Translational Bioinformatics for Immunogenomics

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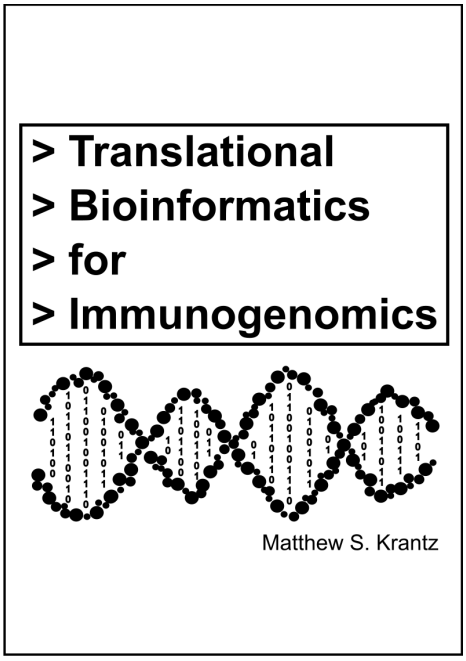
Welcome

This is the website for **"Translational Bioninformatics for Immunogenomics."**



Introduction

Part I

Human Leukocyte Antigens

Background

HLA is located on chromosome 6 in the Major Histocompatibility Complex (MHC).

HLA Class I

HLA class I molecules are expressed by healthy nucleated cells.

HLA Class II

HLA class II molecules are expressed by professional antigen-presenting cells (APC)–dendritic cells, macrophages, and B cells.

HLA Nomenclature

1 Functional Divergence

Heterozygosity of HLA class I genes is associated with better outcomes after HIV infection. This is thought to be due to a greater repertoire of HIV peptides presented and cytotoxic T cell response. However, looking at HLA class I allotype alone does not take into account differences in actual peptide repertoire. Viard et al. (2024) developed a metric to measure this difference, termed "functional divergence." Functional divergence predicts the peptide repertoire as a continuum. They showed that greater functional divergence was associated with better HIV outcomes. Functional divergence may be relevant to other diseases where HLA heterozygosity confers advantage, such as infection, vaccination, and immunotherapy.

You can download functional divergence estimates for pairwise combinations of HLA-A, HLA-B, and HLA-C alleles from their article's Supplementary Materials. The functional divergence measure ranges from 0 (i.e., smallest functional divergence) to 1 (i.e., greatest functional divergence).

2 HLA Imputation Programs

Name	Programming Language	Input Data	Output	Reference
SNP2HLA	Command line interface	PLINK binary format	HLA class I and II alleles	Jia et al. (2013)
HIBAG	R	Plink binary format	HLA class I and II alleles	Zheng et al. (2014)

Part II

Killer Cell Immunoglobulin-like Receptors

Background

KIR is located on chromosome 19 (19q13.4) in the Leukocyte Receptor Complex (LRC). KIR is expressed on the surface of Natural Killer (NK) cells and some T cells. KIR do not undergo somatic rearrangement–a key difference from T-cell receptors. KIR interacts with HLA class I–their cognate ligand–to recognize and destroy unhealthy tissue cells while preventing the same from occurring to healthy cells. Therefore, NK cells play a role in fighting infections, resisting some cancers, pregnancy, and preventing autoimmunity. For further reading and references, I highly recommend the review article by Pollock, Harrison, and Norman on the immunogenetics and co-evolution of KIR and HLA class I.

KIR Locus

Adapted from Pollock, Harrison, and Norman. JACI: In Practice. 2022.

Gene

3DL3

2DS2

2DL2/3

2DL5B

2DS3

2DL1

2DL4

3DL1

3DS1

2DL5A

2DS5

2DS4

2DS1

3DL2

Function

Inhibit.

Activ.

Inhibit.

Inhibit.

Activ.

Inhibit.

Activ.

Inhibit.

Activ.

Inhibit.

Activ.

Activ.

Activ.

Inhibit.

Alleles

228

65

98

47

71

173

112

184

39

44

88

39

33

166

Allotypes

112

22

50

21

23

65

58

92

22

19

38

20

12

115

HLA Class I Ligand Motifs

B7H7

A*11 C1

B*46:01 B*73:01 C1 C2

PVR

?

C2

HLA-G

Bw4+ HLA-A and Bw4+ HLA-B

Bw4+ HLA-B and HLA-F

PVR

C2

A*11 HLA-C

C2

A*3 A*11*

: Adapted from *Pollock, Harrison, and Norman. JACI: In Practice. 2022.*

KIR Diversity

KIR diversity is influenced by gene content variation and sequence variation. Distinct DNA sequences of KIR genes are called "alleles." Distinct polypeptide sequences of KIR genes are called "allotypes." Because different DNA sequences of KIR gene can lead to the same polypeptide, there are more alleles than allotypes for a given KIR gene.

KIR Diversity Concept	Definition
Gene Content Variation	Presence/absence, fusion, duplication
Sequence Variation	May alter ligand affinity or specificity, signal transduction ability, or surface expression (e.g., promoter activity, translation, intracellular trafficking)
Allele	Distinct DNA sequence
Allotype	Distinct polypeptide sequence

NK Cell Education

NK Cell Education (i.e., Arming, Licensing)	Corresponding Pairs of KIR and HLA Class I Ligands	Cytotoxicity and other Effector Abilities
Strong	Many	More
Weak	Few	Less

KIR Nomenclature

Inhibitory KIR

The main role of inhibitory KIR is to prevent cytotoxic NK and T cells from killing tissue cells–unless their HLA class I expression is lost or altered by infection or mutagenesis.

