MACHINE LEARNING FOR BIOLOGICAL NETWORKS

BY

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THESIS

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ABSTRACT

Genetic studies often involve huge number of covariants that interact with each other, in the form of expressions or mutations. It is crucial to mine important covariants associated with different diseases for better clinical treatment. Traditional statistical methods have been successful in testing single covariants, but are limited when studying the joint effect of multiple related genes. Hence, incorporating biological interaction networks becomes a promising approach for genetic association study. On the other hand, the advance of graph learning algorithms has made it possible to build data-driven models for large graph problems. These methods generally fall into two categories: 1) random walk and 2) deep graph neural net. We study how to leverage information from biological networks under these frameworks to solve genetic association problems on large scale. Towards this end, we have applied graph neural network to cancer prognostic prediction. We also develop a network diffusion method for variant association study for Parkinson’s disease. Our results demonstrate the power of graph learning algorithms in biological domain.
To my parents, for their love and support.
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1.1 MACHINE LEARNING IN COMPUTATIONAL BIOLOGY

Computational biology is the science of using biological data to develop algorithms or models in order to understand biological systems and relationships. While biologists conduct \textit{in vivo} and \textit{in vitro} experiments for scientific discoveries, they are facing a large search space of bio-molecule structures, gene sequences, mutations, and many others. On the other hand, these experiments can be prohibitively expensive such that exhaustive search in wet lab is unaffordable. To alleviate this issue, computational biologists design algorithms and models to analyze biological data obtained from wet lab. Their \textit{in silico} work not only reveals the underlying biological relationships from data, but also guides \textit{in vivo} and \textit{in vitro} experiments by proposing high-quality hypotheses, reducing the search space by a significant amount.

In the past, computational biologists have largely focused on designing combinatorial and statistical methods that work on a relatively small amount of data. With the recent development of Next-Generation Sequencing (NGS), massive amount of data is now available for study. It is crucial to design new methods that are both computationally and statistically efficient for analyzing data on a large scale. Machine learning, which has achieved huge success in domains such as vision, natural language processing and social network mining, has become increasingly popular in bioinformatics.

However, new challenges arise for using machine learning techniques in computational biology. First, biological data are typically in a large feature space, which can be genes, proteins or molecule structures, with a relatively small amount of samples. A large portion of machine learning algorithms, including deep learning, are data hungry, meaning that they may fail when the number of features far exceeds the number of data points. It is occasionally possible to do feature selection by expert knowledge, but in most cases people have no idea which part of the features are important for the problem. Essentially, we need to design algorithms that can automatically handle a large feature space and find useful features.

Secondly, biological data have different structures in the form of matrices, sequences and graphs. Even in a single task there may be different data structures involved. For example, a protein interaction network may be constructed from the matrix data of patient mutation profiles. How to incorporate the heterogeneous data structures in machine learning algorithms becomes another critical issue in algorithm design.

In summary, machine learning has become increasingly popular in computational biology. Nevertheless, to leverage its power, we need to carefully design algorithms and models that
overcome the aforementioned challenges. In this thesis, we will focus on handling one specific type of data, namely the biological interaction network.

1.2 BIOLOGICAL INTERACTION NETWORKS

Biological networks are summarized information networks constructed from multiple sources of data. The data source could be from experiments, literature or databases. Protein interaction networks and gene interaction networks are two common examples, and they can translate to each other in the way that genes encode proteins. In a protein interaction network, each node corresponds to a unique protein. Each edge denotes interaction between two proteins. Sometimes a weight is assigned to the edge, indicating the strength of interaction or correlation.

Biological interaction networks serve as databases in graph structure, encoding human knowledge about genes and proteins. So far they have been used for case study and feature selection. However, we hope to design a systematic way to incorporate the network as part of the machine learning algorithm to solve problems that involve modeling protein/gene interactions. To this end, we turn to machine learning on graphs, a subfield of machine learning that specifically deals with graph-type data.

1.3 MACHINE LEARNING ON GRAPHS

Graphs are ubiquitous structures in computer science. Typical examples include social networks, academic citation graphs, knowledge graphs, and biochemical molecule structures. Graphs model the interaction between vertices. In contrast to learning over the classical matrix data, where each data point is assumed to be independent, learning over graph is able to capture the relationship among each pair of nodes, as well as the holistic graph topology.

Learning methods over graphs mainly fall into two categories: 1) random walk-based methods, which is more traditional and has been widely used, 2) deep graph neural network, which appears recently and achieves great performance on large scale problems. Random walk models the diffusion of a probability matrix through the graph. Information is propagated towards neighboring nodes to reflect the graph structure. One famous example is Google’s PageRank, which propagates node importance to rank all websites.

