Impact of Host and Vaccine Characteristics on Immune Responses following Influenza Vaccination

Dissertation proposal by

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PROJECT SUMMARY

Vaccine efficacy for seasonal influenza is typically low, especially when strains used in vaccine formulation are mismatched to circulating strains. Such vaccines often offer even less protection from novel strains – the 2008 seasonal influenza vaccine may have even worsened immune responses to the novel 2009 pandemic strain. The development of a universal influenza vaccine is hindered by a lack of understanding of factors driving variation in the immune response to influenza. We will use longitudinal cohort data from multiple sources to explore how characteristics of vaccines interact with characteristics of the vaccine recipient to clarify potential drivers and mechanisms of the immune response to vaccination. We aim to explore how antigenic differences between the vaccine strain and historical strains (a surrogate for potential future strains) interact with the preexisting immune repertoire of the recipient, and how vaccine dose and prior vaccination history can modulate this relationship. In addition, we will propose metrics for the fair evaluation of vaccine candidates to multiple historical strains, which can allow the selection of an optimal broadly reactive vaccine. Our analysis will allow for improved development and evaluation of universal vaccine candidates.

PROJECT NARRATIVE

Influenza is a respiratory disease that occurs in seasonal epidemics worldwide. The Centers for Disease Control and Prevention recommend annual vaccination against seasonal influenza with a modified vaccine for adults in the United States, but the effectiveness of these vaccines varies greatly across seasons and individuals. Improved understanding of the drivers of the immune response following vaccination is crucial for improving influenza vaccines.

1. SPECIFIC AIMS

Recent concerns about H5N1 influenza spillover events highlight the need for a universal influenza vaccine with the ability to mitigate future pandemic events. Outside of pandemic concerns, seasonal epidemic influenza has a consistently high burden, with vaccine effectiveness typically 50% or less. A combination of rapid antigenic evolution and heterogeneity in individual host response makes developing a durable universal vaccine difficult. The goal of my proposal is to understand how pre-existing immunity affects individual response to the seasonal influenza vaccine, and how this effect is modified by prior immunity, vaccination history, vaccine dose, and recipient demographics. A better understanding of the host immune response will inform the design and evaluation of vaccines which induce a broader, more robust response.

We will use Bayesian hierarchical models, causal inference, and machine learning methods to quantify these effects. Our models will account for interactions between predictors, nonlinear effects, and clustered measurements. We will analyze longitudinal influenza vaccination data from the UGAFluVac cohort study (PI: Ross), wherein individuals provided serum samples, potentially for multiple years, and these samples were tested against a panel of historical viruses. We will also combine the UGAFluVac data with other data sources provided by Ben Cowling, Andrea Sant, and potentially other investigators involved with the DIVERsity study (NIH project number 1R01AI170116-01) or the CIVR-HRP NIH CIVICs site (NIH project number 75N93019C00052).

Aim 1. Develop metrics for the quantification of the total immune response to an influenza vaccine, incorporating both strength and breadth. Using the UGAFluVac data, we will analyze the relationship between immune response and antigenic distance, a measurement of how different the assay strain and the vaccine strain are. We will develop metrics for quantifying the overall strength of the immune response to a panel of heterologous strains, and the breadth of the response – intuitively, how the immune response diminishes as antigenic distance increases. We will also use subsamples of the UGAFluVac data to analyze the robustness of these metrics across differing panels of historical viruses.

Aim 2. Quantify the role of pre-vaccination titer, prior vaccinations, vaccine dose, and antigenic distance on individual vaccine response. Influenza vaccinations provide diminishing boosts for recipients with high antibody titers, called the antibody ceiling effect. However, the threshold and rate of diminishing boosts depend on several other host and vaccine factors, including prior vaccination history, vaccine dose, and antigenic distance. We will use hierarchical statistical models and mechanistic models to disentangle the individual effects and interactions.

Aim 3. Explore how age and vaccine dose interact to affect the antibody response. The UGAFluVac cohort allows participants over the age of 65 to choose whether they receive FluZone standard dose or FluZone high dose, and Andrea Sant's cohort study administered FluZone HD to individuals aged 18 – 49. By combining the two datasets, we can use causal inference and hierarchical modeling techniques to understand the effect of dose, and how this relates to previous mechanistic modeling predictions.

2. SIGNIFICANCE

Influenza rapidly evolves to escape current vaccines through two major mechanisms: antigenic drift and shift (1,2). Antigenic drift is the gradual process of mutation, driven by selective pressure. Antigenic shifts are sudden and abrupt changes in influenza antigens, which occur by recombination with other strains. See Figure 2.1.
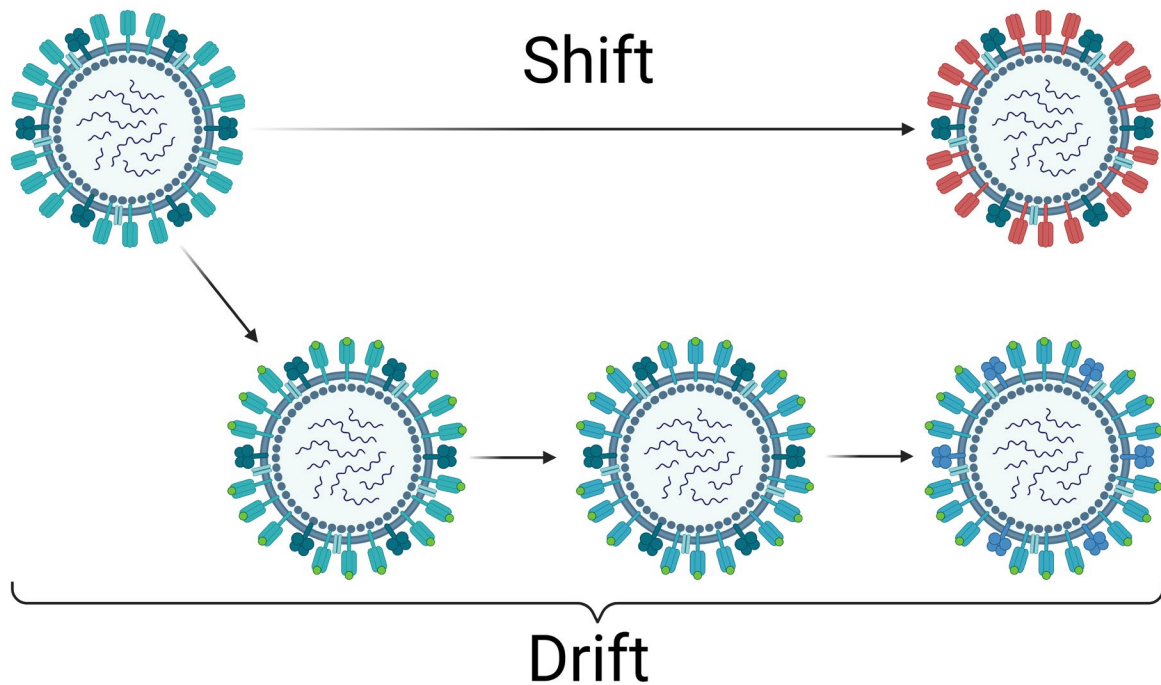
Figure 2.1: Antigenic drift and shift both lead to the emergence of influenza strains with novel antigens that can escape prior immunity. While vaccine escape due to drift is a gradual process that can be palliated by annual reformulation of vaccines, escape due to shift is a relatively quick process that can lead to epidemic or even pandemic spread of influenza before there is time to develop and administer a new vaccine.

Seasonal epidemic influenza has a substantive burden (3–5), especially in children and older adults (6,7). Vaccine effectiveness (VE; a measurement of how protective the vaccine is) varies widely, and is typically lower when strains used in the seasonal vaccine do not match circulating strains (8–10). Even when strains are correctly matched, VE rarely exceeds 50%. The current strategy of predicting circulating strains to determine the formulation of the influenza vaccine also leads to low pandemic preparedness. The spontaneous emergence of new influenza strains, as with the 2009 H1N1 pandemic (11) or the highly pathogenic H5N1 spillover cases in early 2023 (12) also demonstrate the need for a broadly protective influenza vaccine. A *universal* vaccine, which protects against current and future influenza variants has the potential to reduce the burden of seasonal influenza and mitigate future pandemics.

Unfortunately, designing a universal influenza vaccine is challenging (13–15). Understanding the immune response to influenza, in the context of rapid evolution of new variants, is a major landmark for improving vaccine design. Since the immune response to a vaccine depends on an individual's history of infection and vaccination events (Figure 2.2), the susceptible population contains incredibly diverse immune repertoires. Decomposing the immune response to novel influenza strains into understandable effects is an open problem. Dissecting the components of the response would yield key insights into the design of vaccines which are reliably broadly protective for individuals regardless of their immune state at time of vaccination (8).
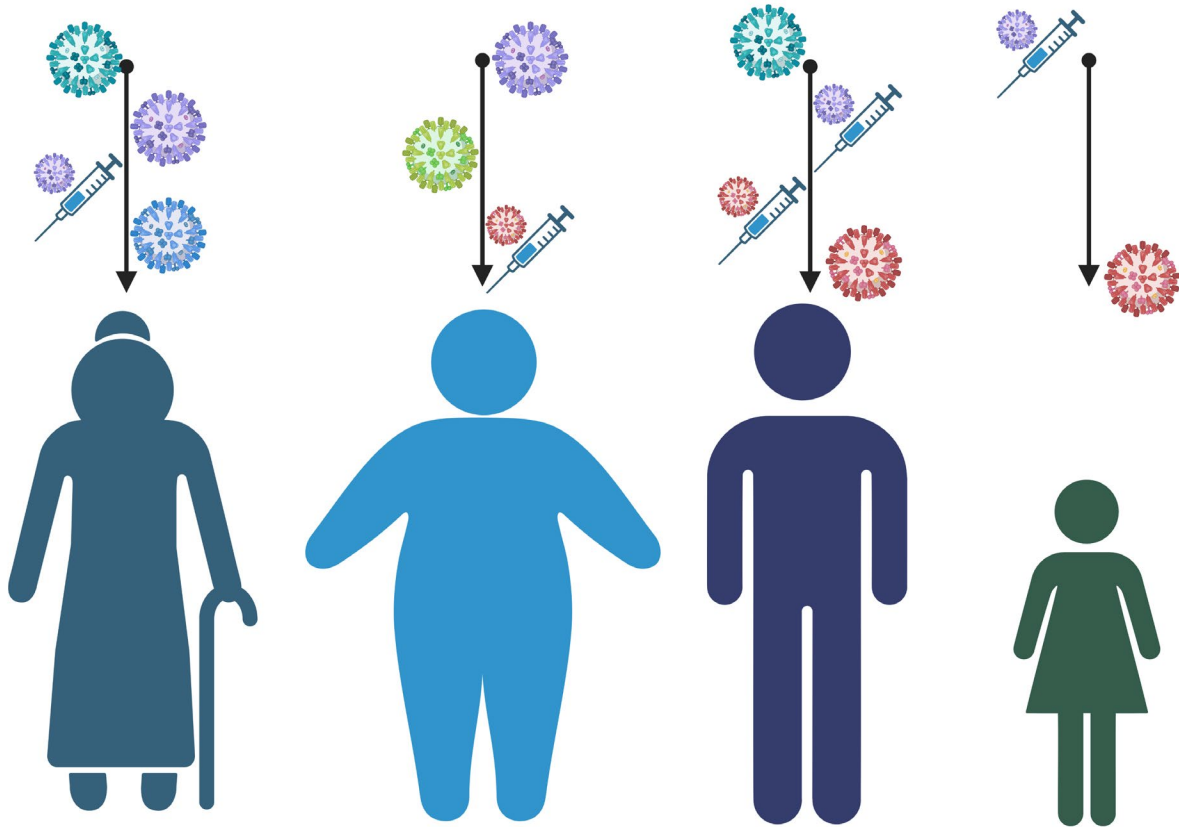
Figure 2.2: While there are some similarities among individuals in influenza exposure, every member of the susceptible population likely has a different pattern of exposure to influenza. Exposure patterns include not only the set and order of influenza strains which an individual has encountered, but also whether a particular exposure was due to infection or vaccination. Each individual also expresses variability in their immune state at the time of exposure, leading to different responses, and thus different immune repertoires, even if the path of exposure is identical.