Activating KIR

Activating KIR help identify diseased cells for destruction by cytoxic NK and T cells. Binding of foreign peptides by HLA class I molecules retained by infected cells may be most critical for activating KIR.

Broad KIR Haplotypes

Broad KIR Haplotype	KIR Copy Number Variation	KIR Gene Organization	Activating KIR
A	Relatively stable	Generally non-variable	Less
B	Extensive	Highly variable	More

KIR Ligand Motifs

Table 2.5: Adapted from *Pollock, Harrison, and Norman. JACI: In Practice. 2022.*

KIR Ligand Motif	HLA-A Allotypes	HLA-B Allotypes	HLA-C Allotypes
A3/A11	A*03, A*11	———	———
Bw4	A*23, A*24, A*32	B*07:27, B*08:02, B*08:03, (B13), B*15:13, B*15:16, B*15:17, B*15:23, B*15:24, B*15:36, B*15:43, B*15:67, B*27:01, B*27:02, B*27:03, B*27:04, B*27:05, B*27:07, B*37, B*38, B*40:13, B*40:19, B*44, B*47, B*49, B*51, B*52, B*53, B*56:07, B*57, B*58, B*59	———
C1	C*01, C*03, C*07, C*08, C*12:02, C*12:03, C*12:06, C*12:08, C*13, C*14, C*16	B*46, B*73	———
C2	C*02, C*03:07, C*04, C*05, C*06, C*12:04, C*12:05, C*12:07, C*14:04, C*15, C*16:02, C*17, C*18	———	———

KIR3DL1 and KIR3DS1

Because of significant non-allelic recombination in the KIR region, the distinction between KIR genes and alleles can be confusing. Specifically, KIR3DL1 and KIR3DS1 are alleles of the same gene. Of the KIR3DS1 allotypes–3DS1*013 and* 014–are observed with the greatest frequency in any population.

3 KIR Allele Imputation Programs

Name	Programming Language	Input Data	Output	Reference
PONG	R	PLINK binary format	KIR3DL1/S1 alleles (Global Model includes 51 alleles)	Harrison, 2022
KIR*IMP	Online portal	HAPSAMPLE format	19 KIR types: 17 loci (presence/absence and copy number) plus 2 extended haplotype classifications (A and B haplotypes)	Vukcevic, 2015

Part III

ERAP

ERAP is located on chromsome 5.

4 ERAP Imputation

Part IV

Drug Allergy

5 Immediate Drug Allergy

5.1 Skin Testing

Concentrations typically employed for drug skin testing are 1:10, 1:100, and full strength.

Skin Prick Testing

Intradermal Testing

6 Delayed Drug Allergy

6.1 Phenotypes

Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) secondary to topical medications is characterized by an eczematous eruption–which typically localized to sites of direct exposure. Depending on the severity and chronicity of ACD, eczematous eruptions can range from localized erythema and edema to vesicularization, crusting and weeping. However, ACD can become generalized to non-exposed sites, referred to as "autoeczematization" or "id reaction."

The differential diagnosis for ACD also includes irritant contact dermatitis as well as other chronic eczematous dermatoses (e.g., atopic dermatitis, psoriasis).

The top 4 drug category causes of ACD are: antibiotics, local anesthetics, corticosteroids, and propylene glycol (techically an excipient).

> **!** Important
>
> Antibiotics (e.g., neomycin, bacitracin, polymyxin B) are the most common cause of ACD. Therefore, it is recommended to use petrolatum or other bland emolients for wound care because have equally low infection rate as bacitracin and other topical antibiotics without the risk of ACD.

Co-sensitization—when sensitized to 2 structurally distinct allergens—often occurs in patients who experience ACD. Therefore, when possible, it is important to test to individual components of a culprit topical drug.

Mimics of Delayed Drug Allergy

- Lichen planus
- Prurigo nodularis
- Psoriasis
- Atopic dermatitis
- Mycosis fungoides

> **Note**
>
> These underlying chronic dermatoses can be exacerbated by drugs but not primarily driven by delayed drug allergy.

6.2 Skin Testing

Table 6.1: Utility of patch and intradermal skin testing for delayed drug allergy reaction types

Reaction	Patch Testing	Intradermal Testing
Maculopapular exanthem (MPE)	Useful if positive	Useful if positive
Acute generalized exanthematous pustulosis (AGEP)	Useful if positive	Useful if positive
Stevens-Johnson Syndrome/Toxic epidermal necrolysis (SJS/TEN)	Low sensitivity but potentially useful if positive	Contraindicated due to concern for potential reactivation
Drug reaction with eosinophilia and systemic symptoms (DRESS)	Useful if positive	Useful if positive
Fixed drug eruption	Useful if applied to the site of reaction	Useful if positive

Reaction	Patch Testing	Intradermal Testing
Allergic contact dermatitis	Useful if positive	Useful if positive
Symmetrical drug-related intertrigenous and flexural exanthema (SDRIFE)	Useful if positive	Useful if positive
Drug-induced organ injury (e.g., kidney, liver)	Not useful	Not useful

> **!** Important
>
> No delayed skin testing method has 100% negative predictive value.

