

<title>

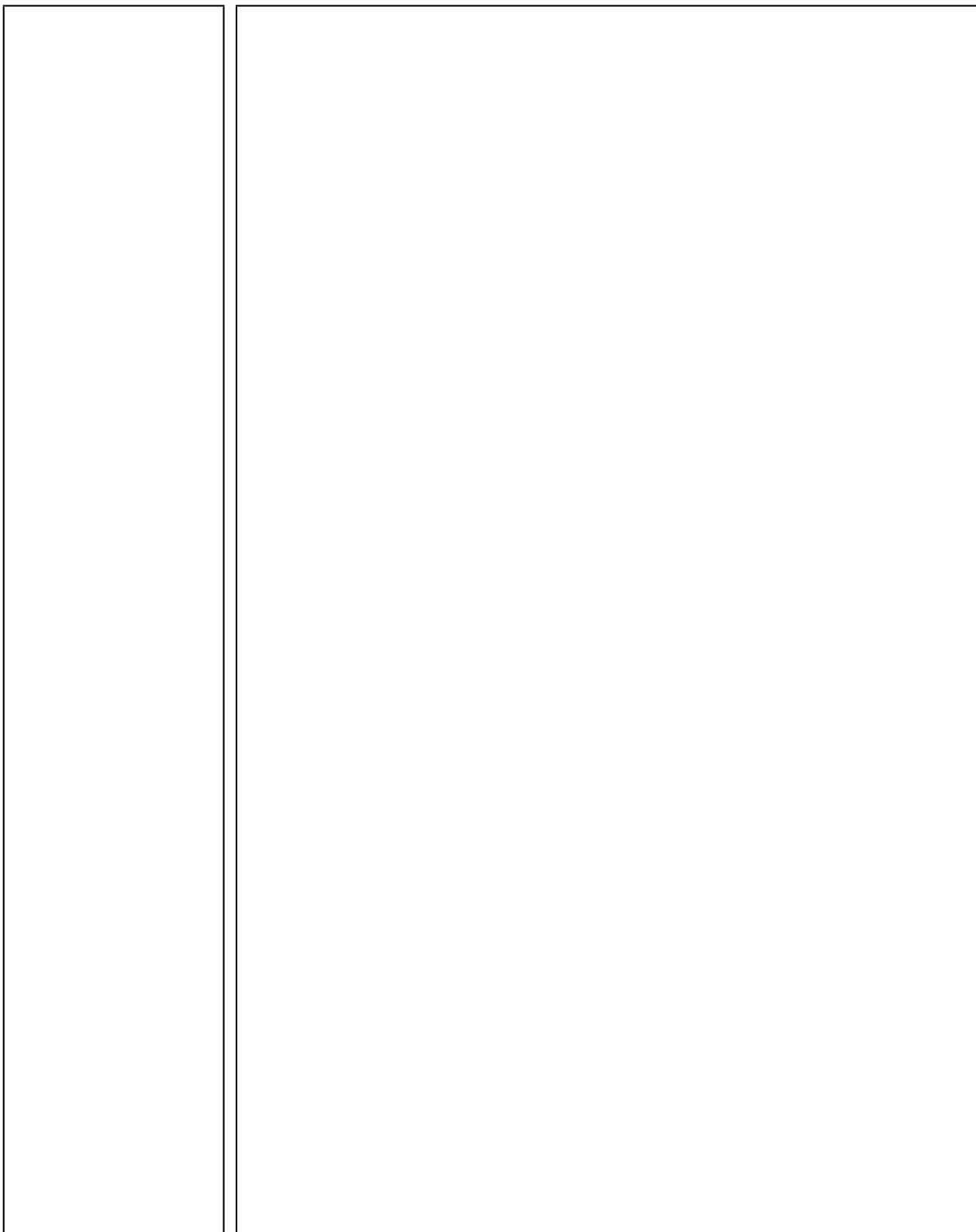
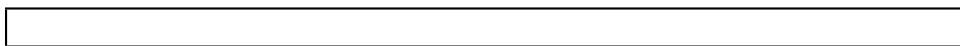
Benjamin Yu Hang Bai

2020-05-19 04:17:03+01:00

https://www.poetsgraves.co.uk/Classic%20Poems/Pope/a_little_learing.htm

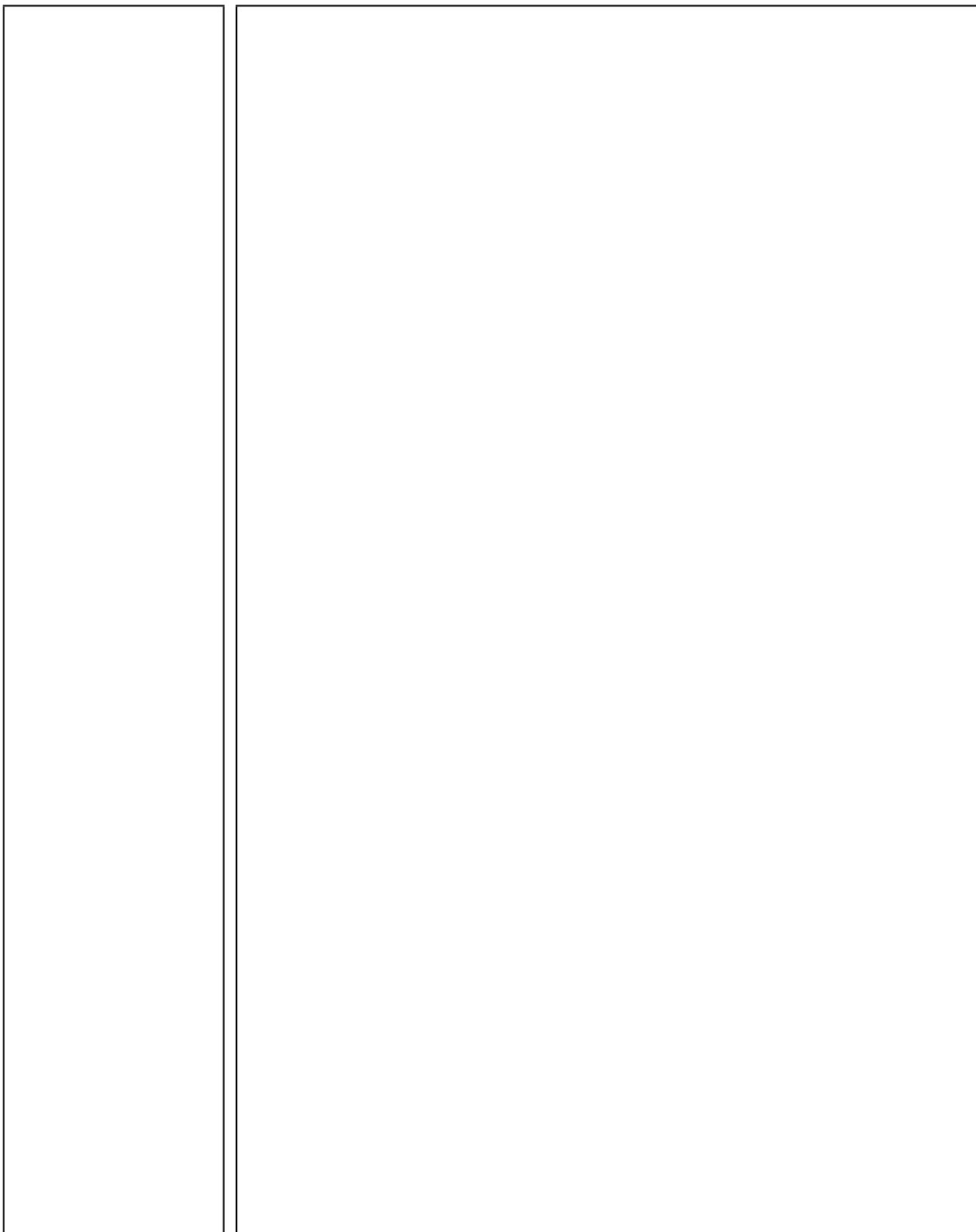
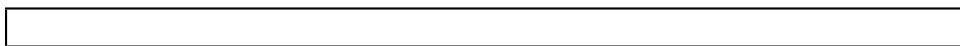
Abstract

<thesis abstract>



Acknowledgements

<acknowledgements>



Contents

List of Figures	xi
List of Tables	xvii
1 Introduction	1
1.1 Structure and diversity of the human genome	1
1.2 Genetic association studies for complex traits	2
1.2.1 From trait to locus: principles of genome-wide association studies	4
1.2.2 From locus to variant: fine-mapping	5
1.2.3 From variant to function: gene prioritisation	5
1.3 Interpretation of genetic associations with molecular phenotypes	6
1.4 The effects of genetic variation on expression: context is key .	8
1.5 Immunity is a complex trait	9
1.5.1 Context-specific immune response eQTLs in vitro . . .	9
1.5.2 <i>in vivo</i> response QTL mapping	10
1.5.3 Immune response to vaccination is a complex trait . .	11
1.5.4 Immune response to biologic therapies is a complex trait	12
1.6 Thesis overview	13
2 Transcriptomic response to influenza A (H1N1)pdm09 vaccine	15
2.1 Introduction	15
2.1.1 Seasonal and pandemic influenza	15
2.1.2 Quantifying immune response to influenza vaccines . .	16
2.1.3 Systems vaccinology of influenza vaccines	16
2.1.4 The Human Immune Response Dynamics (HIRD) study	17

2.1.5	Chapter summary	18
2.2	Methods	18
2.2.1	Existing HIRD study data and additional data	18
2.2.2	Computing baseline-adjusted measures of antibody re- sponse	19
2.2.3	Genotype data generation	20
2.2.4	Genotype data preprocessing	20
2.2.5	Computing genotype principal components as covari- ates for ancestry	24
2.2.6	RNA-seq data generation	24
2.2.7	RNA-seq quantification and filtering	26
2.2.8	Array data preprocessing	28
2.2.9	Differential gene expression	32
2.2.9.1	Per-platform differential gene expression model	34
2.2.9.2	Choice of differential gene expression meta- analysis method	34
2.2.9.3	Prior for between-studies heterogeneity . . .	35
2.2.9.4	Prior for effect size	36
2.2.9.5	Evaluation of priors	36
2.2.9.6	Multiple testing correction	36
2.2.10	Gene set enrichment analysis using blood transcrip- tion modules	38
2.3	Results	38
2.3.1	Extensive global changes in expression after vaccination	38
2.3.2	Innate immune response at day 1 post-vaccination . .	38
2.3.3	Adaptive immune response at day 7 post-vaccination .	40
2.3.4	Expression signatures associated with antibody response	40
2.3.5	Identifying expression signatures for predicting anti- body response [probably cut this section and just add to discussion]	42
2.4	Discussion	42
3	Genetic factors affecting Pandemrix vaccine response	49
3.1	Introduction	49
3.1.1	Genetic factors affecting influenza vaccine response . .	49

3.1.2	Response expression quantitative trait loci for seasonal influenza vaccination	50
3.1.3	Chapter summary	50
3.2	Methods	51
3.2.1	Genotype phasing and imputation	51
3.2.2	Overall strategy for detecting reQTLs	51
3.2.3	Controlling for population structure with linear mixed models	53
3.2.3.1	Estimation of kinship matrices	53
3.2.4	Additional eQTL-specific expression preprocessing . .	54
3.2.5	Estimation of cell type abundance from expression . .	55
3.2.6	Finding hidden covariates using factor analysis	61
3.2.7	eQTL mapping per timepoint	63
3.2.8	Joint eQTL analysis across timepoints	65
3.2.9	Defining shared and response eQTLs	67
3.2.10	Replication of eQTLs in a reference dataset	67
3.2.11	Genotype interactions with cell type abundance	68
3.2.12	TODO Statistical colocalisation	70
3.3	Results	70
3.3.1	Mapping reQTLs to Pandemix vaccination	70
3.3.2	Characterising reQTLs post-vaccination	71
3.3.3	Genotype by cell type interaction effects	75
3.3.4	TODO Genotype by platform interaction effects	75
3.3.5	TODO Colocalisation of reQTLs with known <i>in vitro</i> condition-specific immune eQTLs	76
3.4	Discussion	76
4	Response to live attenuated rotavirus vaccine (Rotarix) in Vietnamese infants	83
4.1	Introduction	83
4.1.1	The genetics of vaccine response in early life	84
4.1.2	Rotavirus and rotarix in Vietnam	84
4.1.3	Known factors that affect rotavirus vaccine efficacy . .	84
4.2	Methods	84
4.2.1	RNA-seq data generation	84
4.2.2	Genotyping	84

4.3	Results	84
4.4	Discussion	84
5	multiPANTS	85
5.1	Introduction	85
5.2	Methods	85
5.2.1	Phenotypes	86
5.2.2	Covariates to use	86
5.2.3	reQTL	86
5.3	Results	86
5.4	Discussion	86
6	Discussion	87
A	Supplementary Materials	89
A.1	Chapter 2	89
A.2	Chapter 3	89
A.3	Chapter 4	90
	Bibliography	91
	List of Abbreviations	109

List of Figures

2.1	Data types, timepoints, and sample sizes. Individuals were vaccinated after day 0 sampling. Antibodies to the vaccine strain were measured by haemagglutination inhibition (HAI) and microneutralisation (MN) assays. Array and RNA-sequencing (RNA-seq) gene expression measured in the peripheral blood mononuclear cell (PBMC) compartment.	19
2.2	Comparison of titre response index (TRI) to HAI (left column) and MN (right column) titres and binary responder/non-responder status (colored) in 166 Human Immune Response Dynamics (HIRD) individuals. Row 1: baseline titres are positively correlated to post-vaccination titres. Row 2: baseline titres are negatively correlated to fold change. Row 3: TRI regresses out the correlation between baseline titre and response. Row 4: TRI is still comparable in ordering to binary response status.	21
2.3	Distribution of TRI, stratified by platform used to measure expression.	22
2.4	Sample filters for missingness and heterozygosity rate. Samples outside the central rectangle were excluded.	23
2.5	HIRD samples (cyan) projected onto principal component (PC)1 and PC2 axes defined by principal component analysis (PCA) of HapMap 3 samples. The first two PCs separate European (CEU, upper-right) from Asian (CHB and JPT, lower-right) and African (YRI, lower-left) individuals.	25
2.6	FastQC sequence quality versus read position for HIRD RNA-seq samples.	26
2.7	FastQC sequence duplication levels for HIRD RNA-seq samples.	27

2.8	FastQC GC profile for HIRD RNA-seq samples.	27
2.9	Distributions of removed short ncRNA and globin counts as a proportion of total counts in RNA-seq samples.	29
2.10	Distribution of the proportion of samples in which genes were detected (non-zero expression). Many genes are not detected in any samples. Vertical line shows 5% threshold below which genes were discarded.	29
2.11	Distribution of gene expressions for RNA-seq samples before and after filtering no expression and low expression genes. Vertical line shown at counts per million (CPM) = 0.5 threshold.	30
2.12	Raw foreground intensities for 173 HIRD array samples. Colored by array processing batch.	30
2.13	Array intensity estimates after VSN normalisation and collapsing of probes to genes. Colored by array processing batch.	31
2.14	First four PCs in the HIRD expression data, colored by platform and batch (left), and timepoint (right).	33
2.15	Gamma prior for τ used for bayesmeta (blue), compared to the empirical distribution of per-gene frequentist metafor::rma estimates for τ , for the day 1 vs. baseline effect (small estimates of $\tau < 0.01$ excluded). Empirical log-normal fit also shown (red).	37
2.16	Normal prior for μ used for bayesmeta (blue), compared to the empirical distribution of per-gene frequentist metafor::rma estimates for τ , for the day 1 vs. baseline effect. The non-scaled normal fit is shown (black), as well as a Cauchy fit (red).	37
2.17	Normalised gene expression for genes differentially expressed between any pair of timepoints (lfsr < 0.05, absolute fold change > 1.5) across HIRD samples, clustered by gene (Manhattan distance metric).	39
2.18	Transcriptomic modules significantly up or downregulated post-vaccination. Size of circle indicates effect size. Color of circle indicates significance and direction of effect (red = upregulation, blue = downregulation).	41

2.19	DGE effect sizes estimated in array vs. RNA-seq. Significance colored by frequentist random effects meta-analysis $FDR < 0.05$. Genes with day 7 expression associated with responder/non-responder status in ⁴⁴ are circled for that contrast.	43
2.20	DGE effect sizes estimated in array vs RNA-seq. Significance colored by Bayesian random effects meta-analysis $lfsr < 0.05$. Genes with day 7 expression associated with responder/non-responder status in ⁴⁴ are circled for that contrast.	43
2.21	Transcriptomic modules enriched in genes with expression associated with antibody response (TRI) at each day. Size of circle indicates effect size. Color of circle indicates significance and direction of effect (red = expression positively correlated with TRI, blue = negative).	44
3.1	Simulated log scale expression in two conditions for six genes (columns) representing six different scenarios: Scenario 0 has no expression quantitative trait locus (eQTL), scenario 1 is a shared eQTL ($\beta = 1$), scenario 2 is a response expression quantitative trait locus (reQTL) where β increases from 0 to 1, scenario 3 is a reQTL where β increases from 0 to 2, scenario 4 is a reQTL where β increases from 1 to 2, and scenario 6 is a reQTL where β increases from 1 to 4. Rows represent the effect of different expression transformations across samples, conducted both within condition, and including both conditions.	56
3.2	Standardised xCell enrichment scores for seven PBMC cell types in array samples.	58
3.3	Standardised xCell enrichment scores for seven PBMC cell types in RNA-seq samples.	59
3.4	Quality of representation (\cos^2) for each input variable in each PC dimension after PCA of xCell scores. Higher \cos^2 represents higher contribution of that variable to that dimension.	60

3.5	Correlation between standardised xCell scores and normalised fluorescence-activated cell sorting (FACS) measurements for a similar immune subset, in the subset of individuals with FACS data.	62
3.6	Correlation of PEER factors to known factors and other possible covariates. Note that PEER factors are not constrained to be orthogonal, so correlations to known factors are expected.	64
3.7	Number of significant eGenes detected on chromosome 1 (hierarchical Bonferroni-Benjamini-Hochberg (BH) FDR < 0.05) as a function of the number of PEER factors included as covariates k.	65
3.8	Clustering of within-timepoint Z scores in the strong mashr subset (random sample of 10000/45962 tests), confirming the presence of strong condition-specific effects.	66
3.9	Effect of HIRD lfsr threshold on GTEx whole blood replication rate (π_1), number of p -values used to compute π_1 , and maximum p -value among those p -values; for shared and reQTL called from the array-only, RNA-seq-only and mega-analysis pipelines. Shaded region for π_1 represents the 5th-95th percentile range of 1000 bootstraps.	69
3.10	Summary of eQTL mapping results at 13570 genes-lead eQTL pairs, with intersections based on significance (lfsr < 0.05). Counts of shared eQTLs and reQTLs; and distribution of INFO score, min MAF across timepoints, and max PVE across timepoints for those lead variants are shown above each intersection.	72
3.11	Z score for difference in effect vs. day 0, of lead eQTLs for all eGenes significant at either day 1 or day 7; versus distance of the lead SNP to the TSS. Direction of effect is aligned so that the beta at day 0 is positive. Points with positive z score are magnified effects post-vaccination, points with negative z scores are dampening and opposite sign effects.	73
3.12	<i>ADCY3</i> , strongest reQTL at day 1.	74

3.13	<i>SH2D4A</i> , strongest reQTL at day 7. Top: Array and RNA-seq expression before merging with ComBat for mega-analysis. Bottom: eQTL effects at each timepoint condition in the mega-analysis.	74
3.14	Multi-trait colocalisation of HIRD reQTL signal at <i>ADCY3</i> (500 Kb window), with QTL studies from IHEC, BLUEPRINT, eQTL Catalogue, and GWAS Catalogue. Plots are colored by colocalised cluster. Black indicates non-colocalised datasets. .	77

LIST OF FIGURES

LIST OF FIGURES

--	--

<h1>List of Tables</h1>	
2.1	Sample descriptive statistics. 47
2.2	HIRD batch balance 48

--	--

Chapter 1

Introduction

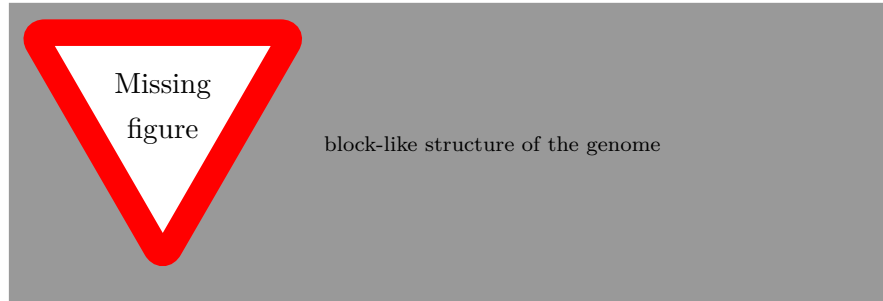
1.1 Structure and diversity of the human genome

- The human genome is almost three billion base pairs (bps) in length, containing 20000-25000 protein-coding genes^{1,2} that span 1-3% of its length. The majority of the remainder is likely dedicated to regulatory activities². A diploid human cell contains two copies of the genome; 46 chromosomes comprised of 23 maternal-parental pairs: 22 pairs of homologous autosomes and one pair of sex chromosomes.
- Variation in the genome between individuals in a population exists in the form of single nucleotide polymorphisms (SNPs), short indels, and structural variants—SNPs and short indels take up the vast majority (> 9990 %) ¹. On average, a pair of human genomes differs by one SNP per 1000-2000 bp³. Each version of a variant is called an allele; an individual has a maternal and parental allele at each variant.
- The many variants in a population are inherited in a smaller number of haplotypes: contiguous stretches of the genome passed through generations via meiotic segregation. The fundamental sources of genetic diversity are mutation and meiotic recombination, generating new alleles and breaking apart haplotypes into shorter ones over time. Variants at physically close positions (loci) on a chromosome are less likely to flank a recombination event, hence more likely to cosegregate on the same haplotype, referred to as genetic linkage. Genetic linkage is one source of linkage disequilibrium (LD): the non-random association of

LD decay just takes a really really long time, but there are evo forces at work too that maintain LD

alleles at two loci, differing from expectation based on their frequencies and the law of independent assortment⁴. LD is often quantified within a population by r^2 , the squared correlation coefficient between alleles⁴.

Recombination events are not distributed uniformly throughout the genome. The genome is a mosaic of blocks delimited by recombination hotspots, characterised by strong LD within blocks, and little LD between blocks^{5,6}. The structure of correlated haplotypes reflects a population's unique evolutionary history, and can be used to trace the demography of human populations back through time⁷.



1.2 Genetic association studies for complex traits

- Variation in human traits arises from an interplay between genetics and environment. Traits for which genetic variation explains a non-zero fraction of phenotypic variation are heritable.
 - Twin studies provide upper bounds on heritability <https://www.nature.com/articles/nrg3243>
- Discovering the specific variants that contribute, through association of genetic variants and phenotypes measured from the same individual, is a mainstay of the field of human genetics. Barring somatic mutation, an individual's genome is fixed at conception, providing a causally upstream anchor. Genetic association studies have intrinsic resistance to backdoor path effects that permeate observational studies of the causes of human phenotypes.
 - What are the benefits of leveraging natural genetic variation? Analogies to RCTs.

insert something sweeping about utility: insights into trait biology and clinical translational potential for disease traits, genetic support target identification

- Under the central dogma, information flows from gene to RNA to protein to phenotype via transcription and translation, thus it is assumed that genetic variants at loci in the genome affect phenotype through by impacting on the function or regulation of target genes. How genetic variation contributes to any heritable trait defines it's genetic architecture: the number of genes affecting that trait; and the allele frequencies, effect sizes, and interactions of trait-associated variants⁸. The number of genes defines a spectrum of traits from monogenic (where inheritance follow simple Mendelian patterns) to polygenic (where inheritance is more complex). Many architectures have been proposed for complex traits; all have in common that the number of genes that affect a complex trait is large (ranging from dozens to many thousands), and the average effect of trait-associated loci is relatively small compared to monogenic traits^{9,10}.
- For decades, linkage analysis was successfully used to map loci affecting Mendelian traits by tracing their cosegregation with the trait through pedigrees. They were not successful for complex traits, as small effect sizes equate to low penetrance in pedigrees¹¹. Genetic association studies were also performed for a variety of traits, focusing on variants in or near candidate genes selected on the basis of prior biological knowledge, but these studies were plagued with reproducibility issues¹².
- Genome-wide association studies (GWAS) systematically test common variants (traditionally defined as $MAF > 1 - 5\%$) selected in an unbiased manner across the genome for association with a trait. Many of the early association studies were on traits now known to be complex. Thousands of robust associations auto-immune, metabolic, and psychiatric disease in particular^{11,13}

The vast majority of these traits are now known to be complex appreciation of polygenicity No one risk locus is sufficient, only raises by a bit

genetic variance most genetic variance is additive⁸ not epistasis

pleiotropy is wide spread¹¹

many replicated across popoulations¹¹



OR vs MAF tam2019BenefitsLimitationsGenomewide

14,15

1.2.1 From trait to locus: principles of genome-wide association studies

As variants on the same haplotype tend to be in strong LD, and the number of haplotypes is comparatively few, it is possible to select a subset of tag variants such that all other known common variants are within a certain LD threshold of that subset. In practice, there is enough redundancy that the number of variants measured on a modern genotyping array (in the order of 10^5 to 10^6) is sufficient to tag almost all common variants^{16,17}. Remaining common variants are indirectly measured through their strong correlation with a tag variant. Furthermore, as unrelated individuals still share short ancestral haplotypes, study samples can be assigned haplotypes from a panel of haplotypes derived from reference samples by matching on the directly genotyped variants. This process of genotype imputation allows ascertainment of variants not directly genotyped¹⁸, and help to recover many rarer variants that are poorly-tagged¹³. Modern imputation panels enable cost-effective GWAS including tens of millions of variants down to frequencies of $\sim 0.01\%$ <https://www.biorxiv.org/content/10.1101/563866v1>.