2.1 Aim 1

There is no universally accepted method for quantifying the overall strength of the immune response induced by a vaccine candidate. Since direct trials of efficacy are expensive, correlates of protection (CoP) are measured instead. Several CoPs are currently used to test the efficacy of influenza vaccine candidates, but there is no consensus on which, if any, are best (16). The most common CoP used in practice is the *hemagglutination inhibition (HAI) titer*. We will focus on HAI titer, but the methods we propose could be applied to any quantitative CoP.

HAI is correlated with protection from influenza, with an individual titer of 1:40 corresponding to approximately 50% protection (17,18). If an individual's reciprocal titer is 40 or greater for a particular strain of influenza, the individual is said to be *seroprotected* against that strain. If a vaccine induces a 4-fold or greater increase in HAI titer, with the final post-vaccination measurement above 40, the individual is said to have *seroconverted* against that strain. Seroconversion and seroprotection are commonly used clinical endpoints for assessing the immunogenicity of an influenza vaccine candidate. An assay using the vaccine strain is said to be *homologous*, while an assay using any other strain is said to be *heterologous*.

Many vaccine studies only measure the immune response to homologous strains, which we will call the **magnitude** of the response. However, to evaluate a broadly reactive universal vaccine candidate, we also need to measure the induced immune response to heterologous strains, which we call the **breadth** of the response.

We can compare universal vaccine candidates using some weighted combination of magnitude and breadth, which we call the **overall strength** of the response.

Previous studies have evaluated the overall strength of individual immune responses after vaccination by running panel of assays using multiple heterologous strains (Figure 2.3). The breadth of the response is then taken as either the count or proportion of strains to which the individual seroconverted (19,20). While this method is easy to quantify in a laboratory setting, the estimates of breadth are biased by the selection of the panel of heterologous strains and do not take *antigenic distance* into account. Antigenic distance is a metric for describing how similar two strains of influenza should appear to the immune system. Using the proportion of seroconverted strains metric, one vaccine candidate could appear to be more broadly protective than another simply because one lab chose a panel of strains which were less antigenically distant on average. Such variation in panels between research groups makes comparing these breadth estimates across studies difficult (21).
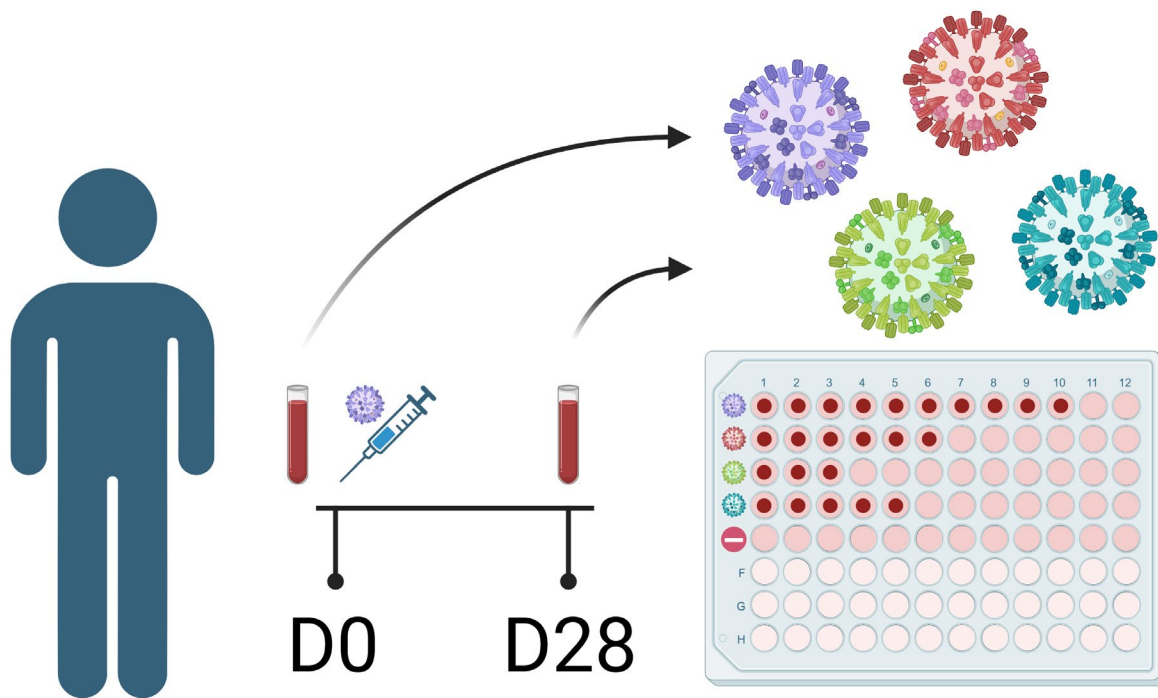

Figure 2.3: A typical experimental setup involves taking serum samples from an individual both before and some time after vaccination. We can then conduct HAI assays using the serum samples against a variety of influenza strains. Changes in the response to this panel of strains after vaccination indicates the degree to which vaccination elicited immune responses to particular strains.

Modern methods for measuring the antigenic distance between strains of influenza will allow us to develop a consistent framework for assessing the breadth of a vaccine candidate. The simplest method for computing antigenic distance is the *time-based* method, where the antigenic distance is taken as the difference in isolation year between strains (22,23). Other methods include *sequence-based* methods, which assess the similarity of the genetic or protein sequence of the two strains (24–28); and *antigen-based methods*, which use immunogenicity data to inform distance between strains (29–33). There is no clear consensus on which measures of distance are most useful for informing vaccine evaluation. An individual's responses to a panel of virus strains can be plotted against antigenic distance, forming an *antibody landscape* (Figure 2.4). While previous work has explored the quantitative comparison of antibody landscapes (22), such approaches have not been widely utilized.
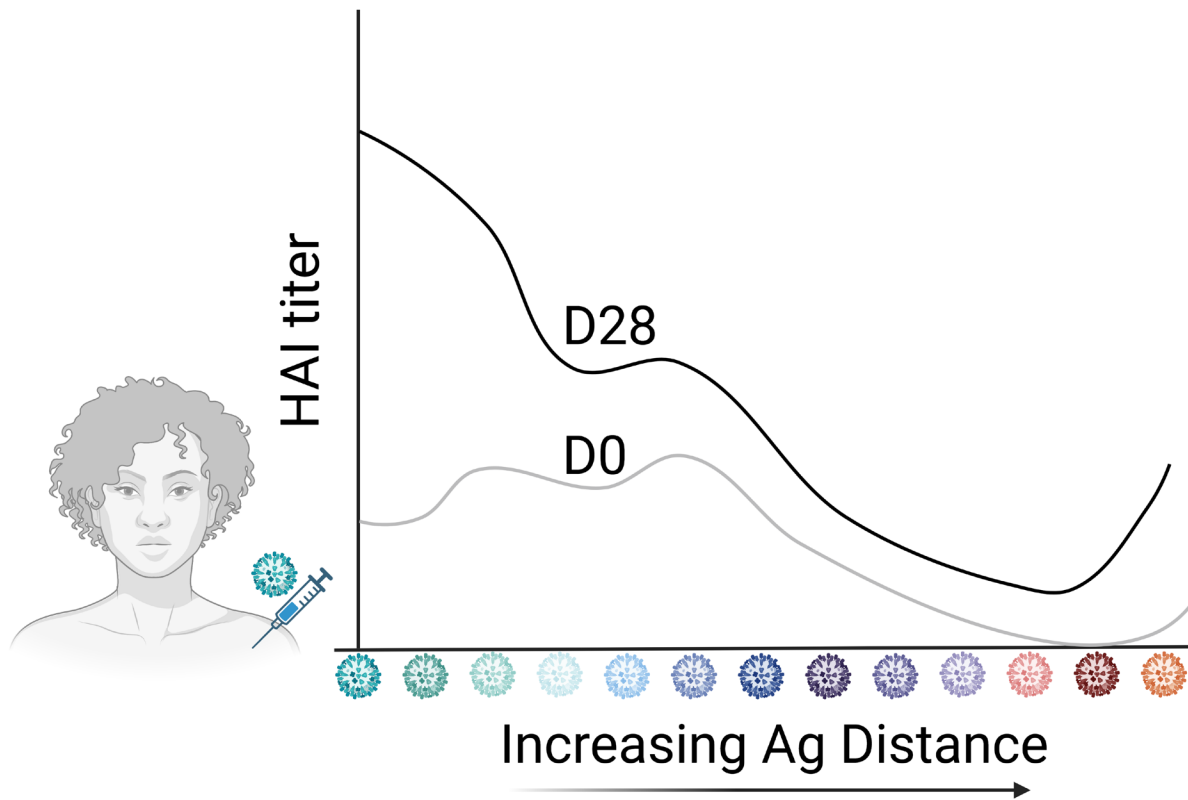
Figure 2.4: We can model an individual's immune response to viral strains as a function of antigenic distance, creating an antibody landscape. A broadly reactive vaccine will have a post-vaccination (for example, day 28) antibody landscape which is higher than the pre-vaccination (day 0) landscape, even for distant strains. A hypothetical universal influenza vaccine would have a post-vaccination landscape that is uniformly higher than the pre-vaccination landscape, as shown here, eliciting a response to all comparable influenza strains.

We will develop methods for quantifying the magnitude, strength, and overall breadth of a vaccine response from individual antibody landscapes. Using our framework for vaccine evaluation, we will compare measures of antigenic distance. We also plan to test the robustness of our metric to the selection of the virus panel in order to compare our framework to the traditional method. We will explore simple regression models, flexible spline models, and functional data analytic techniques for their potential to characterize the magnitude and breadth of the immune response (Figure 2.5). For this aim, we will use longitudinal cohort data with a wide panel of heterologous responses for each individual collected by Ted Ross (34–36).
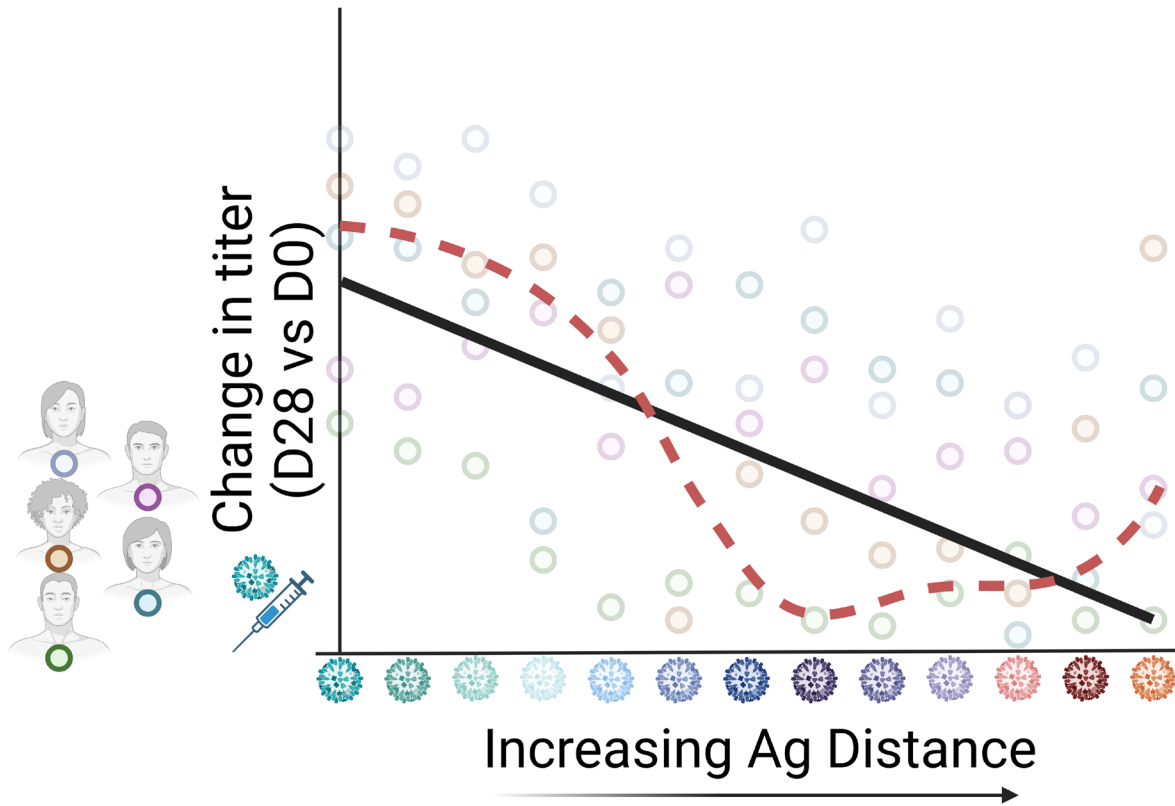
Figure 2.5: In a vaccine cohort study, we can take immune measurements for a panel of heterologous viruses from many individuals. Each of these individuals then has their own antibody landscape, shown here as different colored points for each individual. From these individual landscapes, we can use statistical modeling techniques to infer a typical landscape that might be representative of the response we expect to see from this vaccine candidate. The black line here is a linear statistical model, while the dashed red line is a potential nonlinear statistical model. We then hope to summarize these typical landscapes to obtain metrics for vaccine candidate comparison that balance the magnitude of the response with the breadth of the response. (This figure is conceptual and no real data or fits are shown.)

