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<dedication>

Abstract

<thesis abstract>

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Chapter 1

Introduction

- Variation between humans exists
- The eternal debate: nature vs nurture
- Why study human genetics?
- The structure of the genome and it's variation
- Finding causal anchors
- Leveraging natural G variation.

1.1 A brief overview of genetic association for complex traits

1.1.1 Early days

- Early days, prior to GWAS
- Mendelian genetics, family and linkage studies
- Complex traits and the Common disease, common variant hypothesis
- Twin studies and heritability estimates of complex traits
- Candidate gene studies (Border et al., 2019)
- Appreciation of polygenicity

1.1.2 The advent of GWAS

- 10 years of GWAS
 - "The case of the missing heritability"
 - genotyping arrays
 - common known variants
 - designed to cover tag variants that represent most genetic variation
 - imputation
 - if discovering new var
 - WES (about 40Mbp of the genome)
 - covers more of the genome in terms of bp
 - but lower n, so lower power than array genotyping to do single variant associations
 - why 50x? variable coverage due to pulldown
 - WGS
 - tradeoff between variant capture (n needed to observe variant) and sequencing depth (gives confidence to call variants)
 - 20x ok to call 90% of singletons
 - rare variants, including in nc regions
 - * current discovery biases, finding higher effect size vars first
 - * burden tests (e.g. SAIGE)
 - to get gene, aggregate based on variant consequence scores e.g. vep scores
 - structural variants

1.1.3 Narrowing the signal

- PheWAS¹
 - Fine-mapping

- as sample sizes get larger, and provided that sequencing or imputation can more exhaustively identify all of the candidate SNPs on the haplotype, rare recombination events will pile up, helping to make the causal SNP stand out above the passenger SNPs that usually travel on its haplotype [Huang 2017].
- tag snps: causal snps may not be directly typed, may need to be imputed

1.1.4 Interpretation of genetic associations with molecular studies

- Locus to gene mapping problem
 - nc snps
 - * 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].
- using intermediate/endophenotypes
 - endophenotypes paper
 - expression as an important intermediate
 - * measure by array, rnaseq
 - theory is that genetic variants manifest their effects through these phenotypes, central dogma based
- coloc methods
 - coloc
 - * 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
 - a transcriptional risk score (TRS)
 - for eQTLs, closest gene is often not the best candidate
 - annotation of nc var is functional genomics
 - * e.g. gtex, ENCODE

1.1.5 So what? Translational directions [can cut this whole section]

- 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
 - Drug target prioritisation for disease traits
 - 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

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

- in the dreaded GxE interaction
 - "In genetics, context matters"
 - for both gwas, and molQTLs, context is key
- Architecture varies e.g. across cell type and tissues
 - tissue
 - cell type
 - interaction between cells *in vivo*
 - stimulation conditions
- QTLs can interact with sex and age
- types of context specific QTL
 - ackerman conditional vs dynamic
- 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.
coloc of immune mediated traits is enhanced by context-specific eQTLs

1.3 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

1.3.1 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)."

1.3.2 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.

Posit that eQTLs where the genetic effect of

1.3.2.1 Context-specific immune response eQTLs in vitro

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 confound 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. .

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

One of the first studies to perform **response expression quantitative trait locus (reQTL)** mapping for an immune stimulation was², 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+ monocytes³) and stimulations (IFN γ and LPS⁴).

Take home messages: - reQTLs develop trans-effects on stimulation³
Overall, as the number of experimental systems and stimulations increases,

CHAPTER 1. INTRODUCTION IMMUNE RESPONSE TO VACCINATION

large number of eQTLs are only detected.

1.3.2.2 *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

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

Large cohorts:

1.4 Immune response to vaccination

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.

1.4.1 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

1.4. IMMUNE RESPONSE TO VACCINES

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

1.4.2 Genetic factors affecting vaccine response

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.].

Search for "variation in vaccine response genetics GA Poland" in google scholar

Genetics of adverse events e.g. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4845468/>

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)

Review paper on GWAS for vaccines mooney2013SystemsImmunogeneticsVaccines

CHAPTER 1. INTRODUCTION TO BIOLOGIC THERAPIES

1.5 Immune response to biologic therapies

1.5.1 Genetic factors affecting biologic responses

e.g. PANTS immunogenicity

1.6 Thesis overview

Chapters 1 and 2. Chapter 3. Chapter 4. Chapter 5.

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 mammals. They are classified into antigenically-distinct subtypes by the combination of two surface proteins: **haemagglutinin (HA)** and **neuraminidase (NA)**⁵.

There are three classes of influenza vaccine against seasonal strains in use: inactivated vaccines, **live attenuated influenza vaccine (LAIIV)**, 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^{6,7}. On occasion, a novel subtype against which the majority of the population is immunologically naive can arise, 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, the fourth to occur in the last 100 years⁵.

why? for diff groups of people

add a point that 2009h1n1 is now circulating seasonally

2.1.2 Quantifying immune response to influenza vaccines

Add specific section about pandemrix? or maybe in methods

The 2009 pandemic motivated the rapid development, trialing, and licensing of several novel vaccines⁸. 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 culture⁹. 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)^{10,11}.

2.1.3 Systems vaccinology of influenza vaccines

is there a more recent review?

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 reliable⁶. 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 system¹². 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^{13,14}.

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.¹⁵

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 vaccine¹⁶; kinase CaMKIV expression is also a strong predictor¹⁷, as are genes related to B cell proliferation¹⁸.

For these studies of seasonal influenza vaccines in adults, responses tend to be biased by recall from past vaccination or infection^{16,19}. There have also been few studies of adjuvanted influenza vaccines, despite their superior efficacy in comparison to non-adjuvanted counterparts^{20,21}.

2.1.4 The Human Immune Response Dynamics (HIRD) study

The **Human Immune Response Dynamics (HIRD)** study conducted by Sobolev *et al.* [22] 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 vaccine response even pre-vaccination^{23–26}.

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 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^{27,28}.

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 study²², 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)**¹⁶. 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 coexpressed 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 in²². 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 measured 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).

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

In²², 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 Administration²⁹, and is used in many studies of seasonal influenza vaccines¹³. 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,²² 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^{27,28}.

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

cite appropriate subfig-
ures here

To address these concerns, I computed the **TRI** as defined in Bucasas *et al.* [16]. For each assay, a linear regression was fit with the \log_2 day 63/day -7 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

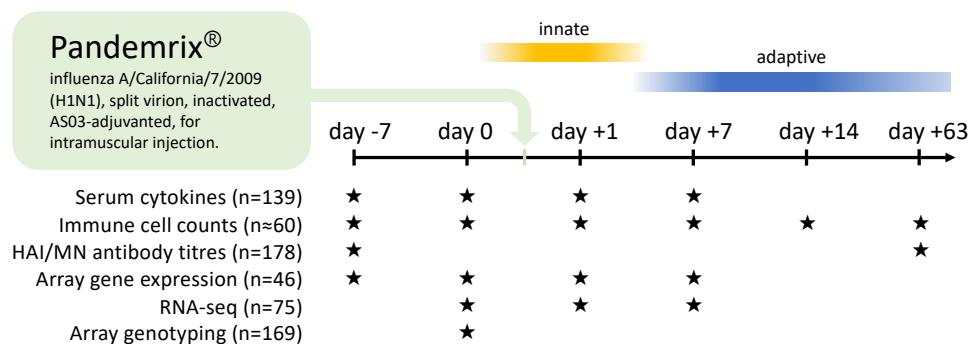


Figure 2.1: Data types, timepoints, and sample sizes. Individuals were vaccinated after day 0 sampling. Antibodies to the vaccine strain were measured by **HAI** and **MN** assays. Array and **RNA-seq** gene expression measured in the **PBMC** compartment.

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](#)).

cite appropriate subfigures here, after adding proper subfigure labels

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*.

Add to collab note that extractions were done at KCL

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 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 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.

*Personal communication with authors.

