HAMdetector: Combining information to detect HLA-associated mutations with a Bayesian regression model

Daniel Habermann[1], ..., Daniel Hoffmann[1]

Abstract

Motivation
The human leukocyte antigen system (HLA) is of paramount importance to combat viral infections by presenting peptides on the cell surface via MHC I. Thus, CD8+ cytotoxic T-Lymphocytes exert a strong selection pressure towards virus variants that escape that immune recognition pathway, e.g. through point mutations that decreases binding of the respective peptide to MHC I.

Reliably identifying HLA-associated mutations is important for understanding viral evolution, but experimental methods like binding assays are prohibitively expensive for large-scale use and fail to recognize other mechanisms of immune escape like proteasomal processing.
One step in finding these mutations is through the statistical analysis of sequence data. However, existing methods are based on null hypothesis significance testing and do not make use of all the available information and therefore have unsatisfactory real-world performance.

Results
Here, we present a Bayesian regression model that is easily extensible to include information from different sources (e.g. epitope prediction software) and makes use of recent advances in Bayesian inference, e.g. by using a sparsifying prior. We show that including this kind of information improves predictive performance considerably over state-of-the-art methods.

Availability and Implementation
The source code of this software is available at `http://github.com` under a permissive MIT license.

Supplementary information
Supplementary data are available at *Bioinformatics* online.

Keywords
human leukocyte antigen system, HLA, multiple sequence alignment, escape mutations, viral escape, Bayesian inference, sparsity, horseshoe, epitope prediction

[1] *Bioinformatics & Computational Biophysics, Faculty of Biology, University of Duisburg-Essen, 45117 Essen, Germany*

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1. Introduction

1.1 The HLA system

One way how the human immune system is able to recognize intracellular viral infections is through the human leukocyte antigen system[1]: In cells with active protein biosynthesis, proteins are continuously synthesized and also degraded by a process called proteasomal degradation, which cleaves proteins into linear peptides of varying length[2].

A small subset of these peptides is presented on the cell surface via receptors called MHC class I (HLA-A, HLA-B and HLA-C in humans). The genomic region encoding for MHC I is known to be highly polymorphic, with more than 20000 different HLA alleles described today[3]. The resulting gene products differ in their binding properties, which means that cells from different individuals present a highly diverse set of peptides on their surface.

Cytotoxic T cells are selected during maturation to only weakly bind to peptide/MHC I complexes when the peptide originated from proteins of the usual proteome, but might be able to strongly bind to complexes of MHC I with peptides which are generated from of a viral protein[4]. Upon activation, T cells induce cytolytic activity and recruit other immune cells [5].

1.2 HLA escape

In this way, the HLA system exerts strong selection pressure towards virus variants that escape T cell recognition[6], for example through a point mutation that results in reduced binding of an immunogenic peptide to MHC I or through a set of mutations that alters the viral protein in such a way that it is cleaved into different peptides that are not recognized by the host's T cell repertoire[7].

The evolutionary events are complex and occur not only on the level of individuals, where a virus adapts to specific features of the host, but also on the population level, because HLA alleles differ in their frequency across geographic regions[8]. Upon transmission to a new host, HLA escape mutations can revert to their wild type, as HLA escape mutations are associated with a reduction in viral replicative capacity[9]. Kawashima et al. [8] describe an escape mutation that is selected by HLA allele HLA-B*51, does not strongly affect viral replicative capacity, and therefore slowly enriches over time in Japan, where HLA-B*51 commonly occurs.

How quickly a given escape mutation is selected upon transmission in a host depends on the magnitude of the reduction in viral replicative capacity, on the strength of selection pressure, and also on the genetic background, e.g. some escape mutations require compensatory mutations which partly attenuate the negative impact on viral replicative capacity.

Studying HLA escape provides a unique opportunity to gain insight into viral evolution, on the host level, but also on the population level. Unfortunately, identifying HLA escape mutations is difficult in practice.