2.2 Aim 2

Several characteristics of the vaccine and the recipient are associated with the immune response to the vaccine (Figure 2.6). In addition to antigenic distance between the vaccine strain and the strain of interest, vaccine dose (37–39), route of administration (40–42), and type (43–45) are associated with the overall strength of the immune response. Promising vaccine candidates have been developed using intranasal, intramuscular, and subdermal routes of administration. Recombinant protein or mRNA vaccines may prove to be superior to the traditional split-inactivated or live attenuated vaccine types.

In addition to vaccine design choices, baseline characteristics of the vaccine recipient (the host) can alter the vaccine response. These characteristics can vary between every member of the susceptible population, or be aggregable at the population level. Genetic differences (46–48), epigenetic modifications, and differential gene expression (35,49–53), all fall into the former category. Aggregable characteristics include sex, obesity, and age.
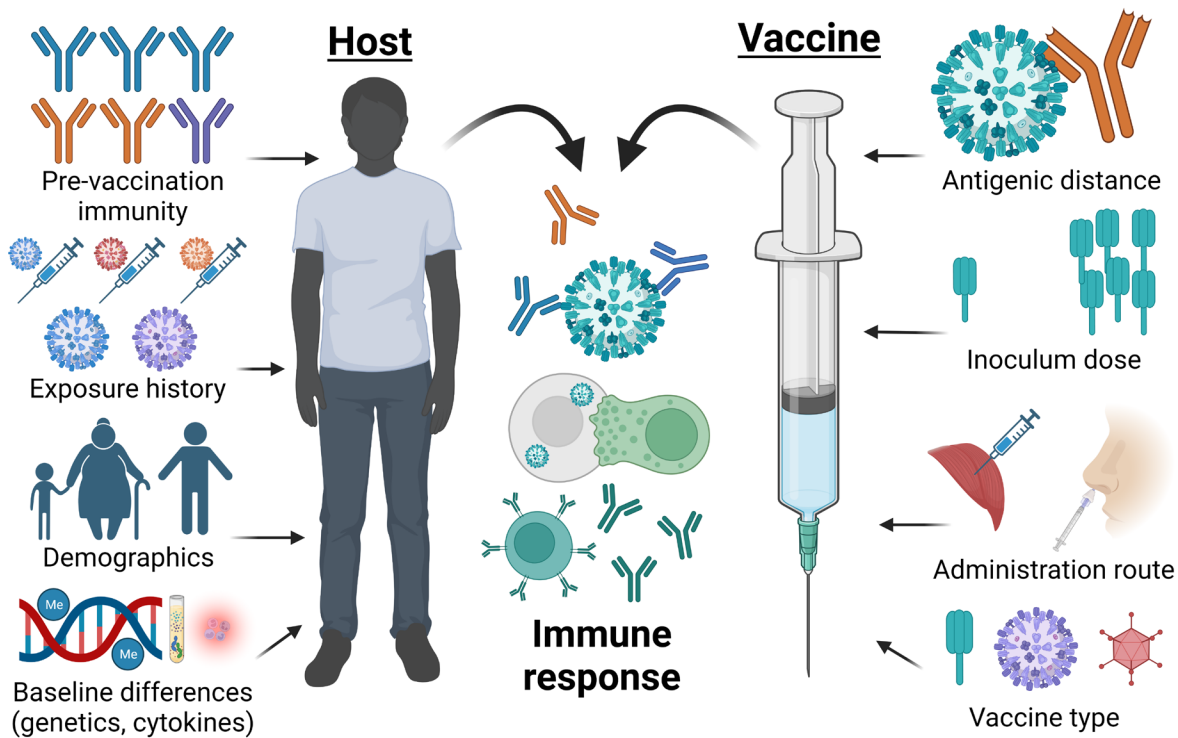
Figure 2.6: Schematic showing the overall drivers to the vaccine response.

Birth sex-associated genetic differences and sex hormone levels may influence the immune response to influenza, although results are ambiguous with no mechanism yet discovered (54–58). Obesity, typically measured through BMI, is associated with a decreased response or with more rapid waning of antibodies (59). As individuals age, they undergo immunosenescence, a gradual decline in immune function and protection (20,60).

In addition to the immunosenescence effect of age, an individual's birth year is predictive of the immune response to specific strains of influenza, because birth year is strongly associated with the first strain of influenza to which an individual is exposed (61–63). The theory of imprinting (also called original antigenic sin) predicts that an individual's first influenza exposure leads to the development of a memory response to that strain. Future exposures then activate the memory response to the original strain (assuming the strains are somewhat similar), which dominates the novel immune response to the new strain. Eventually, influenza antibodies reach a saturation level called the "antibody ceiling", which can vary between individuals (64–66).

One consequence of imprinting and the antibody ceiling is a strong negative relationship between pre-vaccination immunity and the response to a vaccine (44,67). Prior and repeat vaccination also has a strong effect on vaccine response (20,68), potentially independent of the antibody ceiling effect. In fact, the reactivation of the memory response at every exposure to a somewhat similar strain makes the response to vaccination dependent on an individual's entire history of influenza infection and vaccination (23,31,64,69). In addition, the immune response to influenza may be affected by prior exposure to other pathogens, including herpesviruses such as Epstein-Barr virus (70) or cytomegalovirus (71), or through antigen-independent effects which modify the baseline immune state and induce a differential response (72).

While measuring all of these effects simultaneously is impossible, we intend to model the effects of vaccine dose, pre-vaccination titer, prior vaccination, and antigenic distance using data from the cohort studies conducted by Ted Ross. While our analysis will be limited to a single vaccine (Sanofi Pasteur's FluZone, with standard and high dose formulations), we have data on an extensive panel of historical viruses along with reported vaccination history for each patient. We will incorporate predictive machine learning approaches and

hierarchical bayesian modeling to understand the individual contributions of these factors to the overall immune response. We also plan to modify previously-developed mechanistic models for multiple influenza epitope responses (73–75) to include a degree of similarity between epitopes (conceptually representing antigenic distance), and compare the results from the updated model to our data.

2.3 Aim 3

The role of vaccine dose is so important in vaccine response that determining a dose that balances efficacy and side effects is a crucial part of drug approval in the United States (8,13,76). High-dose influenza vaccines are approved for use in older adults and can substantially improve the immune response for older and otherwise immunocompromised individuals (39,77,78). While otherwise healthy recipients generate immune responses to fractional doses of influenza vaccine (79,80), mechanistic models predict that increased dose may be useful in overcoming the negative effects of prior immunity and original antigenic sin (73,74).

These mechanistic models allow for simulation of the immune response to a variety of vaccine doses, and predict that as the dose is increased, the effect of prevaccination titer is suppressed. That is, an individual with a higher prevaccination titer could potentially receive a higher dose than an individual with a low prevaccination titer, and observe the same fold-change in titer as a result of vaccination. To date, a randomized clinical trial comparing standard and high dose formulations of available vaccines has not been conducted in otherwise healthy adults 18 - 49, so these claims have not yet been evaluated in groups that are not elderly or immunocompromised.

We will combine observational data from multiple sources to estimate the effect of dose on influenza vaccine response while controlling for the effects of prevaccination titer, age, and other relevant effects as enumerated previously (Figure 2.7). In this analysis, we specifically plan to focus on the effect of dose on homologous vaccine response by combining the UGAFluVac data, where high dose vaccines were only administered to individuals aged 65 or older, with data collected by Angela Branche and Andrea Sant (44,81). We will obtain the average causal effect of dose, after controlling for age and other confounders, and compare the predictions to those of the mechanistic model, which does not currently account for age. Together, these analyses will provide a better substantive understanding of the effect of dose on the immunogenicity of a standard influenza vaccine.
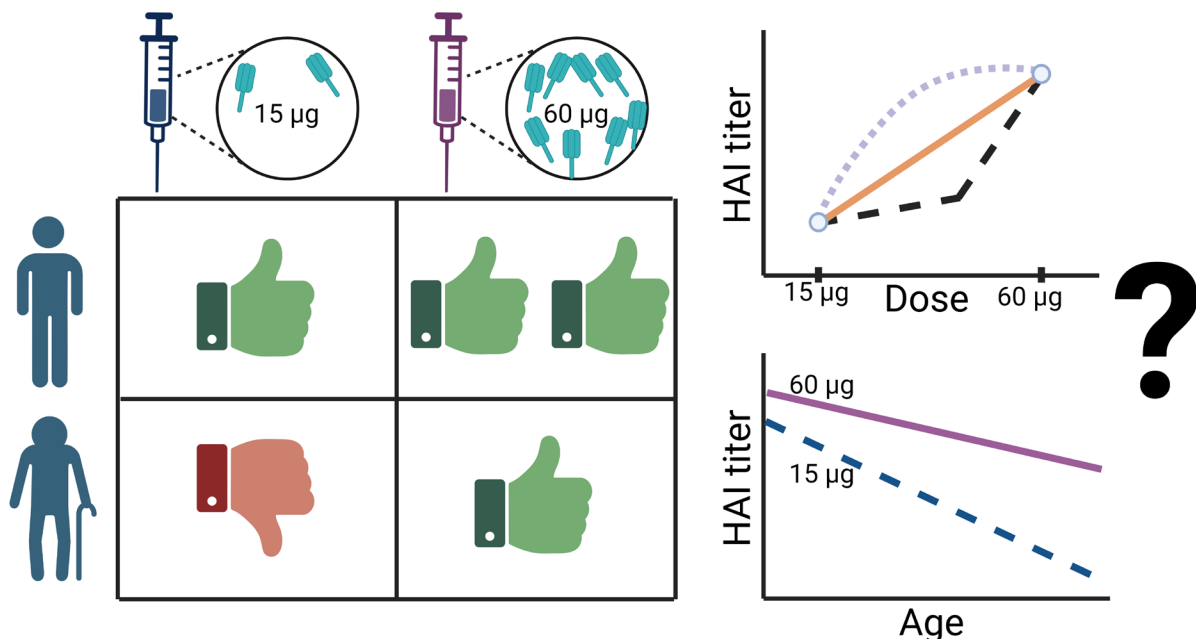

Figure 2.7: High dose (HD) vaccines can induce a stronger immune response in elderly participants than

standard dose (SD). While SD vaccines can elicit a satisfactory immune response in younger participants, an HD vaccine still induces a larger antibody response. However, the exact dose response relationship for intermediate doses (top right graph, showing three potential dose-response relationships) and the relationship between dose and age (bottom right graph, showing one potential dose-age relatiopnship) remain unclear. While we would ideally like to understand the dose-response relationship, we only have data on standard and high dose vaccines. Mechanistic modeling will allow us to predict potential response patterns. We also expect the effect of dose to differ across vaccine formulations.