Table 6.2: Shared characteristics of patch and intradermal testing

Characteristic	Details
Timing	Perform at least 6 to 8 weeks after reaction; and 6 months or later after DRESS
Concomitant medications	Most medications okay to continue, including anti-histamines and beta-blockers. Should be off of steroids for 1 month or prednisone equivalent dose 10 mg/day

Intradermal Testing

Table 6.3: Characteristics of intradermal testing

Characteristic	Details
Testing site	Volar forearm or extensor upper arm
Testing reagents	Must be sterile; often higher concentrations than those used for immediate skin testing

Characteristic	Details
Reading	At 24 hours
Controls	+ None - Saline
Test interpretation	+ Papule present - Negative

Patch Testing

Table 6.4: Characteristics of patch testing

Characteristic	Details
Testing site	Back or upper arm (needs to be hairless)
Testing reagents	1% and 10% of reagent grade product; 10% and 30% of trade product; most commonly used vehicle is petrolatum
Controls	+ None - Petrolatum
Shelf-life of patch test mixes	Most antibiotics at room temperature are stable for 1 to 3 months; check with USP Pharmacopeia for verification
Patches	Finn chambers (can be aluminum or molded plastic)
Tape	Use hypoallergenic paper tape
Reading	At 48 hours (85% of drugs-if will be positive-are positive by this point); 72 hours; 96 hours; and 1 week
Test interpretation	- Negative ? Doubtful reaction + Weak reaction, erythema ++ Strong reaction, erythema, papules, or vesicles +++ Extreme, bullous, ulcerative

6.3 Human Leukocyte Antigen Testing

6.4 Enzyme-linked Immunospot Testing

7 Specific Drugs

7.1 Antibiotics

Cephalosporins

Fluoroquinolones

Macrolides

Background

What is the chemical structure of macrolides?

Macrolides are defined by a large lactone ring, which varies from 12 to 16 carbons, with 1 or more attached sugar chains. Eyrthromycin and clarithromycin have 14 carbons in their lactone rings while azithromycin has 15.

What is the mechanism of action of macrolides?

As 50S ribosomal subunit inhibitors, macrolides exert their bacteriostatic effect by inhibiting protein synthesis.

What is the cross-reactivity pattern macrolide antibiotics?

While not extensively studied, macrolide antibiotics with a different number of carbon atoms in their lactone ring are tolerated by most patients. Macrolide antibiotics are also unlikely to cross-react with macrolide immunosuppressants (e.g., tacrolimus, sirolimus).

What are some infections that use macrolides as first-line therapy?

Clarithyromycin is used as part of combination treatment for *H. pylori*. Azithromycin is a part of the first-line combination therapy for *Mycobacterium avium* complex.

Non-Hypersensitivity Reactions

GI Side Effects

Because macrolides are also agonists for the motilin receptor–stimulating gastric and small intestine motility–they can cause nausea, vomiting, diarrhea, and abdmoninal cramping. Accordingly, erythromycin can be used as a treatment for gastroparesis.

Sensorineural Otoxicity

Macrolides can cause usually, transient sensorineural ototoxicity.

QT Interval Prolongation

Macrolides are associated with QT interval prolongation.

Skin Testing

Skin testing for macrolide immediate hypersensitivity has not been shown to be reliable.

Oral Challenge

For patients with a history of immediate hypersensitivity reaction to a macrolide, graded azithromycin challenge can be performed, starting with azithromycin 25 mg followed by 1 hour observation then 250 mg followed by 2 hour observation.

For patients with a history of non-severe delayed hypersensitivity reaction to a macrolide, single dose azithromycin 250 mg challenge with 2 hour observation can be performed. Patients should be instructed to report any other delayed symptoms, which may occur up to 24 to 48 hours after the challenge dose.

Penicillins

Skin Testing

What is the NPV of penicillin skin testing?

The NPV of penicillin skin testing is $> 95\%$ when performed with only PPL plus penicillin G or with PPL plus full panel of minor derterminants.

Reagent	Description
Penicilloyl polyly-sine (PPL, PrePen®)	• Major antigenic determinant (what 95% of penicillin degrades into); PPL is penicilloyl complexed with polyltysine to constitute a multivalent skin test reagent. Polylysine acts like the carrier for the penicilloyl hapten in vivo.
Minor derter-minant mixture (MDM) Penicillin G Ampicillin	• Penicillin itself, penicilloate, penilloate • No commercially available expect in South America and Spain

> **Note**
>
> Selective IgE-mediated reactions to aminopenicillins are rare in North America (e.g. 3-5% in the United States) versus 25-50% of skin test positive patients in Europe.

References:

Chapter 77 Middleton's Drug Allergy

Sulfa Antibiotics

Vancomycin

7.2 Antiepileptic Drugs

Background

How are antiepileptic drugs (AEDs) broadly categorized?

AEDs can be broadly categorized by their structure: aromatic or non-aromatic.

What defines an "aromatic" versus a "non-aromatic" AED?

Historically, compounds were labeled as aromatic based on their distinctive aromas.

Today, aromatic compounds and AEDs are defined by containing a benzene ring or other benzene-like properties.

Benzene has a sweet odor and is found naturally (e.g., crude oil) and produced as an intermediate for use in plastics, resins, nylons, synthetic fibers.

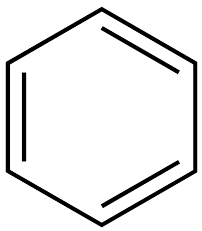


Figure 7.1: 2D skeletal representation of benzene from Wikimedia

Which class of AEDs are associated with the greatest risk of rash and other cutaneous ADRs?

Aromatic AEDs are associated with the greatest occurence of rash and other cutaneous ADRs, including phenytoin, carbamazepine, phenobarbital, lamotrigine, zonisamide, and oxcarbazapine.

In addition, there is greater cross-reactivity amongst aromatic AEDs (40-58%) than non-aromatic AEDs.

Of the AEDs with lower rash risk, most are non-aromatic (e.g. valproate, gabapentin, levetiracetam, pregabalin, vigabatrin, lacosamide, brivaracetam).

Table 7.2: Select aromatic and non-aromatic AEDs. Adapted from Mani et al. Seizure. 2019.