1.3 Identifying HLA-escape mutations

There are several experimental methods available to study HLA escape: Recombinant MHC-I molecules can be used in binding assays. Upon complex formation with a peptide, a change in conformation can be detected with conformation-specific antibodies. This method is relatively fast, but only measures binding affinity of a peptide to MHC-I and does not account for antigen processing or immunodominance, which describes the observation that a peptide may be presented via MHC-I on the cell surface, but does not induce an immune response.

An experimental setup that resembles the conditions in-vitro more closely but is also more time-consuming is to measure CD8+ T cell responses instead. This is usually done by stimulating peripheral blood mononuclear cells with prototype and variant peptides and measuring the secretion of IFN-γ by intracellular cytokine staining and fluorescence-assisted cell sorting.

To analyze CD8+ T cell responses against endogenously processed antigens, it is necessary to generate cell-lines stably expressing the antigen in question and adding antigen-specific CD8+ T cells. This method scales poorly as it requires transfection of cell lines and antigen-specific expansion of CD8+ T cells.

1.4 Computational methods

Because the currently available experimental methods do not scale well enough to analyze whole viral genomes, a useful strategy might be to use annotated sequence data to identify candidate HLA escape mutations that can then be verified experimentally.

As the selection pressure exerted by cytotoxic T cells depends on successful recognition of viral peptides on

the cell surface, escape mutations are often HLA-allele specific and can therefore be detected as HLA-allele dependent footprints in sequence alignments of viral proteins[10]: At certain alignment positions, a replacement might be more frequently observed in sequences from hosts with a specific HLA allele than in sequences from hosts without that HLA allele. By quantifying this difference for all replacement and HLA allele pairs, it is possible to identify replacements that are enriched in sequences coming from hosts with certain HLA alleles, and thus are likely to be HLA escape mutations.

One way of quantifying this enrichment is Fisher's exact test[11]: For a given replacement R_i at alignment position i and HLA allele H, a 2-by-2 contingency table is constructed containing the absolute counts of the number of sequences in the four possible categories (R_i, H), (R_i, $!H$), ($!R_i$, H) and ($!R_i$, H), where $!R_i$ denotes any replacement except R_i, and $!H$ denotes any HLA allele except H.

Fisher's exact test is a conventional null hypothesis significance test (NHST) that generates p-values. In this case, the null hypothesis is that HLA allele H and replacement R_i are independent, and the p-value is the probability of observing a deviation from independence that is at least as extreme as in the data at hand under the assumption that the null hypothesis is true.

Fisher's exact test has the advantage of being easy to apply[12], but also has several disadvantages that are outlined in Carlson et al.[13]. The most striking one is that viral sequences share a common phylogenetic history, and, therefore, treating sequences as independent and identically distributed samples may under- or overestimate effect sizes. In the context of hypothesis testing, this leads to increased false positive and false negative rates.

Another issue of applying Fisher's exact test is that HLA class I loci are located in proximity on chromosome 6 and are therefore in linkage disequilibrium, which means that HLA alleles are not inherited completely independent of each other, i.e. inheritance of one HLA allele correlates with inheritance of another HLA allele. When using a statistical method that tests each HLA allele individually without considering the whole set of alleles present, spurious associations might occur: If HLA allele H_1 is associated with an amino acid replacement R, but H_1 is in linkage disequilibrium with another allele H_2, this also means that we observe an association between replacement R and H_2, even in absence of any underlying escape mechanism.

Correlations can not only occur between HLA alleles, but also between replacements. This kind of codon covariation occurs for example in compensatory mutations that attenuate the negative impact of immune escape mutations. For instance, a compensatory mutation might lead to a conformational change in such a way that the mutated protein resembles the original wild type more closely.

Carlson et al. [13] developed a method called Phylogenetic Dependency Network that accounts for phylogenetic bias, HLA linkage disequilibrium, and codon covariation and is based on null hypothesis significance testing.