3. APPROACH

3.1 Data description

We will combine data from multiple influenza vaccination cohort studies for our analyses. At the time of writing, we have data from two cohort studies conducted by Ted Ross, one study conducted by Andrea Sant and Angela Branche (see (44)), and a collaboration with Ben Cowling. Ben is the PI of several similar studies and has offered to share data with us. The two studies conducted by Ted Ross provide a unique opportunity to study heterologous antibody responses to influenza vaccination, as a wide historical panel of assays was conducted for each individual. Combining Ross' data with data from Sant and Cowling will allow for us to compare the immunogenicity of multiple vaccines, and increase the power of our analyses of host factors.

We will refer to both of Ross' cohort studies conjointly as the **UGAFluVac** study. This data set consists of longitudinal HAI measurements taken at three different study sites. From fall 2013 to spring 2016, participants were recruited at the study site in either Pittsburgh, PA or Stuart, FL. Sample collection is detailed in (34). Briefly, the study is a prospective open cohort design. Participants were adults aged 18 and up who were allowed to repeat each year, and the data includes a unique ID per participant that allows for the identification of longitudinal measurements. Each participant received a pre-vaccination blood draw, and was then administered a split-inactivated standard dose (SD) Fluzone seasonal influenza vaccine (Sanofi Pasteur). Patients aged 65 or older could opt to receive Fluzone High Dose (HD) instead. At the PA study site in 2013, some patients were administered Fluzone intradermal rather than the standard intramuscular Fluzone formulation. Followup whole blood draws were targeted for 21 days post-vaccination.

Processed sera were used for HAI assays following standard protocols. HAI assays were conducted using the homologous strain and a panel of heterologous strains. The starting dilution was 1:10 and assays which did not agglutinate at the starting dilution were coded as 1:5. For all further analyses, we will use the reciprocal titer transformed as analysis titer $= \log_2(\text{raw reciprocal titer}/5)$. This transformation serves to set 0 as the limit of detection on the log scale. Additionally, the following data were collected from patients by a survey prior to vaccination: year of birth, age, gender (the covariate is listed as gender but is coded as male/female only), and race/ethnicity.

In January 2017, the study moved to the University of Georgia in Athens, GA. The paper (36) contains a description of the study, but at the time of writing, no published paper contains a complete description of the study cohort. The study design was similar, with additional covariate information collected: complete date of birth, sex assigned at birth, race/ethnicity, BMI, height, weight, and questions about smoking and comorbidities. Beginning in the 2017-2018 flu season, participants aged 10 and older were also recruited for the study. The HAI assays were conducted in the same way. The post-vaccination time point target was changed to Day 28 beginning in fall 2018. Finally, additional subcohorts were administered other vaccines at certain points during the study. See Figure 3.1 for details.

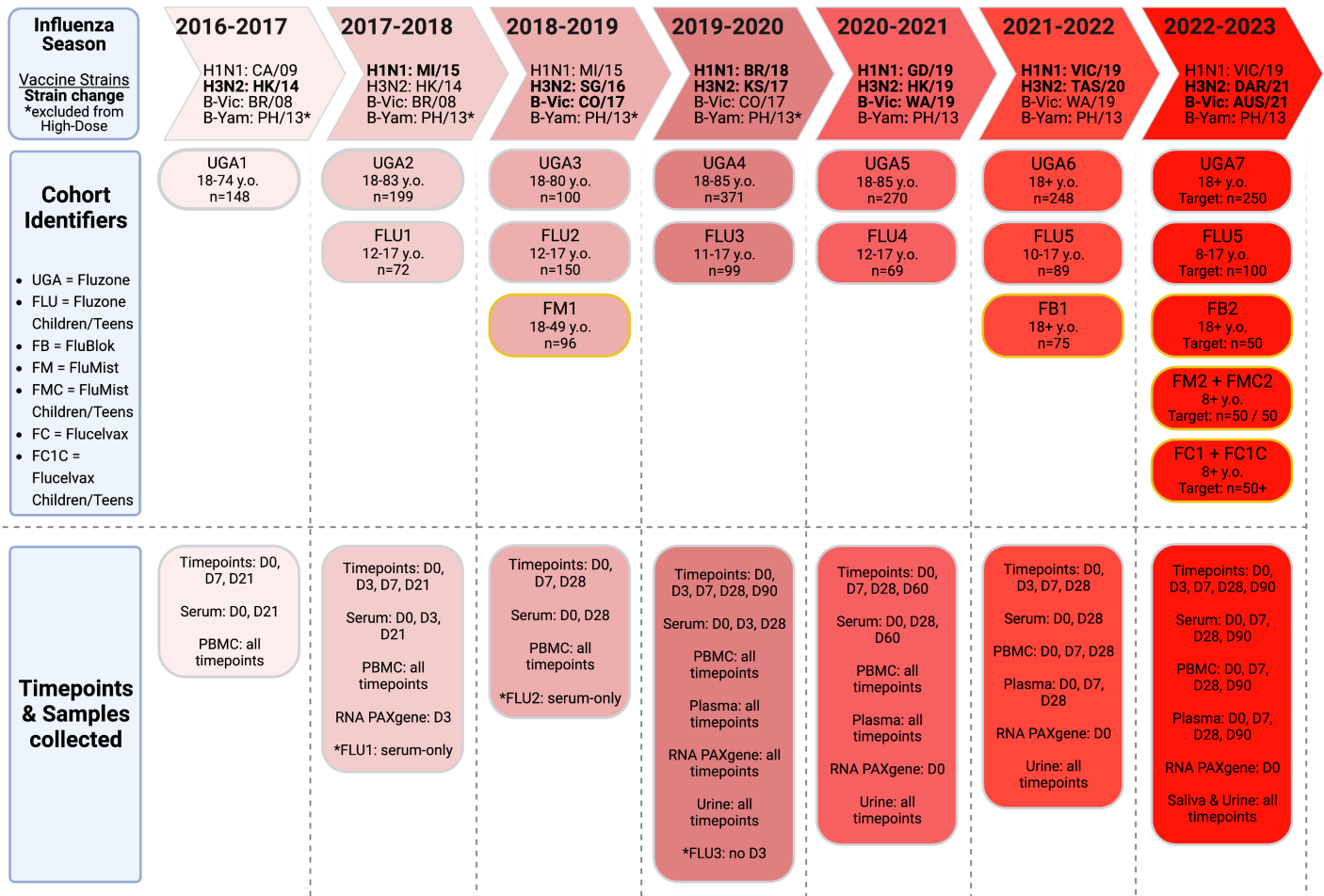|	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022	2022-2023
Influenza Season Vaccine Strains **Strain change** *excluded from High-Dose	H1N1: CA/09 **H3N2: HK/14** B-Vic: BR/08 B-Yam: PH/13*	**H1N1: MI/15** H3N2: HK/14 B-Vic: BR/08 B-Yam: PH/13*	H1N1: MI/15 **H3N2: SG/16** **B-Vic: CO/17** B-Yam: PH/13*	**H1N1: BR/18** **H3N2: KS/17** B-Vic: CO/17 B-Yam: PH/13*	**H1N1: GD/19** **H3N2: HK/19** **B-Vic: WA/19** B-Yam: PH/13*	**H1N1: VIC/19** **H3N2: TAS/20** B-Vic: WA/19 B-Yam: PH/13	H1N1: VIC/19 **H3N2: DAR/21** **B-Vic: AUS/21** B-Yam: PH/13
Cohort Identifiers • UGA = Fluzone • FLU = Fluzone Children/Teens • FB = FluBlok • FM = FluMist • FMC = FluMist Children/Teens • FC = Flucelvax • FC1C = Flucelvax Children/Teens	UGA1 18-74 y.o. n=148	UGA2 18-83 y.o. n=199 FLU1 12-17 y.o. n=72	UGA3 18-80 y.o. n=100 FLU2 12-17 y.o. n=150 FM1 18-49 y.o. n=96	UGA4 18-85 y.o. n=371 FLU3 11-17 y.o. n=99	UGA5 18-85 y.o. n=270 FLU4 12-17 y.o. n=69	UGA6 18+ y.o. n=248 FLU5 10-17 y.o. n=89 FB1 18+ y.o. n=75	UGA7 18+ y.o. Target: n=250 FLU5 8-17 y.o. Target: n=100 FB2 18+ y.o. Target: n=50 FM2 + FMC2 8+ y.o. Target: n=50 / 50 FC1 + FC1C 8+ y.o. Target: n=50+
Timepoints & Samples collected	Timepoints: D0, D7, D21 Serum: D0, D21 PBMC: all timepoints	Timepoints: D0, D3, D7, D21 Serum: D0, D3, D21 PBMC: all timepoints RNA PAXgene: D3 *FLU1: serum-only	Timepoints: D0, D7, D28 Serum: D0, D28 PBMC: all timepoints *FLU2: serum-only	Timepoints: D0, D3, D7, D28, D90 Serum: D0, D3, D28 PBMC: all timepoints Plasma: all timepoints RNA PAXgene: all timepoints Urine: all timepoints *FLU3: no D3	Timepoints: D0, D7, D28, D60 Serum: D0, D28, D60 PBMC: all timepoints Plasma: all timepoints RNA PAXgene: D0 Urine: all timepoints	Timepoints: D0, D3, D7, D28 Serum: D0, D28 PBMC: D0, D7, D28 Plasma: D0, D7, D28 RNA PAXgene: D0 Urine: all timepoints	Timepoints: D0, D3, D7, D28, D90 Serum: D0, D7, D28, D90 PBMC: D0, D7, D28, D90 Plasma: D0, D7, D28, D90 RNA PAXgene: D0 Saliva & Urine: all timepoints

Figure 3.1: Study diagram showing the subcohorts and associated time points collected during the ongoing UGA study. The diagram was created by Michael Carlock, the CIVR-HRP research director.

We will refer to the data provided by Andrea Sant and Angela Branche as the **RocFluVac** study. We have not yet finished cleaning and processing the data, but it contains similar HAI measurements for homologous strains only, participants were administered a variety of vaccines (including Fluzone HD administered to participants aged 18 - 49), and also contains data on T cell responses to the vaccine. See (44) for one brief explanation of the data.

3.2 Aim 1

For this aim, we will use the cohort data collected by Ted Ross.