Aromatic AEDs	Non-aromatic AEDs
• Ethosuximide	• Valproate
• Phenytoin	• Gabapentin
• Phenobarbital	• Topiramate
• Carbamepine	• Levetiracetam
• Oxcarbazepine	• Pregabalin
• Zonisamide	• Vigabatrin
• Lamotrigine	• Lacosamide
• Clobazam	• Brivaracetam
• Felbamate	
• Primidone	
• Eslicarbazepine	
• Perampanel	
• Cannabidiol	

What are some risk factors for developing AED-induced rashes?

- History of previous AED-related skin reaction

- Female > Male (2:1), though difference does not persist after > age 50 years
- Older age
- Low vitamin D concentrations

Reference

Mani R, Monteleone C, Schalock PC, Truong T, Zhang XB, Wagner ML. Rashes and other hypersensitivity reactions associated with antiepileptic drugs: A review of current literature. Seizure. 2019 Oct;71:270-278. doi: 10.1016/j.seizure.2019.07.015. Epub 2019 Jul 24. PMID: 31491658.

7.3 Buproprion

7.4 Iron

Background

Why is intravenous (IV) iron used?

IV iron is used for the treatment of iron deficiency anemia when oral iron is effective or not tolerated.

What IV iron formulations are available in the United States?

Formulations available in the United States include low-molecular-weight iron dextran (LWMID), ferric gluconate, iron sucrose, ferumoxytol, iron isomaltoside, and ferric carboxymaltose.

> **Note**
>
> High-molecular weight iron dextrans were discontinued in the United States due to having a higher rate of hypersensitivity reactions.

Table 7.3: Characteristics of iron formulations available in the United States

Generic name	Iron gluconate	Iron Sucrose	LMWID	Ferric carboxymaltose	Iron isomaltoside	Ferumoxytol
Brand name	Ferrlecit	Venofer	INFeD	Injectafer	Monofer	FeraHeme
Molecular weight (kD)	289-440	30-60	165	150	150	750
Labile iron (% injected dose)	3.3	3.5	2	0.6	1	0.8

Immediate Hypersensitivity Reactions

What is the incidence of anaphylactic reactions with IV iron?

Anaphylactic reactions—when high-molecular weight dextrans are excluded—occur with an incidence of < 1 in 200,000.

Significant differences in reaction risk have not been shown among low-molecular weight iron dextran, iron sucrose, ferric gluconate, and ferric carboxymaltose.

What is mechanism of most IV iron immediate hypersensitivity reactions?

Most IV iron immediate hypersensitivity reactions are mediated through complement-activation related pseudoallergy (CARPA). Rarely, hypersensitivity reactions are IgE-mediated.

Minor Infusion Reactions

What are the symptoms of minor infusion reactions to IV iron?

Symptoms of minor infusion reactions to IV iron include—flushing, chest/back tightness, myalgias—and, importantly, do not have any features of anaphylaxis.

What is considered to be the main driver of minor infusion reactions to IV iron?

Labile, or also called "free," iron is associated with minor infusion reactions to IV iron.

Skin Testing

What is the utility of immediate skin testing for IV iron hypersensitivity reactions?

As most hypersensitivity reactions are non-IgE-mediated—rather via CARPA—skin testing has limited utility for evaluating IV iron hypersensitivity reactions; however, it may help detect the rare patients with IgE-mediated hypersensitivity.

Management

What are some approaches to subsequent IV iron administration in patients with previous IV iron reactions?

Approaches for patients with history of mild to moderate IV iron reactions include: switching to an alternative IV iron formulation, slowing the infusion rate (e.g., 10% of recommended rate during the first 10 to 15 minutes), and/or pre-treatment with non-sedating, second generation antihistamines.

For patients with a history of anaphylactic reactions to IV iron, desensitization can be considered, such as ferric gluconate.

References

Gómez-Ramírez S, Shander A, Spahn DR, et al. Prevention and management of acute reactions to intravenous iron in surgical patients. *Blood Transfusion*. Published online April 10, 2019. doi:10.2450/2018.0156-18

Muñoz M, Gómez-Ramírez S, Bhandari S. The safety of available treatment options for iron-deficiency anemia. Expert Opin Drug Saf 2018; 17: 149-59.

7.5 Local Anesthetics

7.6 Nonsteroidal Anti-Inflammatory Drugs

Background

What is the prevalence of nonsteroidal anti-inflammatory drug (NSAID) allergy?

In the general population, 1.5 - 3.5% of individuals report an NSAID allergy. However, fewer than 20% of those individuals with self-reported NSAID allergy are consistent with a true hypersensitivity.

What are some important therapeutic uses of NSAIDs?

NSAIDs are important for the treatment of acute coronary syndrome, pain treatment, and for certain high-risk pregnancy conditions.

For individuals with chronic back pain, NSAID allergy is a risk factor for increased receipt of opioid prescriptions and development of opioid use disorder.

Management

For non-aspirin exacerbated respiratory disease (AERD) NSAID hypersensitivity, a two-step outpatient NSAID oral challenge has been shown to be a safe and effective approach.

Factors associated with a positive NSAID challenge

- NSAID reaction within the past 5 years
- Prior immediate reaction within 3 hours of NSAID ingestion
- History of reaction to multiple NSAIDs
- Presence of co-morbid chronic spontaneous urticaria

Oral Challenge

Table 7.4: Proposed two-step protocol for outpatient NSAID oral challenge in patients without AERD. Adapted from Li et al. JACI In Practice. 2022

NSAID	Step 1 Dose (60 minute observation)	Step 2 Dose (120 minute observation)
Aspirin	40.5 mg	325 mg
Ibuprofen	50 mg	500 mg
Naproxen	60 mg	600 mg

Because of the potential future need for higher dose of aspirin for management of acute coronary syndrome, the second dose for aspirin oral challenge is 325 mg instead of 81 mg.