1.5 Issues with using p-values as a screening tool

In addition to the aforementioned biological reasons why Fisher's exact test is not suited for the analysis of sequence data, there are also more fundamental statistical issues that universally occur when using p-values as a screening tool[14]:

In the presence of small effect sizes and high variance between measurements, as it is typically the case when working with biological data, statistically significant results can often be misleading and are likely to be in the wrong direction (a so-called type S error) or greatly overestimate an effect (a so-called type M error)[15]. This problem has recently been widely appreciated in the literature in the context of the current „replication crisis", which describes the circumstance that scientific claims with seemingly strong statistical evidence fail to replicate[16].

Another issue that occurs when using p-values as a screening tool is the problem of multiple comparisons. When applying a statistical test, the probability of obtaining a statistically significant result increases with each additional test, even in absence of any real effect. When using p-values as a filter, it is therefore likely to obtain significant effects that are in fact not real. To circumvent this problem, a common strategy is to control the false discovery rate, which is the expected proportion of false positives[17]. These adjustment procedures have the problem that, when performing many of such comparisons, none but the very largest effects remain.

Instead of performing many hypothesis tests and trying to adjust for them, we instead prefer to fit a single, multilevel model that contains all comparisons of interest. When using multilevel models, the problem of multiple comparisons can disappear entirely and also yield more valid estimates[18].

2. Material and methods

When possible, we choose to fit Bayesian models. By using prior information, adding problem-specific structure and partial pooling, the accuracy of estimates can often be noticeably improved[19]. Prior information does not necessarily mean to use external data, even a rough idea about the expected magnitude of estimates is often surprisingly effective.

Additionally, Bayesian statistics provides an accessible way to test models: By comparing data generated under the model's assumptions to the actually observed data, it is possible to identify important aspects of the dataset that the models fails to capture and subsequently improve the model until it is consistent with the observed data[20].

In the context of identifying HLA-associated mutations we propose to improve existing methods by the following additions, which can be broadly divided into additional information and model structure:

Additional information

Binding affinity prediction

HLA-associated mutations are expected to lie more frequently in regions of known epitopes. There are vast experimental binding affinity data available of different peptides and MHC I molecule pairs, and there are well-established computational methods that use these data to extrapolate HLA binding for untested peptides. We show that, by including the outcome of these computational tools as input for a probabilistic model, the prediction of HLA-associated mutations can be improved.

Antigen processing prediction

Similarly, there are also HLA-allele independent effects like antigen processing that influence presentation on MHC I. Second generational tools like MHCFlurry 2.0 use both binding affinity and antigen processing data to improve epitope prediction. For our tool HAMdetector, we use the output of MHCFlurry to benefit from this binding affinity and antigen processing data in order to predict HLA-associated mutations.

Model structure

Sparsity-inducing priors

Recent advantages in Bayesian inference include so-called sparsity-promoting priors. Sparsity-promoting priors convey the a priori expectation that most coefficients in a regression model are close to 0. This assumption also applies to HLA-associated mutations, because the number of epitopes that are restricted by a given HLA allele is typically small compared to the number of all possible epitopes. If no epitope spanning a given alignment position is presented on the MHC I molecule, any association between a replacement and the respective HLA allele is likely to be due to random variation alone (or HLA linkage disequilibrium).

It has been shown that using a sparsity-promoting prior when non-zero coefficients are sparse can drastically improve predictive performance, because the model is better able to differentiate between signal and noise.

We show that a simple logistic regression model with a sparsity-promoting prior on the regression coefficients for the HLA alleles alone already performs roughly on-par with the much more elaborate Phylogenetic Dependency Network approach from Carlson et al. when using the fraction of identified escapes that lie in known HLA epitopes as a benchmark.

Partial pooling of 4-digit HLA alleles

It has recently been appreciated that binding specificities can vary drastically across HLA alleles from the same allele group, e.g. between HLA-B*51:01 and HLA-B*51:03. For predicting HLA associated mutations, there has consequently been a shift to use 4-digit resolution data whenever available. This is not without downsides however, because overall, binding specificities are more similar for alleles in the same group, and, therefore, treating them as completely separate might unnecessarily fragment the available data.

