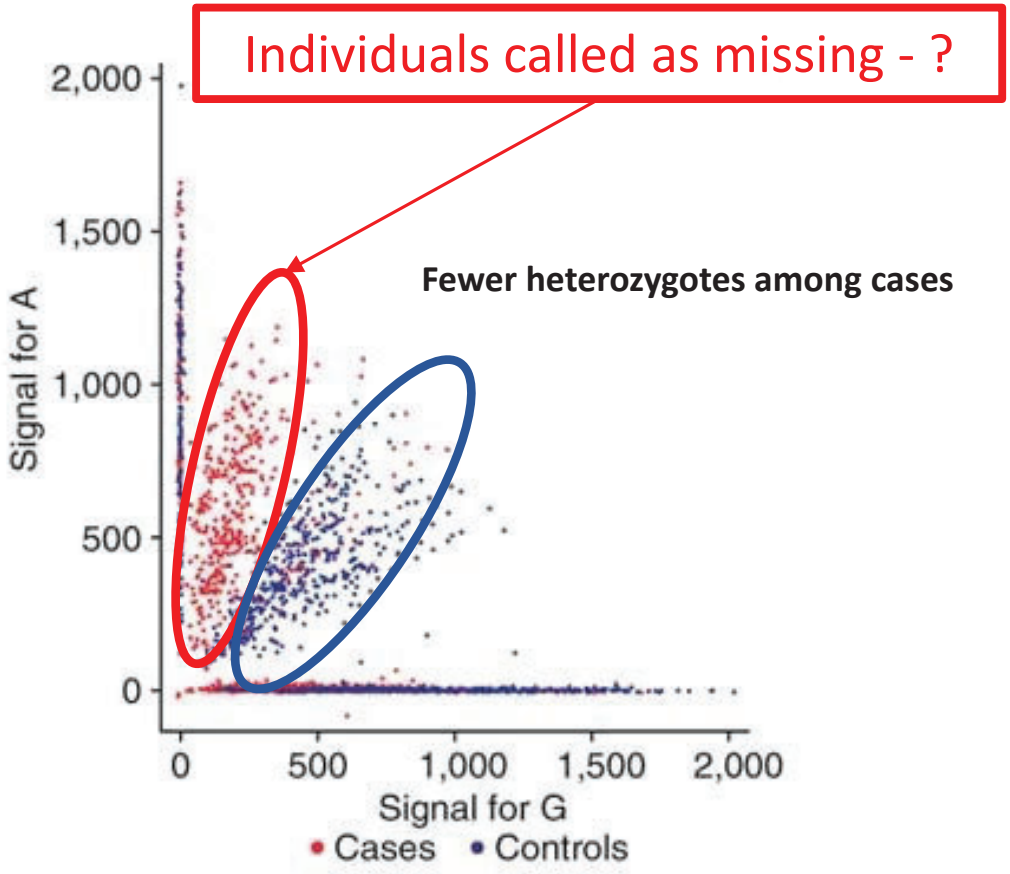

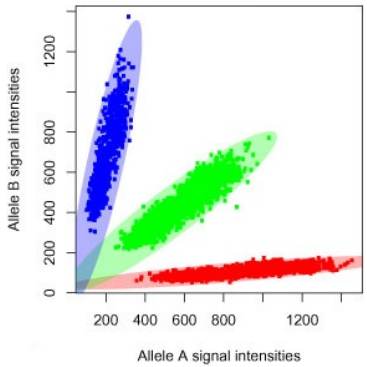

Software for Quality Control

- Specialised software for quality control of genome-wide association studies that can handle scale and complexity of data