Of the 262 NSAID challenges performed in the Li et al study, over 85% had negative challenges. In addition, 76% of patients included in the study reported a history of urticaria, angioedema, or both. For patients experiencing a positive challenge, 45% had their reaction within 3 hours of NSAID ingestion

Reference

Li L, Bensko J, Buchheit K, Saff RR, Laidlaw TM. Safety, Outcomes, and Recommendations for Two-Step Outpatient Nonsteroidal Anti-Inflammatory Drug Challenges. J Allergy Clin Immunol Pract. 2022 May;10(5):1286-1292.e2. doi: 10.1016/j.jaip.2021.11.006. Epub 2021 Nov 17. PMID: 34800703; PMCID: PMC9086081.

7.7 Radiocontrast

Background

How do modern radiocontrasts differ from older radiocontrasts?

Modern radiocontrasts are non-ionic, iodinated and either iso-osmolal or low-osmolality. Older radiocontrasts were high-osmolality–which are no longer used intravenously.

Is radiocontrast allergy related to iodine or seafood allergy?

The radiocontrast molecular structure is responsible for hypersensitivity reactions–not iodine or seafood allergy. Shellfish allergy is secondary to tropomyosin not iodine.

Infusion Reactions

These are also referred to as “toxic” or “chemotoxic” reactions. Characteristic symptoms include transient warmth/flushing, nausea/vomiting, chest pain, metallic taste, hypertension, and/or vasogal signs.

Immediate Hypersensitivity Reactions

The most common immediate hypersensitivity reaction to radiocontrast is mild urticaria and pruritus, occuring in ~0.9% - 3.1% of patients receiving radiocontrast. Anaphylaxis occurs in 0.02% - 0.04% of patients. Of immediate hypersensitivity reactions, 70% occur within 5 minutes of radiocontrast injection and 96% of severe reactions occur within 20 minutes.

Delayed Hypersensitivity Reactions

The most common delayed hypersensitivity reaction to radiocontrast is a maculopapular exanthem–occurring in 1 to 3% of patients.

Skin Testing

Skin prick testing to the culprit and other radiocontrasts followed by intradermal testing–if skin prick testing is negative–can be useful for identifying an alternative radiocontrast agent. A skin test negative radiocontrast alternative has a 95% NPV.

Intravenous Challenge

Intravenous challenge can be performed to radiocontrast with various protocols–such as 1 mL, 5 mL, 15, mL, and 50 mL (cumulative 71 mL) at 60 minute intervals.

Management

The culprit radiocontrast should be avoided, and an alternative radiocontrast should be used, guided by negative skin testing if available. Other measures to decrease risk of recurrent radiocontrast reaction include: lowering the radiocontrast dose, decreasing the injection speed, and pre-treatment with non-sedating, second generation antihistamines and/or corticosteroids.

7.8 Topicals

Antibiotics

The most common topical antibiotics which cause ACD are those found in triple antibiotic ointment—neomycin, polymyxin B, and bacitracin.

> **Important**
>
> Of patients with neomycin ACD, 50% will cross-react with other aminoglycosides such as gentamicin.

Anesthetics

The most common topical anesthetics which cause ACD are lidocaine and benzocaine.

> **Note**
>
> While patch testing can confirm ACD due to a local anesthetic, not all patients will necessarily develop an allergic reaction to the same anesthetic if used intradermally or subcutaneous—which is often done for dental and dermatologic procedures.

Corticosteroids

Most patients with ACD secondary to corticosteroids have a history of atopy. Because corticosteroids are often not considered initially as a possible cause of ACD, there might be increased use and worsening of a patient's dermatitis.

ACD due to corticosteroids may produce an "edge effect" or "doughnut-type" reaction–due to the anti-inflammatory effect of the higher concentration of the corticosteroid in the central area compared to the periphery.

> **Important**
>
> If a patient has ACD due to a topical corticosteroid, you should consider propylene glycol as a potential cause—an excipient found in various topical corticosteroids and one of the top 4 causes of ACD due to drugs.

Propylene Glycol

Propylene glycol—an excipient—may be utilized in topical drugs as a softening agent, solvent, moisturizer, or preservative.

Of patients with propylene glycol ACD, 80% have a history of atopic dermatitis.

> **!** Important
>
> Propylene glycol is present in various topical emolients, corticosteroids, and calcineurin inhibitors.

Table 7.5: Select topical drugs for treatment of atopic dermatitis by propylene glcyol content

Topical	Propylene glycol-containing	Propylene glycol-free
Emolients	• Cetaphil Moisturizing Cream	• Cerave Moisturizing Cream • Eucerin Orginal Healing Cream
Corticosteroids	• Mometasone furoate ointment 0.1%	• Triamcinolone acetonide ointment 0.1% • Hydrocortisone ointment 2.5%
Calcineurin inhibitors	• Pimecrolimus cream 1%	• Tacrolimus ointment 0.03% and 0.1%

References:

https://www.jaad.org/article/S0190-9622(19)33110-X/fulltext

Eye Drops

The eyelids are more susceptible to ACD compared to other facial areas—owing to their thin skin 0.55 mm compared to 2 mm, respectively. Therefore, the eyelids may be the only affected area by a drug that comes in contact with the face.

> **Note**
>
> ACD due to eye drops is primarily caused by antimicrobial preservatives rather than the primary drug. Benzylalkonium chloride is the most commonly implicated preservative in patients with history of eye drop reactions.