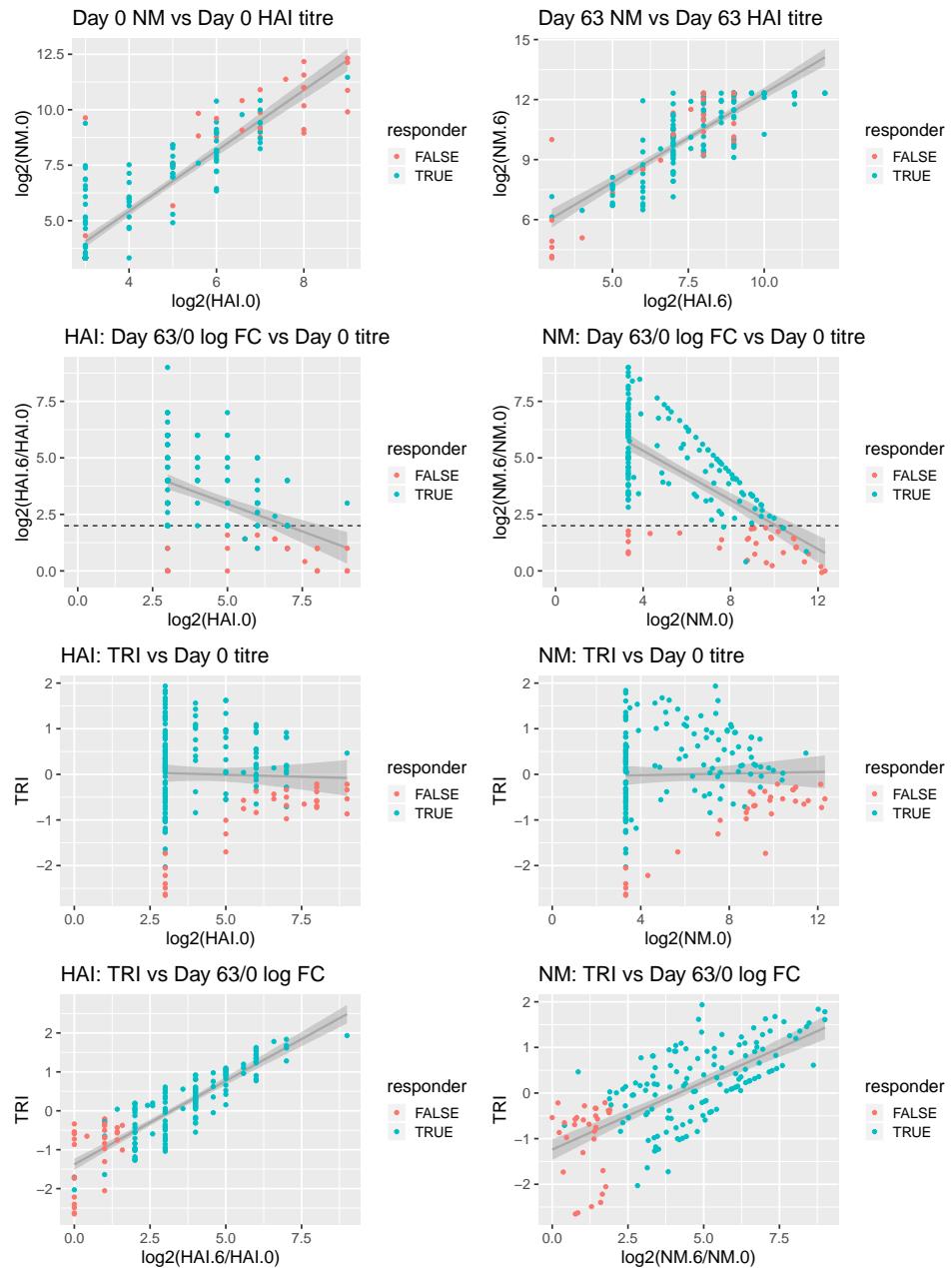


Figure 2.2: Comparison of **TRI** to **HAI** (left column) and **MN** (right column) titres and binary responder/non-responder status (colored) in 166 **HIRD** individuals. Row 1: baseline titres are positively correlated to post-vaccination titres. Row 2: baseline titres are negatively correlated to 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.

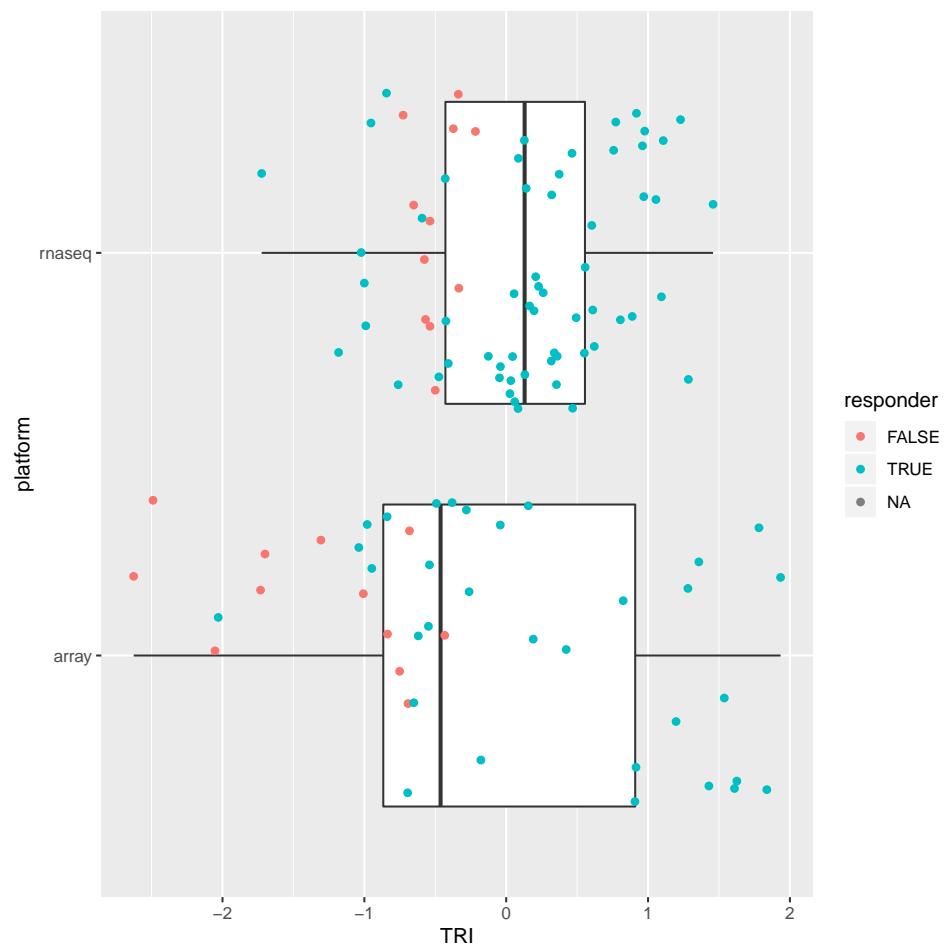


Figure 2.3: Distribution of TRI, stratified by platform used to measure expression.

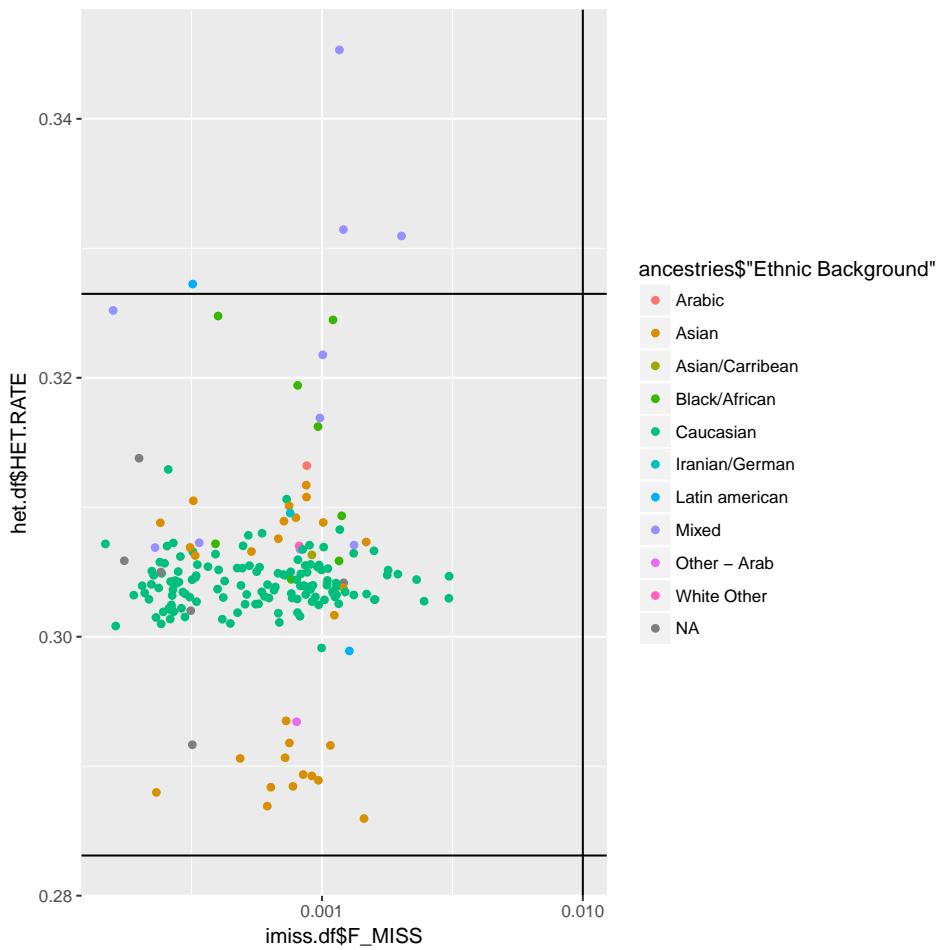


Figure 2.4: Sample filters for missingness and heterozygosity rate. Samples outside the central rectangle were excluded.

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 ancestry) in expression and genetic association studies^{30–32}. Treating HapMap 3 samples 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 covariates for ancestry downstream.