3.2.1 Preliminary results

We have calculated several metrics of antigenic distance, and will compare the year-based method (23), the dominant p-epitope sequence based method (25), and a distance based on antigenic cartography (29,82). To compute the dominant p-epitope distance, the pairwise Hamming distances are computed between the amino acid sequences of each of the five hemagglutinin head epitopes, and these are divided by the length of their respective sequences. The dominant p-epitope measurement is the maximum of each of these probabilities, representing the different between the two strains at their most different epitope.
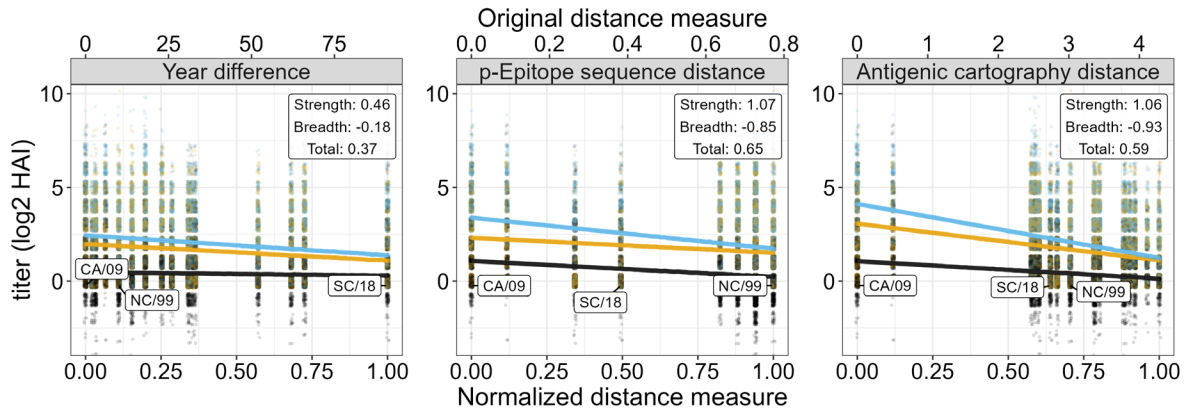
Antigenic cartography analyses were conducted by Amanda Skarlupka, PhD, who continues to work with us on this project. In short, antigenic cartography uses a matrix where each row represents an individual in the study

and each column represents an influenza strain. The cells of the matrix are populated with individual i's titer to strain j. Multidimensional scaling (MDS) is used to reduce the matrix to a specified column dimension, while minimizing the change in the Euclidean distances between measurements. After performing iterative MDS on a variety of target column dimensions, we found that two dimensions was satisfactory, and our maps were similar to those in (29). After calculating the MDS maps, we obtained antigenic distances as the Euclidean distance between map coordinates of the vaccine strain and the other strains in the panel following the method of (82). For the purposes of this analysis, we ignored longitudinal measurements between individuals, and treated each observation of an individual as a unique measurement. Finally, all of the antigenic distance were normalized *for each vaccine strain*. Therefore, each vaccine strain had a distance of 0 with itself, and the most distant historical strain had a distance of 1 with that strain. (We normalized all three of the distance measurements in this way.)

After computing the normalized distance measurements, we fit simple linear regression models with either post-vaccination titer (measuring the absolute immune response post-vaccination) or fold-change in titer (measuring the relative boost post-vaccination) against antigenic distance. Figure 3.2 shows sample linear regression models following this protocol for two vaccine strains.

Our proposed metrics for evaluating the **magnitude**, **breadth**, and **overall strength** are the intercept of the regression line, slope of the regression line, and area under the regression line respectively. The intercept measures how strong the response is to the homologous strain, the slope describes how the response weakens linearly as antigenic distance increases, and the AUC provides a framework for combining the magnitude and breadth measurements into one measurement of overall strength of the vaccine-induced response.
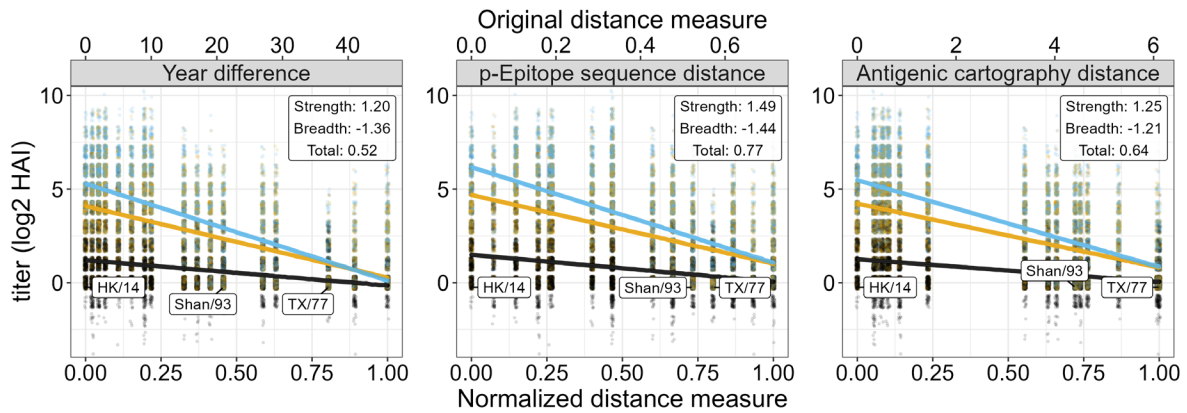
Figure 3.2: Fitted models with titer outcomes and antigenic distance as the only predictor. The metrics reported are for titer increase for simplicity.

As a case study to motivate our framework, we plan to analyze differences in these three metrics between the Fluzone SD and Fluzone HD vaccines, to determine if the high dose vaccine elicits a stronger or broader response in our cohort, as shown in Figure 3.3. Our preliminary results suggests that this effect differs qualitatively across vaccines, and we plan to analyze which vaccines show the most notable differences between strains.
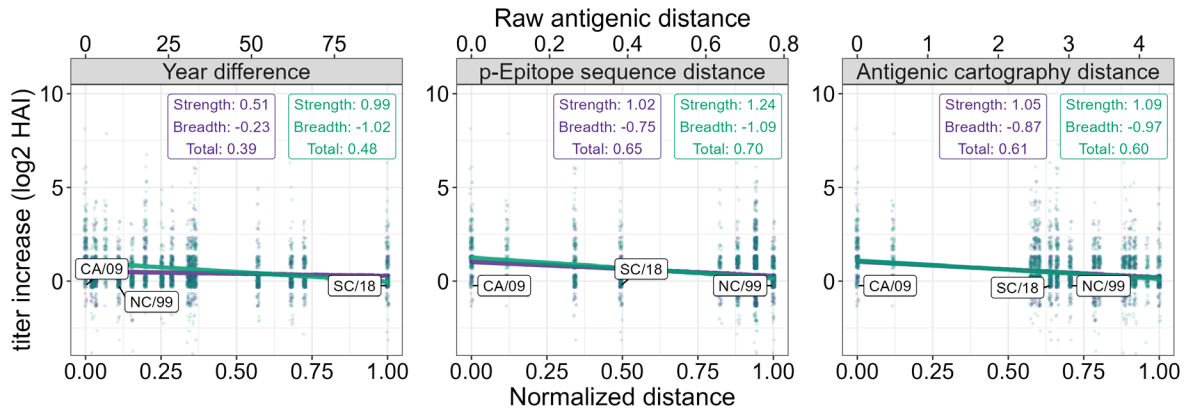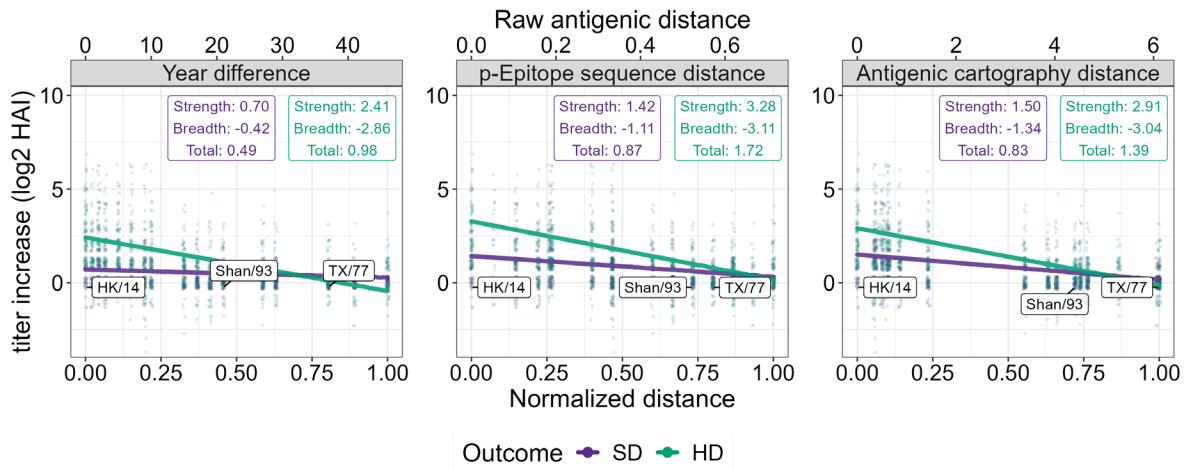
Figure 3.3: Linear models for titer increase vs. antigenic distance, stratified by vaccine dose. The same metrics are reported for both doses.

3.2.2 Proposed studies

Going forward, we plan to consider more flexible models than the simple linear regression models we used in this step of the analysis. We will consider restricted spline GAMs and LOESS to fit a potentially nonlinear effect of antigenic distance, which we would expect in the presence of original antigenic sin if our panel contains strains which are distant enough to confer no cross-protection. We will also implement Bayesian multilevel linear and spline models, in order to incorporate between-subject variability into the overall model fit.

We will then compare our metrics to traditional metrics, notably the mean titer increase, HAI composite score (83), and proportion of seroconverted strains, all calculated for each individual. In order to determine whether our proposed metrics are more robust than traditional metrics, we will subsample measurements from our cohort in order to mimic the use of different virus panels across labs. For k labs, we will subsample p strains from our panel, plus the homologous strain. For each of these panels, we will calculate the metrics for each individual, and then analyze the variability of mean metrics across labs.

Finally, we will explore weighting schema for our metrics. The unweighted AUC assigns equal weight to strains of all antigenic distances, but perhaps we would prefer to weight the response to distant strains higher or lower than similar strains–for example, if we are primarily considered with boosting the response to a specific pandemic strain, we could assign less weight to distant strains. But if we are considering candidates for a

broadly reactive vaccine, we could weight distant strains higher in order to favor vaccine candidates that induce responses to the most distant strains. Examples of weighting schema are shown in Figure 3.4.
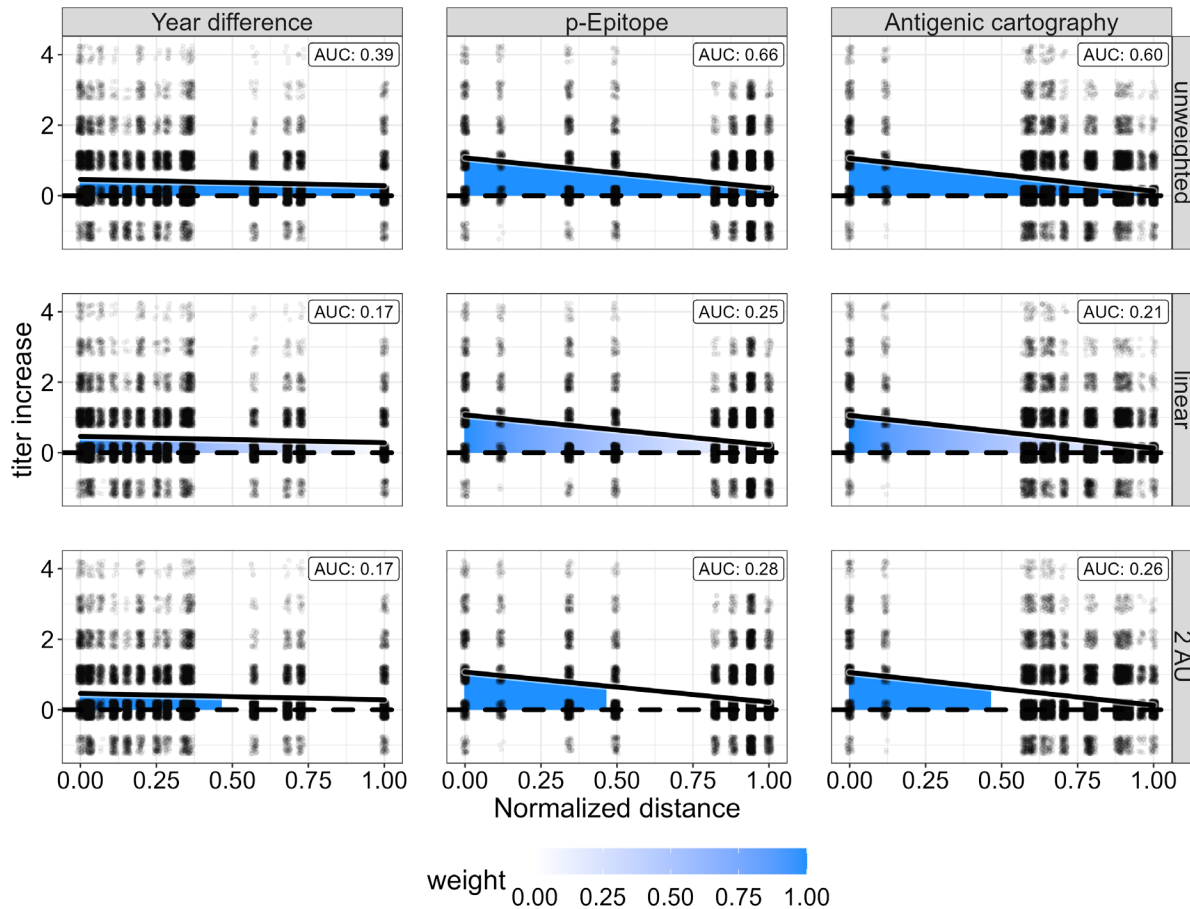

Figure 3.4: Example weighting schema for AUC measurements. This figure shows the responses to the H1N1-California-2009 vaccine in the UGAFluVac study. In the linear weighting scheme, the titer increase is multipled by the reciprocal antigenic distance in order to construct weights that decrease linearly as strains become more distance. In the 2 antigenic unit scheme, all strains which are within two antigenic units on the cartography map (or equivalent with the other distances) are weighted equally, while strains that are further away are not included in calculating the AUC.