Table 7.6: Select commercially-available eye drops for common treatment categories by benzylalkonium chloride content

Category	Benzyalkonium chloride-containing	Benyzlalkonium chloride-free
Antibiotic	• Ciprofloxacin (Ciloxan) • Gatifloxacin (Zymar) • Neomycin/Polymyxin B sulfates/Dexamethasone (Maxitrol) • Ofloxacin (Ocuflox) • Polymyxin B sulfate/Trimethoprim (Polytrim) • Tobramycin (Tobrex)	• Moxifloxacin (Vigamox) • Erythromycin ointment (Ilotycin)
Corticosteroid	• Dexamethasone (Maxidex) • Prednisolone acetate • Prednisolone sodium phosphate	• Loteprednol 0.5% ointment

Category	Benzyalkonium chloride-containing	Benyzlalkonium chloride-free
NSAID	• Keterolac 0.4% (Acular LS) • Ketorolac 0.5% (Acular)	• Keterolac 0.45% (Lotemax)
Gluacoma	• Brinzolamide (Azopt) • Timolol (Timoptic)	• Tafluprost (Zioptan) • Latanoprost (Iyuzeh) • Timolol (Timoptic in Ocudose) • Dorzolamide/Timolol (Cosopt PF)

References:

https://eyewiki.org/Preservatives_in_Topical_Ophthalmic_Medications#Benzalkonium_chloride_(BAK)

Bonus

Part V

Bioinformatic Best Practices

8 Reproducible Data Analysis Workflow

I recommend the tutorial, "A Reproducible Data Analysis Workflow With R Markdown, Git, Make, and Docker" as a starting point for R-based data analyses (Peikert & Brandmaier, 2021).

I also recommend using the integrated development environment (IDE) for R, RStudio.

Component	Description
RMarkdown	Dynamic document generation

- "literate programming paradigm"
- Eliminate copy and paste errors
- `knitr` (export to other formats such documents, presentations)
- `papaja` (APA formatting of results in text)
- `stargazer` (journal ready tables)

Component	Description
Git	Version control tracking • Commits = “snapshots” • Commit Message = Description of update • Repository = Collection of commits • Hash = unique identifier **Note** RStudio has a GUI for Git.
Make	Dependency management
Docker	Containerization • Virtual software environment • Reproduce results independent of host operating system

9 Project Organization

If you would like a video overview of how to organize a project using R Studio, I recommend Ming "Tommy" Tang's tutorial on YouTube, "How to Organize a Computational Biology Project". In his tutorial he references an excellent reference on project organization, "A Quick Guide to Organizing Computational Biology Projects" by Noble (2009).

> **Note**
>
> In RStudio, you should create a "New Project," which creates both a project folder and a .Rproj file (which sets the path for your project working directory). You should use the `here` package to easily build paths to files in a more reliable fashion than using setwd().

Bash Commands to Create Folder Directory Structure for Your R Project

Once you have created your New Project in RStudio and are in `your-r-project-folder` in the Terminal. You can create your `README.md` file and your sub-folder directory structure.

```
touch README.md
mkdir data doc scripts bin outputs
```

Once you have downloaded your raw data to your data folder, you should make the contents of the data folder read-only (non-editable) with the following command: `chmod u-w -R data/`

10 File Naming Conventions

In your README.md, you should define naming conventions for your project files. The main elements for a file naming convention are metadata, separator, and version tracking. I recommend the File Naming Conventions Worksheet (Briney, 2020) to develop your file naming conventions.

Metadata	Separator	Version Tracking
3 to 5 pieces max (e.g. sample ID, date in ISO 8601 format such as YYYY-MM-DD)	Dashes (-), underscore (_), or camel case (i.e., capitalize each word without spaces)	Numeric (e.g., v01) or Status (e.g., raw, processed)

> **Note**
>
> My naming convention for R Markdown analysis files is: "analysis-YYYY-MM-DD-version.Rmd" where version starts with "v01." This is my first analysis file, "analysis-YYYY-MM-DD-v01.Rmd"

11 Package Managers

Homebrew

12 Version Control with Git

You should version control your scripts with Git.

I recommend the Using Git and GitHub with RStudio Cheat-sheet for additional helpful commands.

> **!** Important
>
> As long as you have your raw data backed up and your scripts version controlled, you can reproduce your results!

Verify Git Installation and Version

```
which git # request path to your Git executable
git --version # check your Git version
```

Introduce Yourself to Git

```
git config --global user.name "<username>"
git config --global user.email "<email>"
```

Create a New Repository on GitHub

Go to GitHub to create your new repository, then initialize your repository from the command line.

```
cd </path/to/your-r-project-folder>
echo "# your-r-project-folder" >> README.md
git init
git add README.md
git commit -m "first commit"
git branch -M main
git remote add origin https://github.com/<user.name>/<your-repository>.git
git push -u origin main
```

Add, Commit, and Push Files to Remote Repository

```
git add <file-name>
git commit -m "description"
git push
```

13 Dynamic Document Generation with RMarkdown

- `here` = consistent paths relative to project directory
- `redoc` = bidirectional syncing between RMarkdown and word

14 Dependency Management with Make

- Makefile = a list of recipes
  - All = the target of the recipe
  - then ingredients
  - finally steps to prepare the dish

15 Containerization with Docker

- Docker = sets up a virtual computer (e.g. install software)
  - Then saves the resulting state of the virtual computer = "image"
  - Container = running instance of an image
  - An image can be transferred and executed on any machine that has Docker installed
  - Containers = lightweight
    - Start rapidly
    - Run with little overhead
    - Do no need much storage space

> **Note**
>
> Containers achieve this by reusing large parts of the host operating system.

> **Important**
>
> Software versions should be precisely documented to ensure full computational reproducibility.