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

2.2.6 Genotype phasing and imputation

Prior to imputation, 213277 monomorphic markers 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), then imputation with PBWT (v3.1) using the Haplotype Reference Consortium (r1.1) panel. Markers were lifted-over from GRCh37 to GRCh38 coordinates using CrossMap. Poorly-imputed markers with INFO < 0.4 or missingness > 5% were removed, resulting in 40290981 markers.

2.2.7 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

*<https://imputation.sanger.ac.uk/>

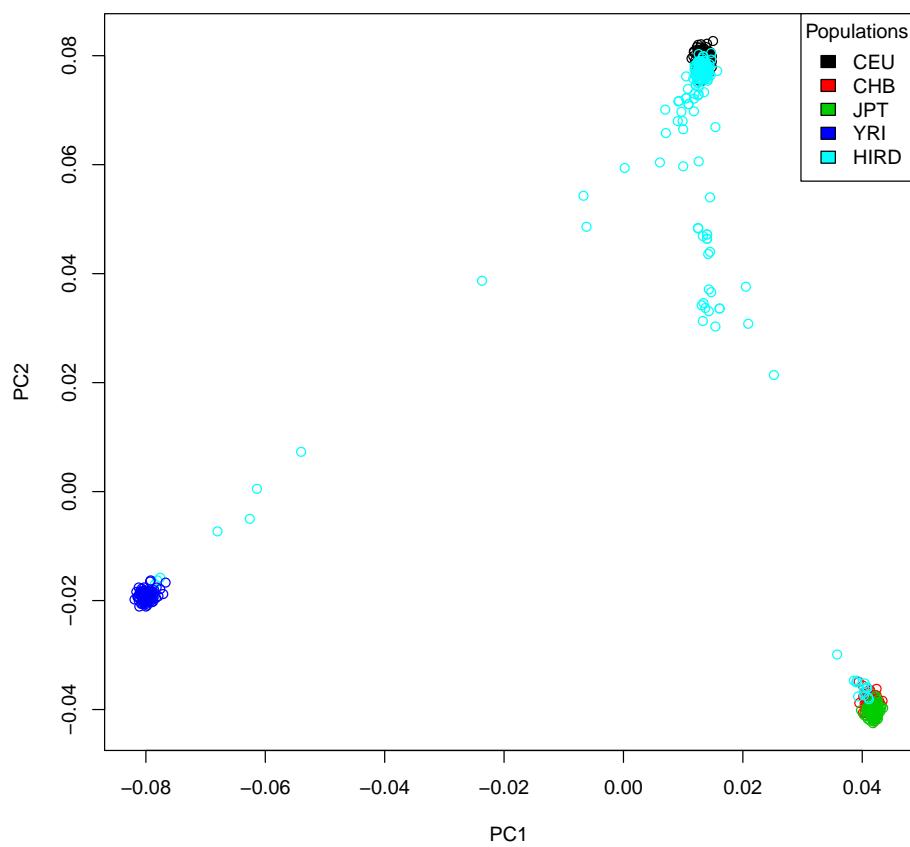


Figure 2.5: HIRD samples (cyan) projected onto PC1 and PC2 axes defined by PCA of HapMap 3 samples. The first two PCs separate European (CEU, upper-right) from Asian (CHB and JPT, lower-right) and African (YRI, lower-left) individuals.

were in the same pool, and then sequenced across multiple lanes as technical replicates (HiSeq 4000, 75bp paired-end).

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

RNA-seq quality metrics were assessed using FASTQC* and Qualimap³³, then visualised with MultiQC³⁴. Sequence quality was high (Fig. 2.6), and duplication levels were low (Fig. 2.7). The unimodal GC-content distribution suggested negligible levels of non-human contamination (Fig. 2.8).

add software versions

2.2.8 RNA-seq quantification and filtering

Reads were quantified against the Ensembl reference transcriptome (GRCh38) using Salmon³⁵ 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 experiments³⁶. Relative transcript abundances were summarised to Ensembl gene-level count estimates using tximport (scaledTPM method) to improve statistical robustness and interpretability³⁷.

Genes with short noncoding RNA biotypes[†] were removed, as they are generally not polyadenylated, and expression estimates can be biased by

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

[†]miRNA, miRNA_pseudogene, miscRNA, miscRNA pseudogene, Mt rRNA, Mt tRNA, rRNA, scRNA, snlRNA, snoRNA, snrRNA, tRNA, tRNA_pseudogene. List from <https://www.ensembl.org/Help/Faq?id=468>

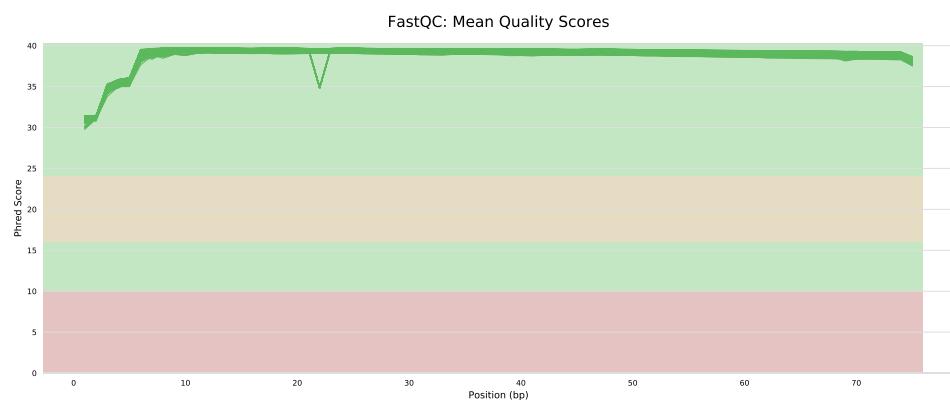


Figure 2.6: FastQC sequence quality versus read position for HIRD RNA-seq samples.

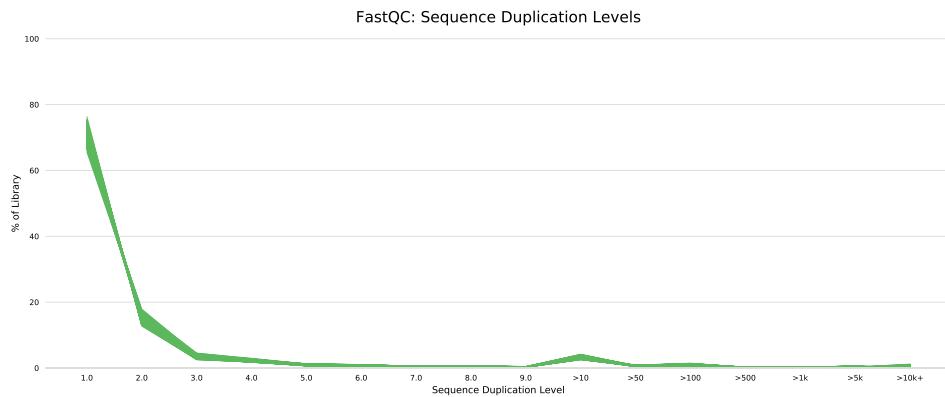


Figure 2.7: FastQC sequence duplication levels for HIRD RNA-seq samples.

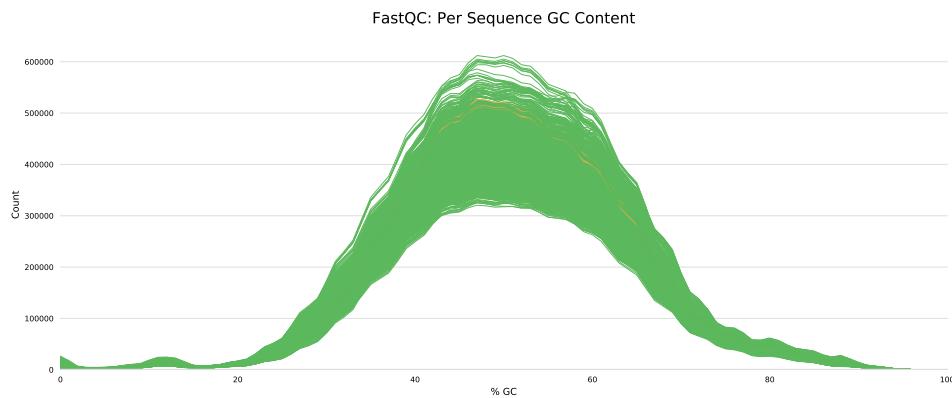


Figure 2.8: FastQC GC profile for HIRD RNA-seq samples.

misassignment of counts from overlapping protein-coding or lncRNA genes³⁸. Globin genes, which are highly expressed in erythrocytes and reticulocytes, cell types expected to be depleted in PBMC³⁹, were also removed. Given the proportion of removed counts at this stage was low for most 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 expressed⁴⁰. 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).

2.2.9 Array data preprocessing

Single-channel Agilent 4x44K microarray (G4112F) data for 173 samples from²² 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).

VSN⁴¹ was used to perform background correction, between-array normalisation, and variance-stabilisation of intensity values, resulting in expression values on a log₂ scale.