Testing such large numbers of variants incurs a massive multiple testing burden, but acknowledging the correlation between variants due to LD, there are only the equivalent of $\sim 10^6$ independent tests in the European genome, regardless of the number of tests actually performed¹⁹. The field has thus converged on a fixed discovery threshold of $0.05/10^6$ =

seems like there is some connection to be made between the tagability of common variation and the feasibility of imputation

5×10^{-8} for genome-wide significance in European populations²⁰, akin* to controlling the family-wise type I error rate at using the Bonferroni correction. Still, for complex traits with small per-variant effects, the required sample sizes are enormous¹³. Total GWAS sample sizes in the millions are no longer an unusual occurrence¹⁵.

1.2.2 From locus to variant: fine-mapping

- By design, a significantly-associated variant from a GWAS needs not be a variant that causally affects the trait, and may only tag a causal variant.
 - Fine-mapping is the process of determining which of the many correlated variants at a GWAS locus are causal.
 - The power to separate causal and tag variants then depends on LD and sample size¹³.
 - State-of-the-art methods provide Bayesian posterior probabilities that associated variants are causal given the LD structure of the region, and can consider the presence of multiple causal variants at the same locus²².
 - Naturally, these methods assign probabilities assuming the causal variant is in the set of variants tested.
 - The causal variant must either be genotyped or imputed denser genotyping e.g. by WGS, and larger imputation panels will help.

1.2.3 From variant to function: gene prioritisation

target gene Even if

Unlike Mendelian traits, reg two-thirds of GWAS-associated loci implicate a gene that is not the nearest gene to the most associated SNP

over 90% nc¹⁴

*The Bonferroni procedure makes no assumptions about the dependence structure of the p -values, and is conservative (i.e. controls the family-wise error rate (FWER) at a stricter level than the chosen α) even for independent tests. In fact it is always conservative unless the p -values have strong negative correlations²¹.

CUT: There is a limit, the strategy of association is fundamentally unsuited to rare variants.

CUT: Missing heritability

CUT: WGS/WES mitigates the coverage bias of GWAS towards known variation.

locus to gene mapping problem

– Even if the causal variant is known

– nc snps regulate

* Genome-wide association studies have successfully identified genetic variants associated with immune-mediated disease, the majority of which are non-coding[10 Years of GWAS Discovery].

e.g. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5062579/> ■

We then used Sherlock, a tool that integrates GWAS and expression quantitative trait locus (eQTL) data, to identify weak GWAS signals that are also supported by eQTL data.

– enriched in reg

IMD, t2d, schizo GWAS hits over-represented in regulatory regions in disease specific tissues

1.3 Interpretation of genetic associations with molecular phenotypes

QTLs¹³

It should be noted that although the effect sizes of individual genetic variants are small in populations, their effect sizes on molecular phenotypes can be large, and the drug effects of gene targets can also be magnified (e.g., statins).

- using intermediate/endophenotypes

– endophenotypes paper²³

- Under the central dogma, information flows from gene to RNA to protein to phenotype via transcription and translation, thus it is assumed that genetic variation affects phenotype through its impact on the function or regulation of genes. direct or indirect

- Gene expression is a fundamental intermediate phenotype

– measure by array, rnaseq

– theory is that genetic variants manifest their effects through these phenotypes, central dogma based

GTEX

<ul style="list-style-type: none"> • eqtl reviw: albert2015RoleRegulatoryVariationa recent review Vandiedonck • coloc methods <ul style="list-style-type: none"> – coloc <ul style="list-style-type: none"> * Under the assumption that the mechanism by which non-coding associations affect disease risk is through their effect on gene expression, a successful way to link associations to their target gene is by statistical colocalisation with eQTL datasets, to determine if the GWAS and eQTL signal share the same causal variant[Co-localization of Conditional eQTL and GWAS Signatures in Schizophrenia]. – TWAS – MR https://www.nature.com/articles/s41576-018-0020-3 Using ge- netic data to strengthen causal inference in observational re- search Transcriptome-wide association studies (TWAS) integrat- ing GWAS and eQTLs data have been proposed to unravel gene-trait associations^{7,9,10}. However, although these studies aim to iden- tify genes whose (genetically predicted) expression is significantly associated to complex traits, they do not aim to estimate the strength of the causal effect and are unable to distinguish causa- tion from horizontal pleiotropy (i.e., when a genetic variant in- fluences multiple phenotypes independently). For this reason, we rather chose to apply a Mendelian randomization (MR) approach to estimate the causal effect of gene expression on complex traits. – a transcriptional risk score (TRS) – causal inference • for eqtls, closest gene is often not the best candidate <ul style="list-style-type: none"> – annotation of nc var is functional genomics <ul style="list-style-type: none"> * e.g. gtex, ENCODE – Drug target prioritisation for disease traits 	
--	--

1.4 The effects of genetic variation on expression: context is key

- in the dreaded GxE statistical interaction
 - "In genetics, context matters"
 - for both GWAS, and molQTLs, context is key
- Architecture varies e.g. across cell type and tissues
- emphasise all these are just interactions with different things
 - tissue
 - cell type
 - interaction between cells in vivo
 - stimulation conditions

review: condition/Cell-type specific methods refer to 2019-11-19
Cell-count specific eQTL mapping papers
- QTLs can interact with sex and age
- types of context specific QTL
 - Ackerman conditional vs dynamic
- why care about reQTLs Expose differences in regulation
- Mechanisms of reQTLs What molecular mechanisms might allow for interaction between Expression quantitative trait locus (eQTL) and different environmental conditions? Four categories of tissue-dependent cis-eQTL effects, and proposed two molecular models.



magnify, dampen, flip

colocalization of immune mediated traits is enhanced by context-specific eQTLs

Longitudinal studies what are we actually being told? differences in regulation over time
genetic effects on change in expression
both appear as an interaction

1.5 Immunity is a complex trait

Is it even plausible that genetic var is important? Brodin: most env paper.

Immune-mediated diseases Heritability of immune parameters and immune-mediated diseases ranges from

Genetic factors affecting the healthy immune system

Why study health? Factors affecting the healthy immune system.

In healthy populations, $\approx 50\%$ variation in immune system driven by non-genetic factors, $\approx 30\text{--}40\%$ variation is driven by genetic variation (Liston and Goris 2018).

"Such systems immunology studies in healthy individuals have revealed that human immune systems are incredibly variable among individuals, but very stable within individuals over time (11), and most of this variation is attributed to non-heritable factors (12)."

Genetic factors affecting immune response to challenge

Given the genetic control of the healthy immune system, one can hypothesise that immune response to challenge may also be influenced by genetic factors.

The need for controlled immune challenge in trials. Studies of natural infection are complicated. clinical trials as an opportunity: Vaccines and drugs used as controlled immune challenge.

Need for systems immunology generate mol phenotypes in the right context

1.5.1 Context-specific immune response eQTLs in vitro

reqtls in immunity

add more pros for in vitro reQTLs here, and find citations

The majority of response eQTL mapping experiments to date have been conducted *in vitro*, where one can precisely adjust both the length and intensity of stimulation. Environmental variables including cell type composition or tissue type that are expected to interact with the eQTL effect and may affect the interaction effect with stimulation can be controlled. The choice of experiment system and stimulation can also be hypothesis-driven, for example, if certain tissues are expected to be more relevant for a specific disease. .

One of the first studies to perform response expression quantitative trait locus (reQTL) mapping for an immune stimulation was²⁴, where eQTLs were mapped separately in monocyte-derived dendritic cells before and after 18h infection with *Mycobacterium tuberculosis*. reQTLs were detected for 198 genes, 102 specific to the uninfected state, and 96 specific to the infected state. These reQTLs were enriched for GWAS SNPs associated with host susceptibility to tuberculosis; this was not observed for eQTLs that were not reQTLs.

Since then, *in vitro* immune reQTL studies have been conducted for a variety of experimental systems (e.g. primary CD14+ monocytes²⁵) and stimulations (IFN γ and LPS²⁶).

Take home messages: - reQTLs develop trans-effects on stimulation²⁵
Overall, as the number of experimental systems and stimulations increases, large number of eQTLs are only detected.

most recent are very high throughput

1.5.2 *in vivo* response QTL mapping

less popular A complementary approach.

in vivo pros choice of context whole organism phenotypes more likely to be repeated measures why is this good

blocking: deliberate aliasing with higher order interactions

Review of *in vivo* mapping. What we learn on top of *in vitro* (Franco et al., 2013)

Large cohorts:

1.5.3 Immune response to vaccination is a complex trait

Vaccination has enormous impact on global health [10.1098/rstb.2013.0433].

Vaccines stimulate the immune system with pathogen-derived antigens to induce effector responses (primarily antigen-specific antibodies) and immunological memory against the pathogen itself. These effector responses are then be rapidly reactivated in cases of future exposure to the pathogen, mediating long-term protection.

Systems vaccinology: from empirical to rational vaccinology

History of vaccine dev [summary of low-throughput immunology e.g. animal models]

- Vaccination coverage in vulnerable populations is below optimal

However, a vaccine that is highly efficacious in one human population may have significantly lower efficacy in other populations. [1 statistic on vaccine efficacy differences e.g. rotavirus] Particularly challenging populations for vaccination include the infants and elderly, pregnant, immuno compromised patients, ethnically-diverse populations, and developing countries. For the majority of licensed vaccines, there is a lack of understanding regarding the molecular mechanisms that underpin this variation in host immune response. Immunological mechanisms that underpin a specific vaccine's success or failure in a given individual are often poorly understood[Immunological mechanisms of vaccination].

rational vacc, where the key is sys vacc

Review of systems vaccinology (pull out of self_viva_copypasta) These systems vaccinology studies often consider longitudinal measurements of the transcriptomic, cellular, cytokine, and antibody immune responses following vaccination[Vaccinology in the era of high-throughput biology].

Systems vaccinology is the application of -omics technologies to provide a systems-level characterisation of the human immune system after vaccine-perturbation. Measurements are taken at multiple molecular levels (e.g. genome, transcriptome, proteome), and molecular signatures that correlate with and predict vaccine-induced immunity are

identified [<http://dx.doi.org/10.1098/rstb.2014.0146>]. Systems vaccinology has been successfully applied to a variety of licensed vaccines [yellow fever, influenza], and also to vaccine candidates against [HIV, malaria], resulting in the identification of early transcriptomic signatures that predict vaccine-induced antibody responses.

Cotugno - dna meth: DNA methylation [52, 53, 54] events

How to use sysvacc to inform better design (A systems framework for vaccine design Mooney2013), and how to move towards personalised vaccinology (<https://doi.org/10.1016/j.vaccine.2017.07.062>).

Overview, including pathogen-side factors

Genetic factors affecting vaccine response

Read this Vaccine.2018August28;36(36):5350-5355. doi:10.1016/j.vaccine.2017.07.062. Search for "variation in vaccine response genetics GA Poland" in google scholar

measles

Relatively few studies have assessed the impact of human genetic variation on responses[Franco, Lareau 2016].

This is despite evidence from genome-wide association studies suggesting such genetic variation influences immune response to vaccines and susceptibility to disease[Systems immunogenetics of vaccines.].

Results from vaccine-related twin studies e.g. in "TWIN STUDIES ON GENETIC VARIATIONS IN RESISTANCE TO TUBERCULOSIS", and (Defective T Memory Cell Differentiation after Varicella Zoster Vaccination in Older Individuals)

Nice summary table for gwas Review paper on GWAS for vaccines mooney2013SystemsImmunogeneticsVaccines

Genetics of adverse events e.g. <https://www.ncbi.nlm.nih.gov/pubmed/18454680>

1.5.4 Immune response to biologic therapies is a complex trait

Expression response to biologics

define what a signature

Genetic factors affecting biologic responses

e.g. PANTS immunogenicity

1.6 Thesis overview

By chapter CCC overview.

Chapter 2

Transcriptomic response to influenza A (H1N1)pdm09 vaccine

2.1 Introduction

2.1.1 Seasonal and pandemic influenza

Influenza is an infectious disease, generally seasonal, caused by the influenza A and influenza B viruses in humans. Influenza A viruses circulate not only in humans, but also in a variety of other birds and mammal hosts. They are classified into antigenically-distinct subtypes by the combination of two surface proteins: haemagglutinin (HA) and neuraminidase (NA)²⁷.

There are three classes of influenza vaccine against seasonal strains in use: inactivated vaccines, live attenuated influenza vaccines (LAIVs), and recombinant HA vaccines. These vaccines confer a degree of strain-specific protection, primarily by raising serum antibodies against the HA and/or NA proteins. Antigenic drift, the accumulation of mutations in these surface proteins over time, necessitates the annual reformulation of seasonal influenza vaccines to reflect circulating strains^{28,29}. On occasion, a novel subtype against which the majority of the population is immunologically naive can arise suddenly (antigenic shift), often from zoonotic origins. A recent example occurred in 2009, when an outbreak of a novel swine-origin strain, eventually termed influenza A (H1N1)pdm09, resulted in a global pandemic,

why? for diff groups of people

add a point that 2009h1n1 is now circulating seasonally, this is a common trend

Add specific section about pandemrix, it's correlates of protection, it's durability? or maybe in methods

Here, add few points about the immunological response to adjuvanted TIVs i.e. what happens after Pandemrix admin? Involve the innate -> B/CD4T response. Goto plotkins

is there a more recent review?

the fourth to occur in the last 100 years²⁷.

2.1.2 Quantifying immune response to influenza vaccines

The 2009 pandemic motivated the rapid development, trialing, and licensing of several novel vaccines³⁰. Immune response to influenza vaccines in clinical trials is evaluated by assays that measure levels of antibodies specific to the vaccine strain(s). The haemagglutination inhibition (HAI) assay measures the levels of serum antibodies specific to the HA surface protein. The related microneutralisation (MN) assay measures levels of antibodies (which may or may not be anti-HA) that neutralise the infectivity of the virus in cell culture³¹. Values from these assays can be compared against thresholds for known correlates of protection: markers that associate with whether an individual is protected from the disease. For example, HAI titres are regarded as the primary correlate of protection for inactivated influenza vaccines. Targets that regulatory agencies expect a licensed vaccine to meet are based on thresholds such as the proportion of trial individuals achieving HAI titres ≥ 40 and seroconversion (≥ 4 -fold increase in titres)^{32,33}.

2.1.3 Systems vaccinology of influenza vaccines

Although HAI titres are accepted as established correlates for inactivated seasonal influenza vaccines, they fail to account for alternate mechanisms such as T cell-mediated protection, and correlates for LAIV and pandemic influenza vaccines are less reliable²⁸. For novel and emerging diseases, there may be no prior knowledge of robust correlates to use in the vaccine development process. In response, the last decade has seen the rise of systems vaccinology studies: the analysis of high-dimensional data measured using multiple technologies in vaccinated individuals, in order to characterise response to vaccination at multiple levels of the biological system³⁴. Such information helps elucidate a vaccine's mode of action, discover "molecular signatures" predictive of vaccine safety and efficacy, and has become an increasingly important part of the modern vaccine development chain^{35,36}.

Various systems vaccinology studies of seasonal influenza vaccines have been conducted, taking longitudinal measurements pre-vaccination, and commonly at some subset of days 1, 3, 7, and 28 post-vaccination. These measurements can be correlated to changes in antibody titres after vaccination

to define signatures of antibody response with potential utility as correlates of protection. One of the earliest such studies by Zhu et al.³⁷ found that expression of type 1 interferon-modulated genes was a signature of response to LAIV. An expression signature including *STAT1*, *CD74*, and *E2F2* correlated with serum antibody titres after vaccination with trivalent inactivated influenza vaccine³⁸; kinase *CaMKIV* expression is also a strong predictor³⁹, as are genes related to B cell proliferation⁴⁰.

For these studies of seasonal influenza vaccines in adults, responses tend to be biased by recall from past vaccination or infection^{38,41}. There have also been few studies of adjuvanted influenza vaccines, despite their superior efficacy in comparison to non-adjuvanted counterparts^{42,43}.

2.1.4 The Human Immune Response Dynamics (HIRD) study

The Human Immune Response Dynamics (HIRD) study conducted by Sobolev *et al.* [44] was conceived with the above limitations in mind. The vaccine studied was Pandemrix, an AS03-adjuvanted, split-virion, inactivated vaccine against the influenza A (H1N1)pdm09 strain, for which the majority of the cohort at the time would be unlikely to have immunological memory. A total of 178 individuals were vaccinated with a single dose of Pandemrix, and longitudinal transcriptomic, cellular, antibody titre, and adverse event phenotypes were collected. Gene expression was profiled using a microarray, and differential gene expression (DGE) analyses detected genes associated with both myeloid and lymphoid effector functions upregulated at day 1, most prominently for genes associated with interferon responses. These early myeloid responses were consistent with studies of unadjuvanted seasonal influenza vaccines, but the interferon gamma-associated lymphoid response was unique to this adjuvanted vaccine.

Genes related to plasma cell development and antibody production were more highly expressed in 23 vaccine responders compared to 18 non-responders at day 7 post-vaccination. However, due to high variability among the vaccine non-responders in variables such as baseline antibody titres, a consensus predictive model that segregated the two groups could not be built, even considering other measures such as frequencies of immune cell subsets and serum cytokine levels, suggesting there was no single contributing factor that led to vaccine failure. This is in contrast to several studies of seasonal influenza vaccines, where certain expression signatures are able to predict

make sure gap and how it is filled is emphed enough

vaccine response even pre-vaccination^{45–48}.

2.1.5 Chapter summary

Transcriptomic measurements in the original HIRD study were restricted to a relatively small number (46/178) of individuals, potentially limiting power to detect a expression signatures associated with antibody response. In addition, the responder vs. non-responder phenotype definition used does not account for variation in pre-existing baseline titres, and the binary definition can result in loss of statistical power^{49–51}.

In this chapter, I integrate the original microarray data from HIRD with RNA-sequencing (RNA-seq) data on a larger subset (75) of newly sequenced individuals from the same cohort using Bayesian random-effects meta-analysis. The overall pattern of expression over time from my meta-analysis agrees with the patterns from the original study⁴⁴, with transient innate immune response at day 1 post-vaccination, progressing to adaptive immune response by day 7.

needs 1 more punchline sentence here

From existing HAI and MN data, I compute a baseline-adjusted, continuous measure of antibody response to vaccination, the titre response index (TRI)³⁸. Effect sizes of genes with expression that correlated with TRI were very dependent on measurement platform (array or RNA-seq), and no robust hits were detected in the meta-analysis. Leveraging the greater power that rank-based gene set enrichment analyses affords, I find modules of co-expressed genes that correlate with antibody response, with the strongest effects observed for adaptive immune modules at day 7, but also in inflammatory modules at baseline.

2.2 Methods

2.2.1 Existing HIRD study data and additional data

The design of the HIRD study is described in⁴⁴. In brief, the study enrolled 178 healthy adult volunteers in the UK. The vaccine dose was administered after blood sampling on day 0; five other longitudinal blood samples were taken on days -7, 0, 1, 7, 14 and 63. Serological responses were medasured on days -7 and 63 using the HAI and MN assays, and various subsets of the cohort were also profiled for serum cytokine levels (Luminex panel, days -7,

0, 1 and 7), immune cell subset counts (fluorescence-activated cell sorting (FACS) panels, all days), and peripheral blood mononuclear cell (PBMC) gene expression (microarray, days -7, 0, 1 and 7). The gene expression microarrays were performed in two batches.

In addition to the existing data, array genotypes were generated for 169 individuals; and RNA-seq data for 75 individuals at days 0, 1, and 7. The sets of individuals with gene expression assayed by microarray and RNA-seq is disjoint, as no biological material for RNA extraction remained for the microarray individuals. An overview of datasets is shown in Fig. 2.1.

2.2.2 Computing baseline-adjusted measures of antibody response

In⁴⁴, Pandemrix responders were defined as individuals with ≥ 4 -fold titre increases in either the HAI or MN assays. This is a threshold for seroconversion set out by the U.S. Food and Drug Administration⁵², and is used in many studies of seasonal influenza vaccines³⁵. The responder status for 166 individuals with both HAI and MN titres available at baseline (day -7) and post-vaccination (day 63) were computed according to this definition. However,⁴⁴ noted there was heterogeneity in the baseline titres of non-responders, citing “glass ceiling” non-responders whose high baseline titres made the fixed 4-fold threshold hard to achieve. Dichotomisation of continuous response variables can also result in loss of statistical power^{49,51}.

To address these concerns, I computed the TRI as defined in Bucasas *et al.* [38]. For each assay, a linear regression was fit with the \log_2 day 63/day -7

atm I'm not using R/NR. wording here implies I am

cite appropriate subfigures here

change score is usually negatively correlated to baseline⁵³. hence TRI, whilst combining, is still not ideal

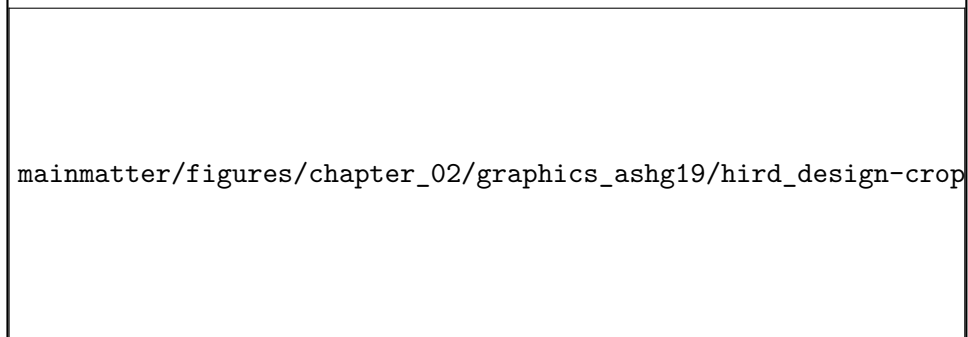


Figure 2.1: Data types, timepoints, and sample sizes. Individuals were vaccinated after day 0 sampling. Antibodies to the vaccine strain were measured by HAI and MN assays. Array and RNA-seq gene expression measured in the PBMC compartment.

cite appropriate subfigures here, after adding proper subfigure labels

Add to collab note that extractions were done at KCL

titre fold change as the response, and the \log_2 day -7 baseline titre as the predictor. The residuals from the two regressions were each standardized to zero mean and unit variance, then averaged. The TRI expresses a continuous measure of change in antibody titres across both assays post-vaccination, compared to individuals with a similar baseline titre, and remains comparable to the binary 4-fold change definition (Fig. 2.2).

Descriptive statistics for the 114 individuals with both gene expression and antibody titre data are presented in Table 2.1. Although the proportion of responders between array (32/44) and RNA-seq (59/70) individuals is similar ($p = 0.1551$, Fisher's exact test), the variance of TRI in array individuals is higher ($p = 0.0002098$, Levene's test), suggesting more extreme antibody response phenotypes are present (Fig. 2.3). The cause of this is unknown, there is a possibility that individuals with more extreme phenotypes were prioritised for array transcriptomics in the original HIRD study*.

2.2.3 Genotype data generation

DNA was extracted from frozen blood using the Blood and Tissue DNeasy kit (Qiagen), and genotyping was performed using on the Infinium CoreExome-24 BeadChip (Illumina). In total, 192 samples from 176 individuals in the HIRD cohort were genotyped at 550601 markers, including replicate samples submitted for individuals where extracted DNA concentrations were low.

2.2.4 Genotype data preprocessing

Using PLINK (v1.90b3w), genotype data underwent the following quality control procedures to remove poorly genotyped samples and markers: max marker missingness across samples $< 5\%$, max sample missingness across markers $< 1\%$, max marker heterozygosity rate within 3 standard deviations of the mean (threshold selected visually to exclude outliers, Fig. 2.4), removal of markers that deviate from Hardy-Weinberg equilibrium (`--hwe` option, $p < 0.00001$).