3.2.3 Expected outcomes

Our primary outcome for this goal will be a set of metrics that we propose for the evaluation of broadly reactive vaccine candidates. These metrics will be calculated on the UGAFluVac data and will be supported by our subsampling analysis to estimate the robustness of our metrics. Developing these metrics will improve our understanding of the functional relationship between antigenic distance and immune response. Finally, our robustness analysis will provide insight into the amount of error induced into metrics for post-vaccination immune response by variability within virus panels used across different research groups.

3.3 Aim 2

For this aim, we will use the UGAFluVac data, specifically the portion collected at the University of Georgia. The PA/FL data does not have prior vaccination history and thus we can only use the UGA data for this aim.

3.3.1 Preliminary results

We first explored the homologous case in order to identify simple first-order effects of each of the covariates of interest. Figure 3.5 shows plots of fold change in titer (titer increase) plotted against available covariates. The only covariate which independently explained a substantial proportion of the variance in the outcome was prevaccination titer. Note that these results do not consider interaction effects (or subgroup specific effects that can be modeled as interactions): for example, we would not expect to see an effect of dose in the entire population since otherwise healthy adults under the age of 65 were not offered high dose vaccine. The R^2 metrics also only capture linear trend–if a predictor acts through a primarily nonlinear (especially true for nonmonotonic effects) the R^2 will drastically underestimate the strength of association between the two variables.
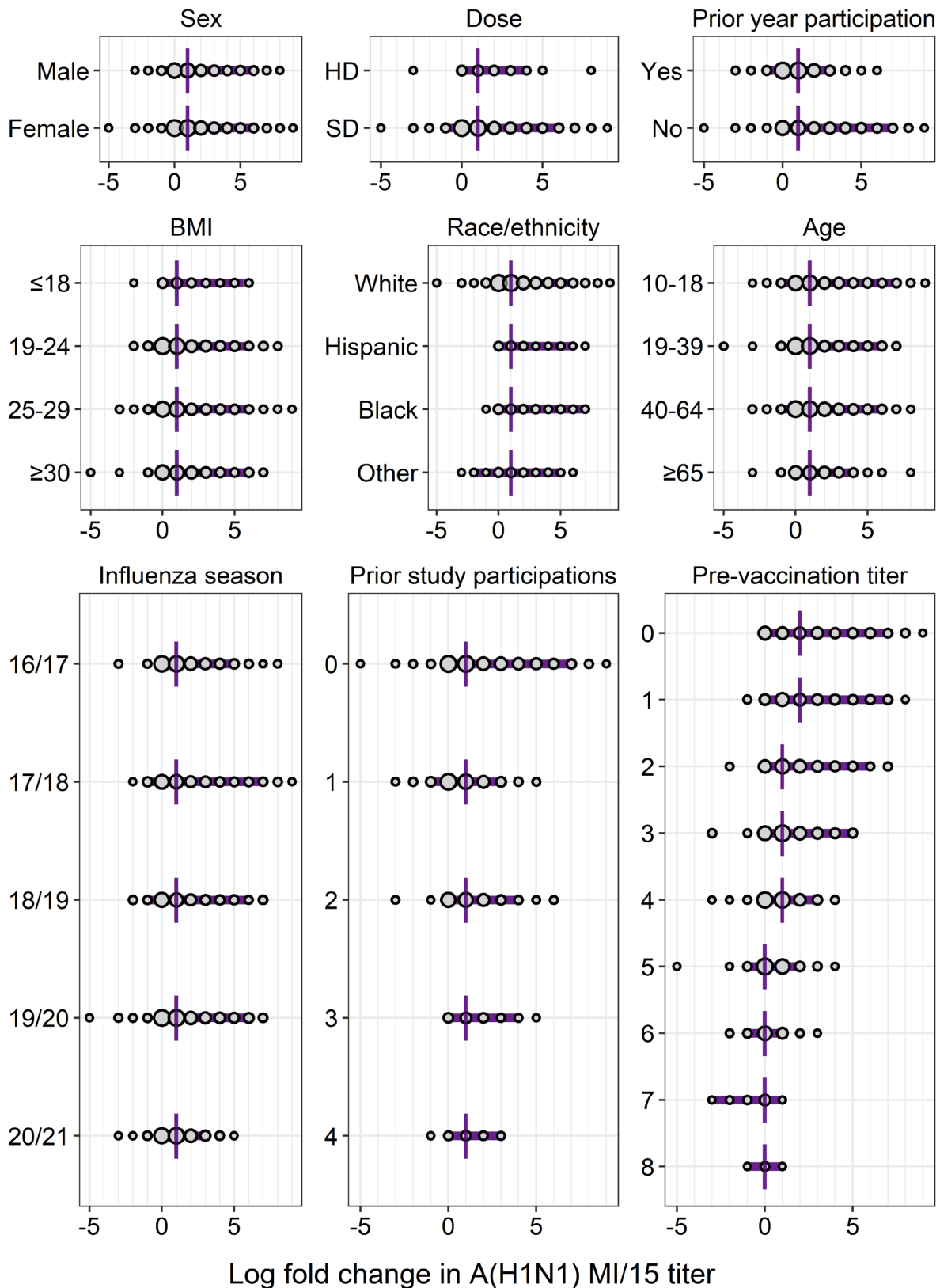
Figure 3.5: Homologous titer increase to the influenza A(H1N1) Michigan-2015 strain in the UGAFluVac data, plotted against a variety of covariates. The size of the point corresponds to the number of overlapping measurements at that location. The purple vertical lines indicate medians, and the horizontal purple lines mark the 2.5th and 97.5th quantiles. While many of the univariate associations with titer increase are weak or difficult to see, many of these effects become much stronger when interactions are taken into effect. These

trends also vary widely across other strains which are not shown.

From our exploratory analyses, we also know that the effect of prevaccination titer on the homologous immune response varies across vaccine strain and dose (Figure 3.6). We fit stratified linear models with both parallel slopes (A and C) and varying slopes (B and D) with these two factors. We further expect to see a third-order interaction between vaccine strain and dose. Note that these preliminary figures show all individuals, and the effect of dose will be further modified by age, since high dose vaccine was only offered to elderly participants.
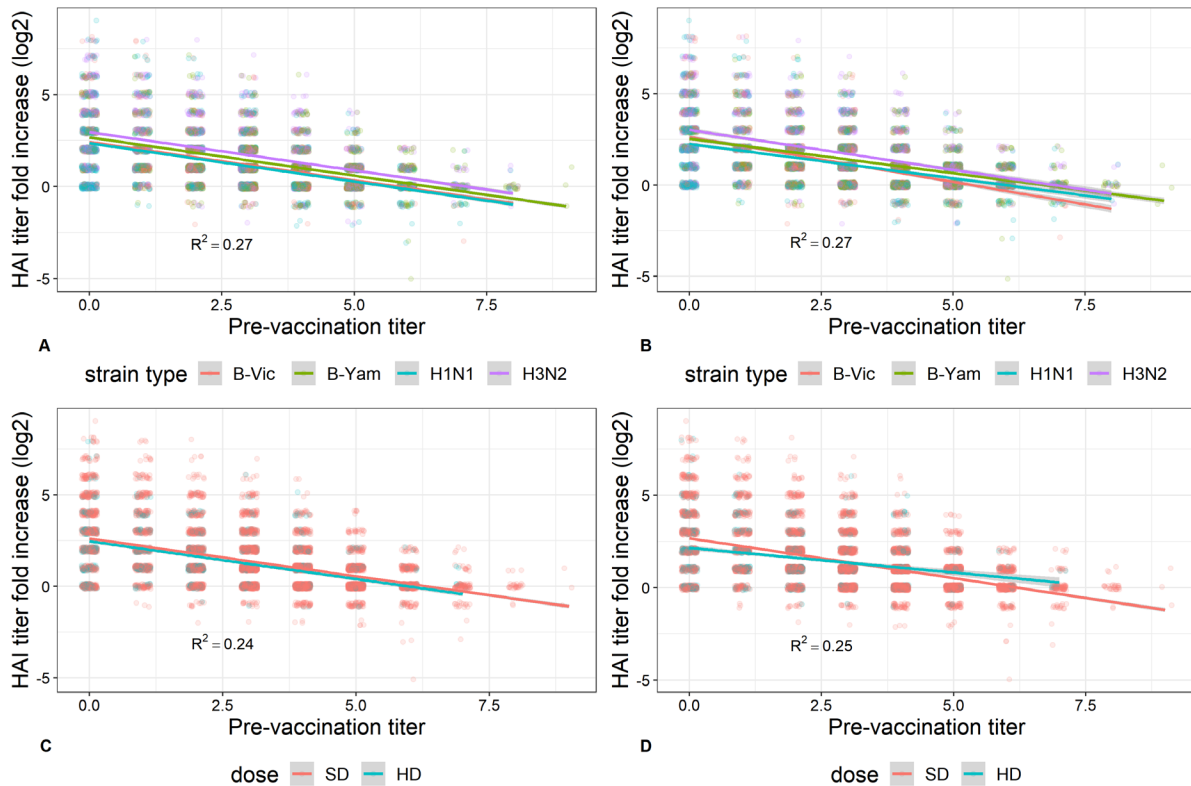
Figure 3.6: Titer increase plotted against pre-vaccination titer. Models are stratified by vaccine strain type (A and B) or by vaccine dose (C and D). Figures A and C show a parallel slopes model where only the intercepts are allowed to vary (corresponding to the model with no interaction term, only main effects for both variables), while Figures B and D show the varying slopes (interaction and main effects) models. The varying slopes models improve R-squared marginally, but there are several limitations to these analyses that may hide larger differences.