Most genes are targeted 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 collapsing⁴²). 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

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

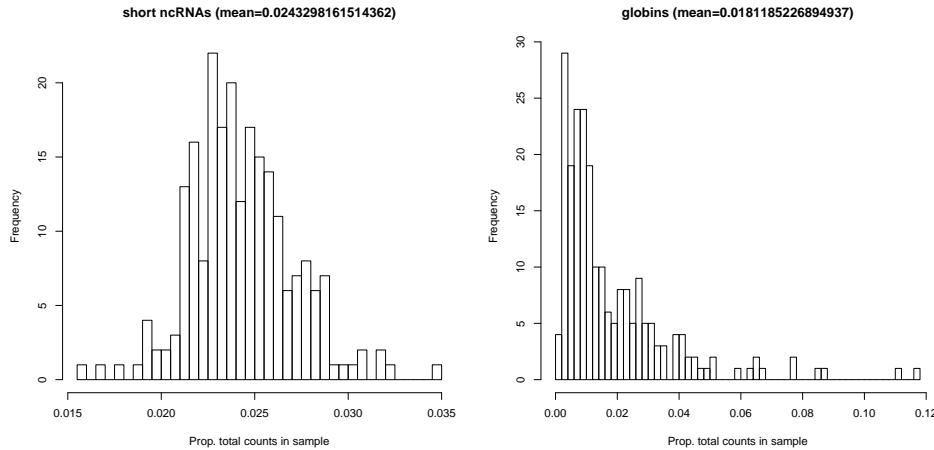


Figure 2.9: Distributions of removed short ncRNA and globin counts as a proportion of total counts in RNA-seq samples.

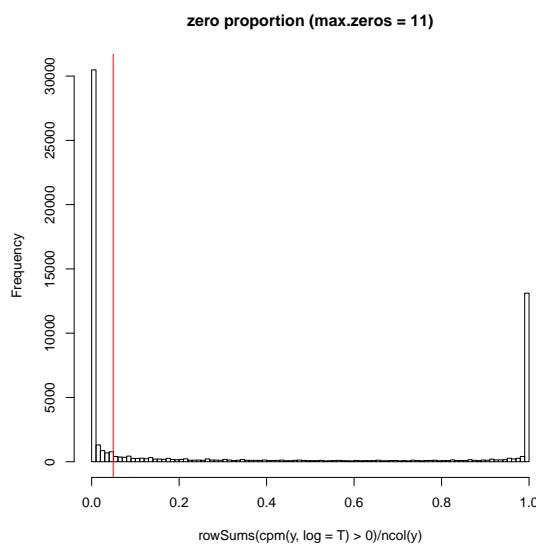


Figure 2.10: Distribution of the proportion of samples in which genes were detected (non-zero expression). Many genes are not detected in any samples. Vertical line shows 5% threshold below which genes were discarded.

CHAPTER 2. TRANSCRIPTOMIC RESPONSE TO INFLUENZA A
2.2. METHODS
(H1N1)PDM09 VACCINE

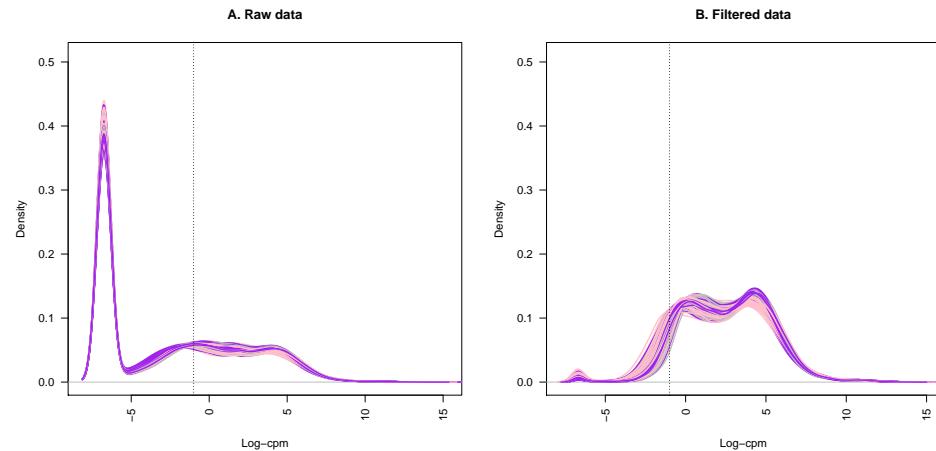


Figure 2.11: Distribution of gene expressions for RNA-seq samples before and after filtering no expression and low expression genes. Vertical line shown at CPM = 0.5 threshold.

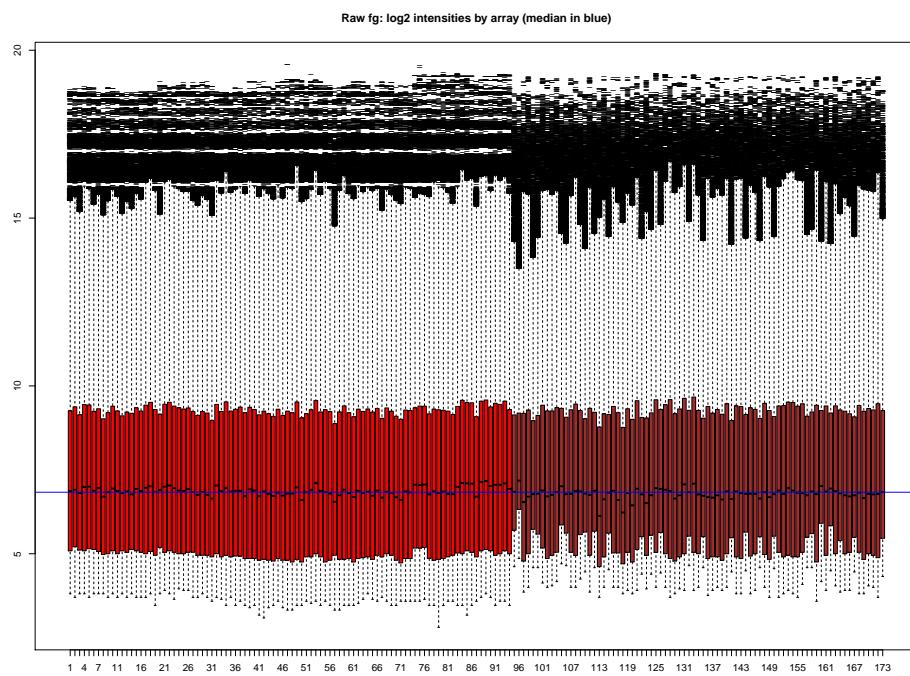


Figure 2.12: Raw foreground intensities for 173 HIRD array samples. Colored by array processing batch.

in the **HIRD** dataset. The final normalised \log_2 intensity values for these 18216 genes over 173 samples is shown in [Fig. 2.13](#).

2.2.10 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-hybridisation⁴³. **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 intensity⁴⁴. There are also differences in the statistical models behind expression quantification and normalisation, as described above.

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.

Regarding the batch effect within the array data, a popular adjustment method is ComBat⁴⁵, which estimates centering and scaling parameters by pooling information across all genes using empirical Bayes. ComBat is the method used in²². In comparisons of microarray batch effect adjustment methods, ComBat performs favourably (vs. five other adjustment packages)⁴⁶ or comparably (vs. batch as a fixed or random effect in the linear model)⁴⁷. However, where batches are unbalanced in terms of sample size⁴⁸ or distribution of study groups that have an impact on expression⁴⁹, 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.

cite relevant preprocessing sections

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

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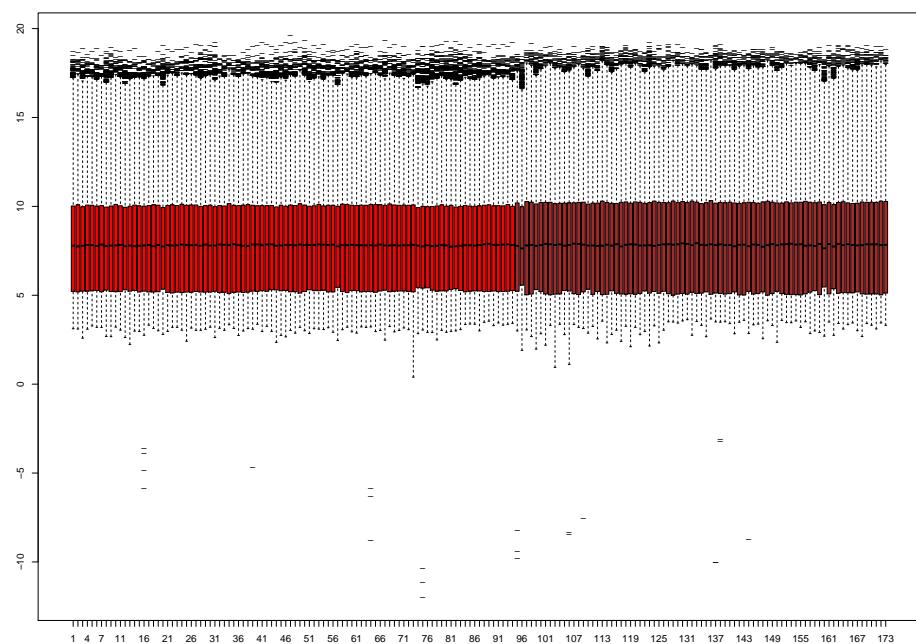


Figure 2.13: Array intensity estimates after VSN normalisation and collapsing of probes to genes. Colored by array processing batch.