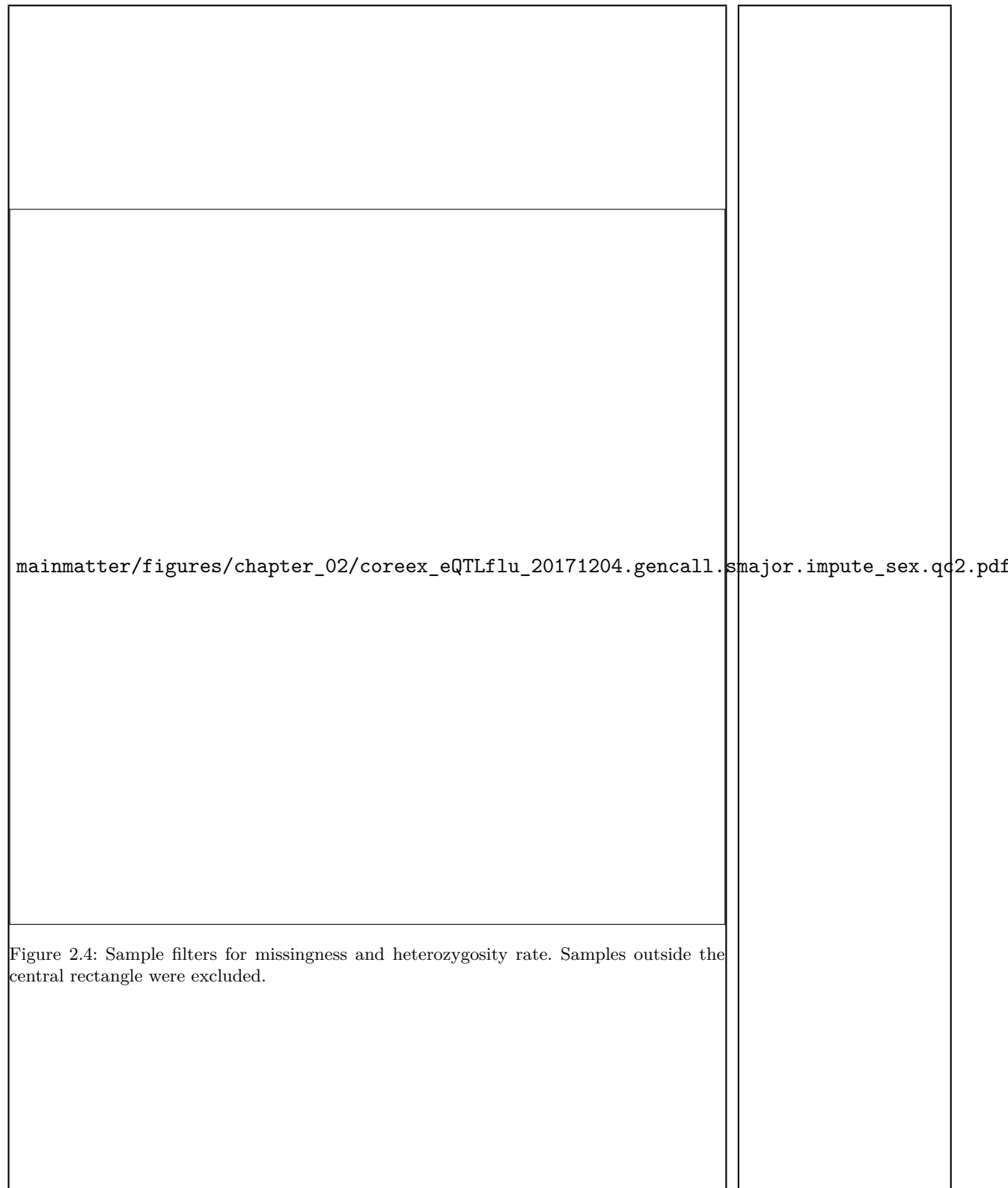
To exclude highly-related individuals and deduplicate replicate samples, pairwise kinship coefficients were computed on minor allele frequency (MAF) < 0.05 pruned genotypes using KING (v1.4). For each pair of samples with

*Personal communication with authors.



mainmatter/figures/chapter_02/compare_phenotype_by_platform.pheno_boxplots.pdf

Figure 2.3: Distribution of TRI, stratified by platform used to measure expression.



pairwise kinship coefficient > 0.177 (first-degree relatives or closer), the sample with lower marker missingness was selected.

After filtering, 169 samples and 549414 markers remained.

2.2.5 Computing genotype principal components as covariates for ancestry

As shown in Table 2.1, the HIRD cohort is multi-ethnic, hence there is potential for confounding by population structure (sample structure due to genetic background) and genetic association studies^{54,55}. Large-scale population structure explains variation in gene expression⁵⁶, so including population structure as covariates can increase power. Treating HapMap 3 samples⁵⁷ as a reference population where the major axes of variation in genotypes are likely to be ancestry, principal component analysis (PCA) was performed using smartpca (v8000) on linkage disequilibrium (LD)-pruned genotypes (PLINK `--indep-pairwise 50 5 0.2`). HIRD sample principal components (PCs) were computed by projection onto the HapMap 3 PCA eigenvectors. For non-genotyped individuals, PC values were imputed as the mean value for all genotyped individuals with the same self-reported ancestry. The top PCs separate samples of European, African and Asian ancestry (Fig. 2.5), hence these PCs can be used as continuous covariates for ancestry downstream.

Add Tracy-Widom statistics for PCs to justify later choice of 4 PCs for covariates

nicer version, copy the peer code, facet the hird and hapmap samples

Can add other fastqc plots e.g. kmers, overrepresented seqs, seq length

2.2.6 RNA-seq data generation

Total RNA was extracted from PBMCs using the Qiagen RNeasy Mini kit, with on-column DNase treatment. RNA integrity was checked on the Agilent Bioanalyzer and mRNA libraries were prepared with the KAPA Stranded mRNA-Seq Kit (KK8421), which uses poly(A) selection. To avoid confounding of timepoint and batch effects from pooling, samples were pooled by library prep plate, ensuring libraries from all timepoints of an individual were in the same pool, and then sequenced across multiple lanes as technical replicates (HiSeq 4000, 75bp paired-end).

RNA-seq quality metrics were assessed using FASTQC* and Qualimap⁵⁸, then visualised with MultiQC⁵⁹. Sequence quality was high (Fig. 2.6), and

*<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>



duplication levels were low (Fig. 2.7). The unimodal GC-content distribution suggested negligible levels of non-human contamination (Fig. 2.8).

2.2.7 RNA-seq quantification and filtering

Reads were quantified against the Ensembl reference transcriptome (GRCh38) using Salmon⁶⁰ in quasi-mapping-based mode, which internally accounts for transcript length and GC composition. To combine technical replicates, as the sum of Poisson distributions remains Poisson-distributed, counts for technical replicates were summed for each sample. The mean number of mapped read pairs per sample after summing was 27.09 million read pairs (range 20.24-39.14 million), representing a mean mapping rate of 80.73% (range 75.57-90.10%), comfortably within sequencing depth recommendations for DGE experiments⁶¹. Relative transcript abundances were summarised to Ensembl gene-level count estimates using tximport (scaledTPM method) to improve statistical robustness and interpretability⁶².

Genes with short noncoding RNA biotypes* were removed, as they are generally not polyadenylated, and expression estimates can be biased by mis-assignment of counts from overlapping protein-coding or lncRNA genes⁶³. Globin genes, which are highly expressed in erythrocytes and reticulocytes, cell types expected to be depleted in PBMC⁶⁴, were also removed. Given the proportion of removed counts at this stage was low for most samples

*miRNA, miRNA_pseudogene, miscRNA, miscRNA_pseudogene, Mt rRNA, Mt tRNA, rRNA, scRNA, snlRNA, snoRNA, snRNA, tRNA, tRNA_pseudogene. List from <https://www.ensembl.org/Help/Faq?id=468>

mainmatter/figures/chapter_02/graphics_firstYearReport/fastqc/mqc_fastqc_per_ba

Figure 2.6: FastQC sequence quality versus read position for HIRD RNA-seq samples.

	
Figure 2.7: FastQC sequence duplication levels for HIRD RNA-seq samples.	
	
Figure 2.8: FastQC GC profile for HIRD RNA-seq samples.	

(Fig. 2.9), poly(A) selection and PBMC isolation procedures were deemed to have been efficient.

Many of the genes in the reference transcriptome are not expressed in PBMC (Fig. 2.10), and many genes are expressed at counts too low for statistical analysis of DGE. Genes were further filtered to require detection (non-zero expression) in at least 95% of samples, and a minimum of 0.5 counts per million (CPM) in at least 20% of samples. The 0.5 CPM threshold was chosen to correspond to approximately 10 counts in the smallest library, where 10-15 counts is a rule of thumb for considering a gene to be robustly expressed⁶⁵. The change in the distribution of gene expressions among samples before and after filtering shows a substantial number of low expression genes are removed (Fig. 2.11).

After the application of all filters, expression values were available for 21626 genes over 223 samples (75/75 individuals on day 0, 73/75 on day 1, and 75/75 on day 7).

2.2.8 Array data preprocessing

Single-channel Agilent 4x44K microarray (G4112F) data for 173 samples from⁴⁴ were downloaded from ArrayExpress*. These arrays were originally processed in two batches, the effect of which is seen in the raw foreground intensities (Fig. 2.12).

VSN⁶⁶ was used to perform background correction, between-array normalisation, and variance-stabilisation of intensity values, resulting in expression values on a \log_2 scale.

Most genes are targetted by multiple array probes; 31208 probes were collapsed into 18216 Ensembl genes using by selecting the probe with the highest mean intensity for each gene (`WGCNA::collapseRows(method=MaxMean)`, recommended for probe to gene collapsing⁶⁷). While it would be optimal to select a collapsing method to maximise the concordance between array and RNA-seq expression values, there were no samples assayed by both platforms in the HIRD dataset. The final normalised \log_2 intensity values for these 18216 genes over 173 samples is shown in Fig. 2.13.

*<https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-2313/>

```
mainmatter/figures/chapter_02/rnaseq_data_setup.per_sample.short_ncRNA_globin_levels_hi
```

Figure 2.9: Distributions of removed short ncRNA and globin counts as a proportion of total counts in RNA-seq samples.

mainmatter/figures/chapter_02/rnaseq_data_setup.gene_zero_prop.pdf

Figure 2.10: Distribution of the proportion of samples in which genes were detected (non-zero expression). Many genes are not detected in any samples. Vertical line shows 5% threshold below which genes were discarded.

mainmatter/figures/chapter_02/rnaseq_data_setup.sample_cpm_density_filtered.pdf

Figure 2.11: Distribution of gene expressions for RNA-seq samples before and after filtering no expression and low expression genes. Vertical line shown at CPM = 0.5 threshold.

mainmatter/figures/chapter_02/array_data_setup.array_intensity_boxplots.pdf

Figure 2.12: Raw foreground intensities for 173 HIRD array samples. Colored by array processing batch.



2.2.9 Differential gene expression

PCA of the expression data reveals although samples separate by experimental timepoint along PC3 (Fig. 2.14d), measurement platform is by far the largest source of variation. Normalisation was also not able to completely remove the batch effect within the array data (Fig. 2.14a). The large platform effect likely stems from systematic technological differences in how each platform measures expression. For example, arrays suffer from ratio compression due to cross-hybridisation⁶⁸. RNA-seq has a higher dynamic range, resulting less bias at low expression levels, but estimates are more sensitive to changes in depth than array estimates are to changes in intensity⁶⁹. There are also differences in the statistical models behind expression quantification and normalisation, as described above.

cite relevant preprocessing sections

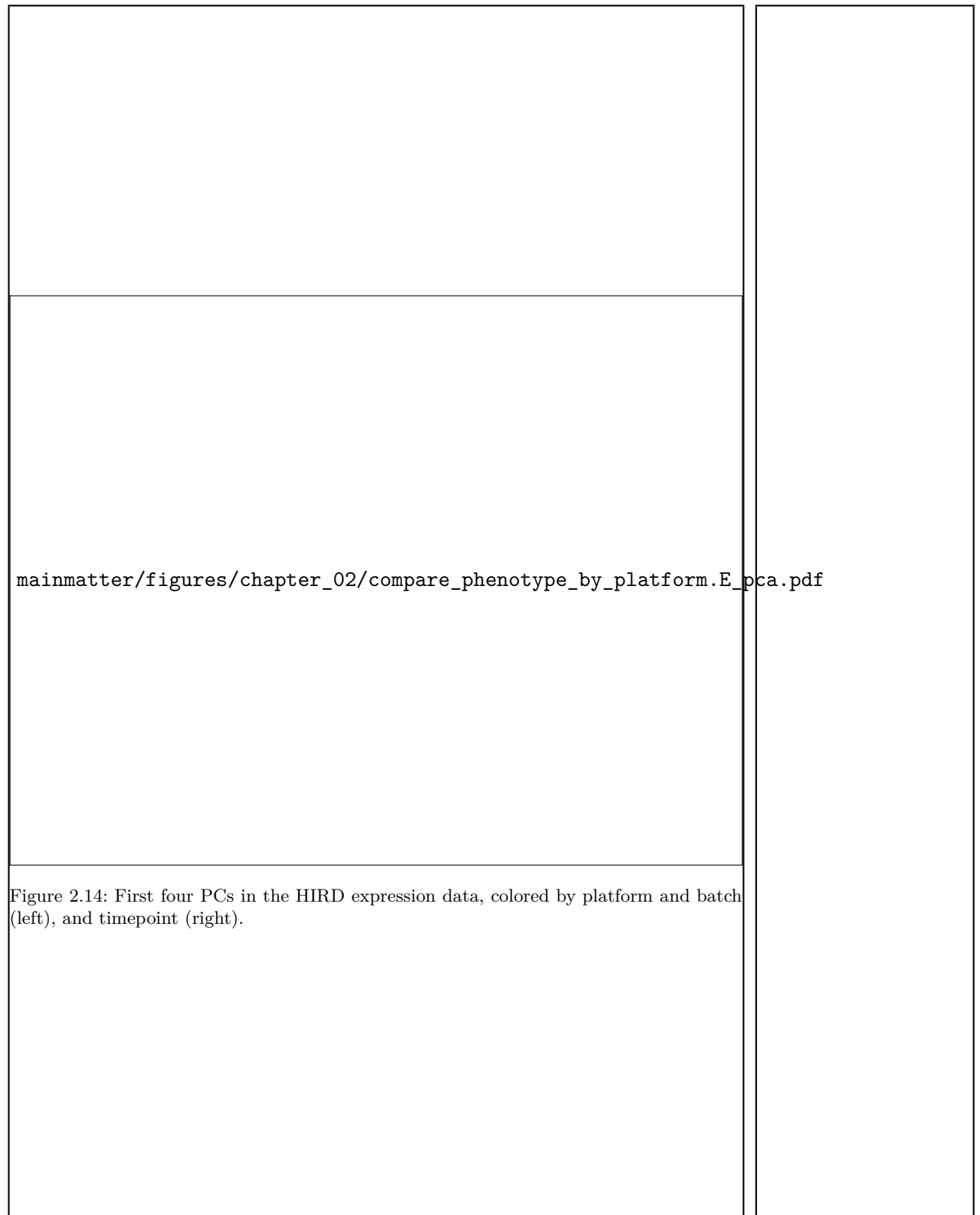
Despite the shortcomings of array data detailed above, the array dataset tends to contain individuals with more extreme antibody response phenotypes (Fig. 2.3), and hence the data should not be excluded. Given the magnitude of the platform effect, I concluded that the appropriate approach should be a two-stage approach that integrates per-platform DGE effect estimates while explicitly accounting for between-platform heterogeneity.

combat does have a pro in that it can do per gene scaling, that fixed fx won't do

Regarding the batch effect within the array data, a popular adjustment method is ComBat⁷⁰, which estimates centering and scaling parameters by pooling information across all genes using empirical Bayes. ComBat is the method used in⁴⁴. In comparisons of microarray batch effect adjustment methods, ComBat performs favourably (vs. five other adjustment packages)⁷¹ or comparably (vs. batch as a fixed or random effect in the linear model)⁷². However, where batches are unbalanced in terms of sample size⁷³ or distribution of study groups that have an impact on expression⁷⁴, ComBat can overcorrect batch differences or bias estimates of group differences respectively. In our data, sample size and timepoint groups are fairly balanced between the two array batches, but the proportion of responders is not Table 2.2, hence I elect not to use ComBat to pre-adjust the array expression data, and model the batches as fixed effects. In practice, results from the DGE analysis were not substantially affected by the choice of whether to use a ComBat pre-adjustment or a fixed effect.

this is not a very precise justification. actually, if I were to color R/NR in the PCA plot, R/NR doesn't really explain a lot of var in global gene expression. that's probably why the results don't change much.

weaken this. combat is



mainmatter/figures/chapter_02/compare_phenotype_by_platform.E_pca.pdf

Figure 2.14: First four PCs in the HIRD expression data, colored by platform and batch (left), and timepoint (right).

2.2.9.1 Per-platform differential gene expression model

For the array data, as⁴⁴ demonstrated no significant global differences in expression between day -7 and day 0, I likewise merge these two timepoints into a single “day 0” baseline timepoint in the following DGE models.

For the RNA-seq data, between-sample normalisation was performed using the trimmed mean of M-values (TMM) method⁷⁵ from edgeR⁷⁶; then variance-stabilisation was performed using voom⁷⁷, resulting in expression values with units of \log_2 CPM.

Linear models were fit using limma⁷⁸, which is computationally fast, and performs well for sufficiently large ($n \geq 3$ per group) sample sizes⁷⁹. For each gene, I fit a model (model 1) with expression as the response variable; with timepoint (baseline, day 1, day 7), TRI, batch, sex, age, and the first 4 genotype PCs as fixed-effect predictors; and individual as a random-effect predictor. Within-individual correlations for the random effect were estimated using limma::duplicateCorrelation. A second model (model 2) was also fit, including 3 additional terms for the interactions between each timepoint and TRI. Contrasts were defined, testing if linear combinations of estimated coefficients are different from zero. From model 1, I defined contrasts for day 1 vs. baseline, day 7 vs. baseline, day 7 vs. day 1, TRI, sex, and age. From model 2, I defined contrasts for the TRI specifically at each of the three timepoints. Corresponding coefficients and standard errors for the contrasts were extracted from the linear models, which represent effect size in units of \log_2 expression fold change per unit change in predictor value.

2.2.9.2 Choice of differential gene expression meta-analysis method

In the section , I concluded that a two-stage meta-analysis approach would be appropriate. This meta-analysis is restricted to 13593 genes assayed by both the array and RNA-seq platforms.

Two popular frameworks for effect size meta-analysis are fixed-effect and random-effects^{80,81}. Given k studies, the fixed-effect model assumes a common population effect size shared across all studies, with observed variation explained only by sampling error. The random-effects model assumes the k study-specific effect sizes are drawn from some distribution with variance τ^2 (standard deviation (SD) τ), representing an additional source of variation termed the between-studies heterogeneity, reducing to the fixed-effect

model when $\tau = 0$. In the HIRD data, there are $k = 2$ 'studies' (array and RNA-seq), where the platform differences described in section contribute to considerable between-studies heterogeneity. The assumption of $\tau = 0$ is unrealistic, hence a random-effects model is more appropriate.

Unfortunately, there is no optimal solution for directly estimating τ in random-effects meta-analyses with small k ⁸³, in the case of $k = 2$ especially⁸⁴. Many estimators are available⁸⁵, but lack of information with small k causes estimation to be imprecise, and often results in boundary values of $\tau = 0$ that are incompatible with the assumed positive heterogeneity^{86,87}. In such circumstances, the most sensible choice may be to incorporate prior information about model hyperparameters in a Bayesian random-effects framework^{85–88}. For this study, I use the implementation in `bayesmeta`⁸², which requires priors for both effect size and between-studies heterogeneity.

2.2.9.3 Prior for between-studies heterogeneity

The choice of prior for between-studies heterogeneity is influential when k is small⁸⁸. Gelman [89] considers the case of $k = 3$, showing that a flat prior places too much weight on implausibly large estimates of τ , and recommends a weakly informative prior that acts to regularise the posterior distribution. Since I assumed zero estimates for τ are unrealistic, I use a weakly-informative gamma prior recommended by⁸⁶, which has zero density at $\tau = 0$, increasing gently as τ increases. This constrains τ to be positive, but still permits estimates close to zero if the data support it. This is in contrast to priors used in other studies from the log-normal (e.g.^{90,91}) or inverse-gamma (e.g.⁹²) families that have derivatives or zero close to zero, thus ruling out small values of τ no matter what the data suggest; and in contrast to half-t family priors (e.g.^{88,89}), which have their mode at zero, and do not rule out $\tau = 0$.

To estimate the appropriate shape and scale parameters for the gamma empirically, a frequentist random-effects model using the restricted maximum likelihood (REML) estimator for τ (recommended for continuous effects⁸⁵) was first for each gene using `metafor::rma`. Genes with small estimates of $\tau < 0.01$ were excluded, and a gamma distribution was fit to the remaining estimates using `fitdistrplus`.

add label

make all the notation in this section consistent with, and add the equation 2.1. The normal-normal hierarchical model,⁸²

2.2.9.4 Prior for effect size

While the choice of prior on τ is influential when k is small, there is usually enough data to estimate the effect size μ such that any reasonable non-informative prior can be used^{87,89}. `bayesmeta` implements both flat and normal priors for μ . Assuming that most genes are not differentially expressed with effect sizes distributed randomly around zero, I selected a normal prior with $N(\mu = 0, \sigma^2)$, over a flat prior. As in the section above, to determine an appropriate scale, a normal distribution with mean $\mu = 0$ was fit to the distribution of effect sizes from the gene-wise frequentist models to empirically estimate σ .

Heavy-tailed Cauchy priors have been proposed for effect size distributions in DGE experiments to avoid over-shrinkage of true large effects in the tails⁹³. Since `bayesmeta` does not implement a Cauchy prior, to avoid over-shrinkage, I flatten the normal prior considerably by scaling up the variance to $N(0, 100\sigma^2)$. This is equivalent to assuming placing a 95% prior probability that effects are less extreme than approximately 20σ .

2.2.9.5 Evaluation of priors

An example of the empirically estimated hyperparameters for the priors for the day 1 vs. baseline contrast are shown in Fig. 2.15 (for τ) and Fig. 2.16 (for μ). For τ , the final prior used was `Gamma(shape = 1.5693, scale = 0.0641)`. This is comparable to⁸⁶'s default recommendation of a `Gamma(shape = 2, scale = λ)` prior where λ is small. For μ , the final prior used was $N(0, (0.3240 \times 10^2))$. The tails of the non-scaled normal fit (black) are light compared to the Cauchy fit (red), which may lead to over-shrinkage, especially since there are many genes with high positive fold changes for the day 1 vs. baseline effect.

2.2.9.6 Multiple testing correction

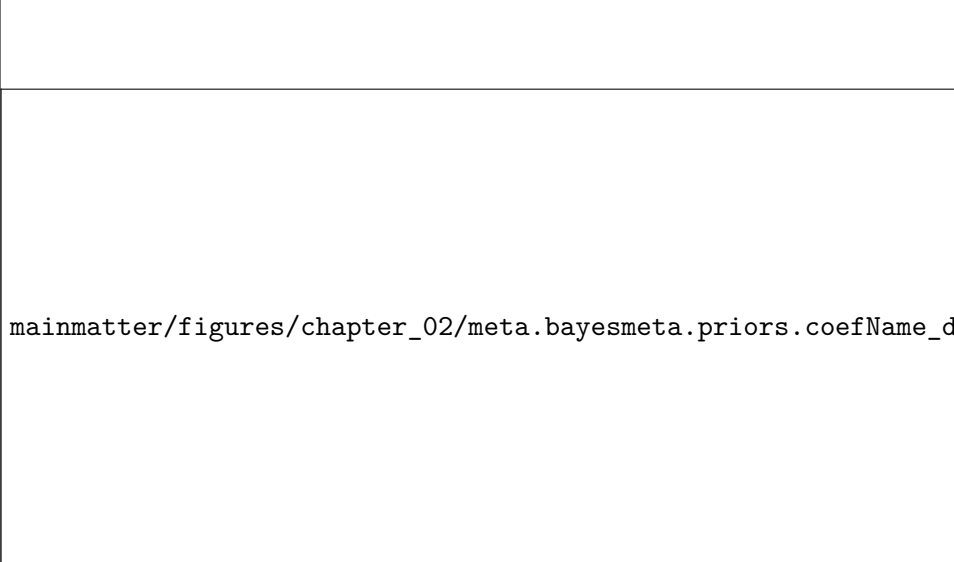
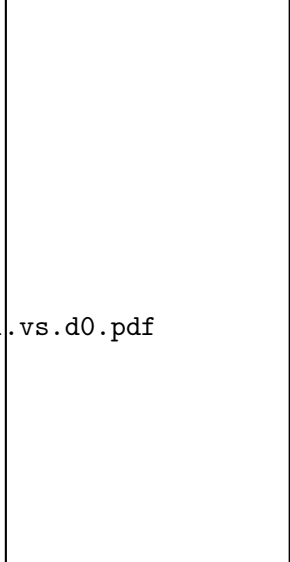
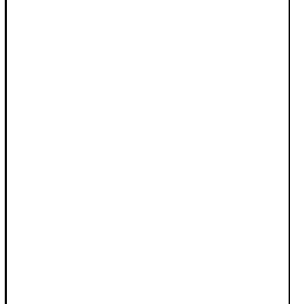
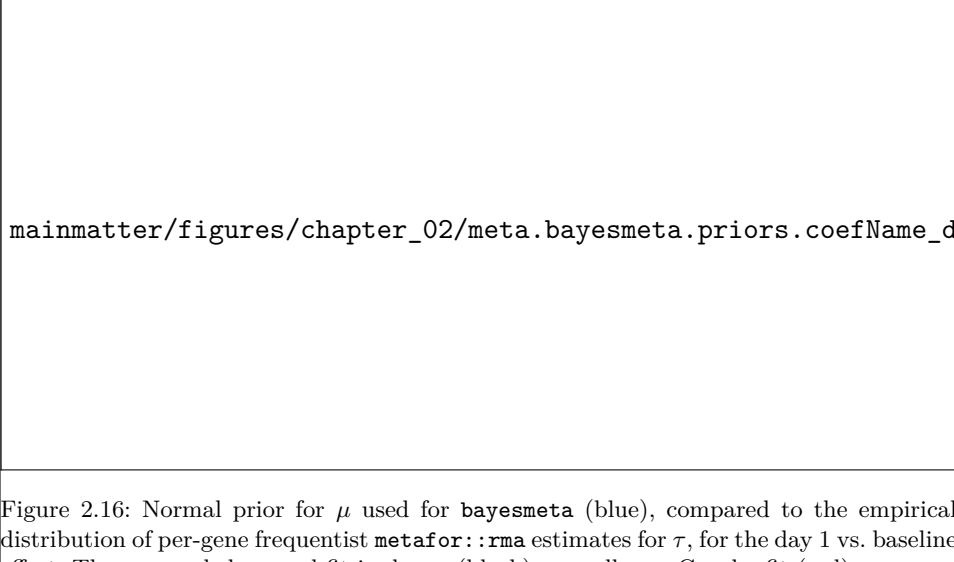
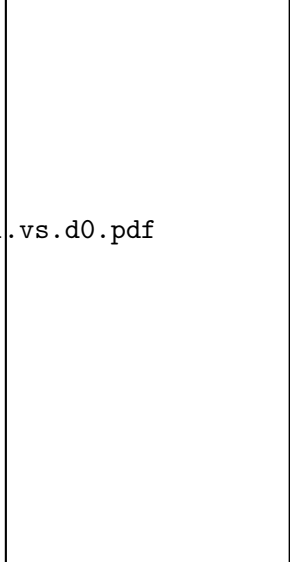

For the frequentist random-effects meta-analysis, nominal gene-wise p -values are converted to false discovery rate (FDR) estimates using the Benjamini-Hochberg (BH) procedure (`p.adjust` in R). For the Bayesian random-effects meta-analysis, posterior effect sizes and standard errors are supplied to `ashr`, which estimates the local false sign rates (lfsrs), which are analogous to FDR, but quantifies the probability of calling the wrong sign for an effect rather

why is this? is it having well powered studies? gelman is vague

the derivation here is `qnorm(0.975, mean=0, sd=1*10) = 1*19.59964`, bit iffy, double check this is correct

could also include a table of all sets of parameters here?

add note on ositive regression dependency²¹

	
<p>Figure 2.15: Gamma prior for τ used for <code>bayesmeta</code> (blue), compared to the empirical distribution of per-gene frequentist <code>metafor::rma</code> estimates for τ, for the day 1 vs. baseline effect (small estimates of $\tau < 0.01$ excluded). Empirical log-normal fit also shown (red).</p>	
	
<p>Figure 2.16: Normal prior for μ used for <code>bayesmeta</code> (blue), compared to the empirical distribution of per-gene frequentist <code>metafor::rma</code> estimates for τ, for the day 1 vs. baseline effect. The non-scaled normal fit is shown (black), as well as a Cauchy fit (red).</p>	

add comment on symmetry

than than the confidence of a non-zero effect⁹⁴.