We further conducted an analysis at the strain-specific level, fitting separate models for each combination of vaccine strain and assay strain represented in the data (Figure 3.7).
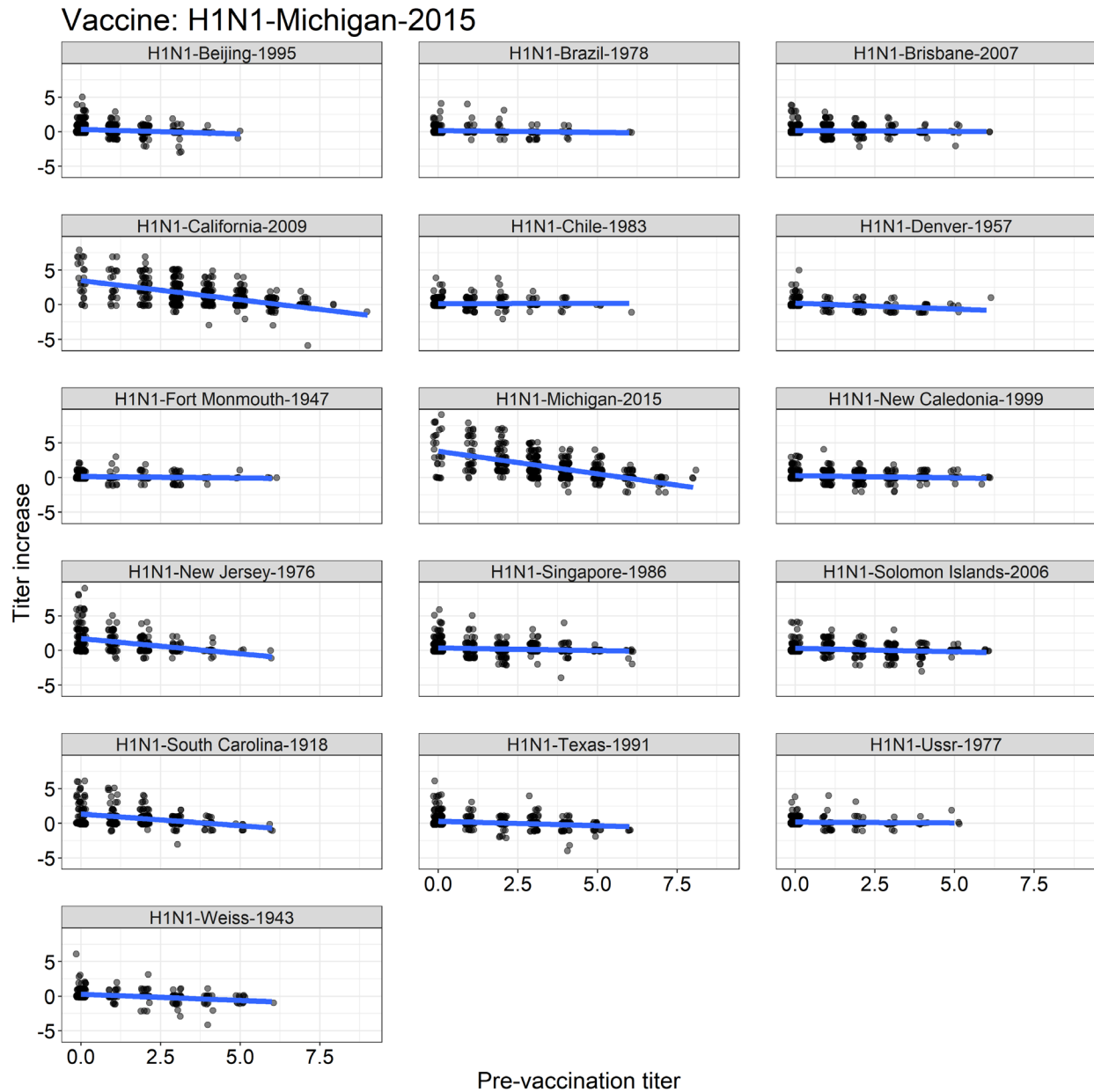
Figure 3.7: Relationship between titer increase and prevaccination titer for each of the assay strains which were used as part of a historical panel. Only assays for individuals who received H1N1-Michigan-2015 containing vaccine are shown for simplicity. We can see that the intercept and slope have the largest magnitude for the homologous strain and for the previous vaccine strain (H1N1-California-2009), and decreases with other assay strains.

We can further stratify these strain specific analyses by other factors like dose (Figure 3.8). These analyses reveal a non-constant modification of the effect of prevaccination titer by dose, which differs across vaccines and across assay strains within vaccines.

Figure 3.8: The same analyses as shown in Figure 3.7, but stratified by vaccine dose. Additionally, only individuals aged 65 and older are included in these models, since younger individuals were not eligible to receive high dose vaccines. Some relationships are distorted by small sample effects, but in general the dose appeared to make little difference for this particular vaccine. However, we see varying, potentially real effects in the MI/15 and CA/09 effects. The effect of dose also varies across vaccine strains (data not shown).

Differences in strain-specific models for the same vaccine demonstrate the interaction between antigenic distance, prevaccination titer, and other stratifying factors (like dose and prior vaccination history). We conducted a preliminary analysis of the strength of this interaction by compiling the slopes of each model (as shown in Figure 3.7, but incorporating data from all vaccine strains that were used in the study), and plotting these slopes against the antigenic distance between the vaccine strain and the assay strain for that model, shown in Figure 3.9. Note that in this framework, vaccines were only compared against assays of the same type. HAI assays for influenza B strains which predated the divergence of the Victoria and Yamagata lineages were compared against both B-Yamagata and B-Victoria vaccines.

For the type A influenza assays, we see a strong positive correlation between antigenic distance and slope of the strain-specific model. As the vaccine strain and the assay strain become more distant, pre-vaccination titer has less of an effect on the amount of boosting produced by the vaccine.
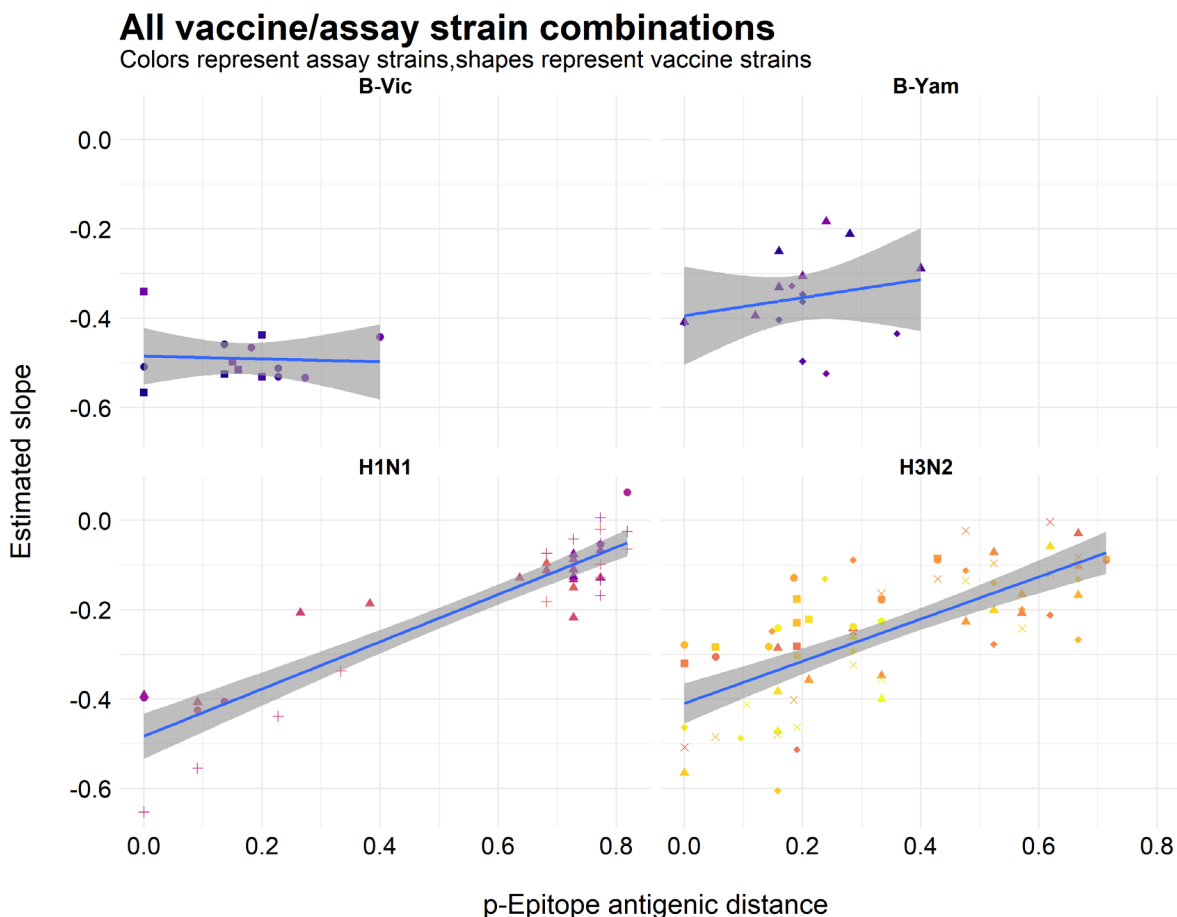

Figure 3.9: Slopes from each of the individual regression analyses (similar to those depicted in Figure 3.7) plotted against the dominant p-Epitope antigenic distance between the vaccine strain and the assay strain. We performed these individual regression analyses for every combination of vaccine strain and assay strain in the UGAFluVac data. Point colors in this plot represent different assay strains, while point shapes represent different vaccine strains.

We also used machine learning models to predict response to the homologous vaccine strain based on all of the covariate data that was available to us. We used permutation variable importance to score the importance of the included predictors (Figure 3.10). We found that prevaccination titer was, by far, the most important covariate in predicting vaccine boosting. However, we also observed differences between influenza seasons, individuals of different ages, and the vaccine strain type. Future models will include antigenic distance measurements instead of indicators for strain types.
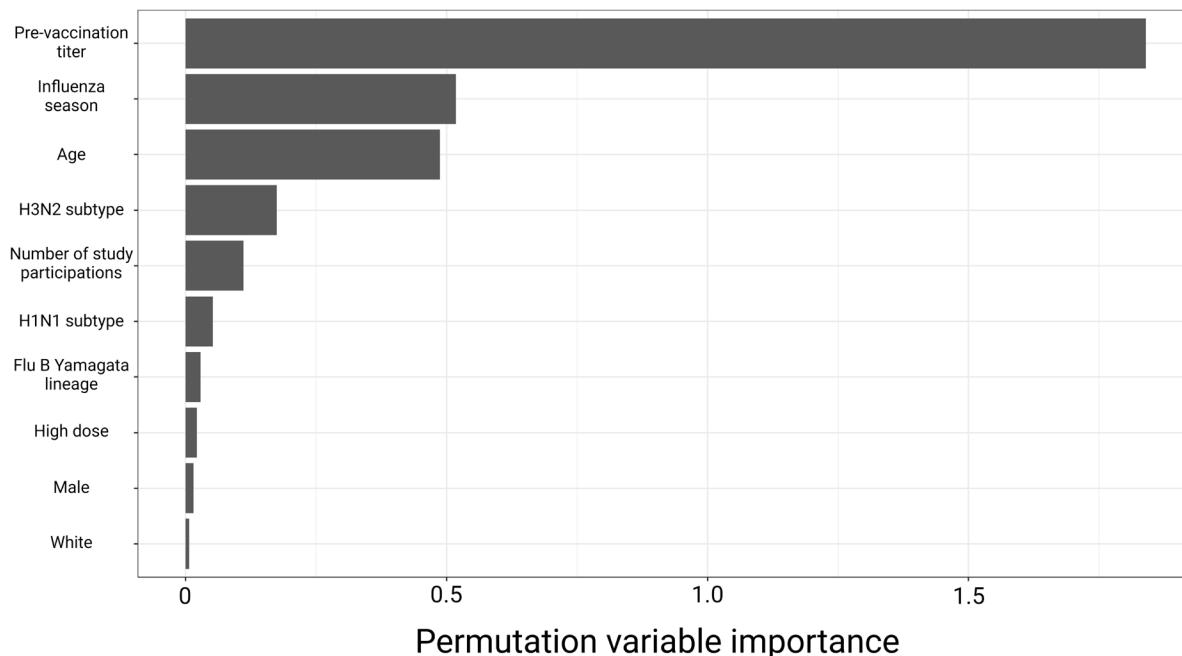
Figure 3.10: Permutation variable importance (VI) for each feature included in the final tuned random forest model. Permutation VI is calculated by randomly permuting the variable of interest (which should destroy any signal), and subtracting the estimated RMSE with the permuted variable from the estimated RMSE when the variable is not permuted. The model was trained using a subset of 70% of the UGAFluVac data, and these RMSE for both the permutated and non-permuted data were calculated using the holdout set of the remaining 30% of data. Despite using a random forest model, which can detect interactions and nonlinear effects, any effects that interact with age or dose may be artificially dampened in this analysis due to class imbalance.