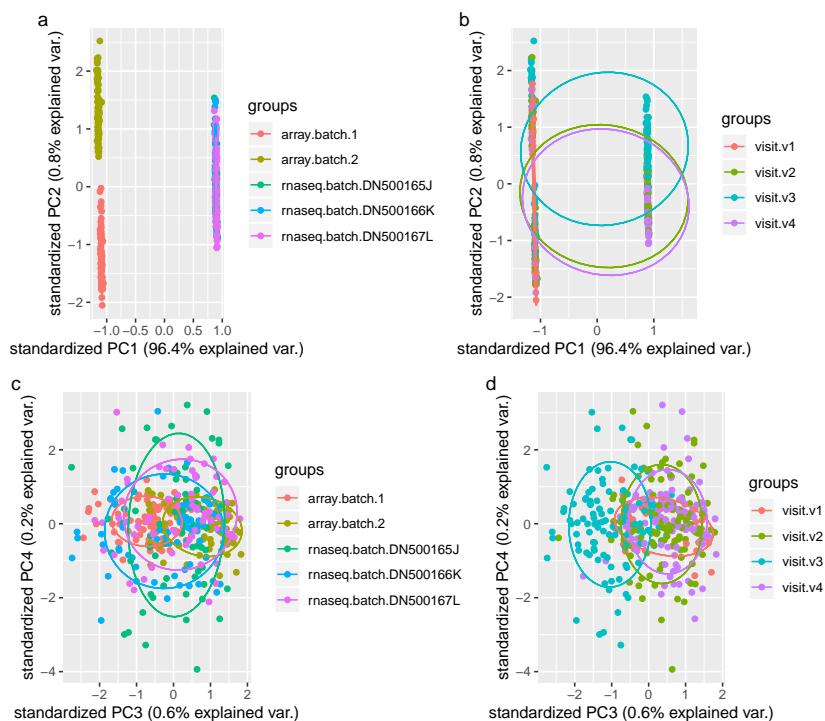


Figure 2.14: First four PCs in the HIRD expression data, colored by platform and batch (left), and timepoint (right).

2.2.10.1 Per-platform differential gene expression model

For the array data, as²² 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) method⁵⁰ from edgeR⁵¹; then variance-stabilisation was performed using voom⁵², resulting in expression values with units of log₂ CPM.

this is DGE specific normalisation, which is why it goes here, not in the preprocessing section

Linear models were fit using limma⁵³, which is computationally fast, and performs well for sufficiently large ($n \geq 3$ per group) sample sizes⁵⁴. 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. 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 log₂ expression fold change per unit change in predictor value.

link to papers justifying sex, age, ancestry as significant effects on immune gene expression

2.2.10.2 Choice of differential gene expression meta-analysis method

add section labels

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^{55,56}. 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 ⁵⁸, in the case of $k = 2$ especially⁵⁹. Many estimators are available⁶⁰, 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^{61,62}. In such circumstances, the most sensible choice may be to incorporate prior information about model hyperparameters in a Bayesian random-effects framework^{60–63}. For this study, I use the implementation in bayesmeta⁵⁷, which requires priors for both effect size and between-studies heterogeneity.

add label

make all the notation in this section consistent with, and add the equation 2.1. The normal-normal hierarchical model,⁵⁷

2.2.10.3 Prior for between-studies heterogeneity

The choice of prior for between-studies heterogeneity is influential when k is small⁶³. Gelman [64] 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 by⁶¹, 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.^{65,66}) or inverse-gamma (e.g.⁶⁷) 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.^{63,64}), 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 effects⁶⁰) 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`.

2.2.10.4 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^{62,64}. `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 tails⁶⁸. 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 .

2.2.10.5 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 $\text{Gamma}(\text{shape} = 1.5693, \text{scale} = 0.0641)$. This is comparable to⁶¹'s default recommendation of a $\text{Gamma}(\text{shape} = 2, \text{scale} = \lambda)$ prior where λ is small. For μ , the final prior used was $N(0, (0.3240 * 10)^2)$. 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.

2.2.10.6 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 than the confidence of a non-zero effect⁶⁹.

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

the derivation here is
 $\text{qnorm}(0.975, \text{mean}=0, \text{sd}=1*10) = 1*19.59964$,
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could also include a table of all sets of parameters here?

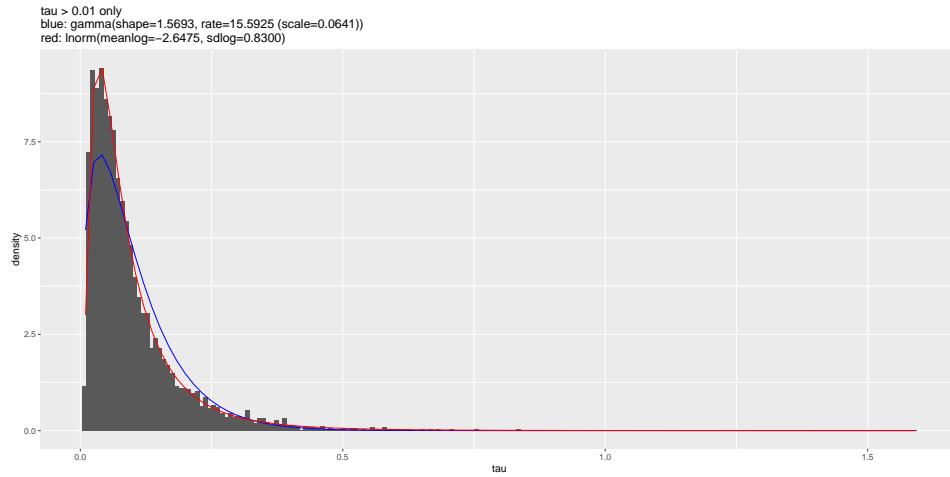


Figure 2.15: Gamma prior for τ used for `bayesmeta` (blue), compared to the empirical distribution of per-gene frequentist `metafor::rma` estimates for τ , for the day 1 vs. baseline effect (small estimates of $\tau < 0.01$ excluded). Empirical log-normal fit also shown (red).

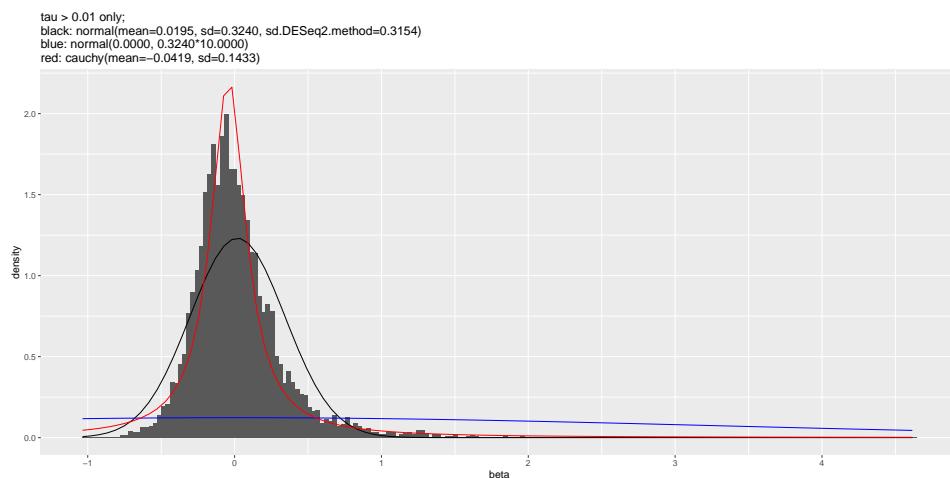


Figure 2.16: Normal prior for μ used for `bayesmeta` (blue), compared to the empirical distribution of per-gene frequentist `metafor::rma` estimates for τ , for the day 1 vs. baseline effect. The non-scaled normal fit is shown (black), as well as a Cauchy fit (red).