2.2.10 Gene set enrichment analysis using blood transcription modules

Gene set enrichment analyses were conducted using `tmod::tmodCERNOtest`⁹⁵, which assesses the enrichment of small ranks within specific sets of genes compared to all genes, when the genes are ranked by some metric—here I used effect sizes from `bayesmeta`. The gene sets used were blood transcription modules (BTMs) from⁹⁶, which are annotated sets of coexpressed genes mined from publicly available human blood transcriptomic data, and provide sets tailored for enrichment analyses in blood cells.

2.3 Results

2.3.1 Extensive global changes in expression after vaccination

To gain an overview of how the transcriptome changes after vaccination, linear models were fit to identify genes differentially expressed at day 1 or day 7 compared to baseline (day -7 and day 0) in the HIRD array and RNA-seq expression data, accounting for covariates such as batch effects, sex, age, TRI, and ancestry. At 13593 genes with expression measured by both platforms, models were fit within each platform, then effect sizes were combined using Bayesian random-effects meta-analysis.

At a $\text{lfsr} < 0.05$ and absolute $\text{FC} > 1.5$ cutoff, 857/13593 genes were differentially expressed between any pair of timepoints, with their expression clustering into three main clusters (Fig. 2.17).

2.3.2 Innate immune response at day 1 post-vaccination

Consistent with global expression at day 1 being markedly different from expression at other timepoints (Fig. 2.14), the highest numbers of differentially expressed genes are observed at day 1, with 644 genes differentially expressed vs. baseline. The majority of these (580/644) were upregulated. The gene with the highest FC increase at day 1 compared to baseline was *ANKRD22* ($\log_2 \text{FC} = 4.49$), an interferon-induced gene in monocytes and dendritic cells (DCs) involved in antiviral innate immune pathways⁹⁷. Other key genes



	in the interferon signalling pathway ⁹⁸ such as <i>STAT1</i> (\log_2 FC = 2.1693060), <i>STAT2</i> (\log_2 FC = 0.9489341), and <i>IRF9</i> (\log_2 FC = 0.8153674) are also up-regulated at day 1. Gene set enrichment analysis using tmod revealed that genes with the high FC increases at day 1 were enriched in modules associated with activated DCs, monocytes, toll-like receptor and inflammatory signalling (Fig. 2.18), confirming that day 1 responses are dominated by signatures of innate immunity. 64 genes were downregulated at day 1, enriched in modules associated with T cells and natural killer (NK) cells, with the largest absolute fold change observed for <i>FGFBP2</i> (\log_2 FC = -0.9141547). For both up and downregulated genes, there was a tendency to return to baseline expression levels by day 7.
can also add MSigDB hallmark sets, which include interferon sets; and of course gene ontology sets	
not sure of interpretation at FGFBP2, it is indeed highly expressed in NKs through https://dice-database.org/genes/FGFBP2	
any point in a table of e.g. top 20 DE genes, or is the gene set analysis already enough?	
change x axis labels to baseline, specify top 10 procedure in figure caption	
finish citing	
	<p>2.3.3 Adaptive immune response at day 7 post-vaccination</p> <p>59 genes were differentially expressed at day 7 vs. baseline, with expression fold changes more modest than those at day 1. The genes with the highest upregulation were the B cell-associated genes <i>TNFRSF17</i> (\log_2 FC = 1.7538617) and <i>MZB1</i> (\log_2 FC = 1.7369668). Plasma cell-specific genes including <i>SDC1</i> (encodes CD138 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5437827/) (\log_2 FC = 1.3673081) and <i>ELL2</i> (https://www.nature.com/articles/ni.1786) (\log_2 FC = 0.8679659) were also prominently upregulated. Strongly enriched modules at day 7 were related to mitosis and cell proliferation, particularly in CD4⁺ T cells (Fig. 2.18). Both the CD4⁺ T cell and plasma cell response are indications of an adaptive immune response at day 7.</p>
	<p>2.3.4 Expression signatures associated with antibody response</p> <p>I also looked for genes which have expression associated with baseline-adjusted antibody response, as quantified by TRI. At the initial frequentist meta-analysis stage, with a significance threshold of FDR < 0.05, 6 genes had expression associated with TRI at baseline, 55 at day 7, and 11 pooling samples across timepoints (Fig. 2.19).⁴⁴ also identified genes with day 7 expression associated with antibody response, where response was defined as a binary phenotype based on 4-fold change (described in section). They reported 62 significant associations at FDR < 0.05, of which 58/62 fall into the 13593 genes considered in my meta-analysis (circled, Fig. 2.19), and 15/58</p>
add label	



Figure 2.18: Transcriptomic modules significantly up or downregulated post-vaccination. Size of circle indicates effect size. Color of circle indicates significance and direction of effect (red = upregulation, blue = downregulation).

replicated, all with the same positive direction of effect (high expression with high TRI). In the Bayesian meta-analysis, no single gene was detected as significantly associated with TRI at $\text{lfsr} < 0.05$ at any timepoint, or when pooling samples across all timepoints (Fig. 2.20).

Significant enrichments were detected at the gene set level; the strongest effects are seen at day 7, where expression of cell cycle, CD4⁺ T cells, and plasma cells are associated with high TRI. At day 0, modules related with inflammatory response in myeloid cells are also associated with high TRI (Fig. 2.21).

figure x labels here should be TRI, not R.vs.NR

2.3.5 Identifying expression signatures for predicting antibody response [probably cut this section and just add to discussion]

2.4 Discussion

There is extensive transcriptomic response to Pandemrix vaccination in the HIRD cohort. Upregulation of genes and modules related to the interferon signalling pathway, monocytes, inflammatory response, and other aspects of innate immunity were detected at day 1. This response is transient, with most such genes returning to baseline expression by day 7. Upregulation of cell cycle/proliferation, activated CD4⁺ T cell, and B (plasma) cell genes and modules were detected at day 7. This is likely a signature indicating the shift to an adaptive immune response, involving CD4⁺ T cell-supported differentiation and proliferation of antibody-secreting plasmablasts and plasma cells¹⁰⁰. These patterns of expression change between timepoints in the RNA-seq data are consistent with the patterns in the array data in the original study⁴⁴, and with expansions of monocyte and plasma cell populations seen in the FACS data at days 1 and 7 respectively in the original HIRD study⁴⁴.

Not sure if there is a biological interpretation of downreg of T cells and NK cells gene sets at day 1, since it could be due to increase in other cell types in the sample. similar findings in⁹⁹ though

lit search for downregulation interpretation paper, and downreg T cell paper

In contrast, I was not able to fully replicate the originally reported single gene-level associations between day 7 expression and antibody response in the RNA-seq data and subsequent and meta-analyses. In⁴⁴, 62 genes were reported as differentially expressed between vaccine responders and non-responders. Although⁴⁴ encodes responder status as a binary phenotype, whereas my analysis uses TRI, this is not the primary difference, as 51/62 genes replicated ($\text{FDR} < 0.05$) using TRI when considering just the array

<p style="font-family: monospace; font-size: 0.9em;">mainmatter/figures/chapter_02/plot_dge_eqtl.DGE.effectSizeComparison.pdf</p>	
<p>Figure 2.19: DGE effect sizes estimated in array vs. RNA-seq. Significance colored by frequentist random effects meta-analysis $FDR < 0.05$. Genes with day 7 expression associated with responder/non-responder status in⁴⁴ are circled for that contrast.</p>	
<p style="font-family: monospace; font-size: 0.9em;">mainmatter/figures/chapter_02/plot_dge_eqtl.DGE.effectSizeComparison.pdf</p>	
<p>Figure 2.20: DGE effect sizes estimated in array vs RNA-seq. Significance colored by Bayesian random effects meta-analysis $lfsr < 0.05$. Genes with day 7 expression associated with responder/non-responder status in⁴⁴ are circled for that contrast.</p>	

mainmatter/figures/chapter_02/compare_dge_eqtl.tmodDotPlot.DGE.TRI.pdf

Figure 2.21: Transcriptomic modules enriched in genes with expression associated with antibody response (TRI) at each day. Size of circle indicates effect size. Color of circle indicates significance and direction of effect (red = expression positively correlated with TRI, blue = negative).

data. The same analysis using only the RNA-seq data replicated 0/62 genes. The majority of the effects for these genes were simply much stronger in the array dataset than in the RNAseq dataset (Fig. 2.19). Given that the range of TRI is higher in the array individuals (Table 2.1), this does not seem unusual that stronger TRI-associated effects are observed there.

58/62 reported hits were measured by both platforms and assessed in the meta-analysis. Only 15/58 signals replicated using frequentist random-effects meta-analysis to combine per-platform estimates. I do not consider these hits as robust, as the REML estimate of between-platform heterogeneity was zero for 8563/13593 for the day 7 TRI contrast overall, and zero for all 15 of these signals. None of these signals replicated in the Bayesian random-effects meta-analysis. The Bayesian meta-analysis is in general more conservative, calling fewer differentially expressed genes compared to the frequentist analysis for all contrasts (Fig. 2.20). Prior information about τ is incorporated, discouraging unrealistic estimates of zero heterogeneity. Given the between-platform heterogeneity coming from both platform-specific technical differences and TRI phenotype differences, relative to the modest effect size distributions compared to between-timepoint DGE comparisons, the data are not well-positioned to identify significant single-gene associations with antibody response.

Expression signatures of antibody response were, however, observed at the gene set level, for modules of coexpressed genes that are associated with TRI as a whole. The strongest effects were observed at day 7, where expression of adaptive immune response modules (cell cycle, stimulated CD4⁺ cell, plasma cell modules) were positively associated with TRI. These are the same modules observed to be upregulated at day 7 compared to baseline; it seems that those individuals with the greatest antibody response to vaccination are most able to upregulate these gene sets by day 7 post-vaccination.

Module associations were also observed pre-vaccination (cell adhesion, enriched in B cells, proinflammatory cytokines, platelet activation), suggesting baseline immune state has some influence on long-term antibody response to Pandemrix. Over the years, a diverse range of gene sets have been found to be baseline predictors of serological response to influenza vaccination: apoptosis⁴⁵; Fc γ receptor-mediated phagocytosis, TREM1 signaling⁴⁶; enriched in B cells, T cell activation⁴⁷; B cell receptor signalling, inflammatory response, platelet activation⁴⁸; several of which I also observe. It should

might have to rerun everything using the original binary R/NR if this line of reasoning isn't strong enough

move numbers to results?

TRI is also not ideal

<div style="background-color: #f9cb9c; border: 1px solid black; padding: 5px; margin-bottom: 10px;"> could comment on phenotype differences too, i.e. HIRD measure antibodies at d63, much later than is popular in the field: d28 usually </div> <div style="background-color: #f9cb9c; border: 1px solid black; padding: 5px; margin-bottom: 10px;"> should probably emphasise sobolev didn't find pre-vacc signatures, and we did. But it's not exactly fair, as sobolev didn't use gene set enrichment as far as i can tell </div> <div style="background-color: #f9cb9c; border: 1px solid black; padding: 5px; margin-bottom: 10px;"> There is also something to be said about 'prediction is not inference'. For use as correlates of protection, as promised by proponents of systems studies, prediction is what is important. </div> <div style="background-color: #f9cb9c; border: 1px solid black; padding: 5px; margin-bottom: 10px;"> found signatures, but so what? Feels like chapter lacks a punchline? </div> <div style="background-color: #f9cb9c; border: 1px solid black; padding: 5px;"> why blood? ready easy supply of immune cells </div>	<p>be noted that comparisons with these signatures from existing influenza systems vaccinology studies should be caveated, as most existing studies are for non-adjuvanted influenza vaccines. Adjuvanted influenza vaccines are considerably more immunogenic, and post-vaccination expression patterns differ to those of non-adjuvanted vaccines^{42,44}. Hence, it is particularly important that the robustness of these observed baseline expression signatures be validated in an independent cohort for a comparable AS03-adjuvanted influenza vaccine.</p> <p>In conclusion, Chapter 2 characterises the expansive changes in PBMC gene expression that follow vaccination with Pandemrix. The dominant trend for all individuals is transient upregulation of the innate immune response at day 1, transitioning into adaptive immunity by day 7. Baseline-adjusted antibody response is correlated with expression of gene sets, particularly adaptive immunity modules at day 7, but also for some modules pre-vaccination. Unfortunately, between-platform variation in expression impedes identification of specific genes that contribute. The fundamental question of why gene expression and antibody responses vary between HIRD individuals remains. Chapter 3 will examine one hypothesis: the impact of <u>common human genetic variation on Pandemrix expression response</u>.</p>
---	--

Table 2.1: Sample descriptive statistics.

	Total n = 114	platform	
		array n = 44	rnaseq n = 70
Gender			
F	72 (63.2%)	27 (61.4%)	45 (64.3%)
M	42 (36.8%)	17 (38.6%)	25 (35.7%)
Age at vaccination years	29.2 (11.8)	32.9 (14.1)	26.8 (9.4)
Ethnic Background			
Asian	14 (12.3%)	5 (11.4%)	9 (12.9%)
Black/African	9 (7.9%)	4 (9.1%)	5 (7.1%)
Caucasian	82 (71.9%)	33 (75%)	49 (70%)
Latin american	2 (1.8%)	1 (2.3%)	1 (1.4%)
Mixed	5 (4.4%)	1 (2.3%)	4 (5.7%)
Other - Arab	1 (0.9%)	0 (0%)	1 (1.4%)
White Other	1 (0.9%)	0 (0%)	1 (1.4%)
log2 HAI 0	4.4 (1.8)	4.2 (1.6)	4.5 (1.9)
log2 HAI 6	7.6 (1.8)	7.4 (2.2)	7.6 (1.5)
log2 HAI ratio	3.2 (1.9)	3.2 (2.4)	3.1 (1.6)
log2 MN 0	6.2 (2.8)	5.4 (2.4)	6.6 (3.0)
log2 MN 6	10.4 (2.0)	9.5 (2.2)	10.9 (1.6)
log2 MN ratio	4.2 (2.3)	4.1 (2.6)	4.3 (2.1)
responder			
FALSE	23 (20.2%)	12 (27.3%)	11 (15.7%)
TRUE	91 (79.8%)	32 (72.7%)	59 (84.3%)
TRI	-0.0 (0.9)	-0.2 (1.2)	0.1 (0.7)

Table 2.2: HIRD batch balance

	Total	1	2	batch	DN500166K	DN500167L
	n = 374	n = 87	n = 79	DN500165J n = 70	n = 69	n = 69
visit						
v1	40 (10.7%)	20 (23%)	20 (25.3%)	0 (0%)	0 (0%)	0 (0%)
v2	114 (30.5%)	24 (27.6%)	20 (25.3%)	24 (34.3%)	23 (33.3%)	23 (33.3%)
v3	109 (29.1%)	21 (24.1%)	20 (25.3%)	22 (31.4%)	23 (33.3%)	23 (33.3%)
v4	111 (29.7%)	22 (25.3%)	19 (24.1%)	24 (34.3%)	23 (33.3%)	23 (33.3%)
responder						
FALSE	80 (21.4%)	12 (13.8%)	36 (45.6%)	11 (15.7%)	9 (13%)	12 (17.4%)
TRUE	294 (78.6%)	75 (86.2%)	43 (54.4%)	59 (84.3%)	60 (87%)	57 (82.6%)
TRI						
	-0.1 (1.0)	-0.1 (1.0)	-0.4 (1.4)	0.1 (0.6)	-0.0 (0.8)	0.2 (0.6)

Chapter 3

Genetic factors affecting Pandemrix vaccine response

3.1 Introduction

3.1.1 Genetic factors affecting influenza vaccine response

Vaccination is the most effective way by which seasonal influenza is controlled²⁸, and the mechanism by which influenza vaccines are efficacious is by raising strain-specific antibodies protective against future infection¹⁰¹. Humoral responses are influenced by vaccine-associated factors (e.g. type, dose, adjuvants), but are also a complex trait influenced by host genetics^{102,103}. Genetic variants associated with antibody response have been detected for vaccines such as hepatitis B, influenza, measles, rubella, and smallpox^{102,104}. For antibody response to seasonal influenza vaccines, studies have implicated genetic variation within cytokine genes, cytokine receptors¹⁰⁵; antigen processing and intracellular trafficking genes¹⁰⁶; immunoglobulin heavy-chain variable region loci¹⁰⁷; and specific human leukocyte antigen (HLA) alleles^{105,108}.

A potential mechanism through which genetic variation can play a causal role in influenza vaccine response is through altering the expression of genes as expression quantitative trait loci (eQTLs). eQTL can have condition-specificity: an interaction between their effect on expression and different environmental contexts such as tissue or cell type^{109,110}. The mechanisms by which eQTLs interact with environment are of great interest; for example, cell type specificity can inform us about how expression is regulated in a cell type specific manner¹¹¹. In a vaccination context, an important sub-

pull in citations from intro

set of environment-interacting eQTLs are response expression quantitative trait loci (reQTLs), defined as an eQTL whose effect interacts with external stimulation or perturbation. reQTL have been observed in many human cell types *in vitro*, or in the whole organism *in vivo*. As the pre- and post-stimulation environments are separated in time, a possible mechanism that leads to the observation of reQTL is a genotype-dependent change in gene expression between timepoints, which may underly genotype-dependent differences in antibody phenotypes.

3.1.2 Response expression quantitative trait loci for seasonal influenza vaccination

reQTL can be mapped considering a vaccine as an *in vivo* immune stimulation, looking for genotype-dependent changes in gene expression in immune cells. Little work has been done on vaccine-stimulated reQTLs, except one study conducted for the seasonal trivalent inactivated influenza vaccine (TIV).¹⁰⁶ collected longitudinal data in 247 European adults: peripheral whole blood gene expression measured at four timepoints (day 0, 1, 3, 14), and antibody titres measured at three timepoints (day 0, 14, 28). They identified 20 genes with a cis-eQTL effect, expression correlation with antibody response, and either post-vaccination differential expression *or* a reQTL effect at that cis-eQTL. Genes involved in intracellular antigen transport and processing were enriched among those 20 genes.

3.1.3 Chapter summary

distinction between expression/ab response is blurry here

The HIRD cohort represents a unique opportunity for detecting genetic contributions to influenza vaccine response. In chapter 2, we observed global changes in gene expression after Pandemrix vaccination, as well as expression signatures correlated to degree of antibody response. For seasonal influenza vaccines, the contribution is small: antibody responses in adults are largely driven by non-genetic influences such as previous influenza vaccination or infection¹¹². As the Pandemrix vaccine is against the pdm09 pandemic strain that was not in seasonal circulation at the time the Human Immune Response Dynamics (HIRD) cohort was recruited (2010-11), with individuals mounting an expression response that was not recall-dominated⁴⁴, the relative contribution of genetic factors to Pandemrix response may be greater.

straighten out tenses

In this chapter, I model the influence of common host genetic variation on longitudinal *in vivo* expression response to Pandemrix. I map cis-eQTL within each timepoint, accounting for ancestry, cell type abundance and unmeasured covariates, then call shared and reQTL effects from a joint model, looking for genes where the lead eQTL has a different effect size pre- and post-vaccination. Many of the strongest reQTL effects involve opposite signed effects on expression for the same variant at different timepoints. I detect a strong day 1 specific reQTL effect at *ADCY3*. Through modelling interaction of reQTL with cell type abundance estimates and statistical colocalisation with cell type specific QTL datasets, the reQTL signal was determined to be a monocyte-specific effect likely driven by increase monocyte abundance at day 1.

1 more sentence to round off context

3.2 Methods

3.2.1 Genotype phasing and imputation

Prior to imputation, 213277 monomorphic variants that provide no information for imputation were removed. Imputation for the autosomes and X chromosome was conducted using the Sanger Imputation Service, which involves pre-phasing with EAGLE2 (v2.4) and imputation with PBWT (v3.1) against the Haplotype Reference Consortium (r1.1) panel <https://www.ncbi.nlm.nih.gov/pubmed/27548312>. Variants were lifted-over from GRCh37 to GRCh38 coordinates using CrossMap. Poorly-imputed variants with INFO < 0.4 or post-imputation missingness > 5% were removed, leaving 40290981 variants.

3.2.2 Overall strategy for detecting reQTLs

Since the aim of this chapter is to identify genetic variation that affects expression response to vaccination, it may seem most direct to model the change in each individual's expression after vaccination as the response variable. This approach has been applied for identification of condition-specific eQTL, typically with the response taking units of log fold change between conditions (e.g.^{113–115}). Although potentially powerful if eQTL effects are small and opposite between conditions¹¹⁴, it is analogous to the “change score” approach, which can suffer from regression to the mean, and increased

<p>the variable is not used as an inclusion/exclusion criterion for the study, otherwise regression to the mean will be strong</p>	<p>uncertainty from the variance sum law if effects between conditions have positive covariance^{53,116}. Instead, I map eQTLs within each of three timepoint conditions (day 0 pre-vaccination, day 1, and day 7), and find reQTLs by <u>looking for eQTLs that have different effects between conditions.</u></p>
<p>Can this really demonstrate genotype-dependent change in gene expression between timepoints? i.e. need understand how the change score/ANCOVA approaches differ from repeated measures ANOVA differ from the interaction/stratified approach I take?</p>	<p>Within each timepoint, recall the the HIRD dataset includes expression measured by both array and RNA-sequencing (RNA-seq). As discussed in subsubsection 2.2.9.2, it is difficult to directly estimate the between-studies heterogeneity when the number of studies is small, and Bayesian meta-analysis was preferred for combining array and RNA-seq differential gene expression (DGE) estimates. That method does not scale to eQTL analysis, where the number of tests is large, in the order of thousands of tests per gene, versus the handful DGE contrasts per gene performed in chapter 2. Instead, I perform a mega-analysis within each timepoint, first merging array and RNA-seq expression estimates into a single matrix with ComBat⁷⁰. For comparison purposes, analyses were also run using in the array and RNA-seq samples separately.</p>
<p>why I didn't just do a mega-analysis in chapter 2 then, given I haven't any evidence if it's better or worse than Bayesian meta-analysis in that context.</p>	<p>Defining whether an eQTL is shared between conditions can be a tricky business. Naively, one can map eQTLs separately in each condition, then assess the overlap of significant associations between conditions. This underestimates sharing due to the difficulty of distinguishing true lack of sharing from missed discoveries from incomplete power within each condition. Condition-by-condition analysis also makes no attempt to borrow information across conditions for mapping shared associations^{117–119}. Counterintuitively, a joint multivariate analysis may be more powerful even when associations are not shared across all conditions¹²⁰.</p>
<p>add -7 note as with ch2</p>	<p>A variety of models have been employed for joint eQTL mapping, including the use of classical multivariate methods such as multivariate analysis of variance (MANOVA)¹²¹, frequentist meta-analyses (e.g. Meta-Tissue¹²², METASOFT), and Bayesian models (e.g. eQtlBma¹¹⁷, MT-HESS, MT-eQTL). Joint mapping has been repeatedly been demonstrated to be more powerful than condition-by-condition analysis, and recent methods are now computationally efficient when scaling to large numbers of conditions and variants tested (e.g. RECOV¹²³, mashr¹¹⁸, HT-eQTL¹¹⁹). In this chapter, I apply mashr¹¹⁸ for the estimation of eQTL effects across my three timepoints. mashr learns patterns of correlation among multiple conditions empirically <u>from condition-by-condition summary statistics, then applies shrinkage to</u></p>

provide improved posterior effect size estimates, and compute measures of significance per condition.