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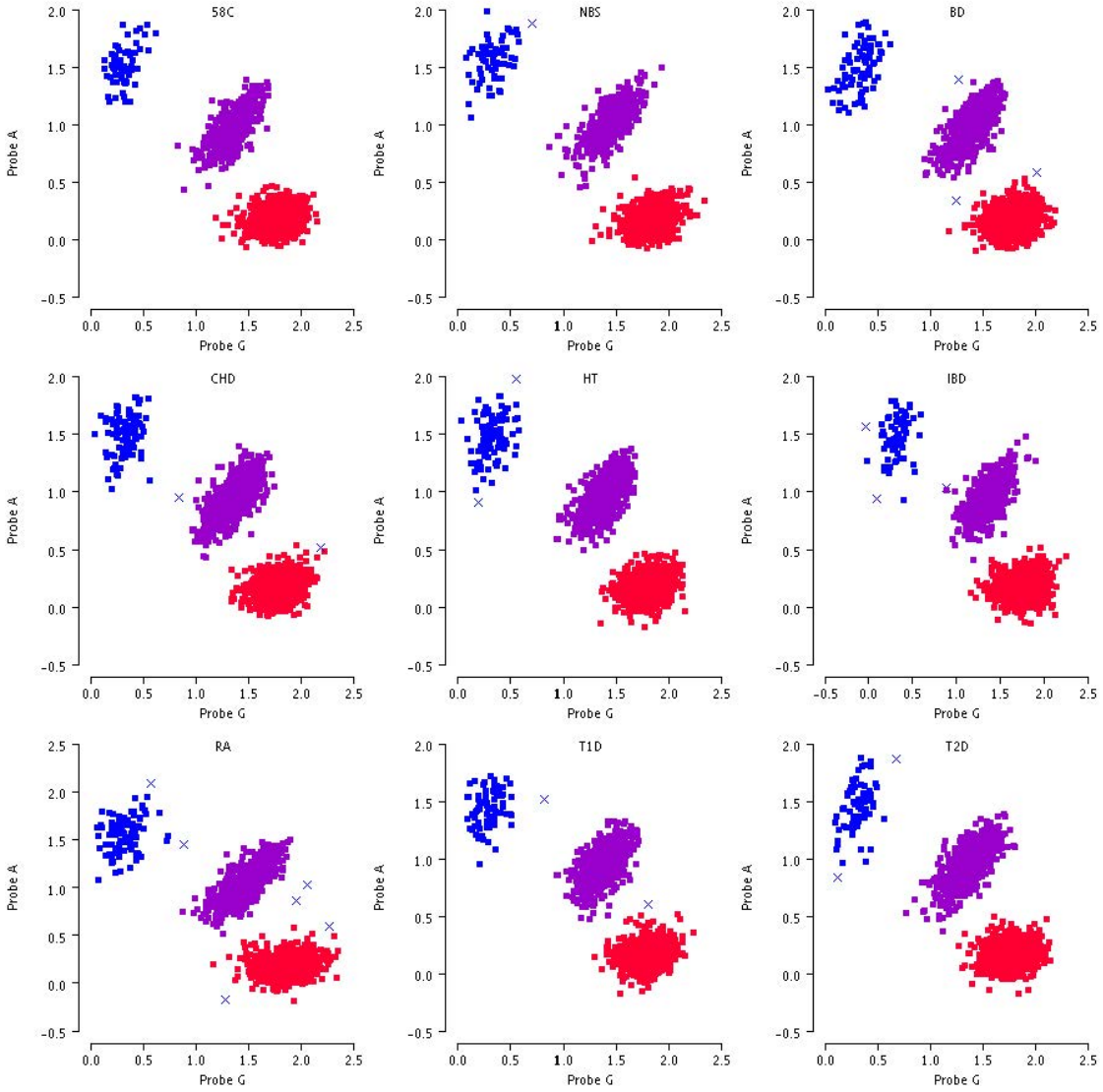
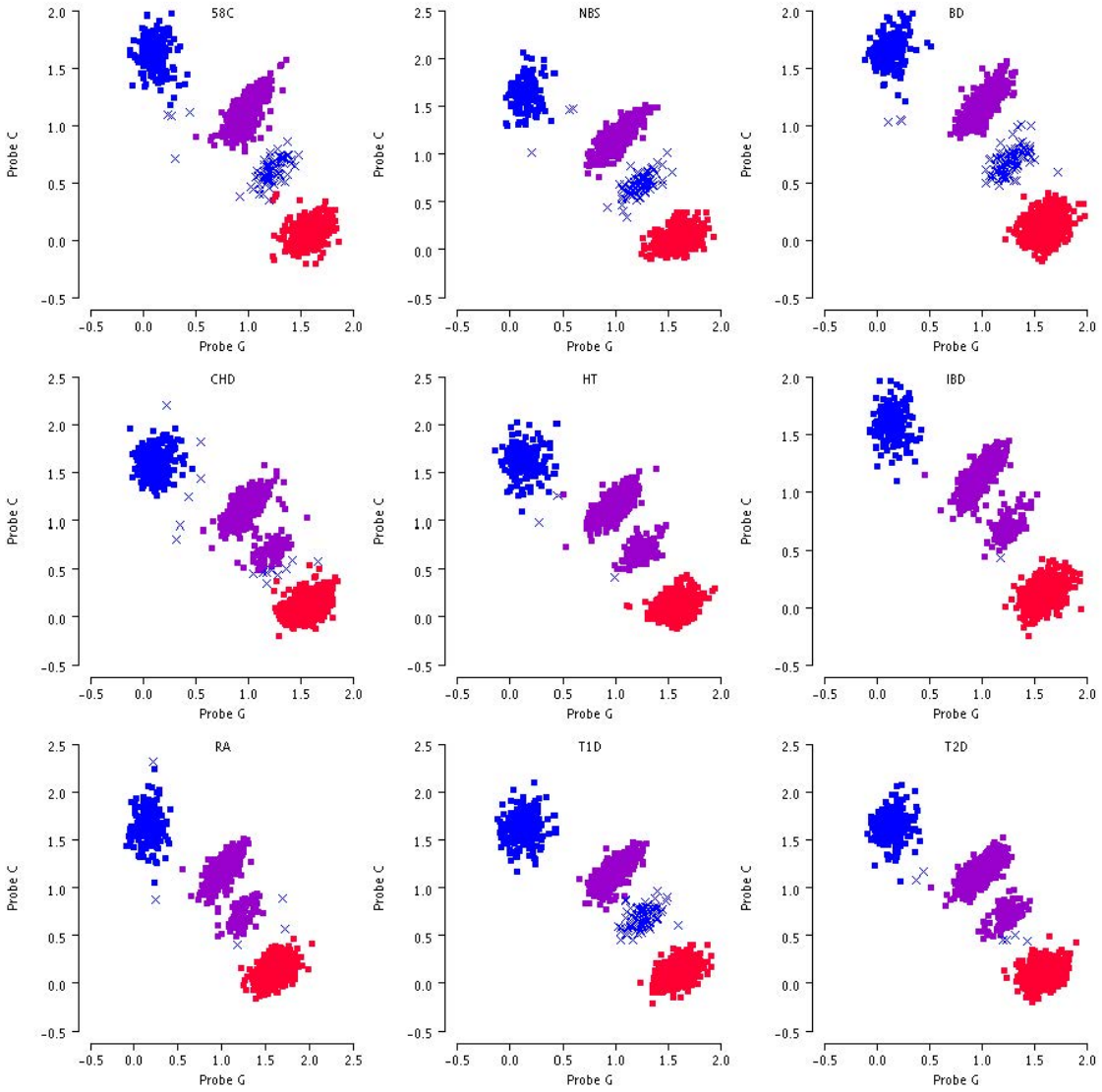
  - **Spoiler alert**: PLINK will be used in practicals

Visual Inspection of Cluster Plots - Good SNP



Visual Inspection of Cluster Plots - Bad SNP



Summary

- QC is an essential step of the analysis
- QC criteria are subjective and vary from one study to another
- Sample QC filters should not be so stringent as to remove the majority of the analysis cohort
- SNP QC filters should eliminate the worst quality markers
- All SNPs demonstrating evidence for association should be followed up with visual inspection of cluster plots