2.2.11 Gene set enrichment analysis using blood transcription modules

Gene set enrichment analyses were conducted using `tmod::tmodCERNOtest`⁷⁰, 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)** from⁷¹, 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 **lfsr** < 0.05 and absolute **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* ($\log_2 \text{FC} = 4.489\,150$), an interferon-induced gene in monocytes and **dendritic cells (DCs)** involved in antiviral innate immune pathways⁷². Other key genes in the interferon signalling pathway⁷³ such as *STAT1* ($\log_2 \text{FC} = 2.169\,3060$), *STAT2* ($\log_2 \text{FC} = 0.948\,9341$), and *IRF9* ($\log_2 \text{FC} = 0.815\,3674$) are also

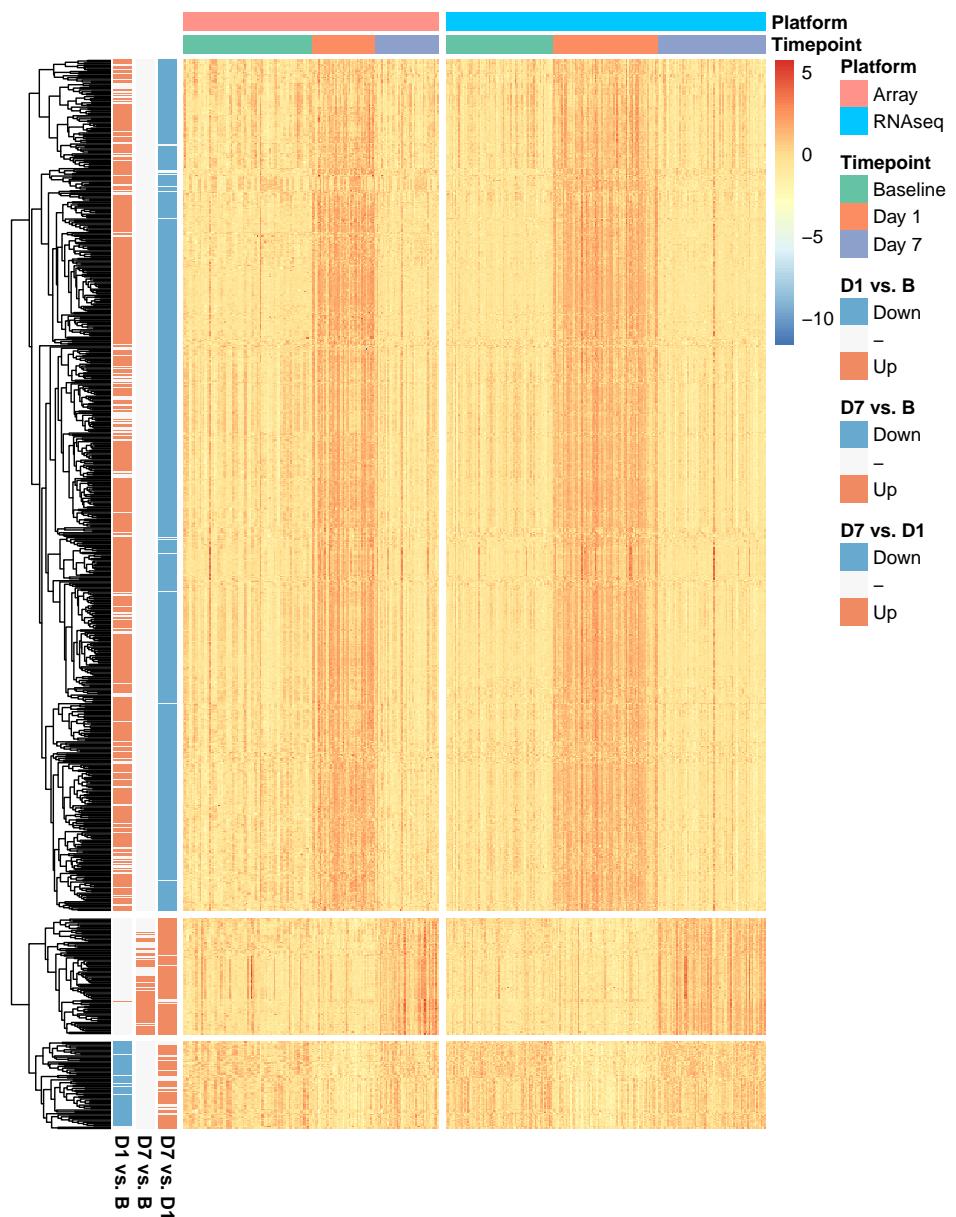


Figure 2.17: Normalised gene expression for genes differentially expressed between any pair of timepoints ($\text{lfsr} < 0.05$, absolute fold change > 1.5) across HIRD samples, clustered by gene (Manhattan distance metric).

upregulated 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 FGFBP2 ($\log_2 \text{FC} = -0.9141547$). 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

2.3.3 Adaptive immune response at day 7 post-vaccination

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 up-regulation were the B cell-associated genes TNFRSF17 ($\log_2 \text{FC} = 1.7538617$) and MZB1 ($\log_2 \text{FC} = 1.7369668$). Plasma cell-specific genes including SDC1 (encodes CD138 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5437827/>) ($\log_2 \text{FC} = 1.3673081$) and ELL2 (<https://www.nature.com/articles/ni.1786>) ($\log_2 \text{FC} = 0.8679659$) 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.

2.3.4 Expression signatures associated with antibody response

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).²² 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 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

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CHAPTER 2. TRANSCRIPTOMIC RESPONSE TO INFLUENZA A (H1N1)PDM09 VACCINE

2.3. RESULTS

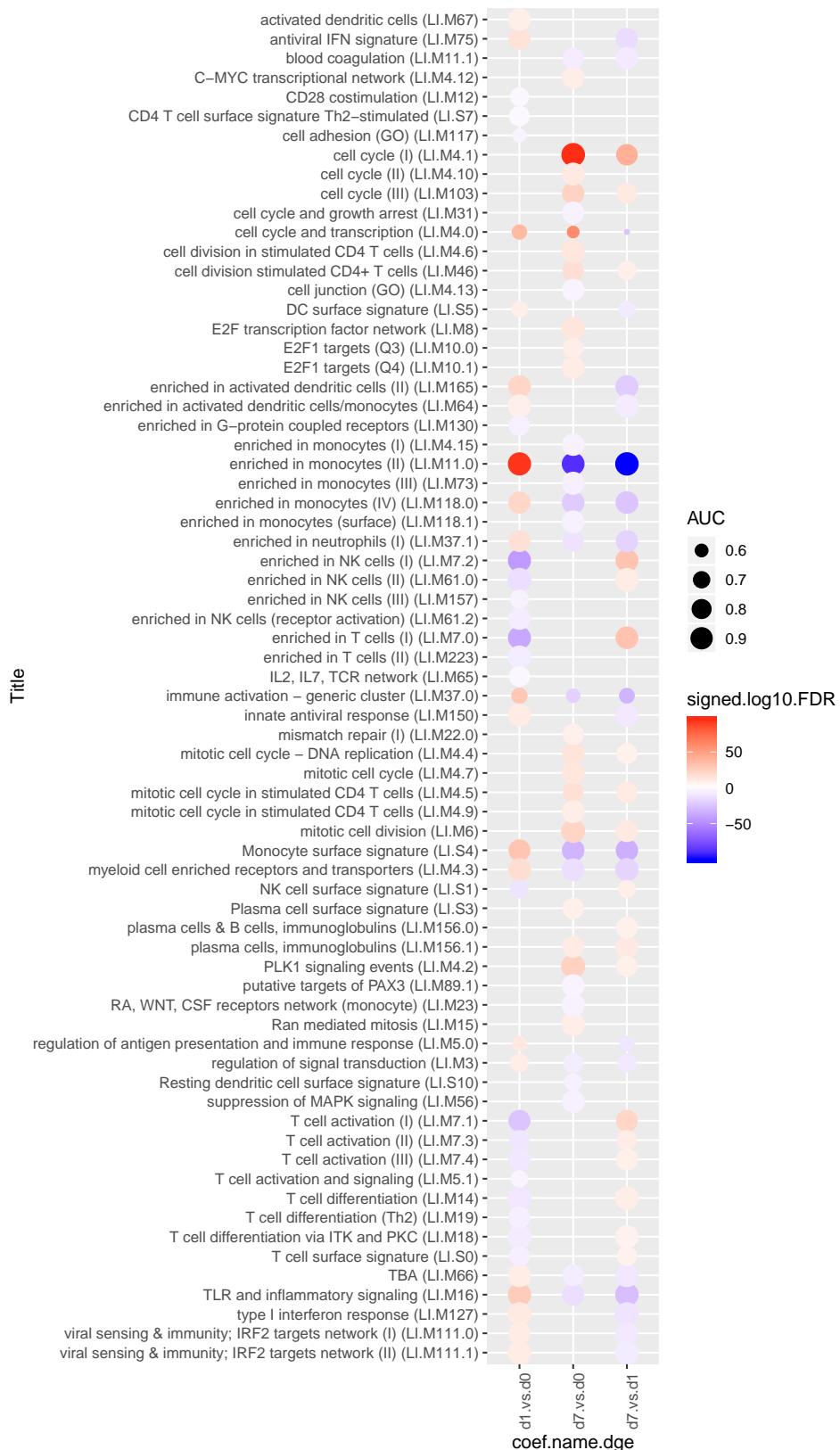


Figure 2.18: Transcriptomic modules significantly up or downregulated post-vaccination. Size of circle indicates effect size. Color of circle indicates significance and direction of effect (red = upregulation, blue = downregulation).

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 cells⁷⁵. These patterns of expression change between timepoints in the **RNA-seq** data are consistent with the patterns in the array data in the original study²², and with expansions of monocyte and plasma cell populations seen in the **FACS** data at days 1 and 7 respectively in the original **HIRD** study²².