3.2.3 Controlling for population structure with linear mixed models

There is population structure due to ancestry in the HIRD cohort, which was incorporated in DGE analyses by treating the top principal components (PCs) of the genotype matrix as continuous covariates for large-scale population structure (subsection 2.2.5). In the context of eQTL mapping (and genetic association studies in general), where the aim is to assess the marginal effect of a single genetic variant on expression, population structure can be correlated with both expression (e.g. through polygenic effects) and the tested variant (e.g. through ancestry-dependent frequency differences). This leads to omitted-variable bias (OVV) from confounding, which if not controlled for, leads to genome-wide inflation of test statistics¹²⁴. An efficient approach is the linear mixed model (LMM) with a random effect that incorporates genetic correlation between individuals, usually in the form of a kinship matrix, into the covariance of that random effect^{55,124,125}. The LMM approach has the advantage of not only modelling large-scale population structure, but also cryptic relatedness (the presence of closely related individuals in a sample assumed to consist of unrelated individuals¹²⁶) due to finer-scale effects such as family structure¹²⁵.

3.2.3.1 Estimation of kinship matrices

When testing a variant for association using LMMs, to avoid loss of power from “proximal contamination”, the kinship matrix used should not include that variant¹²⁷. A simple way to avoid this is to compute a leave-one-chromosome-out (LOCO) kinship matrix using all variants on chromosomes other than the tested variant’s chromosome¹²⁸.

I estimated kinship in the HIRD data from common autosomal variants, using LDAK (5.0), which computes kinship matrices adjusted for bias caused by linkage disequilibrium (LD)¹²⁹. Filtered, pre-imputation sample genotypes from subsection 3.2.1 were pruned to $MAF > 0.05$. A kinship matrix was computed for each autosome, then combined into a single genome-wide matrix using `LDAK --join-kins`. To obtain a LOCO kinship matrix for

add some indication of how much inflation can be reduced by LMMs

add chr1 loco kinship matrix as example, note the estimates for self-relatedness on the diagonals are not constrained to be 1

each autosome, each autosome's kinship matrix was then subtracted from this genome-wide matrix (`LDAK --sub-grm`).

3.2.4 Additional eQTL-specific expression preprocessing

There are a number of transformations often applied to expression data before eQTL mapping, such as the rank-based inverse normal transformation (INT) (e.g. GTEx v8¹³⁰), which conforms often non-normal expression data to an approximately normal distribution, and reduces the impact of expression outliers. In the context of genetic association studies, the practice of applying rank-based INT to phenotypes has been criticised for only guaranteeing approximate normality of residuals when effect sizes are small, and potential inflation of type I error, especially in linear models that include interactions¹³¹. In multi-condition datasets, these transformations are also typically applied within conditions (e.g. within each tissue individually in GTEx v8¹³⁰). Another common transform is standardising (centering and scaling to zero mean and unit variance) (e.g. eQTLGen Consortium¹³²), often done so that effects across genes and studies can be comparably interpreted in units of standard deviation expression¹³³.

I performed simulations to evaluate the effect of these transformations on reQTL detection between a hypothetical baseline and day 1 post-vaccination condition. Expression values on the log scale were simulated with the eQTL slope (beta) set to specific values corresponding to six scenarios for six gene-variant pairs (Fig. 3.1). The simulated scenarios were subjected to rank-based INT (Blom method¹³¹), standardisation (both centering and scaling), scaling-only, and centering-only transformations. Transformations were applied both within each condition and without separating conditions.

The boxed facets in Fig. 3.1 represent undesirable effects of transformations on reQTL calls. For example, rank-based INT induces false shared eQTL effects in scenarios 4 and 5. In general, transformations that scale within condition are not appropriate, as different variance between conditions can be what drives a reQTL effect. Scaling without separating conditions can also be problematic, since the total variance also contributes to the reQTL effect size. For example, scenarios 2 and 4 have the same 1 unit increase in slope pre-transformation (the same fold-change between conditions), but after scaling-only the beta increases are $0.75 - 0 = 0.75$ and $0.8 - 0.4 = 0.4$ respectively—eQTL 4 now looks like a weaker effect.

log scale: as interactions depend on the scale at which departure from additivity is detected

In light of these simulations, I decided that neither rank-based INT nor standardisation were appropriate given my intent of detecting reQTLs between conditions. Only the centering-only transformation avoided both false shared effects and preserves relative reQTL effect sizes between genes. The simple inclusion of an intercept term in the eQTL model already achieves this. Not performing any rank-based transform does lose the advantage of reining in outliers. The expression data have already been preprocessed to remove low-expression outliers in subsection 2.2.7, but automatic outlier exclusion based on standard deviation (SD) thresholds at the eQTL mapping step could be considered in future implementations¹³². Note that many preprocessing steps done prior to this stage in the pipeline (e.g. variance-stabilisation, ComBat batch effect correction) are also expression transformations, but I only consider the preservation of reQTL effects defined from expression values post-adjustment for those technical effects to be important.

3.2.5 Estimation of cell type abundance from expression

Peripheral blood mononuclear cell (PBMC) samples are a mixture of immune cells, and a fixed input of RNA extracted from that mixture is used to estimate expression, so estimates for genes that have cell type specific expression depend on the relative proportions of each cell type in each sample. These proportions shift after Pandemrix vaccination⁴⁴, and eQTL effects can also be cell type specific. As genotype can be assumed to stay constant, it is valid to compare the effect of genotype on expression between multiple time-points to call reQTLs, but changes in cell type abundance influence this by modifying both expression and the effect of genotype on expression. Immune cell abundance also varies naturally between healthy individuals^{112,134}, so it is important to model these effects even at baseline.

Cell type abundance directly measured via fluorescence-activated cell sorting (FACS) are only available for a small subset of HIRD individuals (subsection 2.2.1), so I derived cell type abundance estimates from the expression data as an alternative. Such estimates have previously been used in eQTL analyses from bulk samples where cell type specific effects are expected^{111,135,136}. As the estimates are based on the expression of multiple genes, is not entirely circular to use them as covariates in this way for gene-wise eQTL models. I selected `xCell`¹³⁷, which previously been shown to

add sample sizes and model for expression sim

determine appropriate citations from existing refs in intro

mainmatter/figures/chapter_03/simulate_expression_transforms.pdf

Figure 3.1: Simulated log scale expression in two conditions for six genes (columns) representing six different scenarios: Scenario 0 has no eQTL, scenario 1 is a shared eQTL ($\beta = 1$), scenario 2 is a reQTL where β increases from 0 to 1, scenario 3 is a reQTL where β increases from 0 to 2, scenario 4 is a reQTL where β increases from 1 to 2, and scenario 6 is a reQTL where β increases from 1 to 4. Rows represent the effect of different expression transformations across samples, conducted both within condition, and including both conditions.

outperform other deconvolution methods for cell type specific eQTL mapping in blood¹¹¹. **xCell** computes enrichment scores based on the expression ranks of approximately 10000 signature genes derived from purified cell types, works for both array and RNA-seq expression data, and implements “spillover compensation” to reduce dependency of estimates between related cell types¹³⁷. **xCell** was originally developed for tumor samples, so many of the built-in cell types are not expected in PBMC. Reviewing the literature to find which broad classes of peripheral blood cell types are commonly-expected in the PBMC compartment^{136,138,139}, I selected 7/64 of the built-in cell types: CD4⁺ T cells, CD8⁺ T cells, B cells, plasma cells, natural killer (NK) cells, monocytes, and dendritic cells (DCs). Array and RNA-seq data from subsection 2.2.8 and subsection 2.2.7 were processed through **xCell** separately. The large batch effect present in the array expression was first removed using ComBat. Finally, enrichment scores were standardised, so that a score of zero estimates the average abundance of that cell type across all timepoints (Fig. 3.2 and Fig. 3.3).

As with actual cell type abundances, the enrichment scores are correlated. Multicollinearity will be a problem for interpreting effect size estimates when these scores are used as predictors downstream. To prune the number of scores, I performed a principal component analysis (PCA) of the cell type scores across samples, determined the number of principal components that exceed the eigenvalues-greater-than-one rule of thumb¹⁴⁰, then selected only the one cell type with the highest contribution for each of those components. In both array and RNA-seq datasets, the number of components retained was three, and the selected cell types were monocytes, NK cells, and plasma cells (Fig. 3.4). The choice to use the actual cell type scores over principal components directly as covariates is a sacrifice of orthogonality for interpretability.

Scores were validated against FACS measurements in the subset of individuals that had them. Depending on each panel’s gating strategy for each cell subset, the FACS data were in units of either absolute counts, or percentage of the previously gated population. A rank-based INT was applied within each panel and cell subset, so that the transformed measure could be compared between individuals for each subset (¹⁴¹ takes a similar approach for cell abundance data using a quantile-based INT). Missing values were imputed with **missForest**, a random forest imputation method suitable for

add comment on existence of chosen cell types in samples, and clustering by visit

no need for both size and color, use one for contribution percent

add info on the markers used for the chosen FACS counterparts

mainmatter/figures/chapter_03/get_xCell_estimates.dataset_array.plots.pdf

Figure 3.2: Standardised xCell enrichment scores for seven PBMC cell types in array samples.



mainmatter/figures/chapter_03/get_xCell_estimates.dataset_array.plots.pdf

(a) Array estimates.

mainmatter/figures/chapter_03/get_xCell_estimates.dataset_rnaseq.plots.pdf

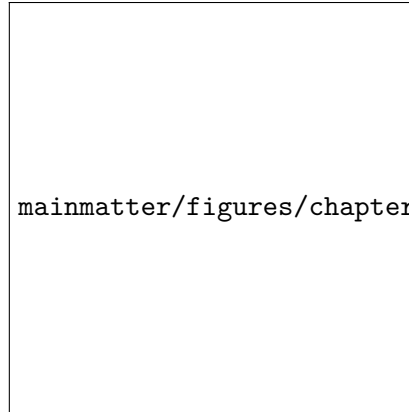
high-dimensional data where $p \gg n$. Although the increase in xCell score for monocytes at day 1 and plasma cells at day 7 reflect the increases in these cell types observed by⁴⁴, overall correlation between xCell and FACS was weak (Fig. 3.5). Weighing the downside of having imperfect estimates of cell type abundance against the downsides of not accounting for abundance, or excluding samples without FACS measures, I chose to continue the analysis using the xCell scores.

3.2.6 Finding hidden covariates using factor analysis

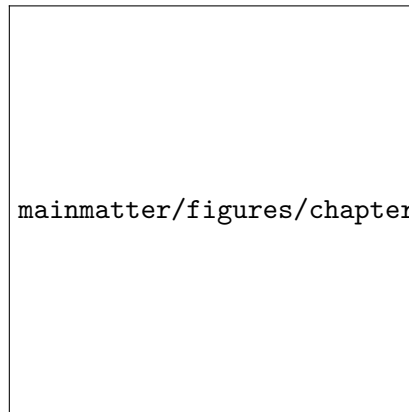
Apart from cell type abundance, a myriad of other unmeasured variables contribute to expression variation. Hidden determinants of expression variation were learnt using PEER¹⁴². As recommended by¹⁴², between-sample normalisation and variance stabilisation RNA-seq data was performed using DESeq2::vsn. ComBat was applied to first merge array and RNA-seq data into a single log scale expression matrix per timepoint, treating the largest global effects on expression—the two array batches and three RNA-seq library prep pools (Fig. 2.14)—as known batch effects. Given known covariates (intercept, sex, four genotype PCs from subsection 2.2.5 representing ancestry, and the three xCell scores estimated above), PEER was used to estimate additional hidden factors that explain variation in expression matrix. Factors are assumed to be unmeasured covariates that have global effects on a large fraction of genes, whereas a cis-eQTL will typically only have local effects, so including factors as covariates should not introduce dependence with the genotype term, but should soak up some of residual variation, improving power to detect cis-eQTLs. The analysis was run per timepoint, otherwise global changes in expression between timepoints induced by the vaccine would be recapitulated as factors.

Correlating the estimated factors to a larger set of known covariates reveals many correlations with xCell estimates, indicating that cell type abundance does indeed have substantial global effects on the expression matrix. There is little correlation with known array or RNA-seq batch effects, indicating ComBat did an adequate job of removing batch- and platform-dependent global effects on expression (Fig. 3.6). Note that I did not leave this adjustment for PEER to perform, as ComBat estimates centering and scaling factors per gene to adjust for batch effects, whereas the use of PEER factors represent a mean-only adjustment. Given the severity of the batch

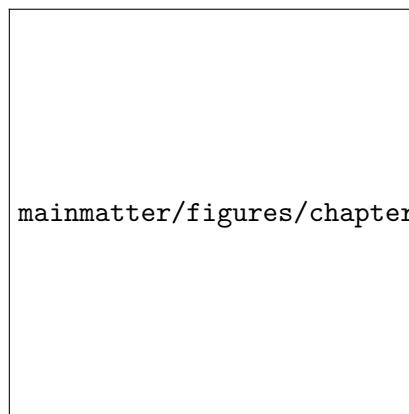
get subset size



(a) Monocytes.



(b) NK cells.



(c) Plasma cells.

Figure 3.5: Correlation between standardised xCell scores and normalised FACS measurements for a similar immune subset, in the subset of individuals with FACS data.

effect in this dataset, especially between platforms, mean-only adjustment may be insufficient⁷³.

3.2.7 eQTL mapping per timepoint

The performance of various software implementations of LMMs specialised for genetic association studies are highly comparable; the specific choice of implementation can usually be made on the basis of computational efficiency⁵⁵.

I map eQTLs within each timepoint using LIMIX¹⁴³, which implements univariate and multivariate LMMs with one or more random effects.

Imputed genotype probabilities were converted to alternate allele dosages using bcftools (1.7-1-ge07034a). Variants with sample AC < 15 within each timepoint were excluded.

At each of 13570 genes, at all cis-variants within within $\pm 1Mb$ of the transcription start site (TSS), I fit the following model to map eQTL:

$$Y = 1 + sex + \sum_{i=1}^4 PC_i + \sum_{i=1}^3 xCell + \sum_{i=1}^k PC_i + \beta G + \mathbf{u} + \epsilon \quad (3.1)$$

where the eQTL effect size of interest is the slope of the genotype fixed effect β , the average additive effect of the alternate allele⁸; and \mathbf{u} is a random effect with zero mean and covariance matrix proportional to the LOCO kinship matrix*.

PEER factors are automatically weighted such that the variance of factors tends to zero as more factors are estimated, hence continuing to add more and more factors as covariates will not continue to improve eQTL detection power, and eventually the model degrees of freedom will be depleted. To optimise k, the number of factors to include as covariates[†], Per-timepoint eQTL mapping was performed in chromosome 1, iteratively increasing the number of factors until the number of eQTLs detected plateaus. I settled on a final choice of $k = 10$ factors for pre-vaccination, 5 factors for day 1, and 5 factors for day 7 (Fig. 3.7).

*For chromosome X variants, no LOCO matrix is available from LDAK, so the matrix for chromosome 1 is used.

†I avoid the commonly-performed two-stage approach of treating PEER residuals as expression phenotypes, as the degrees of freedom seen downstream will be incorrect, which can have a substantial effect on estimates at this modest sample size.

remake this with only top k factors, and prune the possible covariates

add approximate MAFs, then cite hierarch paper

add note on treating x chrom variants with caution

lift proper vector notation from limix, then redo this with a timepoint subscript

add formulation of the 0-mean random effect to show exactly how the kinship matrix is used¹⁴⁴

note stacking of kinship for day -7 repeated measures

i leave the pcs in to guard against unusually differentiated markers, where random effect alone may not be enough¹²⁴

mainmatter/figures/chapter_03/peer_mega/peer.factor_cor_matrix.v2.pdf

Figure 3.6: Correlation of PEER factors to known factors and other possible covariates. Note that PEER factors are not constrained to be orthogonal, so correlations to known factors are expected.

<div style="border: 1px solid black; height: 240px; margin-bottom: 10px;"></div> <div style="font-family: monospace; font-size: 0.9em;"> mainmatter/figures/chapter_03/count_eGenes.signif_eGenes_vs_PEER_n.dataset_mega.chr_chr </div>	<div style="border: 1px solid black; height: 240px; margin-bottom: 10px;"></div> <div style="background-color: #fde9d9; padding: 5px; border-radius: 5px; font-size: 0.8em;"> recheck if did I do a SNPs only filter </div>
<p>Figure 3.7: Number of significant eGenes detected on chromosome 1 (hierarchical Bonferroni-Benjamini-Hochberg (BH) FDR < 0.05) as a function of the number of PEER factors included as covariates k.</p>	
<h3>3.2.8 Joint eQTL analysis across timepoints</h3> <p>Joint analysis was conducted with <code>mashr</code>¹¹⁸, at 40197618 gene-variant pairs (mean of 2962 tests per gene) for which summary statistics from within timepoint mapping were available in all three timepoint conditions. The <code>mashr</code> model incorporates multiple canonical (the identity matrix etc.) and data-driven covariance matrices to represent patterns of effects across conditions (in this case, 3x3 matrices). Data-driven covariance matrices are derived by dimension reduction of a strong subset of tests likely to have an effect in at least one condition. I took the most significant variant per gene per condition, which ensures strong condition-specific effects are included (Fig. 3.8), then further filtered to only nominally significant tests, resulting in a strong subset of 45962 tests.</p> <p>The <code>mash</code> model was trained on a random subset of 200000 tests, using the Exchangeable Z-scores model¹¹⁸. The correlation of null tests between conditions, important to account for due to the repeated measures structure of the data, was estimated using <code>mashr::estimate_null_correlation</code>. The fitted model was used as a prior to compute posterior effects and standard errors for all tests through shrinkage. A condition-specific Bayesian measure of significance local false sign rate (<code>lfsr</code>) is returned, which can be interpreted</p>	

mainmatter/figures/chapter_03/mash_mega/mashr.strong_subset_zval_heatmap.cisDis

Figure 3.8: Clustering of within-timepoint Z scores in the strong mashr subset (random sample of 10000/45962 tests), confirming the presence of strong condition-specific effects.

as the the probability given the data, that the declared sign of the effect is incorrect.

3.2.9 Defining shared and response eQTLs

Many of the tested variants for each gene will be in high LD. To unambiguously select a lead eQTL variant per gene, I selected the variant with the lowest lfsr in any condition, breaking ties by highest imputation INFO, highest MAF, shortest distance to the TSS, and genomic coordinate. Sharing was then evaluated for that gene-variant pair across all three conditions.

Thresholding on the lfsr is not appropriate for determining sharing, as the difference between significant and non-significant effect estimates in two conditions is not necessarily significant^{145,146,118} provides a heuristic that two effects are shared by magnitude if they have the same sign, and are also within a factor of 2 of one another, but this does not consider the posterior standard error of the estimates. Between a pair of effects in two conditions, I compute a z-score for the difference in effects^{145,147}:

$$z = \frac{\beta_x - \beta_y}{\sqrt{\sigma_x^2 + \sigma_y^2 - 2\sigma^2(x, y)}} \quad (3.2)$$

This strategy has been applied to call reQTLs by¹⁴⁸, assuming posterior pairwise covariance of effects is zero $\sigma^2(x, y)$. Unlike a test for difference implemented using a genotype-condition interaction term in a joint regression model, homoscedasticity of errors is not assumed for all conditions¹⁴⁷. A Wald test p -value for the difference can be computed, as under the null hypothesis of zero difference, asymptotically $z \sim \mathcal{N}(0, 1)$. I use nominal p -value < 0.05 as a heuristic threshold (like the mashr recommended 2-fold threshold) to define reQTL effects that are strong, rather than a formal measure of significance. Effects are only compared if at least one of the two effects has lfsr < 0.05 , to avoid sharing being driven by null effects.

3.2.10 Replication of eQTLs in a reference dataset

To validate the eQTL mapping approach, I estimate the replication of significant eQTLs in a large independent reference. Due to the lack of large sample size eQTL maps specific to PBMC, I use the GTEx v8 whole blood dataset as my reference dataset (n=670, 51.2% eGene rate). For lead variants called

move lfsr explanation prior to ashr in dge chapter

not sure whether this is conservative or anti-conservative

as significant in the HIRD dataset at a given lfsr threshold, I lookup the nominal p -value for that variant in GTEx (where the variant exists in both datasets). I applied `qvalue::qvalue_truncp` to estimate the proportion of those GTEx nominal p -values that are null (π_0), then compute a measure of replication $\pi_1 = 1 - \pi_0$.

The mega-analysis has comparable replication rate to RNA-seq-only analysis for shared eQTLs at moderately stringent lfsr thresholds up to 10^{-5} (Fig. 3.9). Past this, as the π_1 procedure assumes a well-behaved p -value distribution in $[0, 1]$, reliability declines due to the number of p -values being too small*, or the maximum p -value being too far from 1. The numbers of reQTLs were too low to assess replication using this method, and one might not expect them to replicate in a baseline dataset such as GTEx whole blood, especially for those reQTLs significant only at post-vaccination timepoints. As the mega-analysis has a higher eGene rate (50.8 % vs. 29.9 %) compared to the RNA-seq-only analysis, with similar replication, I assume this represents a power advantage from having larger a sample size, rather than technical effects from merging the expression data.

RNAseq does test about 7000 more genes though...

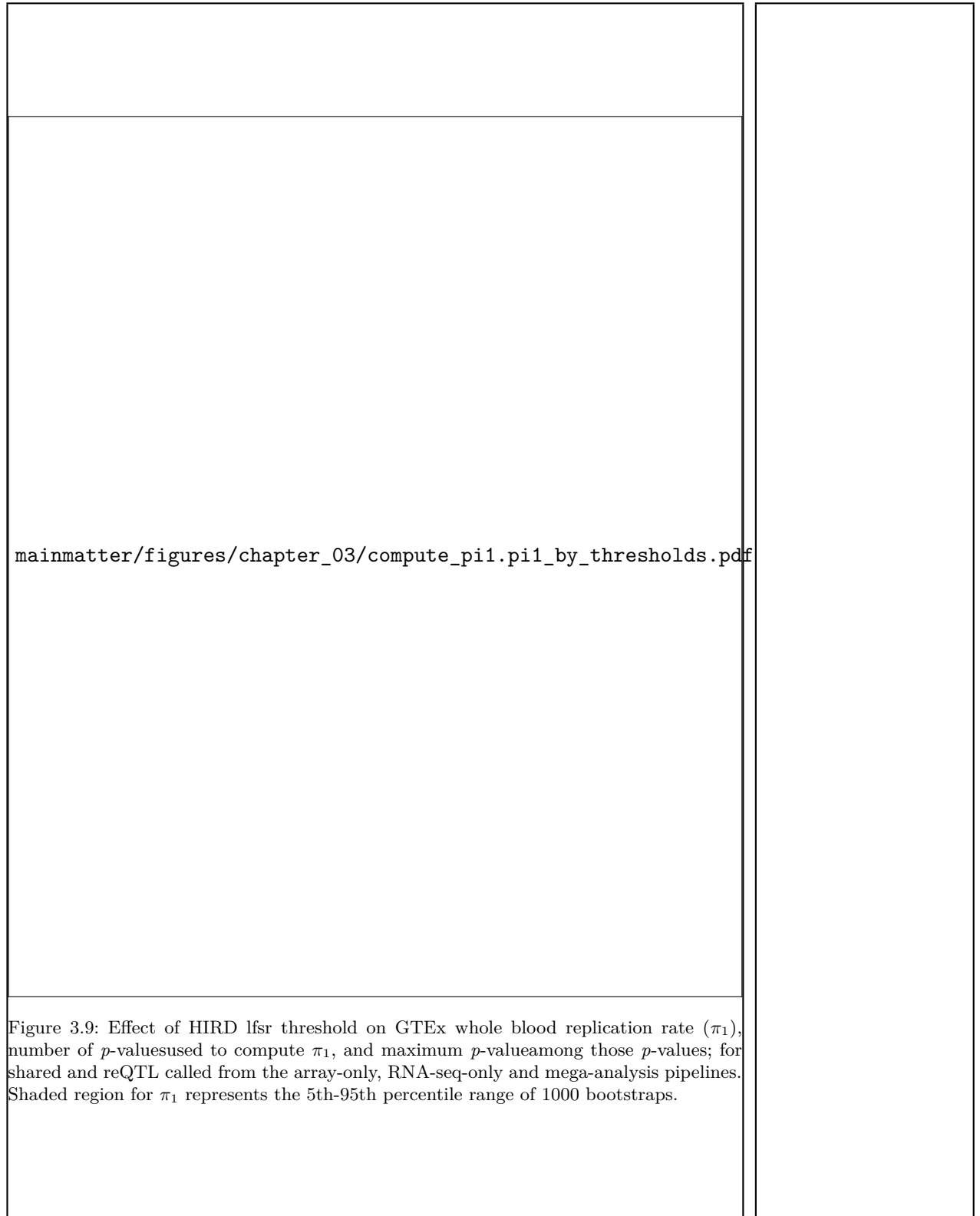
be more specific: "moderator", not 'modify'

point is, doesn't make sense to assume the genotype effect is the same at all levels of cell type abundance

3.2.11 Genotype interactions with cell type abundance

If the abundance of a particular cell type does truly modify the eQTL effect, then an interaction term between genotype and cell type abundance is required, otherwise the regression slope of the eQTL will represent an average across the abundance range for that cell type; one can not correct for this modification just by including the main effect for cell type abundance. Given the modest sample size, I use the two-step approach used by others^{135,136,148,149}, where tests for interaction are only performed at a subset of tests, often the lead eQTL variant for each gene. The key to the two-stage approach is that if the estimates for the interaction effect are sufficiently independent from the estimates of the main effect from main-effect only models, the type I error can be controlled based on the number of interactions that are actually tested, rather the number of interactions that could have been tested for^{149,150}. It is unclear whether this assumption holds, as the size of the main effect may contribute to power for detecting interaction effects. As the main purpose of the interaction analyses is scanning for cell

*<https://github.com/StoreyLab/qvalue/pull/6#commitcomment-26277751>



type effects at detected reQTLs, I chose to test for interactions only at the lead eQTL variant for each gene with a significant main eQTL, then apply the BH false discovery rate (FDR), as used by others^{148,149}.