In Bayesian statistics, it is not required to chose one extreme or the other: Instead of either treating all HLA alleles from the same allele group as identical or as completely separate, partial pooling of the estimates allows something in between: By partial pooling, estimates do

share some information across each other, but they are also allowed to vary if necessary.

In the context of our model this means that observing a relevant association between a replacement and an HLA allele also makes the model more inclined to estimate relevant associations between that replacement and all other alleles from that allele group. The degree of this partial pooling can be estimated from the data, so if we do observe strong similarity of HLA alleles in a given allele group the estimates are more influenced by each other than if they do not.

3. Implementation

Model backbone

We chose a logistic regression model as a backbone for two reasons: First, because it is easily extensible and secondly, because the coefficients are interpretable as expected increases on the log-odds scale, identical to the familiar interpretation of standard logistic regression models. We also chose to fit the model in a Bayesian fashion using Stan, mostly because it allows us to make use of the hierarchical structure in the data and the possibility to use posterior predictive checks as a way of model checking. Consider the following notation:

$$y_{ij} \sim \text{bernoulli}(\theta_{ij}) \tag{1}$$
$$\theta_{ij} = \text{inv\_logit}(\beta_{0_j} + X_i \beta_{\text{HLA}_j}) \tag{2}$$

,

where y_{ij} is the binary encoded observation at sequence i for replacement j, θ_{ij} is the estimated probability that we observe replacement j in sequence i, X_i is the binary encoded (row) vector of HLA annotations for sequence i, and β_{HLA_j} is the (column) vector of HLA regression coefficients for replacement j and inv_logit is the logistic link function. Note that, if we observe N different amino acid states at a certain alignment position, each of those are considered in turn to be the "replacement".

The regression coefficients β_{HLA_j} are the main focus of interest, as they quantify the association between the occurrence of replacement j and each of the observed HLA alleles. They are in units of log-odds, so a regression coefficient of somewhere around 2 means that when comparing sequences with and without that particular HLA allele, the log-odds of observing replacement j in presence of that HLA allele is increased by 2.

Reasoning on the log-odds can sometimes be unintuitive. A useful approximation to interpret logistic regression coefficients on the probability scale is the so-called divide-by-4 rule, which means that a regression coefficient of 2 corresponds to an expected increase on the probability scale of about 2/4 = 50%.

Including phylogeny

- sequence data shares common phylogenetic history and are therefore not i.i.d, cite Brumme paper
- standard approach: multivariate normal intercept where covariance matrix is based on shared branch lengths
- this approach is computationally too expensive because of current limitations in Stan. Would be possible by better inferencealgorithms, see this recently published paper: https://proceedings.neurips.cc/paper/2020/file/673de96b04fa3adcae1aacda704217ef-Paper.pdf but probably omit that?
- Our approach is close to the one in Carlson et al.
- slight tweak: use phylogeny estimation tools for tree likelihoods p(tree | sequences), then bayes rule to invert that conditional probability. Optimize branch lengths for each replacement, but keep tree topology fixed
- advantages: faster (raxml well optimized)
- disadvantages: ad-hoc, close to conventional multivariate normal approach with off-diagonal elements set to 0, therefore not as flexible. But: should be good enough, phylogeny seems to be not that important (probably omit that?)
- how to include p(replacement | tree) in the model: just add additional intercept
- add coefficient for that information, so model can decide how to make use of that information.
- logit(p(replacement|tree)) used because it cancels out with the logistic link function. therefore: phylogeny information as baseline in absence of any HLA effects

- calibration plots to show that estimated phylogenetic probabilities are reasonably well calibrated.
- meaning: for sequences with an expected probability of observing the replacement of x%, we really do see that x% of the sequences have that replacement.

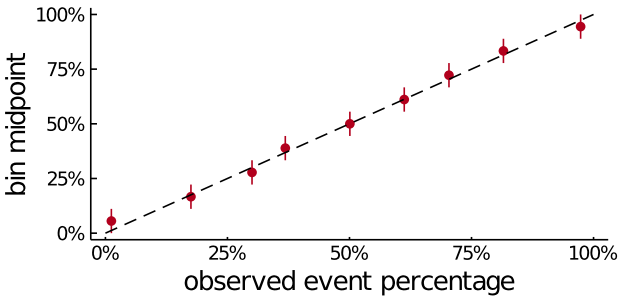

Figure 1. Calibration plot and how it works.