Rocker Project

- Docker images of pre-configured selected version of R from MRAN
- rocker/verse = RStudio and tidyverse

- `liftR` = way to automatically identify dependencies and automatically generate a Docker image

Part VI

Genotype Imputation

Michigan Imputation Server

The Michigan Imputation Server is a free next-generation genotype imputation platform. You can learn more about the Michigan Imputation Server by visiting their Getting Started documentation. The 1000 Genomes Phase 3 (Version 5) Reference Panel is available on the Michigan Imputation Server.

TOPMed Imputation Server

The TOPMed Imputation Server is another free next-generation genotype imputation platform developed by the University of Michigan and powered by data from the TOPMed Program investigators. You can learn more about the TOPMed Imputation Server by visiting their Getting Started documentation. The TOPMed Version 3 Reference Panel was released in December 2023.

Reference Panels

Reference Panel	Genome Assembly	No. of Samples	Sites (chr1-22)	Chr.	Imputation Server
1000 Genomes Phase 3 (Version 5)	GRCh37/hg19	2,504	49,143,605	22, X	Michigan Imputation Server
TOPMed (Version 3)	GRCh38/hg38	97,256	345,600,184	22, X	TOPMed Imputation Server

Genome Assemblies

The Genome Reference Consortium (GRC) is the main source of human genome assembly data. The most recent human

genome assembly version is GRCh38, released in 2013. The "h" in "GRCh" stands for "human." The GRC also maintains genome assembly data for rat (r), mouse (m), zebrafish (z), and chicken (g for gallus). Major updates, called "versions", are released every few years. Minor updates are called "patches" and are released more frequently.

GRCh38 is referred to as "hg38" in the University of California Santa Cruz (UCSC) Genome Browser. The "hg" stands for "human genome." Before the GRCh38 genome assembly, the version numbers of the GRC and UCSC Genome Browser genome assemblies did not match. For example, when the GRCh37 genome assembly was released in 2009, the UCSC Genome Browser version was "hg19." Therefore, to minimize confusion, starting with the GRCh38 genome assembly, the UCSC Genome Browser version number was matched as "hg38."

GRC Version	UCSC Version	Year Released	Genome Coverage	Alternate Haplotypes
GRCh37	hg19	2009	~92.5%	3 regions with 9 alternate loci
GRCh38	hg38	2013	95%	178 regions with 261 alternate loci

Part VII

Presenting Your Medical Research

Font

You should use a sans-serif font like Arial to maximize readability. "Serifs" are extending features at the end of letters. Times New Roman is a serif font.

Table 15.3: Sans-serif versus serif fonts[1]

Font	Illustration	Examples
Sans-serif font		Arial, Calibri, Helvetica
Serif font		Times New Roman, Georgia, Garamond
Serifs (colored in red)		–

Font Size

[1]Font images are recreated by User:Stannered, original by en:User:Chmod007 - en:Image:Serif and sans-serif 01.png, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2058303

Slide Section	Font Size
Title	36 – 44
Text (e.g., Bullets, Figures, Tables)	24 – 28
References	20 – 24

Word Count

The fewer words, the better. A rule to follow is the 7×7 rule: no more than 7 lines and no more than 7 words per line.

Timing

You should estimate approximately 1 minute per slide.

Figures

I recommend creating your figures as Scalable Vector Graphics (SVG). The main advantages of the SVG format include always maintaining its resolution and smaller file size than pixel-based image formats (e.g., JPEG).

Some tools that you can use to get started creating SVG include Microsoft PowerPoint (subscription), Adobe Illustrator (subscription), draw.io (free), and Inkscape (free). Draw.io is best for diagrams and flowcharts. Inkscape is better for flexible drawings. Both draw.io and Inkscape are integrated with Bioicons, an open-source extension which includes >1700 icons for scientific illustrations.

In Microsoft PowerPoint, you can create an SVG file by selecting all shapes, right-clicking, choosing "Save as Picture", and then picking "SVG" as the "Save as Type."

References

Cite references at the bottom of your slides as you present information.

> **Format**
>
> Last Name. *Journal Abbreviation*. Year.

Equipment

Laptop

Bring your own laptop to presentations in case there isn't a desktop computer for you to use, or it is not functioning, reliable or frustratingly slow.

Hub

What is worse than not being able to connect your laptop to the correct cable? While a good host for a presentation should have a hub (or dongle if that's your preferred terminology), you can come prepared with your own too–particularly important if you have a laptop with only USB-C ports and no HDMI port.

There are lots of options for hubs. If you are looking for a recommendation, I've found that Anker usually has a selection of high-quality and affordable hubs.

USB Drive

Do you want the entire audience to see your most recent emails when you login to download the PowerPoint you emailed yourself? No. Me either. To avoid this, bring your presentation loaded onto a USB drive, which should ideally have both USB-A and USB-C ports. Or, you can also avoid this by using your own laptop—where the presentation should already be downloaded.

Presentation Remote

I don't feel as strongly about bringing your own presentation remote as your own laptop, hub, and USB drive–but I think it is another piece of equipment to consider. This helps keep you from being tethered to and white knuckling the podium during your talk.

One option to consider is the Logitech Spotlight Presentation Remote–which includes features such as magnification, vibration alerts for time management (e.g., 5 minutes left), 3 hours use from 1 minute of charging, and connection by USB receiver or Bluetooth–in addition to slide advancement.

Part VIII

On Being a Physician-Scientist

Building Accountability

Semester Plan by Week

Every Friday, you should schedule yourself 30 minutes to plan your next week. Weekly Planning meeting 30 minutes. If wait you wait until Monday, you won't start your week with momentum. 3 hours/deep work per day.