Models in interactions between genotype and other predictors were fit using `lme4qt1`. The model specification identical to Equation 3.1, with the addition of three interaction terms between genotype and each xCell score. Significance is assessed using the likelihood-ratio test versus the nested model with no interaction terms.

3.2.12 TODO Statistical colocalisation

- if adding coloc analysis, add <https://github.com/jrs95/hyprcoloc> methods here

3.3 Results

3.3.1 Mapping reQTLs to Pandemix vaccination

Within each timepoint condition (day 0 pre-vaccination, day 1, and day 7), cis-eQTLs ($\pm 1\text{Mb}$ of the TSS) were mapped using `LIMIX`, then joint analysis of effects was done using `mashr` to obtain posterior effect size and standard errors. At $\text{lfr} < 0.05$, 6887/13570 genes (50.8%) were eGenes (genes with a significant eQTL) in at least one timepoint. To sidestep the issue of multiple tested variants per gene being in LD, the most significant eQTL variant across all timepoints was selected as the lead variant for each eGene, then reQTLs were defined by comparing the effect size of this lead eQTL between each pair of timepoints. Most eQTLs were shared across timepoints; 1154/6887 (16.8%) eQTLs were classified as reQTLs between any pair of timepoints (nominal p difference < 0.05).

Fig. 3.10 illustrates the difference between calling sharing using a significance threshold versus difference in betas approach. For instance, day 0 was the timepoint with the largest number of eGenes, reflecting the larger sample size compared to other timepoints. Although there are 1427 eGenes significant at only day 0, there are only 646/1427 reQTL among them, as the effect size at day 0 does not differ significantly when compared to day 1 or day 7 for the remainder. The strongest eQTLs with the highest proportion

add note here that although peer is correlated with xcell, interactions are only formed with xcell, so the interaction term can be interpreted per unit of genotype increase when xcell=0

this analysis is incomplete, and is one of the things I would suggest to round off this chapter

if it would be interesting to compare the sharing estimate condition by condition approach to mashr, then redo and pull in eigenmt-bh values

of variance explained (PVE)* are shared between timepoints, highlighting the power advantage for mapping shared effects granted by joint analysis.

3.3.2 Characterising reQTLs post-vaccination

As detection power is greatest at day 0, I focus on eQTLs that are reQTLs between day 0 and either day 1 or day 7 post-vaccination, and are significant at the corresponding timepoint: 819 reQTL between day 1 and day 0, and 1002 reQTL between day 7 and day 0 (Fig. 3.11). Gene set enrichment analysis on the eGenes targets for these sets of reQTLs did not detect any significant enrichments (`gprofiler2`, `gSCS` adjusted $p < 0.05$). Many of the reQTL that satisfy this criteria have opposite effects pre- and post-vaccination—as `lfsr` quantifies uncertainty in the sign of the effect, I do not compare the sign unless the reQTL is also significant at day 0. Shared eQTLs are enriched close to the TSS, whereas reQTLs are distributed across the `cis-` window.

The strongest reQTL at day 1 was for *ADCY3* (p difference = 8.68×10^{-6} , BH FDR = 0.118), where the reQTL variant explained approximately 1.9 % of expression variation at day 0, increasing to 14.1 % at day 1 (Fig. 3.12). At day 7 the strongest reQTL was at *SH2D4A* (p difference = 1.37×10^{-6} , BH FDR = 0.0175). Here, the reQTL variant explained similar amounts of expression variation at day 0 (8.2 %) and day 7 (9.0 %), with opposite directions of effect (Fig. 3.13). Both *ADCY3* and *SH2D4A* have moderately high percentile expression at all timepoints, and are not differentially expressed post-vaccination. Overall, compared to genes without reQTL, reQTLs were less likely be differentially expressed post-vaccination at day 1 (26.5 % for reQTL vs. 42.3 %, Fisher’s test $p < 2.20 \times 10^{-16}$), and no significant difference was observed at day 7 (2.2 % for reQTL vs. 1.4 %, Fisher’s test $p = 0.0509$). Only 5/68 (13.2 %) genes with reQTLs that explain more variation at day 1 were upregulated at day 1 vs. day 0; 5/226 (2.2 %) for day 7 vs. day 0.

*TODO: add to methods <https://journals.plos.org/plosone/article/file?type=supplementary&id=info:doi/10.1371/journal.pone.0120758.s001>

actually, i’ve found that my PVE approximation is basically rescaled $\text{abs}(Z)$, so pve is a bit pointless if we already have z , and doesn’t really help with comparability between genes with diff var/MAF

requiring signif post-vaccination may not be correct, as it excludes many dampening effects

the lack of any positional enrichment makes me concerned for false positives? check with ASE?

expand this to plot 1, list top 5 damp, flip, amp at each timepoint

note anything in lit about any of the 30

double check denoms

convert to subfigures

mainmatter/figures/chapter_03/compare_dge_eqtl.upset.pdf

Figure 3.10: Summary of eQTL mapping results at 13570 genes-lead eQTL pairs, with intersections based on significance ($\text{lfr} < 0.05$). Counts of shared eQTLs and reQTLs; and distribution of INFO score, min MAF across timepoints, and max PVE across timepoints for those lead variants are shown above each intersection.



	<div>  </div> <div> <p>Figure 3.12: <i>ADCY3</i>, strongest reQTL at day 1.</p> </div> <div>  </div> <div> <p>Figure 3.13: <i>SH2D4A</i>, strongest reQTL at day 7. Top: Array and RNA-seq expression before merging with ComBat for mega-analysis. Bottom: eQTL effects at each timepoint condition in the mega-analysis.</p> </div>
--	--

3.3.3 Genotype by cell type interaction effects

Given that many reQTLs are not explained by differential expression post-vaccination, the presence of cell type-specific eQTL effects was considered as an alternate explanation. As described in subsection 3.2.5, **xCell** enrichment scores were used to approximate abundance of seven PBMC cell types from the expression data. After pruning highly correlated cell types to avoid multicollinearity, standardised scores for monocytes, NK cells and plasma cells were tested for genotype interactions. Within-timepoint full eQTL models including the genotype main effect, the three cell type abundance main effects, and three cell type-genotype interaction terms, were fit using `lme4qt1`, then compared to a nested model excluding the three interaction terms.

Significant cell type interactions were detected at 16/1154 reQTLs (BH FDR < 0.05) in any timepoint, including *ADCY3* at day 1 ($\chi^2(3) = 26.3$, likelihood ratio test (LRT) BH FDR = 9.54×10^{-5}). Although the genotype effect size was 0.256 (SE = 0.0334) in the nested model, the estimate in the full model was -0.00722 (0.0666); with the three cell type-genotype interaction term estimates being: monocyte=0.213 (0.0490), NK cells= -0.00920 (0.0447), and plasma cells=0.0162 (0.0663). The small magnitude of the genotype main effect in the full model vs. the nested model indicates the eQTL effect is driven largely by the monocyte score (or a cell type that is highly correlated with monocyte score, see Fig. 3.4). In the case where the monocyte score is zero (representing an average abundance across all samples, as scores are standardised), the effect of increasing genotype dosage on *ADCY3* expression is minimal.



expression vs monocyte xCell score, colored by genotype, to visually prove the point

3.3.4 TODO Genotype by platform interaction effects

- Perhaps using platform specific effects as a filter for reQTLs.

siunitx permits uncertainties

gene set enrichment for cell type interacting genes to further validate xCell score usefulness

Need to consider Nikos' comment that there are too many (1069/13570 significant BH FDR) genotype-platform interactions to use mega-analysis. Consider filtering.

this analysis is incomplete, and is one of the things I would suggest to round off this chapter

3.3.5 TODO Colocalisation of reQTLs with known *in vitro* condition-specific immune eQTLs

- Colocalisation is used to understand the molecular basis of GWAS associations (of a variety of human disease traits) (Giambartolome, 2014)
- Here the inverse: coloc is used to understand the biological relevance of observed reQTL by coloc with known immune QTL
- In a 500 Mb window around the lead *ADCY3* variant rs916485, **hyprcoloc** to colocalise with existing datasets and fine map.
- Day 1 HIRD colocs with BLUEPRINT and Fairfax monocytes (both stim and non stim), but not with Quach or Schmiedel monocytes (Fig. 3.14)
 - Biases from ethnicity-derived differences in LD?
 - Also, priors need tuning?
- **hyprcoloc** fine maps the signal to rs13407914 (credible set size=1, PP = 1), an intronic variant 45064 bp downstream of the TSS.

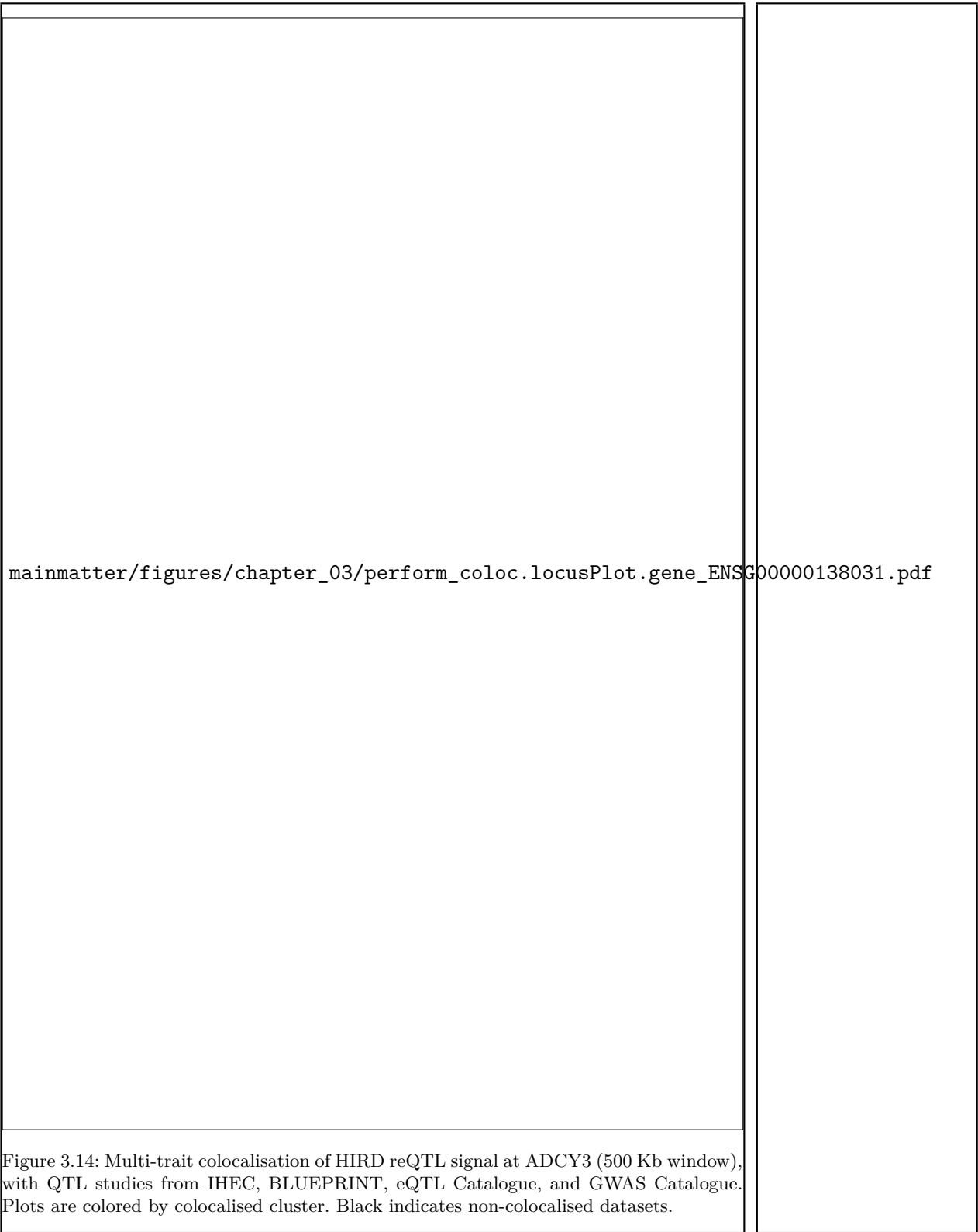
FYI the IBD/T cell coloc fine maps to chr2:24935139 T C (rs713586) with PP=1

add obesity GWAS

3.4 Discussion

In the HIRD cohort, eQTL were detected for 50.8 % of genes in at least one timepoint, day 0, day 1, or day 7. Even in a joint mapping framework, defining reQTL by set significance thresholds, or change in the amount of expression variation explained, will miss classifying equal but opposite effect sizes. I account for the direction and magnitude of effect sizes, defining reQTL strength as the difference in effect size between timepoints. Most eQTL are shared between conditions and replicate well in GTEx whole blood; 16.8 % of lead eQTL for each eGene were reQTL that differed in effect size between timepoints.

Multiple independent eQTLs are present for a large fraction of eGenes¹⁵¹. As the lead variant for reQTL assessment for each eGene was chosen based on significance across all conditions, I can not detect reQTL that are masked



by a stronger shared eQTL at that gene. This is not expected to be uncommon, as the effective sample size for shared eQTLs is usually large due to borrowing of information across conditions. Secondary eQTL signals tend to be weaker, more distal to the TSS, more likely to be enriched in enhancers rather than promoters, and importantly, more context-specific¹⁵². The proportion of genes with reQTL I detect based on only the lead signal likely represents a lower bound.

Given the larger global changes in expression vs. baseline at day 1 compared to day 7 described in chapter 2, the larger number of reQTLs detected at day 7 was unexpected (819 vs. 1002). Opposite sign effects among reQTL post-vaccination were common. Prevalance of opposite sign effects between pairs of conditions has been previously described in multi-tissue studies. In¹⁵³, the proportion of opposite sign effects as a percentage of all eGenes was 7.4 % (48 tissues); in HIRD, I find 39/6887 (0.6 %) at day 1, and 211/6887 (3.1 %) at day 7. In¹⁵⁴, the proportion of opposite sign effects as percentage of all reQTLs was 4.4 % (5 tissues); in HIRD, I find 39/819 (4.8 %) at day 1, and 211/1002 (21.1 %) at day 7. The enrichment of opposite sign effects in HIRD is also apparent at day 7. The strongest reQTL at day 7 is one such opposite sign effect; *SH2D4A* has constitutive expression in T cells, B cells, macrophages, and DCs, encoding a adapter protein involved in intracellular signal transduction*. An approach for validating these opposite sign reQTL using the existing HIRD RNA-seq data is allele-specific expression (ASE) (e.g.¹⁵⁵), where one would expect true opposite sign reQTL effects would also be recapitulated as opposite directions of expression imbalance.

The strongest reQTL detected at day 1 was *ADCY3*, a membrane-bound enzyme that catalyses the conversion of ATP to the second messenger cAMP¹⁵⁶. Genome-wide association studies (GWAS) have identified *ADCY3* as a candidate gene for diseases such as obesity¹⁵⁶ and IBD¹⁵⁷. *ADCY3* has been identified as a target for reQTLs in multiple studies involving stimulated blood immune cells: in PBMC 24h post-infection with rhinovirus¹⁵⁸, in whole blood *in vivo* day 1 after vaccination with seasonal TIV¹⁰⁶, and in whole blood after stimulation with *M. leprae* antigen for 26-32 h¹⁵⁹. Given the diversity of stimulations and tissue types, the effect is likely a consequence of general immune activation, rather than a Pandemrix-specific response.

*<https://doi.org/10.1111/j.1600-065X.2009.00829.x>

I'm not exactly sure why at the moment. Enrichment analyses so far have not turned up much. Up regulation of cell cycle TFs is a possibility.

Statistical colocalisation suggests that the day 1 reQTL signal identified here is likely to be a monocyte-specific effect—and independent to the IBD signal, which colocalises with T cell and macrophage datasets. The proportion of monocytes in the PBMC increase at day 1, supported by both FACS⁴⁴ measurements, and an increase in monocyte xCell score. Expression of *ADCY3* is not monocyte-specific, as despite the increase in monocyte proportion, no upregulation is observed at day 1. Colocalisation is also not restricted to stimulated monocytes, hence the signal could be hypothesised to result simply from the increased proportion of the bulk sample taken up by monocytes, rather than a upregulation-driven increase in detection power, or a vaccine-induced activation of the locus at day 1.

Changes in relative abundances for many cell types occur in the bulk PBMC samples after vaccination. I accounted for the effect of abundance on mean expression including xCell scores and PEER factors as fixed effects in the model, and also considered the effect of abundance on the genotype effect using interaction terms between xCell scores and genotype. Due to the modest sample size, and computational requirements for `lme4qt1`, I focused only whether reQTLs that have a detectable main effect may be driven by cell type interactions, testing only for interactions at significant lead reQTL. Compared to FACS measurements in a cohort subset, the xCell scores used above were only weakly correlated. Some discrepancy is expected, as the cell types as defined in the xCell signatures do not directly correspond to the combinations of surface markers used for FACS. The FACS gating strategy also meant that for some cell populations, the only available FACS measure was a proportion of the previously gated population, whereas xCell attempts to estimate scores that represent proportions of the whole mixture. The accuracy of the built-in signatures is lower when applied to the expression matrix for a stimulated state, likely because the enrichment-based method can not distinguish differential expression of signature genes due to stimulation from actual changes in cell abundance. Nevertheless, as assuming a single genotype where cell-type specific slopes are likely is inappropriate, so xCell scores were used as a best approximation. At 16/1154 reQTLs, the genotype effect was detected to interact with abundance of one or more of the tested cell types (or a correlated cell type). At the day 1 *ADCY3* reQTL, the genetic effect can be mainly attributed to the monocyte score-genotype interaction term, further supporting the hypothesis that it is monocyte-specific.

add lfsr.dge

need to consider: if this kind of thing is what bulk in vivo reQTL can find, they what is the additional value over FACS?

A pressing question remains: what molecular mechanisms underlie the *ADCY3* reQTL, and indeed the remainder of the reQTLs? Power differences due to condition-specific expression are unlikely to explain a large proportion of reQTLs. As in^{136,148}, the overlap between differentially expressed genes and genes with reQTL was poor, and reQTL were not more likely to be differentially expressed compared to genes without reQTL. One mechanism by which cis-eQTL affect expression is through their impact on transcription factor (TF) binding affinity to motifs in promoters and enhancers¹⁶⁰. Immune cells, including monocytes, are regulated by cell type specific TFs¹⁶¹. Cell type specific expression of different TFs have been proposed as a model for explaining magnifying, dampening and opposite reQTL effects; for example, opposite effects can result from TFs regulating the same gene, that are activating in one cell type and suppressive in another¹⁵⁴. There is evidence that TF activity is important for *in vivo* immune reQTL:¹⁵⁸ found rhinovirus reQTLs were ENCODE ChIP-seq peaks for the TFs *STAT1* and *STAT2*, and¹³⁶ found interferon and anti-IL6 drug reQTLs likely disrupt *ISRE* and *IRF4* binding motifs. Rather than condition-specific expression of the eGene, what may be condition-specific is the expression of TFs whose activity is affected by the reQTL*.

Finally, I address the prospect that common genetic variation may explain some variation in antibody response to Pandemrix. I have indirectly demonstrated genotype-dependent effects on expression response by identifying reQTLs with differing effect size between timepoints, but have not yet to determined resulting genotype-dependent differences in antibody phenotypes. Some of the identified reQTLs will undoubtedly affect genes whose expression or post-vaccination expression change correlates with antibody response, but correlation is not transitive¹⁶², and a formal tests such as the causal inference test (CIT)¹⁶³ are required to distinguish mediation of genotype-antibody associations through gene expression from competing models.¹⁰⁶ attempted this, but concluded that they had insufficient power with a greater sample size and comparable study design to HIRD. The HIRD cohort is also too small for a direct GWAS of Pandemrix antibody response.

*A cursory scan of TF motifs disrupted by the location of the fine-mapped *ADCY3* reQTL intronic variant rs13407913 on <https://ccg.epfl.ch/snp2tfbs/snpviewer.php>, does indeed show several motifs (for NR2C2, HNF4A, HNF4G, NR2F1) where the PWM score is higher for the ALT allele, consistent with the direction of effect for the day 1 reQTL.

A suitable approach for prioritising reQTL that contribute to the antibody response to Pandemrix will be to leverage external genetic associations to similar phenotypes, for example, colocalisation with existing GWAS summary statistics for antibody response to a similar type of adjuvanted, inactivated vaccine.

add 1 concluding line

Overall I feel like the chapter is too descriptive, and falls short of making biological insights into Pandemrix response. Any additional analyses would hope to address that.

--	--

Chapter 4

Response to live attenuated rotavirus vaccine (Rotarix) in Vietnamese infants

4.1 Introduction

Summary

Rotavirus vaccine efficacy is lower in LMICs than EU and NA. Protective response to many vaccines is linked with genetic variation. Hypothesis: difference in efficacy is due to differences in genetic variation.

Aim: identify genetic and transcriptomic markers associated with Rotarix protective response primary outcome will be Rotarix vaccine failure events secondary outcomes will be antibody responses and genotypic characterization of the infection virus in Rotarix failure events

4.1.1 The genetics of vaccine response in early life

4.1.2 Rotavirus and rotarix in Vietnam

4.1.3 Known factors that affect rotavirus vaccine efficacy

4.2 Methods

4.2.1 RNA-seq data generation

Stranded RNAseq AUTO with Globin Depletion (>47 samples) uses the NEB Ultra II directional RNA library kit for the poly(A) pulldown, fragmentation, 1st and 2nd strand synthesis and the flowing cDNA library prep (with some minor tweaks e.g. at during the PCR we use kapa HiFi not NEB's Q5 polymerase). Between the poly (A) pulldown and the fragmentation we use a kapa globin depletion kit (it's very similar to their riboerase kit but the rRNA probes are swapped for globin ones).

4.2.2 Genotyping

We will also use the SNP data to accurately impute ABO blood groups and secretor status.

4.3 Results

Transcriptomic response to rotavirus vaccination (pre- vs. post-, prime vs. boost dose, responders vs. non-responders)

Genetic contribution to transcriptomic response

4.4 Discussion

Chapter 5

multiPANTS

5.1 Introduction

Why do some people not respond?

- Explore time series transcriptomic

- Multilevel model where individual is a RE, Find out optimal spline degree. Then work out if genetics changes trajectories for any gene i.e. DGE models with a snp as predictor First need to eQTL scan in general with mashr and find the snps in the most reQTLish genes, since this modelling is probably expensive

- Creating composite features to conduct genetic associations on.

- Identifying signatures of response.

- What is the hypothesis???

5.2 Methods

Can be biased due to dropouts, especially if dropout related to treatment being studied

Responder analysis is a 4-fold loser. It fails to give the needed clinical interpretation, has poor statistical properties, increases the cost of studies because binary outcomes require significantly higher sample sizes, and raises ethical issues because more patients are required to be randomized than would be needed were the endpoint and analysis to be fully efficient.

- immunomods

- In the IFX+ADA cohort, DE PR vs PNR baseline PR vs PNR and w14

n patients with data for each number of visits

5.2.1 Phenotypes

5.2.2 Covariates to use

Sex Age BMI Age of Onset Crohn's Surgery Ever Immunomodulator Current Smoker PCA Proportions of the 6 cell types: CD4+ T cells, CD8+ T cells, B cells, NK cells, monocytes, and granulocytes

5.2.3 reQTL

ANCOVA vs repeated measures vs mixed model

5.3 Results

5.4 Discussion

Chapter 6

Discussion

summary of chapters Tie ch 2 to 3 using baseline predictors? A response eqtl is not always a response eqtl

Limitations, and the perfect study.

gene signatures, rise and fall

Cost-effectiveness and clinical implementation if you can identify NRs, what are you going to do about it?

Finer and finer context

More timepoints Allele-specific expression changes dynamically during T cell activation in HLA and other autoimmune loci

More conditions e.g. 250 e.g. StructLMM Identifies eQTLs with GxE, where the number of environments in E is large (modelled as a random effect)

as datasets and conditions get larger, proportion of eGenes is going to be 100pc, then the question is what are the most relevant ones

Era of single cell. 1st Single-cell RNA sequencing identifies celltype-specific cis-eQTLs and co-expression QTLs <https://www.nature.com/articles/s41588-018-0089-9>

"Single-cell eQTLGen Consortium: a personalized understanding of disease" <https://arxiv.org/abs/1909.12550>

Optimal design of single-cell RNA sequencing experiments for cell-type-specific eQTL analysis <https://www.biorxiv.org/content/biorxiv/early/2019/09/12/766972.full.pdf>

Single-cell genomic approaches for developing the next generation of immunotherapies Ido Yofe, Rony Dahan and Ido Amit

reQTL detection: bulk, sorted, sc current sc will only detect highly ex-

pressed genes

More GWAS

will everything be associated as n continues to increase sensitive to the smallest differences in case/control

complementary to GWAS

Deep phenotyping

disease specific biobanks e.g. ibd bioresource/predicct

PheWAS¹⁶⁴ PheWAS has the advantage of identifying genetic variants with pleiotropic properties.

Translational directions

- Why care?
 - polygenic scores, prs: marker for diagnosis
 - * use in the clinic
 - e.g. polygenic background can modify penetrance
 - * but challenges from:
 - ancestry effects
 - need expanding into global populations, global biobanks e.g. Gains from Africa H3Africa, japanese biobanks
 - non-ancestry effects
 - pathway analysis: "the great hairball gambit"
 - pathway prs
 - * challenge is variant to gene assignment/mapping
 - e.g. restrictions to fine mapped eQTLs
 - Understand mech. of causal genes: molecular pathogenesis
 - how to drug a complex disease with no single 'candidate gene'?
 - * e.g. of successful GWAS -> drug target
 - drug targets with genetic support are more likely
 - * building allelic series
 - vaccines: the cellular response
 - unification immunology and vaccine dev: deep phenotyping, small cohorts achieved -> larger cohorts human genetics and gwas: large cohorts achieved -> deeper phenotyping

Appendix A

Supplementary Materials

A.1 Chapter 2

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

A.2 Chapter 3

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque

cursus luctus mauris.

