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Benjamin Yu Hang Bai

November 5, 2019

<dedication>

Abstract

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Acknowledgements

pipelines

oucru team

research assistants/research managers

friends cuams churchill MCR, badminton

stackexchange publication quality dialogue, model for future peer review?

Contents

List of Figures

List of Tables

List of nomenclature

eQTL expression quantitative trait locus

Chapter 1

Introduction

1.1 Why study human genetics?

Systems biology:

Linkage, GWAS (10 years), burden tests

Candidate genes (Border et al., 2019)

“molecular QTLs”

expression quantitative trait locus (eQTL) eQTL eQTL eQTLs EQTLs

coloc, fine mapping

TWAS, PheWAS[1], MR

pathway analysis

Poster child gwas-eqtl-drug target

1.2 The genetics of immune healthy

Innate immunity is germline encoded

General genetics of immune response, including disease e.g. Immune Response Mainly Heritable?

1.3 Infectious diseases

1.4 Controlled immune perturbation

1.5 The genetics of immune response to perturbation

Innate immunity is germline encoded

General genetics of immune response, including disease e.g. Immune Response Mainly Heritable?

1.6 Host factors affecting vaccine response

Overview, including pathogen-side factors

Review of systems vaccinology

How to use sysvacc to inform better design[Mooney2013a], and how to move towards personalised vaccinology (https://doi.org/10.1016/j.vaccine.2017.07.062).

1.7 The genetics of vaccine response

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

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

Results from vaccine-related twin studies e.g. in "TWIN STUDIES ON GENETIC VARIATIONS IN RESISTANCE TO TUBERCULOSIS", and[Qi2016]

Review paper on GWAS for vaccines[Mooney2013]

1.8 The genetics of drug response

1.9 Thesis overview

By chapter overview of knowns and unknowns

Chapter 2

Transcriptomic response to pandemic influenza H1N1/09 vaccine (Pandemrix)

2.1 Introduction

2.1.1 Response to inactivated influenza vaccines

2.1.2 The H1N1 virus, Pandemrix, and Pandemrix response

Intro to the virus (Characteristics of Swine-Origin 2009 A(H1N1), DOI: 10.1126/science.1176225)

Pandemrix, as one of several vaccines licensed: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/pandemic-influenza/2009-h1n1-influenza-pandemic/medicines-authorised-during-pandemic

Relationship to seasonal H1N1. ...a single dose of monovalent 2009 H1N1 vaccine was recommended in adults, but young children were recommended to receive 2 doses (reviewed by [3••]). It is likely that a single dose was sufficient to induce immunity in adults because prior exposure to seasonal H1N1 viruses had immunologically primed the population. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3224079/ "Seasonal influenza vaccine provides priming for A/H1N1 immunization." https://www.ncbi.nlm.nih.gov/pubmed/20371459 Demonstration in a mouse model: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3024675/

- more variation will be explained by history of exposure rather than

genetics, so may be harder to detect

2.1.3 Response to AS03

2.1.4 the narcolepsy controversy

Prevacc signatures of Tri Using larger transcriptomic dataset Are they genetic

2.2 Methods

2.2.1 The Human Immune Response Dynamics (HIRD) cohort

2.2.2 TRI

In clinical studies seroprotection is normally defined as a specific antibody titer or antibody titer increase (seroconversion).22

2.2.3 RNAseq

Do we have enough reads for RNAseq analysis? https://www.ncbi.nlm.nih.gov/pubmed/24434847 and doi:10.1093/bioinformatics/btt688

2.2.4 DGE

Batch effect correction (see batch effects tag) Combat is best here LM, LMM, Combat were comparable In some cases, Combat overcorrects But main issue is unbalanced design, which affects even 2-way anova. Rather than 2-step, Safest is to use a covariate, which seems to at least create appropriate conficence intervals (1e)

Why combine -7 and 0? See Sobolev: (a) Observed values of multivariate statistic t (m.v.t.) quantifying global PBMC gene-expression dissimilarity in comparison of two study days (red dots) to values expected when days are randomly assigned between groups.

Should we meta-analyse?

