

Translational Bioinformatics for Immunogenomics

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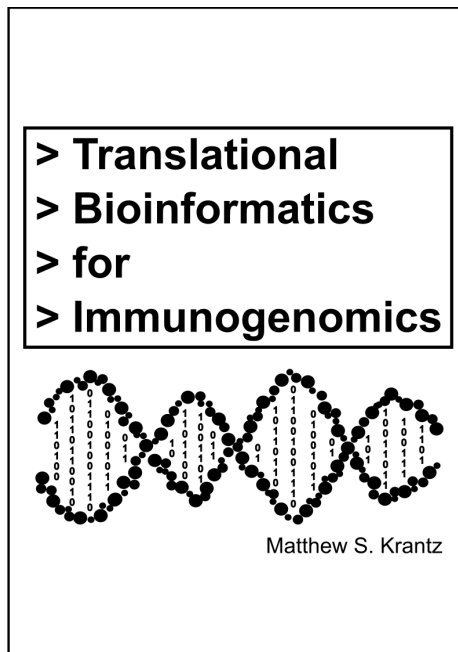
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Welcome

This is the website for “Translational Bioninformatics for Immunogenomics.”



Introduction

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

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).

HLA Imputation Programs

Name	Programming		Output	Reference
	Language	Input Data		
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)

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

B46:01 B73:01 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

A3 A11

: 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 cytotoxic 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 0.4: Adapted from *Pollock, Harrison, and Norman. JACI: In Practice. 2022.*

KIR Ligand Motif	HLA-A Allotypes	HLA-B Allotypes	HLA-C Allotypes
	A3/A11A*03, A*11		

same gene. Of the KIR3DS1 allotypes—3DS1013 and 014—are observed with the greatest frequency in any population.

KIR Allele Imputation Programs

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

ERAP

ERAP is located on chromosome 5.

Epistatic Interactions

KIR-HLA

Epistatic interactions between KIR and HLA are associated with ankylosing spondylitis ([Hanson, 2020](#))

Bonus

Drug Allergy

Immediate Drug Allergy

Skin Testing

Skin Prick Testing

Intradermal Testing

Delayed Drug Allergy

Skin Testing

Table 0.1: 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

Reaction	Patch Testing	Intradermal Testing
Fixed drug eruption	Useful if applied to the site of reaction	Not useful
Drug-induced organ injury (e.g., kidney, liver)	Not useful	Not useful

! Important

No delayed skin testing method has 100% negative predictive value.

Table 0.2: 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 0.3: 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
Reading	At 24 hours
Controls	+ None - Saline

Characteristic Details	
Test inter-	+ Papule present
pretation	- Negative

Patch Testing

Table 0.4: 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

Specific Drugs

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. Erythromycin 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?

Clarithromycin 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 abdominal cramping. Accordingly, erythromycin can be used as a treatment for gastroparesis.

Sensorineural Ototoxicity

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

Sulfa Antibiotics

Vancomycin

Bupropion

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 0.5: 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

Generic name	Iron gluconate	Iron Sucrose	LMW dextran	Ferric carboxymaltose	Iron isomaltoside	Ferumoxylol
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](https://doi.org/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.

Local Anesthetics

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, occurring 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.

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		Chr. Imputation Server
	As- sem- bly	No. of Sites Sam- (chr1- ples 22)	
1000 Genomes Phase 3 (Version 5)	GRCh37	27,041,149,143,160,522, X	Michigan Imputation Server

Reference Panel	Genome As- sem- bly	No. of Sites Sam- ples (chr1- 22)	Chr.Imputation Server
TOPMed (Version 3)	GRCh38	15,600,182	TOPMed Imputation Server X

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 Re- leased	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

Bioinformatic Best Practices

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](#)).

Project Organization

Bash Commands to Create Folder Directory Structure for Your R Project

```
cd </path/to/parent/directory>
mkdir <your-r-project-folder>
cd <your-r-project-folder>
touch README.md
mkdir data doc src 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/`

Version Control with Git

I recommend the [Using Git and GitHub with RStudio Cheat-sheet](#) for additional helpful commands.

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
```

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)

Example

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”

Application Containers with Docker

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 0.1: Sans-serif versus serif fonts¹

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

Font Size

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.

¹Font 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>

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.

On Being a Physician-Scientist

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](#)

NCFDD

[NCFDD](#)

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

References

- 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>.
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