Including HLA linkage disequilibrium and codon covariation

- HLA linkage disequilibrium is accounted for by considering all HLA alleles of a sample at once.
- If strong correlations between HLA alleles: marginal posterior for both of them overlap with 0
- Codon covariation is not accounted for because we are mainly interested in HAMs.
- Model could be extended to include codon covariation by including them as additional predictors. This is possible because we use the horseshoe prior.

Including sparsity assumptions

- one of the recent advances in Bayesian inference: development of spacial priors to give model useful properties
- we expect most HLA coefficients to be 0, because no epitope spanning that region is presented on the cell surface, therefore no selection pressure and no association with possible replacements
- this is prior knowledge that should be incorporated in the model
- leads to better inferences because uncertainty of the regression coefficients does not propagate into observations.
- works by placing most probability mass very close to 0, but with large tails for non-zero coefficients.
- show formula
- global shrinkage parameter is very small and shrinks most coefficients to 0, local shrinkage parameter is very large and allows some coefficients to escape that shrinkage
- prior can be specified in terms of expected number of non-zero coefficients
- in our case, degree of sparsity can be well estimated from the data because all replacements share the same global shrinkage parameter.
- useful addition for logistic regression models: regularized horseshoe to have some regularization for non-zero coefficients. this helps to deal with issues of separability and colinearity.
- effect of horseshoe prior compared to standard logistic regression model: refer to model testing section. In our benchmark, the horseshoe prior alone is more effective than the model with phylogeny.
- maybe include comparison of marginal posteriors with and without horseshoe prior

Including epitope prediction software

- Vast experimental data available.
- HAMs are much more likely to lie in known epitopes
- epitope prediction software works reasonably well and can be included into as input to a probabilitic model
- MHCFlurry is used for epitope prediction and Antigen processing prediction: Input matrix (dimensions: #replacements x #HLA alleles) contains a 1 if that position is either expected to be inside a predicted epitope or related to antigen processing, 0 otherwise
- uses some thresholds, but this is not so bad because we just use it as input for a probabilistic model. Maybe play around with different options? The Brumme paper uses some offsets for defining the region of known epitopes, maybe this does make a difference.

- including this kind of information can drastically improve predictive performance, as it is relevant external data.
- Bayesian inference allows us to include informationen from different sources in a consistent manner.
- this information may be imprecise or wrong, but the model is able to learn how much to trust this data if parameterized correctly.
- epitope prediction / antigen processing escape is information about the expected degree of sparsity, i.e. some coefficients are more likely to be non-zero than others.
- can be included into the model by varying the local shrinkage parameter term.
- larger standard deviation of the local shrinkage parameters means that it is more likely that this coefficient is non-zero.
- show formula

Partial pooling of 4-digit HLA alleles

- (this still has to be implemented, but should be a nice feature. If this is going to be too time-consuming I can just leave it out)
- realized through allele group specific intercepts, e.g. HLA-B51:01 and HLA-B51:03, ..., share a common intercept.
- show formula

Full model specification

- show all formulas
- all models were implemented in Stan. Code is available online in two versions: one optimized for readability, one optimized for speed with multithreading and GPU support
- data can be analyzed using a custom Julia package (tested on linux).

Prior justification

- justify priors, mostly based on the expected scales. Compare to some Betancourt/Gelman papers to see how it is done there.

4. Results

- to show that model works well in several real-world scenarios, versions of the model are run on different datasets: large Brumme HIV, smaller Arevir HIV, larger Düsseldorf HBV, HDV by Michael Roggendorf, Rongge small HIV
- MCMC convergence diagnostics reveal no sampling issues
- general posterior predictive checks are applied to show that model explains data well
- two forms of model checking: external testing through comparisons of known epitopes and leave-one-out cross-validation.