1 - Reflect on the prior week and how you did

2 - Set up skeleton for week (5 min)

- Write/Design/Analysis in Mornings
- Meetings in Afternoon

3 - Brain dump of things to get done; Map steps (15 min)

4 - Tasks meet time (10 min)

You should keep and update your semester plan. Here is a link to an NCFDD example.

> **!** Important
>
> We drastically underestimate how long research and writing tasks will take. Multiplying your initial estimate by 1.5x - 2.5x might get you closer to realistically how long a particular task will take.

Task Tracking

Don't need to track every last minute/hour. Just track the "deep work" hours. Cal Newport, Deep Work.

Harvest Time app. Link to website.

Freedom - block email during "deep work" time.

Mentor Meetings

Could also be a peer.

Have an agenda and take notes for the meeting.

Daily Writing Practice

Why is it that the most important academic activity for tenure, promotion, and professional reputation—writing—has the least amount of built-in accountability?

If you are a physician-scientist, you are a writer; therefore, you should write everyday (Monday - Friday).

Table 15.5: Built-in accountability and importance for tenure, promotion, and professional reputation by activity

Less	**Built-in accountability**	*More*
• Writing	-> **Activity** <-	• Service
• Articles		• Teaching
• Grants		• Clinic/Consults
More	**Importance for tenure, promotion, and professional reputation**	*Less*

> **Note**
>
> The most important part of your promotion—writing—has the least accountability.

Table 15.6: Limiting beliefs to cultivating a daily writing practice. Adapted from NCFDD.

Belief	Reality
"I need huge blocks of time."	"*Both unrealistic and untrue.* You can productively write in 30 minute blocks!"
"I must be inspired to write."	"*No, you don't.* If you put it on your calendar, you can show up to write just like you show up to meetings you don't want to attend."
"Writing is what I do when I'm done thinking."	"*Writing is thinking.*"

Research on Daily Writing

Table 15.7: Adapted from Boice (1989)

Participant Groups	Draft Pages Written per Year
No change	17
Wrote daily and recorded progress	64
Wrote daily, recorded progress, and were accountable	157

Time Target for Daily Writing

Your goal should be to spend 3 or more hours per week on scholarly writing. So, if you write 30 minutes Monday to Friday, you are already at 2.5 hours!

Tips for Daily Writing

Schedule your writing in your calendar like any other meeting or clinical duty.

You should write first thing in the morning. Knock out the most important daily task for your career first!

Map complex goals to attainable steps.

Use a timer, stop when the timer goes off (to avoid slipping back into writing in huge chunks).

Leave yourself a "breadcrumb," so you can pick up where you left off.

Give yourself a treat after writing.

Do a reflection at the end of the week (Friday) on how your writing went.

Benefits of Daily Writing

Writing daily helps align your time with your evaluation criteria (e.g. 80% research and 20% clinical).

Table 15.8: Benefits of daily writing. Adapted from NCFDD

Benefit	Description
Productivity Shift	Leads to slow, steady productivity and fewer feelings of anxiety over meeting writing expectations
Mental Shift	Writing is the most important part of your success; therefore, it is your top priority.
Behavior Shift	You write everyday and find a way to be accountable that works for you!

Academic Medicine Jobs

AAMC Faculty Salary Report

Looking to get an idea of academic faculty salaries? The annual AAMC Faculty Salary Report compiles academic faculty salaries by rank, degree, department/specialty, medical school type, region, and more. This is often available for free through your university library. Get to know your librarian!

Tenure-Track Offer Letters

What goes into a tenure-track offer letter? The Burroughs Wellcome Fund provides a comprehensive list of offer letter components in their article, "Academic Tenure-Track Offer Letters."

NIH Loan Repayment Program

NIH Loan Repayment Program

Online Resources

Edge for Scholars

Edge for Scholars

National Center for Faculty Development and Diversity

The National Center for Faculty Development and Diversity (NCFDD) provides practical resources for academic researchers. I recommend signing up for their Monday Motivator Newsletter and watching their Core Curriculum videos.

Professional Organizations

American Physician Scientists Association

Suggested Readings

Not Discussed

Not Discussed by Michael Stein

Publishing Your Medical Research

Publishing Your Medical Research by Daniel W. Byrne

Deep Work by Cal Newport

References

Boice, Robert. 1989. "Procrastination, Busyness and Bingeing." *Behaviour Research and Therapy* 27 (6): 605–11. https://doi.org/https://doi.org/10.1016/0005-7967(89)90144-7.

Jia, Xiaoming, Buhm Han, Suna Onengut-Gumuscu, Wei-Min Chen, Patrick J. Concannon, Stephen S. Rich, Soumya Raychaudhuri, and Paul I. W. De Bakker. 2013. "Imputing Amino Acid Polymorphisms in Human Leukocyte Antigens." Edited by Jianming Tang. *PLoS ONE* 8 (6): e64683. https://doi.org/10.1371/journal.pone.0064683.

Noble, William Stafford. 2009. "A Quick Guide to Organizing Computational Biology Projects." Edited by Fran Lewitter. *PLoS Computational Biology* 5 (7): e1000424. https://doi.org/10.1371/journal.pcbi.1000424.

Viard, Mathias, Colm O'hUigin, Yuko Yuki, Arman A. Bashirova, David R. Collins, Jonathan M. Urbach, Steven Wolinsky, et al. 2024. "Impact of HLA Class I Functional Divergence on HIV Control." *Science* 383 (6680): 319–25. https://doi.org/10.1126/science.adk0777.

Zheng, X, J Shen, C Cox, J C Wakefield, M G Ehm, M R Nelson, and B S Weir. 2014. "HIBAG—HLA Genotype Imputation with Attribute Bagging." *The Pharmacogenomics Journal* 14 (2): 192–200. https://doi.org/10.1038/tpj.2013.18.