3.3.2 Proposed studies

First, we will expand and refine our preliminary machine learning approach to include all predictors of interest. We will apply similar models to quantify the predictive power of each variable using permutation importance. For the final models, we will perform more in-depth feature engineering to ensure that predictors have optimal predictive power while retaining biological meaning. These models can learn nonlinear and interaction effects without prior specification and thus will allow us to understand how well all we can expect inferential models to capture trends in the outcome. We will also fit models using both the post-vaccination titer and the titer increase as outcomes, to determine if the drivers of the raw immune response are less influenced by pre-vaccination titer. We will also fit separate models for elderly participants in order to gain a better understanding of the effect of vaccine dose in this subgroup.

After we understand how much of the post-vaccination titer can be explained by the data we have, we will build Bayesian hierarchical models to take advantage of the clustered structure of the data. These hierarchical models will be allow us to make inferences about the relationships between variables, rather than only quantify the predictive power. Furthermore, we can implement random effects in these models to partially absorb unmeasured confounding, which is not possible within a predictive machine learning framework. However, we can compare the overall predictive power of our inferential models to the best predictive models.

Finally, we will modify previously developed mechanistic models (73,74) which model steric hindrance between multiple epitopes of the same antigen. The model for steric hindrance is mathematically similar to how we would model differences in binding avidity between antigenically distance strains, so by incorporating a notion of antigenic similarity, we can use these models to predict how the relationship between dose, prior

immunity, and vaccine response varies with antigenic distance. We will compare the model predictions with the predictions made by our machine learning and inferential models. A notable limitation of the mechanistic models in this case is that we will not be able to make predictions about the effect of serial repeated vaccinations as we hope to do for the hierarchical models.

3.3.3 Expected outcomes

Our work on this aim will produce fitted inferential models, with estimates of the strength of the effect for pre-vaccination titer, prior vaccination history, and other drivers of the immune response. Our models will also provide estimates for the strength of interactions between these covariates. We will also produce a set of tuned predictive models. Estimates of the vaccine response for each individual will be computed from the inferential and the predictive models, and by comparing the two sets of predictions, we will estimate the performance of our inferential models. Finally, we will update the mechanistic models and compare patterns in mechanistic model results to the patterns observed in our data.

3.4 Aim 3

For this aim, we will combine the UGAFluVac data, RocFluVac data, and any applicable data provided by Ben Cowling.

3.4.1 Preliminary results

While working with the UGAFluVac data, we have constructed a small DAG exploring only the necessary adjustment variables that affect the causal pathway between dose and post-vaccination titer (Figure 3.11). In this reduced framework, the total and direct causal effects of dose would be the same, and the only factor we would need to adjust for to obtain the true causal estimate of dose is age. Although age may be a common cause of several other factors that affect post-vaccination titer, vaccine dose is a quality of the intervention (vaccination) and thus the only factors that affect vaccine dose are factors that determine participation in the study.
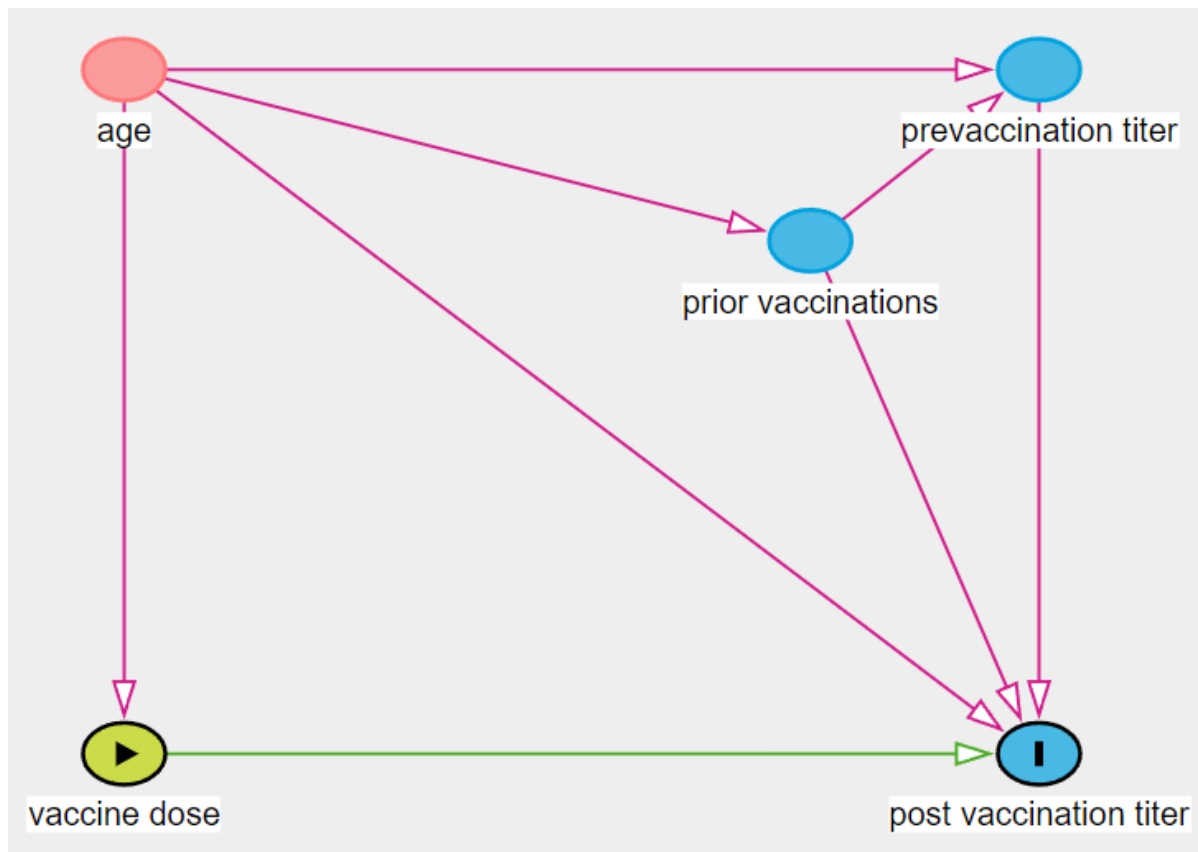
Figure 3.11: A directed acyclic graph (DAG) showing a graphical causal model for the effect of dose (the exposure, in green) on post-vaccination titer (the outcome, in blue with an I symbol). Age is a confounder and is colored pink in the DAG.

We have also conducted preliminary comparisons of the UGAFluVac and RocFluVac data, primarily to compare the patterns in antibody responses observed in the UGAFluVac data with the T cell responses observed in the RocFluVac data (Figure 3.12). We also have HAI data for the RocFluVac data, and thus we can compare the effect of dose on the immune response in the 18 - 49 year olds in the RocFluVac study with the effect in the 65+ year olds in the UGAFluVac study.
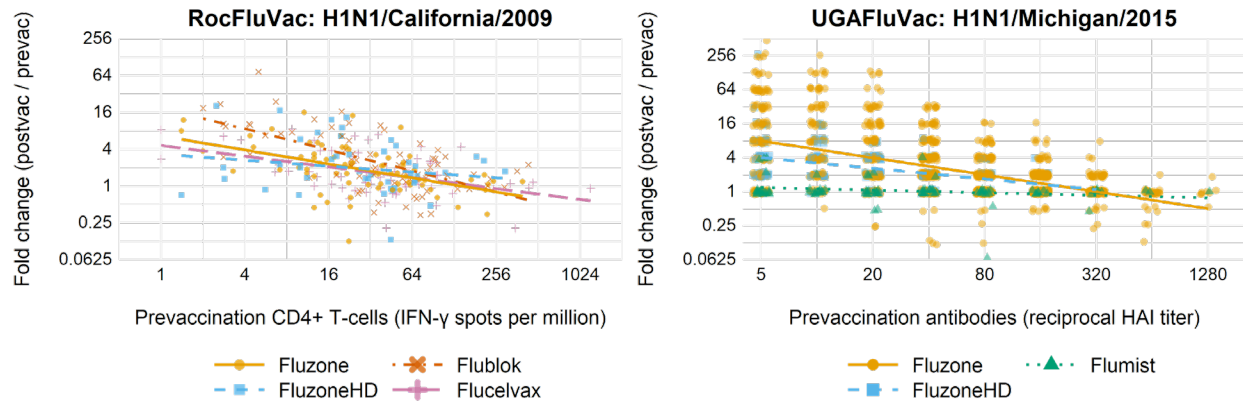

Figure 3.12: Antibody immune response measured via HAI in the UGAFluVac cohort compared to T cell immune response measured via Elispot in the RocFluVac cohort.

3.4.2 Proposed studies

First, we will ensure that our theoretical causal model is accurate, and we will update our model to include other common causes which are observed in both data sets. Our causal model will be formulated as a DAG, which we will use to generate a minimal sufficient adjustment set for the causal effect of vaccine dose on post-vaccination titer and on titer increase. We will analyze the conditional independencies of the DAG by comparing the observed standardized covariance matrix to the covariance matrix predicted by the DAG in order to determine how consistent our data are with the identified DAG.

We will apply traditional regression methods using either cluster-robust standard error estimation or hierarchical modeling to obtain a causal estimate of the effect of dose, using a minimally sufficient adjustment set identified by our DAG. We will use quantitative bias analysis methodology to estimate the effect of unmeasured confounding on our estimate. Finally, we will obtain the same estimate using a targeted maximum likelihood estimation (TMLE) framework, which is doubly robust to model misspecification, and we can compare the TMLE estimate to the regression estimate.

We will explore the interaction of age and dose by estimating the causal effect of dose in both the UGAFluVac and RocFluVac studies separately and comparing these estimates to the combined study estimate. We note that differences in these estimates could be due to sampling variation, systematic variation between the two source populations, or due to the interaction effect with age. We can furthermore estimate interaction effects in all three situations.

3.4.3 Expected outcomes

The outcomes of this aim will be estimates of the effect of vaccine dose on post-vaccination titer, controlling for age. We will estimate the amount of confounding bias in our causal effects, and compare the estimates

from the two individual studies to the overall estimate. The individual study and confounding analyses will allow us to understand the limitations of our causal estimates.

3.5 Timeline

Figure 3.13 shows the expected timeline for our project. The expected completion date for our project is Friday, March 14, 2025, and a dissertation draft will be sent to the committee no later than the following Monday. My dissertation defense will be planned for early April to accommodate Graduate School deadlines.

Aim	Objectives	2023 Summer	2023 Fall	2024 Spring	2024 Summer	2024 Fall	2025 Spring
*	Obtain and prepare all data sources	▓					
1	Regression analyses and modeling extensions	▓	▓	▓			
1	Robustness and subsampling analysis		▓	▓			
2	Machine learning modeling		▓	▓			
2	Hierarchical inferential modeling		▓	▓	▓		
2	Mechanistic modeling			▓	▓	▓	
3	Causal modeling and theoretical framework				▓	▓	
3	Formal statistical analysis					▓	▓
*	Final dissertation writing					▓	▓

Figure 3.13: Proposed project timeline. We expect the majority of research to be conducted during the 2023-2024 academic year, extending into 2025 as necessary. All research will be complete by March 1, 2025 in order to finalize results for the written dissertation.

Acknowledgements

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