Convergence diagnostics

- inference was done with MCMC, using Stan.
- One step in Bayesian workflow: checking inference with convergence diagnostics.
- Stan code is available online.
- All model fits show no signs of inference issues, all Rhats less than 1.01, effective sample size for all model parameters greater than 300.

Posterior predictive checks

- Bayesian modeling allows a simple yet effective strategy to test models: By simulating data under the model's assumptions and comparing simulated and observed data, model misspecifications can be identified and show possible model improvements.
- One way to perform posterior predictive checks for models with binary outcomes: calibration plots.
- cite Gelman dog shock PPC paper
- show calibration plot
- calibration plots are similar to the one showed in phylogeny, but this time test the predictions of the whole model, and not just the phylogeny component
- show example plot, PPCs for all the models are shown in the supplementary

Comparison to list of known epitopes

- When testing models, we prefer to evaluate them on real-world data whenever possible
- comparing against real-world data is not straight forward for testing HAMs, because: 1.) if a tool identifies a HLA-associated mutation which is not yet documented, this could be either because it is a false positive, or a yet unknown HAM that has not been documented before. 2.) Viewing every not identified HAM that is listed in the literature as a false negative is not correct either, because what is documented are escape mutations, which do not necessarily have to show in a sequence alignment. 3.) What is and is not a HAM is not a binary decision, so the true positive / false negative framework does not work as well.
- To circumvent these issues and still compare against real-work data, we chose the following strategy:
- for each model, all evaluated replacements are ranked by decreasing confidence of being an HLA associated mutations.
- Then, a list of known epitopes is used to create a plot of the cumulative number of replacements in known epitopes vs the corresponding rank. The assumption for this kind of model check is that we expect to see an enrichment of replacements that are in known epitopes and that this enrichment is stronger for better performing models.
- We compare several different models on all data sets available.
- Observations:
- The logistic regression model without any additional information performs about as well as Fisher's exact test.
- The horseshoe prior alone is a drastic improvement over Fisher's exact test and the logistic regression model with non-sparsifying priors, even though it does not include any external information. The benefit comes from providing the model the information that most coefficients are expected to be very close to 0.
- Models improve as we add additional information
- Adding epitope prediction does improve predictive performance considerably. Note that the model only uses epitope prediction software and does not utilize any information of experimentally confirmed epitopes, which we only use for model evaluation. Therefore, our method even works in absence of any additional epitope information.
- add plot

4.1 Leave-one-out cross-validation

- In addition to testing the model against real-world epitopes, we also perform leave-one-out cross-validation
- unlike classical application of cross-validation, we are not that interested in the absolute magnitude of the prediction errors, i.e. how well we can estimate the occurrence of a replacement at a given alignment position, because the main objective of the model is to quantify the contribution of the HLA alleles.
- But, LOO gives useful insight into how the accuracy of predictions change as we include more information in the model.
- Performing LOO has historically been challenging to apply for Bayesian models, because fitting the model N times is infeasible because of computational constrains.
- Recently, Pareto-smoothed importance sampling has gained popularity. It approximates the LOO posterior from the full posterior and has the advantage that it allows to perform LOO-CV by only fitting the model once has good diagnostics to see if that approximation is unreliable.
- cite PSIS paper
- For Bayesian models, it is possible to use elpd as quantification of model performance. elpd is defined as -formula here-, i.e. the predictive density at the observed data point.
- This has the advantage over other performance measures like classification accuracy that it not only takes the location of the predictive distribution into account (i.e. the number of correct predictions), but also the width (i.e. how confident the model is in its predictions).
- Table shows LOO results for one dataset of serveral

different models: A classical logistic regression model, similar to the one first used by Moore et al, a logistic regression model with horseshoe prior, a logistic regression model with horseshoe prior and phylogeny, and the complete model with horseshoe prior, phylogeny and epitope information.