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. In²², 62 genes were reported as differentially expressed between vaccine responders and non-responders. Although²² 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 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

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 in⁷⁴ though

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

move numbers to results?

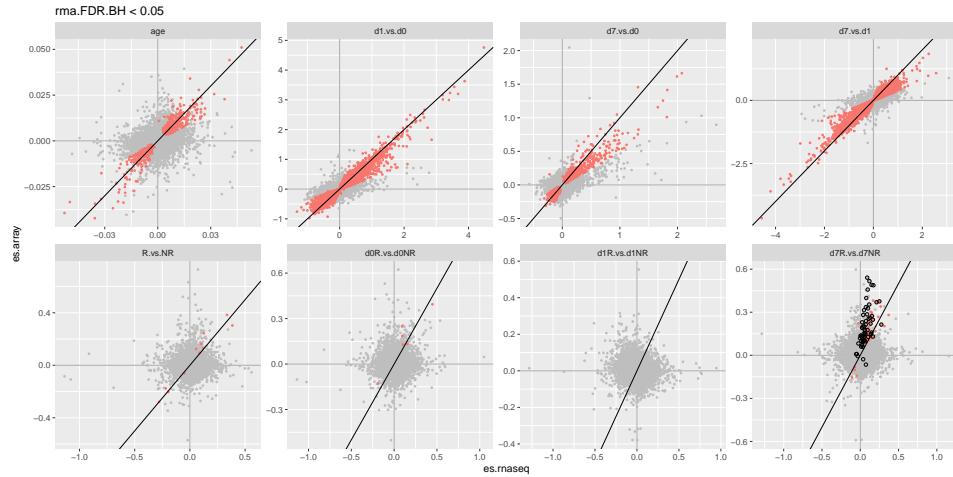


Figure 2.19: DGE effect sizes estimated in array vs. RNA-seq. Significance colored by frequentist random effects meta-analysis FDR < 0.05. Genes with day 7 expression associated with responder/non-responder status in²² are circled for that contrast.

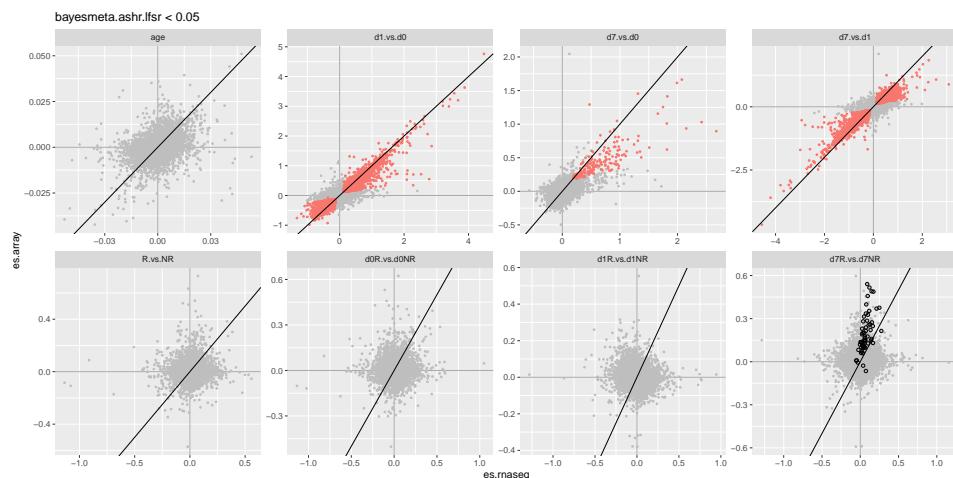


Figure 2.20: DGE effect sizes estimated in array vs RNA-seq. Significance colored by Bayesian random effects meta-analysis lfsr < 0.05. Genes with day 7 expression associated with responder/non-responder status in²² are circled for that contrast.

CHAPTER 2. TRANSCRIPTOMIC RESPONSE TO INFLUENZA A
2.4. DISCUSSION (H1N1)PDM09 VACCINE



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).

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: apoptosis²³; Fc γ receptor-mediated phagocytosis, TREM1 signaling²⁴; enriched in B cells, T cell activation²⁵; B cell receptor signalling, inflammatory response, platelet activation²⁶; several of which I also observe. It should 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

CHAPTER 2. TRANSCRIPTOMIC RESPONSE TO INFLUENZA A
2.4. DISCUSSION **(H1N1)PDM09 VACCINE**

could comment on phenotype differences too, i.e. HIRD measure antibodies at d63, much later than is popular in the field: d28 usually

should probably emphasize 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

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.

found signatures, but so what? Feels like chapter lacks a punchline?

considerably more immunogenic, and post-vaccination expression patterns differ to those of non-adjuvanted vaccines^{20,22}. 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.

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 common human genetic variation on Pandemrix expression response.

Table 2.1: Sample descriptive statistics.

| | Total n = 114 | array n = 44 | platform 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) |

CHAPTER 2. TRANSCRIPTOMIC RESPONSE TO INFLUENZA A
2.4. DISCUSSION **(H1N1)PDM09 VACCINE**

Table 2.2: HIRD batch balance

| | Total n = 374 | 1 n = 87 | 2 n = 79 | batch DN500165J n = 70 | DN500166K n = 69 | DN500167L 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

[The influence of host genetics on vaccines response has also been explored] Vaccine-induced antibody response is a complex trait, with heritability estimates ranging from ... [e.g. seaonsal influenza 10.1016/j.vaccine.2008.07.065 Poland e.g. smallpox e.g. measeks 10.1080/21645515.2015.1119345.]

Narcolepsy controversy (evidence for genetics)

A potential mechanism through which genetic variation can affect vaccine response is through altering the expression of nearby genes (cis-eQTLs). In the case of inactivated trivalent influenza vaccine, genetic variation in membrane trafficking and antigen processing genes was associated with both transcriptomic and antibody responses in patients after vaccination [Franco]. [summary of Sobolev findings]

In this study, we model the influence of host genetics on longitudinal transcriptomic and antibody responses to Pandemrix, *in vivo*.

also, we have phenotype data, *in vivo*

[main aim: how much variation in response is genetic?] [other aims: assess differences to seasonal influenza vaccines] [summary of main results] Why Sobolev? More variation will be explained by history of exposure rather than genetics, so may be harder to detect.

Knowns Sobolev: R vs NR, inconsistent variation in why people are NR
Prevacc signatures of Tri Using larger transcriptomic dataset Are they

genetic

Good points of our study Repeated measures in vivo perturbation

Utility of genetics: allows coloc How does common genetic variation affect response to vaccine?

eQTL becomes more or less important after perturbation: Tells you something about the mechanism of perturbation. Either expression regulatory activation/repression (signalling cascade -> TFs, chromatin remodelling etc.)

3.1.1 Genetic factors affecting influenza vaccine response

Impact of host genetic polymorphisms on vaccine induced antibody response

3.1.2 Context-specific immune response QTLs for influenza vaccine response

if change in expression vs d0 is under genetic control, we should see change in effect size of eqtl vs d0

Summarise Franco et al

3.1.3 Chapter summary

Given the large changes in expression in ch2, detect context-specific fx.

3.2 Methods

3.2.1 Genotype imputation

why exclude x chrom? As is standard for imputation, we excluded all X-linked SNPs for the following reasons: (i) the X chromosome has to be treated differently from the autosomes; (ii) it cannot be predicted which allele is active on the X chromosome, (iii) testing males separately from females results in different sample sizes and power. Imputation of SNPs in the HapMap CEU population was performed using either MACH46 or IMPUTE47. All SNPs with a MAF <0.01 were excluded from analysis. In total, up to 2.11 million genotyped or imputed SNPs were analyzed.

3.2.2 Estimation of cell type abundances

FACS data norm; imputation; scaling

deconv

decon eqtl decon2 has an interesting method: no genotype main effect requires full data i.e. it's an eqtl mapper

cell type interaction terms from proxy genes

Why impute for cell counts but not for eQTL? expression matricse are mostly complete, and we only exclude genes based on low expression in RNAseq we cannot drop whole panels so easily like we can drop genes

Note, the use of gene signatures for deconv in stimulated samples does not distinguish upreg from prolif either if expression goes up, the method will detect more of the signature i.e. it may correct away some signal of upregulation

3.2.3 Mapping cis-eQTLs with LMM

lmms: use a kinship matrix to scale the sample-sample genetic covariance
see: 2018-11-16 notes in log

this is good background

Choice of lmm method for various methods, see 2018-03-05 and 2018-07-25
in log

for discussion of how lmm implementation doesn't matter (Eu-ahsunthornwattana et al., 2014)

Can also refer to previous notes in "2017_Book_SystemsGenetics"

why including known covariates: why not a two stage approach?

Why not mapping on deltas? (if we are interested in the direct question of G on change) ackermann: change scores are prone to increased noise from franco: "We attempted analyses with an approach similar to that proposed by the reviewers in the course of our work, but found that the approach that was ultimately chosen to explore the day differences was the most powerful. Specifically, utilizing a pairwise comparison (difference) between time points as the substrate for the eQTL analysis would lead to an increase in the technical variance of the phenotype, as the sum of two independent (technical) errors has twice the variance of an individual measurement. "

NOTE: peer factors would need to be computed on the foldchange phenotype

The final model:

3.2.3.1 Expression normalisation

2018-03-15 in log

Rank-based int: heavily used in genetics, Although criticised: "Rank-Based Inverse Normal Transformations are Increasingly Used, But are They Merited?"