A.3 Chapter 4

Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

Bibliography

1. 1000 Genomes Project Consortium *et al.* A Global Reference for Human Genetic Variation. *Nature* **526**, 68–74. doi:10.1038/nature15393 (Oct. 1, 2015).
2. The ENCODE Project Consortium. An Integrated Encyclopedia of DNA Elements in the Human Genome. *Nature* **489**, 57–74. doi:10.1038/nature11247 (Sept. 2012).
3. The International SNP Map Working Group. A Map of Human Genome Sequence Variation Containing 1.42 Million Single Nucleotide Polymorphisms. *Nature* **409**, 928–933. doi:10.1038/35057149 (Feb. 2001).
4. Slatkin, M. Linkage Disequilibrium — Understanding the Evolutionary Past and Mapping the Medical Future. *Nature Reviews Genetics* **9**, 477–485. doi:10.1038/nrg2361 (June 2008).
5. Wall, J. D. & Pritchard, J. K. Haplotype Blocks and Linkage Disequilibrium in the Human Genome. *Nature Reviews Genetics* **4**, 587–597. doi:10.1038/nrg1123 (Aug. 2003).
6. The International HapMap Consortium. A Second Generation Human Haplotype Map of over 3.1 Million SNPs. *Nature* **449**, 851–861. doi:10.1038/nature06258 (Oct. 2007).
7. Karczewski, K. J. & Martin, A. R. Analytic and Translational Genetics. *Annual Review of Biomedical Data Science* **3**. doi:10.1146/annurev-biodatasci-072018-021148 (July 20, 2020).
8. Visscher, P. M. & Goddard, M. E. From R.A. Fisher’s 1918 Paper to GWAS a Century Later. *Genetics* **211**, 1125–1130. doi:10.1534/genetics.118.301594 (Apr. 2019).
9. Gibson, G. Rare and Common Variants: Twenty Arguments. *Nature reviews. Genetics* **13**, 135–145. doi:10.1038/nrg3118 (2011).

10. Boyle, E. A., Li, Y. I. & Pritchard, J. K. An Expanded View of Complex Traits: From Polygenic to Omnigenic. *Cell* **169**, 1177–1186. doi:10.1016/j.cell.2017.05.038 (June 2017).
11. Visscher, P. M., Brown, M. A., McCarthy, M. I. & Yang, J. Five Years of GWAS Discovery. *The American Journal of Human Genetics* **90**, 7–24. doi:10.1016/j.ajhg.2011.11.029 (Jan. 2012).
12. Hirschhorn, J. N., Lohmueller, K., Byrne, E. & Hirschhorn, K. A Comprehensive Review of Genetic Association Studies. *Genetics in Medicine* **4**, 45–61. doi:10.1097/00125817-200203000-00002 (Apr. 2002).
13. Visscher, P. M. *et al.* 10 Years of GWAS Discovery: Biology, Function, and Translation. *The American Journal of Human Genetics* **101**, 5–22. doi:10.1016/j.ajhg.2017.06.005 (July 2017).
14. Gallagher, M. D. & Chen-Plotkin, A. S. The Post-GWAS Era: From Association to Function. *The American Journal of Human Genetics* **102**, 717–730. doi:10.1016/j.ajhg.2018.04.002 (May 2018).
15. Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G. & Meyre, D. Benefits and Limitations of Genome-Wide Association Studies. *Nature Reviews Genetics*. doi:10.1038/s41576-019-0127-1 (May 8, 2019).
16. The International HapMap Consortium. A Haplotype Map of the Human Genome. *Nature* **437**, 1299–1320. doi:10.1038/nature04226 (Oct. 2005).
17. Barrett, J. C. & Cardon, L. R. Evaluating Coverage of Genome-Wide Association Studies. *Nature Genetics* **38**, 659–662. doi:10.1038/ng1801 (June 2006).
18. Das, S., Abecasis, G. R. & Browning, B. L. Genotype Imputation from Large Reference Panels. *Annual Review of Genomics and Human Genetics* **19**, 73–96. doi:10.1146/annurev-genom-083117-021602 (Aug. 31, 2018).
19. Pe'er, I., Yelensky, R., Altshuler, D. & Daly, M. J. Estimation of the Multiple Testing Burden for Genomewide Association Studies of Nearly All Common Variants. *Genetic Epidemiology* **32**, 381–385. doi:10.1002/gepi.20303 (May 2008).

APPENDIX A. BIBLIOGRAPHY

20. Jannot, A.-S., Ehret, G. & Perneger, T. $P < 5 \times 10^{-8}$ Has Emerged as a Standard of Statistical Significance for Genome-Wide Association Studies. *Journal of Clinical Epidemiology* **68**, 460–465. doi:10.1016/j.jclinepi.2015.01.001 (Apr. 2015).
21. Goeman, J. J. & Solari, A. Multiple Hypothesis Testing in Genomics. *Statistics in Medicine* **33**, 1946–1978. doi:10.1002/sim.6082 (May 2014).
22. Schaid, D. J., Chen, W. & Larson, N. B. From Genome-Wide Associations to Candidate Causal Variants by Statistical Fine-Mapping. *Nature Reviews Genetics* **19**, 491–504. doi:10.1038/s41576-018-0016-z (Aug. 2018).
23. Goldman, D. & Ducci, F. Deconstruction of Vulnerability to Complex Diseases: Enhanced Effect Sizes and Power of Intermediate Phenotypes. *The Scientific World JOURNAL* **7**, 124–130. doi:10.1100/tsw.2007.210 (2007).
24. Barreiro, L. B., Tailleux, L., Pai, A. A., Gicquel, B., Marionni, J. C. & Gilad, Y. Deciphering the Genetic Architecture of Variation in the Immune Response to Mycobacterium Tuberculosis Infection. *Proceedings of the National Academy of Sciences* **109**, 1204–1209. doi:10.1073/pnas.1115761109 (Jan. 24, 2012).
25. Fairfax, B. P. *et al.* Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression. *Science* **343**, 1246949–1246949. doi:10.1126/science.1246949 (Mar. 7, 2014).
26. Fairfax, B. P. & Knight, J. C. Genetics of Gene Expression in Immunity to Infection. *Current Opinion in Immunology* **30**, 63–71. doi:10.1016/j.coi.2014.07.001 (Oct. 2014).
27. Krammer, F. *et al.* Influenza. *Nature Reviews Disease Primers* **4**. doi:10.1038/s41572-018-0002-y (Dec. 2018).
28. Houser, K. & Subbarao, K. Influenza Vaccines: Challenges and Solutions. *Cell Host & Microbe* **17**, 295–300. doi:10.1016/j.chom.2015.02.012 (Mar. 2015).
29. Sautto, G. A., Kirchenbaum, G. A. & Ross, T. M. Towards a Universal Influenza Vaccine: Different Approaches for One Goal. *Virology Journal* **15**. doi:10.1186/s12985-017-0918-y (Dec. 2018).

30. Broadbent, A. J. & Subbarao, K. Influenza Virus Vaccines: Lessons from the 2009 H1N1 Pandemic. *Current Opinion in Virology* **1**, 254–262. doi:10.1016/j.coviro.2011.08.002 (Oct. 2011).
31. Klimov, A. *et al.* in *Influenza Virus* (eds Kawaoka, Y. & Neumann, G.) 25–51 (Humana Press, Totowa, NJ, 2012). doi:10.1007/978-1-61779-621-0_3.
32. Plotkin, S. A. Correlates of Protection Induced by Vaccination. *Clinical and Vaccine Immunology* **17**, 1055–1065. doi:10.1128/CVI.00131-10 (July 2010).
33. Cox, R. Correlates of Protection to Influenza Virus, Where Do We Go from Here? *Human Vaccines & Immunotherapeutics* **9**, 405–408. doi:10.4161/hv.22908 (Feb. 2013).
34. Pulendran, B. Systems Vaccinology: Probing Humanity’s Diverse Immune Systems with Vaccines. *Proceedings of the National Academy of Sciences* **111**, 12300–12306. doi:10.1073/pnas.1400476111 (2014).
35. Hagan, T., Nakaya, H. I., Subramaniam, S. & Pulendran, B. Systems Vaccinology: Enabling Rational Vaccine Design with Systems Biological Approaches. *Vaccine* **33**, 5294–5301. doi:10.1016/j.vaccine.2015.03.072 (2015).
36. Raeven, R. H. M., van Riet, E., Meiring, H. D., Metz, B. & Kersten, G. F. A. Systems Vaccinology and Big Data in the Vaccine Development Chain. *Immunology* **156**, 33–46. doi:10.1111/imm.13012 (Jan. 2019).
37. Zhu, W. *et al.* A Whole Genome Transcriptional Analysis of the Early Immune Response Induced by Live Attenuated and Inactivated Influenza Vaccines in Young Children. *Vaccine* **28**, 2865–2876. doi:10.1016/j.vaccine.2010.01.060 (Apr. 2010).
38. Bucasas, K. L. *et al.* Early Patterns of Gene Expression Correlate With the Humoral Immune Response to Influenza Vaccination in Humans. *The Journal of Infectious Diseases* **203**, 921–929. doi:10.1093/infdis/jiq156 (Apr. 2011).
39. Nakaya, H. I. *et al.* Systems Biology of Vaccination for Seasonal Influenza in Humans. *Nature Immunology* **12**, 786–795. doi:10.1038/ni.2067 (July 10, 2011).

APPENDIX A. BIBLIOGRAPHY

40. Tan, Y., Tamayo, P., Nakaya, H., Pulendran, B., Mesirov, J. P. & Haining, W. N. Gene Signatures Related to B-Cell Proliferation Predict Influenza Vaccine-Induced Antibody Response. *European Journal of Immunology* **44**, 285–295. doi:10.1002/eji.201343657 (Jan. 2014).
41. Nakaya, H. I., Li, S. & Pulendran, B. Systems Vaccinology: Learning to Compute the Behavior of Vaccine Induced Immunity. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* **4**, 193–205. doi:10.1002/wsbm.163 (Mar. 2012).
42. Wilkins, A. L. *et al.* AS03- and MF59-Adjuvanted Influenza Vaccines in Children. *Frontiers in Immunology* **8**. doi:10.3389/fimmu.2017.01760 (Dec. 13, 2017).
43. Tregoning, J. S., Russell, R. F. & Kinnear, E. Adjuvanted Influenza Vaccines. *Human Vaccines & Immunotherapeutics* **14**, 550–564. doi:10.1080/21645515.2017.1415684 (Mar. 4, 2018).
44. Sobolev, O. *et al.* Adjuvanted Influenza-H1N1 Vaccination Reveals Lymphoid Signatures of Age-Dependent Early Responses and of Clinical Adverse Events. *Nature Immunology* **17**, 204–213. doi:10.1038/ni.3328 (Jan. 4, 2016).
45. Furman, D. *et al.* Apoptosis and Other Immune Biomarkers Predict Influenza Vaccine Responsiveness. *Molecular Systems Biology* **9**, 659. doi:10.1038/msb.2013.15 (Apr. 16, 2013).
46. Tsang, J. S. *et al.* Global Analyses of Human Immune Variation Reveal Baseline Predictors of Postvaccination Responses. *Cell* **157**, 499–513. doi:10.1016/j.cell.2014.03.031 (Apr. 10, 2014).
47. Nakaya, H. I. *et al.* Systems Analysis of Immunity to Influenza Vaccination across Multiple Years and in Diverse Populations Reveals Shared Molecular Signatures. *Immunity* **43**, 1186–1198. doi:10.1016/j.immuni.2015.11.012 (Dec. 2015).
48. HIPC-CHI Signatures Project Team & HIPC-I Consortium. Multicohort Analysis Reveals Baseline Transcriptional Predictors of Influenza Vaccination Responses. *Science Immunology* **2**, eaal4656. doi:10.1126/sciimmunol.aal4656 (Aug. 25, 2017).

49. Cohen, J. The Cost of Dichotomization. *Applied Psychological Measurement* **7**, 249–253. doi:10.1177/014662168300700301 (June 1983).
50. Senn, S. Dichotomania: An Obsessive Compulsive Disorder That Is Badly Affecting the Quality of Analysis of Pharmaceutical Trials, 14 (2005).
51. Fedorov, V., Mannino, F. & Zhang, R. Consequences of Dichotomization. *Pharmaceutical Statistics* **8**, 50–61. doi:10.1002/pst.331 (Jan. 2009).
52. Food and Drug Administration. *Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines* (Jan. 6, 2007), 20.
53. Clifton, L. & Clifton, D. A. The Correlation between Baseline Score and Post-Intervention Score, and Its Implications for Statistical Analysis. *Trials* **20**. doi:10.1186/s13063-018-3108-3 (Dec. 2019).
54. Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A. & Reich, D. Principal Components Analysis Corrects for Stratification in Genome-Wide Association Studies. *Nature Genetics* **38**, 904–909. doi:10.1038/ng1847 (Aug. 2006).
55. Eu-ahsunthornwattana, J. *et al.* Comparison of Methods to Account for Relatedness in Genome-Wide Association Studies with Family-Based Data. *PLoS Genetics* **10** (ed Abecasis, G. R.) e1004445. doi:10.1371/journal.pgen.1004445 (July 17, 2014).
56. Brown, B. C., Bray, N. L. & Pachter, L. Expression Reflects Population Structure. doi:10.1101/364448 (July 8, 2018).
57. The International HapMap 3 Consortium. Integrating Common and Rare Genetic Variation in Diverse Human Populations. *Nature* **467**, 52–58. doi:10.1038/nature09298 (Sept. 2010).
58. Okonechnikov, K., Conesa, A. & García-Alcalde, F. Qualimap 2: Advanced Multi-Sample Quality Control for High-Throughput Sequencing Data. *Bioinformatics* **32**, btv566. doi:10.1093/bioinformatics/btv566 (Oct. 1, 2015).

APPENDIX A. BIBLIOGRAPHY

59. Ewels, P., Magnusson, M., Lundin, S. & Källér, M. MultiQC: Summarize Analysis Results for Multiple Tools and Samples in a Single Report. *Bioinformatics* **32**, 3047–3048. doi:10.1093/bioinformatics/btw354 (Oct. 1, 2016).
60. Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression. *Nature Methods* **14**, 417–419. doi:10.1038/nmeth.4197 (Apr. 6, 2017).
61. Liu, Y., Zhou, J. & White, K. P. RNA-Seq Differential Expression Studies: More Sequence or More Replication? *Bioinformatics* **30**, 301–304. doi:10.1093/bioinformatics/btt688 (Feb. 1, 2014).
62. Soneson, C., Love, M. I. & Robinson, M. D. Differential Analyses for RNA-Seq: Transcript-Level Estimates Improve Gene-Level Inferences. *F1000Research* **4**, 1521. doi:10.12688/f1000research.7563.2 (Feb. 29, 2016).
63. Zhao, S., Zhang, Y., Gamini, R., Zhang, B. & von Schack, D. Evaluation of Two Main RNA-Seq Approaches for Gene Quantification in Clinical RNA Sequencing: polyA+ Selection versus rRNA Depletion. *Scientific Reports* **8**. doi:10.1038/s41598-018-23226-4 (Dec. 2018).
64. Min, J. L. *et al.* Variability of Gene Expression Profiles in Human Blood and Lymphoblastoid Cell Lines. *BMC Genomics* **11**, 96. doi:10.1186/1471-2164-11-96 (2010).
65. Chen, Y., Lun, A. T. L. & Smyth, G. K. From Reads to Genes to Pathways: Differential Expression Analysis of RNA-Seq Experiments Using Rsubread and the edgeR Quasi-Likelihood Pipeline. *F1000Research* **5**, 1438. doi:10.12688/f1000research.8987.2 (Aug. 2, 2016).
66. Huber, W., von Heydebreck, A., Sultmann, H., Poustka, A. & Vingron, M. Variance Stabilization Applied to Microarray Data Calibration and to the Quantification of Differential Expression. *Bioinformatics* **18**, S96–S104. doi:10.1093/bioinformatics/18.suppl_1.S96 (Suppl 1 July 1, 2002).
67. Miller, J. A. *et al.* Strategies for Aggregating Gene Expression Data: The collapseRows R Function. *BMC Bioinformatics* **12**, 322. doi:10.1186/1471-2105-12-322 (2011).

68. Draghici, S., Khatri, P., Eklund, A. & Szallasi, Z. Reliability and Reproducibility Issues in DNA Microarray Measurements. *Trends in Genetics* **22**, 101–109. doi:10.1016/j.tig.2005.12.005 (Feb. 2006).
69. Robinson, D. G., Wang, J. Y. & Storey, J. D. A Nested Parallel Experiment Demonstrates Differences in Intensity-Dependence between RNA-Seq and Microarrays. *Nucleic Acids Research*, gkv636. doi:10.1093/nar/gkv636 (June 30, 2015).
70. Johnson, W. E., Li, C. & Rabinovic, A. Adjusting Batch Effects in Microarray Expression Data Using Empirical Bayes Methods. *Biostatistics* **8**, 118–127. doi:10.1093/biostatistics/kxj037 (Jan. 1, 2007).
71. Chen, C. *et al.* Removing Batch Effects in Analysis of Expression Microarray Data: An Evaluation of Six Batch Adjustment Methods. *PLoS ONE* **6** (ed Kliebenstein, D.) e17238. doi:10.1371/journal.pone.0017238 (Feb. 28, 2011).
72. Espín-Pérez, A., Portier, C., Chadeau-Hyam, M., van Veldhoven, K., Kleinjans, J. C. S. & de Kok, T. M. C. M. Comparison of Statistical Methods and the Use of Quality Control Samples for Batch Effect Correction in Human Transcriptome Data. *PLOS ONE* **13** (ed Krishnan, V. V.) e0202947. doi:10.1371/journal.pone.0202947 (Aug. 30, 2018).
73. Zhang, Y., Jenkins, D. F., Manimaran, S. & Johnson, W. E. Alternative Empirical Bayes Models for Adjusting for Batch Effects in Genomic Studies. *BMC Bioinformatics* **19**. doi:10.1186/s12859-018-2263-6 (Dec. 2018).
74. Nygaard, V., Rødland, E. A. & Hovig, E. Methods That Remove Batch Effects While Retaining Group Differences May Lead to Exaggerated Confidence in Downstream Analyses. *Biostatistics*, kxv027. doi:10.1093/biostatistics/kxv027 (January Aug. 13, 2015).
75. Evans, C., Hardin, J. & Stoebel, D. M. Selecting Between-Sample RNA-Seq Normalization Methods from the Perspective of Their Assumptions. *Briefings in Bioinformatics* **19**, 776–792. doi:10.1093/bib/bbx008 (Sept. 28, 2018).

APPENDIX A. BIBLIOGRAPHY

76. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data. *Bioinformatics* **26**, 139–140. doi:10.1093/bioinformatics/btp616 (Jan. 1, 2010).
77. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. Voom: Precision Weights Unlock Linear Model Analysis Tools for RNA-Seq Read Counts. *Genome Biology* **15**, 1–17 (2014).
78. Ritchie, M. E. *et al.* Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies. *Nucleic Acids Research* **43**, e47–e47. doi:10.1093/nar/gkv007 (Apr. 20, 2015).
79. Soneson, C. & Delorenzi, M. A Comparison of Methods for Differential Expression Analysis of RNA-Seq Data. *BMC Bioinformatics* **14**. doi:10.1186/1471-2105-14-91 (Dec. 2013).
80. Cohn, L. D. & Becker, B. J. How Meta-Analysis Increases Statistical Power. *Psychological Methods* **8**, 243–253. doi:10.1037/1082-989X.8.3.243 (2003).
81. Borenstein, M., Hedges, L. V., Higgins, J. P. & Rothstein, H. R. A Basic Introduction to Fixed-Effect and Random-Effects Models for Meta-Analysis. *Research Synthesis Methods* **1**, 97–111. doi:10.1002/jrsm.12 (Apr. 2010).
82. Röver, C. Bayesian Random-Effects Meta-Analysis Using the Bayesmeta R Package (Nov. 23, 2017).
83. Bender, R. *et al.* Methods for Evidence Synthesis in the Case of Very Few Studies. *Research Synthesis Methods*. doi:10.1002/jrsm.1297 (Apr. 6, 2018).
84. Gonnermann, A., Framke, T., Großhennig, A. & Koch, A. No Solution yet for Combining Two Independent Studies in the Presence of Heterogeneity. *Statistics in Medicine* **34**, 2476–2480. doi:10.1002/sim.6473 (July 20, 2015).
85. Veroniki, A. A. *et al.* Methods to Estimate the Between-Study Variance and Its Uncertainty in Meta-Analysis. *Research Synthesis Methods* **7**, 55–79. doi:10.1002/jrsm.1164 (Mar. 2016).

86. Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A. & Liu, J. A Non-degenerate Penalized Likelihood Estimator for Variance Parameters in Multilevel Models. *Psychometrika* **78**, 685–709. doi:10.1007/s11336-013-9328-2 (Oct. 2013).
87. Friede, T., Röver, C., Wandel, S. & Neuenschwander, B. Meta-Analysis of Few Small Studies in Orphan Diseases. *Research Synthesis Methods* **8**, 79–91. doi:10.1002/jrsm.1217 (Mar. 2017).
88. Seide, S. E., Röver, C. & Friede, T. Likelihood-Based Random-Effects Meta-Analysis with Few Studies: Empirical and Simulation Studies. *BMC Medical Research Methodology* **19**. doi:10.1186/s12874-018-0618-3 (Dec. 2019).
89. Gelman, A. Prior Distributions for Variance Parameters in Hierarchical Models (Comment on Article by Browne and Draper). *Bayesian Analysis* **1**, 515–534. doi:10.1214/06-BA117A (Sept. 2006).
90. Pullenayegum, E. M. An Informed Reference Prior for Between-Study Heterogeneity in Meta-Analyses of Binary Outcomes: Prior for between-Study Heterogeneity. *Statistics in Medicine* **30**, 3082–3094. doi:10.1002/sim.4326 (Nov. 20, 2011).
91. Turner, R. M., Jackson, D., Wei, Y., Thompson, S. G. & Higgins, J. P. T. Predictive Distributions for Between-Study Heterogeneity and Simple Methods for Their Application in Bayesian Meta-Analysis: R. M. TURNER ET AL. *Statistics in Medicine* **34**, 984–998. doi:10.1002/sim.6381 (Mar. 15, 2015).
92. Higgins, J. P. T. & Whitehead, A. Borrowing Strength from External Trials in a Meta-Analysis. *Statistics in Medicine* **15**, 2733–2749. doi:10.1002/(SICI)1097-0258(19961230)15:24<2733::AID-SIM562>3.0.CO;2-0 (Dec. 30, 1996).
93. Zhu, A., Ibrahim, J. G. & Love, M. I. Heavy-Tailed Prior Distributions for Sequence Count Data: Removing the Noise and Preserving Large Differences. *Bioinformatics* **35** (ed Stegle, O.) 2084–2092. doi:10.1093/bioinformatics/bty895 (June 1, 2019).
94. Stephens, M. False Discovery Rates: A New Deal. *Biostatistics*, kxw041. doi:10.1093/biostatistics/kxw041 (Oct. 17, 2016).

APPENDIX A. BIBLIOGRAPHY

95. Weiner 3rd, J. & Domaszewska, T. Tmod: An R Package for General and Multivariate Enrichment Analysis. doi:10.7287/peerj.preprints.2420v1 (2016).
96. Li, S. *et al.* Molecular Signatures of Antibody Responses Derived from a Systems Biology Study of Five Human Vaccines. *Nature Immunology* **15**, 195–204. doi:10.1038/ni.2789 (Dec. 15, 2013).
97. Bin, L., Li, X., Feng, J., Richers, B. & Leung, D. Y. M. Ankyrin Repeat Domain 22 Mediates Host Defense Against Viral Infection Through STING Signaling Pathway. *The Journal of Immunology* **196**, 201.4 LP –201.4 (1 Supplement May 1, 2016).
98. Schneider, W. M., Chevillotte, M. D. & Rice, C. M. Interferon-Stimulated Genes: A Complex Web of Host Defenses. *Annual Review of Immunology* **32**, 513–545. doi:10.1146/annurev-immunol-032713-120231 (Mar. 21, 2014).
99. Nakaya, H. I. *et al.* Systems Biology of Immunity to MF59-Adjuvanted versus Nonadjuvanted Trivalent Seasonal Influenza Vaccines in Early Childhood. *Proceedings of the National Academy of Sciences* **113**, 1853–1858. doi:10.1073/pnas.1519690113 (Feb. 16, 2016).
100. Murphy, K. & Weaver, C. *Janeway's Immunobiology* 9th edition. 904 pp. (Garland Science/Taylor & Francis Group, LLC, New York, NY, 2016).
101. Nauta, J. J., Beyer, W. E. & Osterhaus, A. D. On the Relationship between Mean Antibody Level, Seroprotection and Clinical Protection from Influenza. *Biologicals* **37**, 216–221. doi:10.1016/j.biologicals.2009.02.002 (Aug. 2009).
102. Linnik, J. E. & Egli, A. Impact of Host Genetic Polymorphisms on Vaccine Induced Antibody Response. *Human Vaccines & Immunotherapeutics* **12**, 907–915. doi:10.1080/21645515.2015.1119345 (Apr. 2, 2016).
103. Mentzer, A. J., O'Connor, D., Pollard, A. J. & Hill, A. V. S. Searching for the Human Genetic Factors Standing in the Way of Universally Effective Vaccines. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140341–20140341. doi:10.1098/rstb.2014.0341 (May 11, 2015).