- Results are as expected, models with more information have higher predictive accuracy. Note that this is not due to overfitting, because LOO approximates model performance on unseen data. Additionally, the results are consistent across all datasets (see appendix).
- Note how the model with horseshoe prior alone already has a much higher elpd than the standard logistic regression model, even though it does not use any additional external data. This is because including the sparsity assumptions allows the model to better separate signal from noise and the uncertainty of the close-to-zero coefficients does not propagate into uncertainty of the estimated y.
- Including phylogeny further improved model performance, as the assumption of i.i.d data does not hold for sequences that share a common phylogenetic history.
- Perhaps surprisingly, the model with epitope prediction performs roughly as well as the model without. This is true from a perspective of predictive accuracy. However, in determining which HLA alleles are associated with the replacement, adding epitope prediction is highly useful, as shown in the previous section. This can be explained by the fact that a.) a given HLA allele is only restrincting a subset of all position in a sequence alignment and b.) the benefit of accurately identifying a relevant HLA allele is only relevant for the samples which do have that allele, which is only a small subset of all available samples. Therefore, looking at predictive accuracy alone is misleading in that regard, because we are more interested in the predictors than in the predictions itself.

4.2 A real-world example

- We had the opportunity to test our model against a set of false positives that were identified by Fisher's exact test. On the HDV dataset, Fisher's exact test was applied to a set of HLA annotated sequences and x statistically significant results were reported. The identified positions were experimentally validated and y positions turned out to be false-positives, where Fisher's exact test showed extremely low p-values but experimental validation failed (how?).
- This kind of data is rare because negative results are usually not published in the literature.
- We ran HAMdetector on the same dataset, the posterior probabilities are shown in table x.
- Note that a larger posterior probability denotes that the model is more confident in that HLA alleles being a HAM.
- For most of the false positives we observe low posterior probabilities, which means that the model correctly identifies these alleles as not being relevant.
- X of the false positives remain, which means that the model is not perfect, but a strong improvement over fisher's exact test.
- these results were published previously using a preliminary version of HAMdetector, cite Roggendorf manuscript.

4.3 Rongge dataset

- We ran HLAdetector on a set of X HIVSequences sampled in China. X associations have a posterior mass greater than Y.
- Maybe show a plot of marginal posteriors.

5. Discussion

- We present HAMdetector, a Bayesian model to identify HLA-associated mutations in a sequence alignment
- We propose to use models that include as much information as possible and have shown that including sparsity and epitope prediction software can achieve better performance than existing methods.
- The main advantage of using a logistic regression backbone is that it is easy to modify and the coefficients are interpretable as changes on the log-

odds scale, like with a standard logistic regression model.

- We can believe the general model structure can also be used in other contexts, for example in identifying associations in Sequence data and other features.
- Maybe some additional points?

References

[1] Ronald N. Germain. MHC-dependent antigen processing and peptide presentation: Providing ligands for t lymphocyte activation. *Cell*, 76(2):287–299, jan 1994.

[2] Alfred L Goldberg, Paolo Cascio, Tomo Saric, and Kenneth L Rock. The importance of the proteasome and subsequent proteolytic steps in the generation of antigenic peptides. *Molecular Immunology*, 39(3-4):147–164, oct 2002.

[3] James Robinson, Jason A. Halliwell, James D. Hayhurst, Paul Flicek, Peter Parham, and Steven G. E. Marsh. The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Research*, 43(D1):D423–D431, nov 2014.

[4] S. Murata, K. Sasaki, T. Kishimoto, S. i. Niwa, H. Hayashi, Y. Takahama, and K. Tanaka. Regulation of c8+ t cell development by thymus-specific proteasomes. *Science*, 316(5829):1349–1353, jun 2007.

[5] John T. Harty, Amy R. Tvinnereim, and Douglas W. White. CD8+ t cell effector mechanisms in resistance to infection. *Annual Review of Immunology*, 18(1):275–308, apr 2000.

[6] Persephone Borrow, Hanna Lewicki, Xiping Wei, Marc S. Horwitz, Nancy Peffer, Heather Meyers, Jay A. Nelson, Jean Edouard Gairin, Beatrice H. Hahn, Michael B.A. Oldstone, and George M. Shaw. Antiviral pressure exerted by HIV-l-specific cytotoxic t lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nature Medicine*, 3(2):205–211, feb 1997.