3.2.3.2 Finding hidden confounders with PEER

Why RANKINT before PEER? "Many statistical tests rely on the assumption that the residuals of a model are normally distributed [1]. In genetic analyses of complex traits, the normality of residuals is largely determined by the normality of the dependent variable (phenotype) due to the very small effect size of individual genetic variants [2]. However, many traits do not follow a normal distribution." "applying rank-based INT to the dependent variable residuals after regressing out covariates re-introduces a linear correlation between the dependent variable and covariates, increasing type-I errors and reducing power."

PEER: expression PCs: if too many, will explain away the signal Not a problem with cis-eQTLs, but trans might have more global effects

GWAS on PEER factors would pick up trans fx, cell count QTL effects

Unlike PCs, PEER factors are not constrained to be orthogonal: adding more and more factors will not explain more of the variance Also, they are weighted

why include genetic PCs see stegle 2012 PEER paper: if PCs are not included, they can be recapitulated in the factors

3.2.4 eQTL mapping with mixed models

Sample AC thresh note they are dosages. if they were not, use ac thresh to estimate number of hom minor expected

3.2.5 eQTL meta-analysis

Why not do a mega analysis? Using a fixed effect assumes mean diff between rnaseq and array and forces the slope to the average. Adding a Gxplatform interaction again leads to diff effect sizes problem.

meta-analysis? can't do a bayesian, which would be ideal. also, small n for array

Restricted to non-full bayesian methods. For small k, Sidik MVa or Ruhkin RBp recommended. Sidik-Jonkman estimator, also called the ‘model error variance estimator’, is implemented in metafor (SJ method).

Starts with an init estiamte of $ri=\sigma^2_i/\tau^2_i$ i.e. ratio of study-specific and between-studies het variance, then updates.

They recommend using Hedges [1], to init, but this is bad???

We use mode of gamma as an apriori estiamte of tau.

compuationally challenging Note we can't just meta the top eqtls from RNAseq as a shortcut , as there is no guarantee the top would have been the top from a meta analysis in the beginning

3.2.5.1 Joint mapping with mashr

In the same period that condition specific eQTL mapping was getting started (as discussed in section...), tools were being created to identify these locitoools were being created to identify these loci review: condition/Cell-type specific methods refere to 2019-11-19 Cell-count specific eQTL mapping papers PANAMA, LIMMI

How much sharing is expected? - overestimates of specificity? e.g. (fair-fax2014InnateImmuneActivity More than half of cis-eQTLs identified, involving hundreds of genes and associated pathways, are detected specifically in stimulated monocytes.)

Simple, mixed models, joint models, multilocus models; Ending with why we chose mashr

normally eqtls use perms for FDR

used for smoothing, info sharing, fdr

mashr beats out stuff it compared to in the paper e.g. metasoft

Choice of strong effects If there is a particular condition with much greater power, choosing the lowest p value for each gene across all conditions could bias strong effects towards including just condition-specific effects for that particular condition. how to ensure condition specific effects are present? look at heatmap of strong subset

lfsr:

3.2.6 Defining shared and response eQTLs

beta-comparison approach from Sarah Kim-Hellmuth 2017 note they correct for FDR

3.3 Results

3.3.1 Overview of eQTLs at each timepoint

3.3.1.1 Estimation of eQTL sharing

Look at diff in beta, not multiples, other 0->1 will be inf

3.3.1.2 TODO Replication of shared eQTLs in whole blood

3.3.2 Characterising re-eQTLs at each timepoint

Ranking metrics: PVE: prefers large maf and high betas since it squares the beta. even if the beta does not change so much. ignores sign. beta: p: ignores sign Z score:

3.3.3 The mechanism of reQTLs

3.3.4 TODO Colocalisation of re-eQTLs with known context-specific immune QTLs

Colocalisation with known associations; 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 expression variation

Choice of method; Coloc and assumptions; Hypercoloc and assumptions

3.3.5 TODO Disruption of binding site motifs as a model for re-eQTLs

See models from Fu et al, Unraveling the Regulatory Mechanisms Underlying Tissue-Dependent Genetic Variation of Gene Expression

3.4 Discussion

Current limitations; Confounded by changes in immune cell proportions in bulk PBMCs; Unclear connection to vaccine biology e.g. what genesets/pathways/cell types are driving the observed transcriptomic and eQTL response?;

3.4.1 DGE vs eqtl

Why dge eqtl overlap poor? Peer accounts for cell prop means.....

It must be said, overlap is not rigorous Formal Mediation analysis required

3.4.2 limitations: The mechanism of reQTLs

3.4.3 Conditional eQTL effects

Confounding by multiple causal variants?; No conditional eQTL analysis to disentangle conditional effects; Are re qtls more likely to be distal and secondary?

CHAPTER 3. GENETIC FACTORS AFFECTING PANDEMRIX

3.4. DISCUSSION

VACCINE RESPONSE

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.

5.2 Methods

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 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

5.3 Results**5.4 Discussion**

Chapter 6

Discussion

Tie ch 2 to 3 using baseline predictors?

Limitations, and the perfect study.

A response eqtl is not always a response eqtl

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 expressed genes

Cost-effectiveness and clinical implementation

if you can identify NRs, what are you going to do about it?

Deep phenotyping

disease specific biobanks e.g. ibd bioresource/predict

unification immunology and vaccine dev: deep phenotyping, small cohorts

achieved -> larger cohorts human genetics and gwas: large cohorts achieved

-> deeper phenotyping

CHAPTER 6. DISCUSSION

Appendix A

Supplementary Materials

A.1 Chapter 2

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer 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

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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.

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List of Abbreviations

BH Benjamini-Hochberg

BTM blood transcription module

CPM counts per million

DC dendritic cell

DGE differential gene expression

eQTL expression quantitative trait locus

FACS fluorescence-activated cell sorting

FC fold change

FDR false discovery rate

HA haemagglutinin

HAI haemagglutination inhibition

HIRD Human Immune Response Dynamics

LAI live attenuated influenza vaccine

LD linkage disequilibrium

lfsr local false sign rate

MAF minor allele frequency

MN microneutralisation

NA neuraminidase

NK natural killer

PBMC peripheral blood mononuclear cell

PC principal component

PCA principal component analysis

REML restricted maximum likelihood

reQTL response expression quantitative trait locus

RNA-seq RNA-sequencing

SD standard deviation

TMM trimmed mean of M-values

TRI titre response index

List of Abbreviations

List of Abbreviations

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Todo list

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| define what a signature is | 8 |
| why? for diff groups of people | 11 |
| add a point that 2009h1n1 is now circulating seasonally | 11 |
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| is there a more recent review? | 12 |
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| atm I'm not using R/NR. wording here implies I am | 15 |
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| Add to collab note that extractions were done at KCL | 16 |
| Add Tracy-Widom statistics for PCs to justify later choice of 4 PCs for covariates | 20 |
| Can add other fastqc plots e.g. kmers, overrepresented seqs, seq length | 22 |
| add software versions | 22 |
| cite relevant preprocessing sections | 27 |
| combat does have a pro in that it can do per gene scaling, that fixed fx won't do | 27 |
| 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. | 27 |
| this is DGE specific normalisation, which is why it goes here, not in the preprocessing section | 30 |
| link to papers justifying sex, age, ancestry as significant effects on immune gene expression | 30 |
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| make all the notation in this section consistent with, and add the equation 2.1. The normal-normal hierarchical model, ⁵⁷ | 31 |
| why is this? is it having well powered studies? gelman is vague | 32 |
| the derivation here is qnorm(0.975, mean=0, sd=1*10) = 1*19.59964, bit iffy, double check this is correct | 32 |
| could also include a table of all sets of parameters here? | 32 |
| can also add MSigDB hallmark sets, which include interferon sets; and of course gene ontology sets | 36 |
| not sure of interpretation at FGFBP2, it is indeed highly expressed in NKs through https://dice-database.org/genes/FGFBP2 . . . | 36 |
| any point in a table of e.g. top 20 DE genes, or is the gene set analysis already enough? | 36 |
| change x axis labels to baseline, specify top 10 procedure in figure caption | 36 |
| finish citing | 36 |
| add label | 36 |
| figure x labels here should be TRI, not R.vs.NR | 38 |
| 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 ⁷⁴ though . . . | 38 |
| might have to rerun everything using the original binary R/NR if this line of reasoning isn't strong enough | 38 |
| move numbers to results? | 38 |
| could comment on phenotype differences too, i.e. HIRD measure antibodies at d63, much later than is popular in the field: d28 usually | 42 |
| 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 enrichment as far as i can tell | 42 |
| 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. | 42 |
| found signatures, but so what? Feels like chapter lacks a punchline? . | 42 |
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| add automatic rounding to x decimal places using num and sisetup . | 73 |