In conclusion, we found that underpowered studies play a very substantial role in meta-analyses reported by Cochrane reviews, since the majority of meta-analyses include no adequately powered studies. In meta-analyses

including two or more adequately powered studies, the remaining underpowered studies often contributed little information to the combined results, and could be left out if a rapid review of the evidence is required.

2.2.5 Meta-analysis

Whilst there is a slew of literature on metanalyasis of rnaseq adn array (e.g. metaMA), combining platforms is fraught with difficultiy. different probes, different tech -> diff stat models

Why expected het? platform effect (ratio compression, differences in preprocessing to genes). different sets of samples (more extreme in array)

exmplaes of e.g. random effects model of approx 24 datasets: e.g. sva: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3617154/

Alternative is: CorMotif first applies limma (Smyth, 2004) to each study separately. CorMotif for microarray data since it was motivated by the microarray analysis in the SHH study. However, the idea behind CorMotif is general, and it should be straightforward to develop a similar framework for RNA-seq data.

Or MetaVolcano: vote counting, REM (note small k),

Sweeny tests diff ks: Methods to increase reproducibility in differential

or complicated R package, CBM ("Cross-platform Bayesian Model"), also see CBM paper for discussion of difficulties of combinding platform

cannot acually use CBM, as it operates on expressions, with a binary case vs control, so no covariates same limitation for cormotif, although it takes any number of groups

rankprod (focus on case/control design), mayday seasight

Choice of meta-analysis method

Two schools of thought for frequentist meta-analysis: fixed-effect, or in the presence of het, random-effects.

We have het, so def use random effects.

How to estimate het? Many methods to estimate het, but

The problem: we only have k=2, and MLE estimates of tau are not very good with k=2. Highly imprecise, and often: boundary estimate problems.

and We know 0 het is inappropriate.

Bayesian random-effects meta is attractive. But What priors should we use?

Prior for tau.

A general rec is: Use distribution in the half-t family e.g. Cauchy (df=1) when the number of groups is small and in other settings where a weakly-informative prior is desired. In their 3-schools examples, choose a value of scale just higher than expected, this is to weakly constrain the posterior, and not to actually represent prior knowledge. - Warn against inverse-gamma(e, e), as it can influence the posterior mean.

But weak priors are not recommended, as k is small, so there is little information in the data.

We can get empirical distribution of many genes. fit a default reml model, exclude 0 ests. Advantage of getting the correct parameter scale for our data. So use Empirical Bayes: aside: empirical bayes is popular for high dim data e.g. edgeR, DESeq2, limma-voom, combat (method of moments)

Papers that fit empirical datasets for tau2: Most of these are inverse-gamma/log-t family Fit inverse gamma distribution on method of moments estimates from 18 gastroenterology trials with similar endpoints. This paper has described the distribution of the between-study variance amongst Cochrane reviews published between 2008 and 2009, and investigating a binary outcome. A log-normal distribution incorporating the association between the between-study variance and the pooled effect size gave the best fit. Predictive distributions are presented for nine different settings, defined by type of outcome and type of intervention comparison. For example, for a planned meta-analysis comparing a pharmacological intervention against placebo or control with a subjectively measured outcome, the predictive distribution for heterogeneity is a log-normal (2.13, 1.582) distribution, which has a median value of 0.12. Model selection based on the deviance information criterion (DIC) [8] led to the choice of the log-t model for t2. (5df) The priors are derived as log-normal distributions for the between-study variance, applicable to meta-analyses of binary outcomes on the log odds-ratio scale.

We choose gamma: as Density at tau=0 is 0, but increases linearly from 0, so values close to 0 are still permitted if the data suggests it. For lognormal/inverse gamma, they have a derivative of 0 at tau=0, so they rule out small tau no matter what the data suggest. For The exponential and half-Cauchy families, for example, do not decline to zero at the boundary, so

they do not rule out posterior mode estimates of zero.

Prior for logFC

Not as much discussion in the lit: There is Typically enough data to estimate this to use a non informative prior. Even Friede Uses noninformative flat.

Two choices in bayesmeta are uniform and normal. We know Mean is 0: most genes are not DE. so flat prior makes no sense

To avoid overshrinking, could consider heavy-tailed priors (e.g. cauchy) for mu rather than normal, but this is not possible in bayesmeta. Cauchy 2.5 DEseq/apeglm: prior on logfc, cauchy with scale adapted.