[7] Jonathan W. Yewdell and Ann B. Hill. Viral interference with antigen presentation. *Nature Immunology*, 3(11):1019–1025, nov 2002.

[8] Yuka Kawashima, Katja Pfafferott, John Frater, Philippa Matthews, Rebecca Payne, Marylyn Addo, Hiroyuki Gatanaga, Mamoru Fujiwara, Atsuko Hachiya, Hirokazu Koizumi, Nozomi Kuse, Shinichi Oka, Anna Duda, Andrew Prendergast, Hayley Crawford, Alasdair Leslie, Zabrina Brumme, Chanson Brumme, Todd Allen, Christian Brander, Richard Kaslow, James Tang, Eric Hunter, Susan Allen, Joseph Mulenga, Songee Branch, Tim Roach, Mina John, Simon Mallal, Anthony Ogwu, Roger Shapiro, Julia G. Prado, Sarah Fidler, Jonathan Weber, Oliver G. Pybus, Paul Klenerman, Thumbi Ndung'u, Rodney Phillips, David Heckerman, P. Richard Harrigan, Bruce D. Walker, Masafumi Takiguchi, and Philip Goulder. Adaptation of HIV-1 to human leukocyte antigen class i. *Nature*, 458(7238):641–645, feb 2009.

[9] Philippa C. Matthews, Andrew Prendergast, Alasdair Leslie, Hayley Crawford, Rebecca Payne, Christine Rousseau, Morgane Rolland, Isobella Honeyborne, Jonathan Carlson, Carl Kadie, Christian Brander, Karen Bishop, Nonkululeko Mlotshwa, James I. Mullins, Hoosen Coovadia, Thumbi Ndung'u, Bruce D. Walker, David Heckerman, and Philip J. R. Goulder. Central role of reverting mutations in HLA associations with human immunodeficiency virus set point. *Journal of Virology*, 82(17):8548–8559, jul 2008.

[10] C. B. Moore. Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science*, 296(5572):1439–1443, may 2002.

[11] R. A. Fisher. On the interpretation of χ^2 from contingency tables, and the calculation of p. *Journal of the Royal Statistical Society*, 85(1):87, jan 1922.

[12] Bettina Budeus, Jörg Timm, and Daniel Hoffmann. SeqFeatR for the discovery of feature-sequence associations. *PLOS ONE*, 11(1):e0146409, jan 2016.

[13] Jonathan M. Carlson, Zabrina L. Brumme, Christine M. Rousseau, Chanson J. Brumme, Philippa Matthews, Carl Kadie, James I. Mullins, Bruce D. Walker, P. Richard Harrigan, Philip J. R. Goulder, and David Heckerman. Phylogenetic dependency networks: Inferring patterns of CTL escape and codon covariation in HIV-1 gag. *PLoS Computational Biology*, 4(11):e1000225, nov 2008.

[14] Valentin Amrhein and Sander Greenland. Remove, rather than redefine, statistical significance. *Nature Human Behaviour*, 2(1):4–4, sep 2017.

[15] Andrew Gelman and John Carlin. Beyond power calculations. *Perspectives on Psychological Science*, 9(6):641–651, nov 2014.

[16] John P. A. Ioannidis. Why most published research findings are false. *PLoS Medicine*, 2(8):e124, aug 2005.

[17] Yoav Benjamini and Yosef Hochberg. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1):289–300, jan 1995.

[18] Andrew Gelman, Jennifer Hill, and Masanao Yajima. Why we (usually) don't have to worry about multiple comparisons. *Journal of Research on Educational Effectiveness*, 5(2):189–211, 2012.

[19] Andrew Gelman. Bayesian statistics then and now. *Statistical Science*, 25(2):162–165, may 2010.

[20] Jonah Gabry, Daniel Simpson, Aki Vehtari, Michael Betancourt, and Andrew Gelman. Visualization in bayesian workflow. *Journal of the Royal Statistical Society: Series A (Statistics in Society)*, 182(2):389–402, jan 2019.