104. Dhakal, S. & Klein, S. L. Host Factors Impact Vaccine Efficacy: Implications for Seasonal and Universal Influenza Vaccine Programs. *Journal of Virology* **93** (ed Coyne, C. B.) doi:10.1128/JVI.00797-19 (Aug. 7, 2019).
105. Poland, G. A., Ovsyannikova, I. G. & Jacobson, R. M. Immunogenetics of Seasonal Influenza Vaccine Response. *Vaccine* **26**, D35–D40. doi:10.1016/j.vaccine.2008.07.065 (Sept. 2008).
106. Franco, L. M. *et al.* Integrative Genomic Analysis of the Human Immune Response to Influenza Vaccination. *eLife* **2**, e00299. doi:10.7554/eLife.00299 (July 16, 2013).
107. Avnir, Y. *et al.* IGHV1-69 Polymorphism Modulates Anti-Influenza Antibody Repertoires, Correlates with IGHV Utilization Shifts and Varies by Ethnicity. *Scientific Reports* **6**, 20842. doi:10.1038/srep20842 (Aug. 16, 2016).
108. Moss, A. J. *et al.* Correlation between Human Leukocyte Antigen Class II Alleles and HAI Titers Detected Post-Influenza Vaccination. *PLoS ONE* **8** (ed Sambhara, S.) e71376. doi:10.1371/journal.pone.0071376 (Aug. 9, 2013).
109. Albert, F. W. & Kruglyak, L. The Role of Regulatory Variation in Complex Traits and Disease. *Nature Reviews Genetics* **16**, 197–212. doi:10.1038/nrg3891 (Apr. 2015).
110. Vandiedonck, C. Genetic Association of Molecular Traits: A Help to Identify Causative Variants in Complex Diseases. *Clinical Genetics*. doi:10.1111/cge.13187 (Dec. 1, 2017).
111. Kim-Hellmuth, S. *et al.* Cell Type Specific Genetic Regulation of Gene Expression across Human Tissues. *bioRxiv*. doi:10.1101/806117 (Oct. 17, 2019).
112. Brodin, P. *et al.* Variation in the Human Immune System Is Largely Driven by Non-Heritable Influences. *Cell* **160**, 37–47. doi:10.1016/j.cell.2014.12.020 (Jan. 2015).
113. Maranville, J. C. *et al.* Interactions between Glucocorticoid Treatment and Cis-Regulatory Polymorphisms Contribute to Cellular Response Phenotypes. *PLoS Genetics* **7** (ed Gibson, G.) e1002162. doi:10.1371/journal.pgen.1002162 (July 7, 2011).

APPENDIX A. BIBLIOGRAPHY

114. Ackermann, M., Sikora-Wohlfeld, W. & Beyer, A. Impact of Natural Genetic Variation on Gene Expression Dynamics. *PLoS Genetics* **9** (ed Wells, C. A.) e1003514. doi:10.1371/journal.pgen.1003514 (June 6, 2013).
115. Shpak, M. *et al.* An eQTL Analysis of the Human Glioblastoma Multiforme Genome. *Genomics* **103**, 252–263. doi:10.1016/j.ygeno.2014.02.005 (Apr. 2014).
116. Allison, P. D. Change Scores as Dependent Variables in Regression Analysis. *Sociological Methodology* **20**, 93. doi:10.2307/271083 (1990).
117. Flutre, T., Wen, X., Pritchard, J. & Stephens, M. A Statistical Framework for Joint eQTL Analysis in Multiple Tissues. *PLOS Genet* **9**, e1003486. doi:10.1371/journal.pgen.1003486 (May 9, 2013).
118. Urbut, S. M., Wang, G., Carbonetto, P. & Stephens, M. Flexible Statistical Methods for Estimating and Testing Effects in Genomic Studies with Multiple Conditions. *Nature Genetics*. doi:10.1038/s41588-018-0268-8 (Nov. 26, 2018).
119. Li, G., Jima, D., Wright, F. A. & Nobel, A. B. HT-eQTL: Integrative Expression Quantitative Trait Loci Analysis in a Large Number of Human Tissues. *BMC Bioinformatics* **19**. doi:10.1186/s12859-018-2088-3 (Dec. 2018).
120. Stephens, M. A Unified Framework for Association Analysis with Multiple Related Phenotypes. *PLoS ONE* **8** (ed Emmert-Streib, F.) e65245. doi:10.1371/journal.pone.0065245 (July 5, 2013).
121. Kim, S. *et al.* Characterizing the Genetic Basis of Innate Immune Response in TLR4-Activated Human Monocytes. *Nature Communications* **5**. doi:10.1038/ncomms6236 (Dec. 2014).
122. Sul, J. H., Han, B., Ye, C., Choi, T. & Eskin, E. Effectively Identifying eQTLs from Multiple Tissues by Combining Mixed Model and Meta-Analytic Approaches. *PLoS Genetics* **9** (ed Schork, N. J.) e1003491. doi:10.1371/journal.pgen.1003491 (June 13, 2013).
123. Duong, D. *et al.* Applying Meta-Analysis to Genotype-Tissue Expression Data from Multiple Tissues to Identify eQTLs and Increase the Number of eGenes. *Bioinformatics* **33**, i67–i74. doi:10.1093/bioinformatics/btx227 (July 15, 2017).

124. Price, A. L., Zaitlen, N. A., Reich, D. & Patterson, N. New Approaches to Population Stratification in Genome-Wide Association Studies. *Nature Reviews Genetics* **11**, 459–463. doi:10.1038/nrg2813 (July 2010).
125. Golan, D., Rosset, S. & Lin, D.-Y. in Borgan, Ø., Breslow, N. E., Chatterjee, N., Gail, M. H., Scott, A. & Wild, C. J. *Handbook of Statistical Methods for Case-Control Studies* (eds Borgan, Ø., Breslow, N., Chatterjee, N., Gail, M. H., Scott, A. & Wild, C. J.) 1st ed., 495–514 (Chapman and Hall/CRC, June 27, 2018). doi:10.1201/9781315154084-27.
126. Astle, W. & Balding, D. J. Population Structure and Cryptic Relatedness in Genetic Association Studies. *Statistical Science* **24**, 451–471. doi:10.1214/09-STS307 (Nov. 2009).
127. Listgarten, J., Lippert, C., Kadie, C. M., Davidson, R. I., Eskin, E. & Heckerman, D. Improved Linear Mixed Models for Genome-Wide Association Studies. *Nature Methods* **9**, 525–526. doi:10.1038/nmeth.2037 (June 2012).
128. Lippert, C., Listgarten, J., Liu, Y., Kadie, C. M., Davidson, R. I. & Heckerman, D. FaST Linear Mixed Models for Genome-Wide Association Studies. *Nature Methods* **8**, 833–835. doi:10.1038/nmeth.1681 (Oct. 2011).
129. Speed, D., Hemani, G., Johnson, M. R. & Balding, D. J. Improved Heritability Estimation from Genome-Wide SNPs. *The American Journal of Human Genetics* **91**, 1011–1021. doi:10.1016/j.ajhg.2012.10.010 (Dec. 2012).
130. Aguet, F. *et al.* The GTEx Consortium Atlas of Genetic Regulatory Effects across Human Tissues. *bioRxiv*. doi:10.1101/787903 (Oct. 3, 2019).
131. Beasley, T. M., Erickson, S. & Allison, D. B. Rank-Based Inverse Normal Transformations Are Increasingly Used, But Are They Merited? *Behavior Genetics* **39**, 580–595. doi:10.1007/s10519-009-9281-0 (Sept. 2009).

APPENDIX A. BIBLIOGRAPHY

132. Vösa, U. *et al.* Unraveling the Polygenic Architecture of Complex Traits Using Blood eQTL Meta-Analysis. *bioRxiv*. doi:10.1101/447367 (Oct. 19, 2018).
133. Qi, T. *et al.* Identifying Gene Targets for Brain-Related Traits Using Transcriptomic and Methyloomic Data from Blood. *Nature Communications* **9**. doi:10.1038/s41467-018-04558-1 (Dec. 2018).
134. Brodin, P. & Davis, M. M. Human Immune System Variation. *Nature Reviews Immunology* **17**, 21–29. doi:10.1038/nri.2016.125 (Jan. 2017).
135. Westra, H.-J. *et al.* Cell Specific eQTL Analysis without Sorting Cells. *PLOS Genetics* **11** (ed Pastinen, T.) e1005223. doi:10.1371/journal.pgen.1005223 (May 8, 2015).
136. Davenport, E. E. *et al.* Discovering in Vivo Cytokine-eQTL Interactions from a Lupus Clinical Trial. *Genome Biology* **19**. doi:10.1186/s13059-018-1560-8 (Dec. 2018).
137. Aran, D., Hu, Z. & Butte, A. J. xCell: Digitally Portraying the Tissue Cellular Heterogeneity Landscape. *Genome Biology* **18**. doi:10.1186/s13059-017-1349-1 (Dec. 2017).
138. Kleiveland, C. R. in *The Impact of Food Bioactives on Health* (eds Verhoeckx, K. *et al.*) 161–167 (Springer International Publishing, Cham, 2015). doi:10.1007/978-3-319-16104-4_15.
139. Van der Wijst, M. G. P. *et al.* Single-Cell RNA Sequencing Identifies Celltype-Specific Cis-eQTLs and Co-Expression QTLs. *Nature Genetics* **50**, 493–497. doi:10.1038/s41588-018-0089-9 (Apr. 2018).
140. Kanyongo, G. Y. The Influence of Reliability on Four Rules for Determining the Number of Components to Retain. *Journal of Modern Applied Statistical Methods* **5**, 332–343. doi:10.22237/jmasm/1162353960 (Nov. 1, 2005).
141. Astle, W. J. *et al.* The Allelic Landscape of Human Blood Cell Trait Variation and Links to Common Complex Disease. *Cell* **167**, 1415–1429.e19. doi:10.1016/j.cell.2016.10.042 (Nov. 2016).

142. Stegle, O., Parts, L., Piipari, M., Winn, J. & Durbin, R. Using Probabilistic Estimation of Expression Residuals (PEER) to Obtain Increased Power and Interpretability of Gene Expression Analyses. *Nature protocols* **7**, 500–507. doi:10.1038/nprot.2011.457 (Feb. 16, 2012).
143. Lippert, C., Casale, F. P., Rakitsch, B. & Stegle, O. LIMIX: Genetic Analysis of Multiple Traits. doi:10.1101/003905 (May 22, 2014).
144. Sul, J. H., Martin, L. S. & Eskin, E. Population Structure in Genetic Studies: Confounding Factors and Mixed Models. *PLOS Genetics* **14** (ed Barsh, G. S.) e1007309. doi:10.1371/journal.pgen.1007309 (Dec. 27, 2018).
145. Schenker, N. & Gentleman, J. F. On Judging the Significance of Differences by Examining the Overlap Between Confidence Intervals. *The American Statistician* **55**, 182–186 (2001).
146. Gelman, A. & Stern, H. The Difference Between “Significant” and “Not Significant” Is Not Itself Statistically Significant. *The American Statistician* **60**, 328–331. doi:10.1198/000313006X152649 (Nov. 2006).
147. Clogg, C. C., Petkova, E. & Haritou, A. Statistical Methods for Comparing Regression Coefficients Between Models. *The American Journal of Sociology* **100**, 1261–1293 (1995).
148. Kim-Hellmuth, S. *et al.* Genetic Regulatory Effects Modified by Immune Activation Contribute to Autoimmune Disease Associations. *Nature Communications* **8**. doi:10.1038/s41467-017-00366-1 (Dec. 2017).
149. Peters, J. E. *et al.* Insight into Genotype-Phenotype Associations through eQTL Mapping in Multiple Cell Types in Health and Immune-Mediated Disease. *PLOS Genetics* **12** (ed Plagnol, V.) e1005908. doi:10.1371/journal.pgen.1005908 (Mar. 25, 2016).
150. Kooperberg, C. & LeBlanc, M. Increasing the Power of Identifying Gene \times Gene Interactions in Genome-Wide Association Studies. *Genetic Epidemiology* **32**, 255–263. doi:10.1002/gepi.20300 (Apr. 2008).

APPENDIX A. BIBLIOGRAPHY

151. Zeng, B. *et al.* Comprehensive Multiple eQTL Detection and Its Application to GWAS Interpretation. *Genetics* **212**, 905–918. doi:10.1534/genetics.119.302091 (July 2019).
152. Dobbyn, A. *et al.* Landscape of Conditional eQTL in Dorsolateral Prefrontal Cortex and Co-Localization with Schizophrenia GWAS. *The American Journal of Human Genetics* **102**, 1169–1184. doi:10.1016/j.ajhg.2018.04.011 (June 2018).
153. Mizuno, A. & Okada, Y. Biological Characterization of Expression Quantitative Trait Loci (eQTLs) Showing Tissue-Specific Opposite Directional Effects. *European Journal of Human Genetics* **27**, 1745–1756. doi:10.1038/s41431-019-0468-4 (Nov. 2019).
154. Fu, J. *et al.* Unraveling the Regulatory Mechanisms Underlying Tissue-Dependent Genetic Variation of Gene Expression. *PLoS Genetics* **8** (ed Gibson, G.) e1002431. doi:10.1371/journal.pgen.1002431 (Jan. 19, 2012).
155. Kumasaka, N., Knights, A. J. & Gaffney, D. J. Fine-Mapping Cellular QTLs with RASQUAL and ATAC-Seq. *Nature Genetics* **48**, 206–213. doi:10.1038/ng.3467 (Feb. 2016).
156. Wu, L., Shen, C., Seed Ahmed, M., Östenson, C.-G. & Gu, H. F. Adenylate Cyclase 3: A New Target for Anti-Obesity Drug Development: ADCY3 and Anti-Obesity. *Obesity Reviews* **17**, 907–914. doi:10.1111/obr.12430 (Sept. 2016).
157. McGovern, D. P., Kugathasan, S. & Cho, J. H. Genetics of Inflammatory Bowel Diseases. *Gastroenterology* **149**, 1163–1176.e2. doi:10.1053/j.gastro.2015.08.001 (Oct. 2015).
158. Çalışkan, M., Baker, S. W., Gilad, Y. & Ober, C. Host Genetic Variation Influences Gene Expression Response to Rhinovirus Infection. *PLOS Genetics* **11** (ed Gibson, G.) e1005111. doi:10.1371/journal.pgen.1005111 (Apr. 13, 2015).
159. Manry, J. *et al.* Deciphering the Genetic Control of Gene Expression Following Mycobacterium Leprae Antigen Stimulation. *PLOS Genetics* **13** (ed Sirugo, G.) e1006952. doi:10.1371/journal.pgen.1006952 (Aug. 9, 2017).

160. Pai, A. A., Pritchard, J. K. & Gilad, Y. The Genetic and Mechanistic Basis for Variation in Gene Regulation. *PLoS Genetics* **11** (ed Lapalainen, T.) e1004857. doi:10.1371/journal.pgen.1004857 (Jan. 8, 2015).
161. Choudhury, M. & Ramsey, S. A. Identifying Cell Type-Specific Transcription Factors by Integrating ChIP-Seq and eQTL Data-Application to Monocyte Gene Regulation. *Gene Regulation and Systems Biology* **10**, GRSB.S40768. doi:10.4137/GRSB.S40768 (Jan. 2016).
162. Langford, E., Schwertman, N. & Owens, M. Is the Property of Being Positively Correlated Transitive? *The American Statistician* **55**, 322–325 (2001).
163. Millstein, J., Zhang, B., Zhu, J. & Schadt, E. E. Disentangling Molecular Relationships with a Causal Inference Test. *BMC Genetics* **10**. doi:10.1186/1471-2156-10-23 (Dec. 2009).
164. Verma, A. & Ritchie, M. D. Current Scope and Challenges in Phenome-Wide Association Studies. *Current Epidemiology Reports* **4**, 321–329. doi:10.1007/s40471-017-0127-7 (Dec. 2017).

--

<h1>List of Abbreviations</h1> <p>AC allele count</p> <p>ASE allele-specific expression</p> <p>BH Benjamini-Hochberg</p> <p>bp base pair</p> <p>BTM blood transcription module</p> <p>CIT causal inference test</p> <p>CPM counts per million</p> <p>DC dendritic cell</p> <p>DGE differential gene expression</p> <p>eQTL expression quantitative trait locus</p> <p>FACS fluorescence-activated cell sorting</p> <p>FC fold change</p> <p>FDR false discovery rate</p> <p>FWER family-wise error rate</p> <p>GWAS genome-wide association study</p> <p>HA haemagglutinin</p> <p>HAI haemagglutination inhibition</p>	
---	--

HIRD	Human Immune Response Dynamics
HLA	human leukocyte antigen
INT	inverse normal transformation
LAIV	live attenuated influenza vaccine
LD	linkage disequilibrium
lfsr	local false sign rate
LMM	linear mixed model
LOCO	leave-one-chromosome-out
LRT	likelihood ratio test
MAF	minor allele frequency
MANOVA	multivariate analysis of variance
MN	microneutralisation
NA	neuraminidase
NK	natural killer
OVb	omitted-variable bias
PBMC	peripheral blood mononuclear cell
PC	principal component
PCA	principal component analysis
PVE	proportion of variance explained
REML	restricted maximum likelihood
reQTL	response expression quantitative trait locus
RNA-seq	RNA-sequencing

SD standard deviation

SNP single nucleotide polymorphism

TF transcription factor

TIV trivalent inactivated influenza vaccine

TMM trimmed mean of M-values

TRI titre response index

TSS transcription start site

spell-check

make sure package ver-
sions are in, and package
names are monospace

add automatic rounding
to x decimal places using
num and sisetup

collaboration note in ital-
ics at start of each chap-
ter

LD decay just takes a really really long time, but there are evo forces at work too that maintain LD	1
Figure: block-like structure of the genome	2
insert something sweeping about utility: insights into trait biology and clinical translational potential for disease traits, genetic support target identification	2
Figure: OR vs MAF tam2019BenefitsLimitationsGenomewide	3
seems like there is some connection to be made between the tagability of common variation and the feasibility of imputation	4
CUT: There is a limit, the strategy of association is fundamentally unsuited to rare variants.	5
CUT: Missing heritability	5
CUT: WGS/WES mitigates the coverage bias of GWAS towards known variation.	5
Figure: magnify, dampen, flip	8
add more pros for in vitro reQTLs here, and find citations	10
define what a signature is	12
why? for diff groups of people	15
add a point that 2009h1n1 is now circulating seasonally, this is a common trend	16
Add specific section about pandemrix, it's correlates of protection, it's durability? or maybe in methods	16
Here, add few points about the immunological response to adjuvanted TIVs i.e. what happens after Pandemrix admin? Involve the innate -> B/CD4T response. Goto plotkins	16
is there a more recent review?	16
make sure gap and how it is filled is emphed enough	18

needs 1 more punchline sentence here	18
atm I'm not using R/NR. wording here implys I am	19
cite appropriate subfigures here	19
change score is usually negatively correlated to baseline ⁵³ . hence TRI, whilst combining, is still not ideal	19
cite appropriate subfigures here, after adding proper subfigure labels .	20
Add to collab note that extractions were done at KCL	20
Add Tracy-Widom statistics for PCs to justify later choice of 4 PCs for covariates	24
nicer version, copy the peer code, facet the hird and hapmap samples	24
Can add other fastqc plots e.g. kmers, overrepresented seqs, seq length	24
add software versions	26
cite relevant preprocessing sections	32
combat does have a pro in that it can do per gene scaling, that fixed fx won't do	32
this is not a very precise justification. actually, if I were to color R/NR in the PCA plot, R/NR doesn't really explain a lot of var in global gene expression. that's probably why the results don't change much.	32
weaken this. combat is used multiple times in ch3	32
be more specific about how combat works i.e. estimates factors per gene per batch?	32
this is DGE specific normalisation, which is why it goes here, not in the preprocessing section	34
link to papers justifying sex, age, ancestry as significant effects on immune gene expression	34
add equation from ch3. especially justify having TRI in as predictor .	34
add section labels	34
add label	35
make all the notation in this section consistent with, and add the equation 2.1. The normal-normal hierarchical model, ⁸²	35
why is this? is it having well powered studies? gelman is vague	36
the derivation here is $qnorm(0.975, mean=0, sd=1*10) = 1*19.59964$, bit iffy, double check this is correct	36
could also include a table of all sets of parameters here?	36
add note on ositive regression dependency ²¹	36
add comment on symmetry	38

can also add MSigDB hallmark sets, which include interferon sets; and of course gene ontology sets	40
not sure of interpretation at FGFBP2, it is indeed highly expressed in NKs through https://dice-database.org/genes/FGFBP2 . . .	40
any point in a table of e.g. top 20 DE genes, or is the gene set analysis already enough?	40
change x axis labels to baseline, specify top 10 procedure in figure caption	40
finish citing	40
add label	40
figure x labels here should be TRI, not R.vs.NR	42
Not sure if there is a biological interpretation of downreg of T cells and NK cells gene sets at day 1, since it could be due to increase in other cell types in the sample. similar findings in ⁹⁹ though . . .	42
lit search for downregulation interpretation paper, and downreg T cell paper	42
might have to rerun everything using the original binary R/NR if this line of reasoning isn't strong enough	45
move numbers to results?	45
TRI is also not ideal	45
could comment on phenotype differences too, i.e. HIRD measure anti- bodies at d63, much later than is popular in the field: d28 usually	46
should probably emph sobolev didn't find prevacc signatures, and we did. But it's not exactly fair, as sobolev didn't use gene set en- richment as far as i can tell	46
There is also something to be said about 'prediction is not inference'. For use as correlates of protection, as promised by proponents of systems studies, prediction is what is important.	46
found signatures, but so what? Feels like chapter lacks a punchline? .	46
why blood? ready easy supply of immune cells	46
pull in citations from intro	50
distinction between expression/ab response is blurry here	50
straighten out tenses	50
1 more sentence to round off context	51
the variable is not used as an inclusion/exclusion criterion for the study, otherwise regression to the mean will be strong	52

Can this really demonstrate genotype-dependent change in gene expression between timepoints? i.e. need understand how the change score/ANCOVA approaches differ from repeated measures ANOVA differ from the interaction/stratified approach I take?	52
why I didn't just do a mega-analysis in chapter 2 then, given I haven't any evidence if it's better or worse than Bayesian meta-analysis in that context.	52
add -7 note as with ch2	52
add some indication of how much inflation can be reduced by LMMs .	53
add chr1 loco kinship matrix as example, note the estimates for self-relatedness on the diagonals are not constrained to be 1	54
log scale: as interactions depend on the scale at which departure from additivity is detected	54
add sample sizes and model for expression sim	55
determine appropriate citations from existing refs in intro	55
add comment on existence of chosen cell types in samples, and clustering by visit	57
no need for both size and color, use one for contribution percent . . .	57
add info on the markers used for the chosen FACS counterparts	57
get subset size	61
remake this with only top k factors, and prune the possible covariates	63
add approximate MAFs, then cite hierarch paper	63
add note on treating x chrom variants with caution	63
lift proper vector notation from limix, then redo this with a timepoint subscript	63
add formulation of the 0-mean random effect to show exactly how the kinship matrix is used ¹⁴⁴	63
note stacking of kinship for day -7 repeated measures	63
i leave the pcs in to guard against unusually differentiated markers, where random effect alone may not be enough ¹²⁴	63
recheck if did I do a SNPs only filter	65
move lfsr explanation prior to ashr in dge chapter	67
not sure whether this is conservative or anti-conservative	67
RNAseq does test about 7000 more genes though...	68
be more specific: "moderator", not 'modify'	68

point is, doesn't make sense to assume the genotype effect is the same at all levels of cell type abundance	68
add note here that although peer is correlated with xcell, interactions are only formed with xcell, so the interaction term can be inter- preted per unit of genotype increase when xcell=0	70
this analysis is incomplete, and is one of the things I would suggest to round off this chapter	70
if it would be interesting to compare the sharing estimate condition by condition approach to mashr, then redo and pull in eigenmt-bh values	70
actually, i've found that my PVE approximation is basically rescaled abs(Z), so pve is a bit pointless if we already have z, and doesn't really help with comparability between genes with diff var/MAF	71
requiring signif post-vaccination may not be correct, as it excludes many dampening effects	71
the lack of any positional enrichment makes me concerned for false positives? check with ASE?	71
expand this to plot 1, list top 5 damp, flip, amp at each timepoint	71
note anything in lit about any of the 30	71
double check denoms	71
convert to subfigures	71
siunitx permits uncertainties	75
gene set enrichment for cell type interacting genes to further validate xCell score usefulness	75
Figure: expression vs monocyte xCell score, colored by genotype, to visually prove the point	75
Need to consider Nikos' comment that there are too many (1069/13570 significant BH FDR) genotype-platform interactions to use mega- analysis. Consider filtering.	75
this analysis is incomplete, and is one of the things I would suggest to round off this chapter	76
FYI the IBD/T cell coloc fine maps to chr2:24935139 T C (rs713586) with PP=1	76
add obesity GWAS	76

I'm not exactly sure why at the moment. Enrichment analyses so far have not turned up much. Up regulation of cell cycle TFs is a possibility.	78
add lfsr.dge	79
need to consider: if this kind of thing is what bulk in vivo reQTL can find, they what is the additional value over FACS?	79
harmonise terminology for 'opposite'	80
note coloc doesn't distinguish pleiotropy from mediation?	80
add 1 concluding line	81
Overall I feel like the chapter is too descriptive, and falls short of mak- ing biological insights into Pandemrix response. Any additional analyses would hope to address that.	81
spell-check	112
make sure package versions are in, and package names are monospace	112
add automatic rounding to x decimal places using num and sisetup . .	112
collaboration note in italics at start of each chapter	112