But bayesmeta is normal. So weaken further to place more prior on larger values. This means less shrinkage.

Also: we will shrink again with ashr. which can fit a more complicated distr (mixture?)

So We use a very weak normal prior, scaled to each coef, as we still want some scaling based on parameter scales. Equiv to saying 95pc chance that effect is within log2FC of 20.

Sobolev2016

2.3 Results

2.3.1 Comparison to Sobolev R vs. NR

Stuff from 1st year report.

2.3.2 modules

The reduced efficacy of vaccination has also been linked to excessive inflammation for influenza,31 yellow fever,32 tuberculosis,33 and hepatitis B34 vaccines.

Chapter 3

Genetic contribution to Pandemrix vaccine response

3.1 Introduction

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 <Context-specific QTLs>

types of conditinoal QTL ackerman conditional vs dynamic

Review of stimulation condition QTL mapping, invitro and invivo what models used? did they use change scores for longitudinal?

ackermann: change scores are prone to increased noise

QTLs can interact with sex and age

Mechanisms:

Review of in vivo mapping. Franco, Caliskan Rhinovirus, Davenport

decon eqtl requires full data i.e. it's an eqtl mapper

3.1.2 condition/Cell-type specific methods

interaction terms

deconvolution

3.1.3 genotype qc

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.

3.1.4 Mapping quantitative trait loci

Why not use indicator for rnaseq/array? Peer factors get very tricky to put in?

Why not mapping on deltas? 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. "

including known covariates: why not a two stage approach?

expression PCs: if too many, will explain away the signal

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

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

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 cor-

relation between the dependent variable and covariates, increasing type-I errors and reducing power."

PEER: Not a problem with cis-eQTLs, but trans might have more global effects

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

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)

LDAK kinship matrix construction http://dougspeed.com/method-overview/

Note: can be negative https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6157025/ LDAK version 4.9 [3] and IBDLD version 3.33 [4] were used to derive 2 empirical kinship matrices based on the GAW20 genotype data. For LDAK, in principle, this kernel should correspond to a genetic relationship matrix; in practice, however, we observed that LDAK estimates of self-relatedness were widely spread around their expectation of 1 (Fig. 1a). For IBDLD the estimates of self-relatedness were closer to 1 (Fig. (Fig.1b).1b). The empirical kinship estimate matrices from LDAK and IBDLD were postprocessed to remove negative nonzero values and scaled to have a diagonal equal to 1.

Can also refer to previous notes in "2017_Book_SystemsGenetics"

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

3.1.5 why also cell counts?

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

3.1.6 Meta-analysis

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=sigma2i/tau2i 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.

3.1.7 Sharing

3.1.8 Colocalization

Due to the increasingly abundant

For example, ran

Coloc and assumptions

Hypercoloc and assumptions

large numbers of traits

Confounding by multiple causal

Fine mapping

Chapter 4

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

4.1 Introduction

4.1.1 The genetics of vaccine response in early life

4.1.2 Vietnam specific variation (i.e. not in eu)

4.2 Methods

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).

Chapter 5

multiPANTS

5.1 Introduction

Limitations, and the perfect study.
Era of single cell.

Appendix

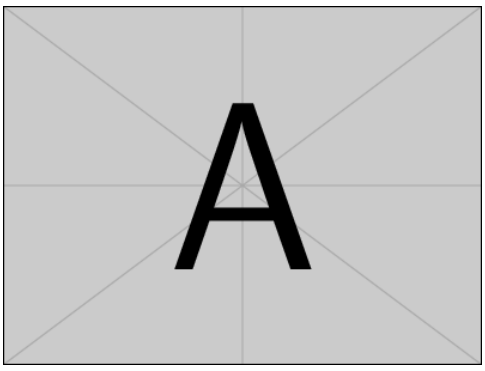

Figure 1: example-image-a

Scratch space

Todo list

Bibliography

1. Verma, A. & Ritchie, M. D. Current Scope and Challenges in Phenome-Wide Association Studies. *Current Epidemiology Reports* **4,** 321–329 (Dec. 2017).