Deep graph neural networks takes the philosophy of both random walk and graph convolution. Each node is represented by a low dimensional vector. Every time the node vector
is updated by neighborhood aggregation, which propagates the information from its neighboring node vectors. Optionally, the node vectors can be further aggregated to obtain a graph-level representation for classifying the whole graph.

In our study, we use random walk and graph neural network for two different problems respectively. We first apply graph neural network to cancer prognostic prognosis, where we use gene expressions over the interaction network to predict patient survival. We also devise a random walk-based method for neural degenerative disease stratification. We will describe these two studies in Chapter 2 and 3.
CHAPTER 2: CANCER PROGNOSIS PREDICTION

2.1 INTRODUCTION

We study the biological problem of cancer prognosis prediction, which falls into the larger category of survival analysis. Given certain features of a cancer patient, such as information about gene expression and somatic mutation, the task is to predict the patient’s survival time. Several machine learning frameworks have been developed for prognosis prediction, including Cox-proportional hazards regression (Cox-PH)[1], Cox-boost[2], Cox-nnet[3], and random survival forest[4]. However, none of these models is able to incorporate prior biological knowledge that would be helpful to prognosis prediction. In particular, the protein-protein interaction networks (PPI networks) carry rich relational information about genes. It is promising to make improvement on prognosis prediction by incorporating PPI networks into the classical learning frameworks for survival analysis.

We start by reviewing both the classical statistical model for prognostic prediction, and the recently developed graph learning techniques that are potentially applicable to our problem. Then we will describe our adapted method, followed by experiment results and analysis. In section 2.2 we will establish a general Cox-PH framework for survival analysis and introduce existing methods on the framework. In section 2.3 we review graph learning techniques and discuss about applicability of these methods to our problem. We conducted experiments on a real world cancer dataset with two different types of graph neural networks, of which the details are described in section 2.4 and 2.5. To adjust the existing graph neural network architecture to our problem, we design two novel loss functions based on the classical partial log likelihood, which increase memory efficiency and allow training over arbitrarily large population scale. Experiment results are presented and analyzed in section 2.6. We compare our methods against baselines and discuss why they succeed or fail.

2.2 SURVIVAL ANALYSIS IN CANCER GENOMICS

In this section we introduce the Cox-PH model and its extensions [1].

2.2.1 Cox proportional hazards regression

Let $T$ be the random variable indicating the survival time of the patient. The hazard function $h(t)$ is the (probability) density of death at time $t$ conditioned on survival until
time \( t \) or later. Formally,

\[
h(t) = \lim_{\Delta t \to 0} \frac{\Pr(t \leq T < t + \Delta t)}{\Delta t \cdot \Pr(T > t)} \tag{2.1}
\]

One can think of the hazard function as measuring the likelihood of death right after time \( t \) given the patient is still alive at time \( t \).

Since we care about the survival of multiple patients, \( h(t) \) should be different across patients. Let \( x_i \) be the feature vector of patient \( i \), in general \( h(t|x_i) \) is a function of both \( t \) and \( x_i \), i.e. \( h(t|x_i) = f(t, x_i) \).

The Cox proportional hazards regression models \( h(t|x_i) \) as

\[
h(t|x_i) = h_0(t) e^{\theta_i} \tag{2.2}
\]

\[
\theta_i = x_i^T \beta. \tag{2.3}
\]

Here, \( h_0(t) \) is the baseline hazard at time \( t \), corresponding to the hazard when \( x_i = 0 \). \( \beta \) is the parameter estimating the importance of each feature in \( x_i \). Notice that \( \beta \) is independent of time. Hence the cox model separates the dependence on \( t \) and \( x_i \) apart from each other. Specifically, the hazard ratio defined as \( h(t|x_i)/h_0(t) \) is time invariant.

Typically the data for survival contains both the feature vector \( x_i \) and the survival time \( t_i \) for each patient. A patient record is censored if death does not occur during the time of observation. In this case, we do not know the survival time of the patient. We only know it is longer than the observation duration. Let \( C(i) \) be the indicator of a record being censored or not. \( C(i) = 1 \) if record \( i \) is uncensored, and \( C(i) = 0 \) if censored.

The probability of the event (or death) occurring at time \( t_i \) with patient \( i \) is

\[
l_i(\beta) = \frac{h(t_i|x_i)}{\sum_{j: t_j \geq t_i} h(t_i|x_j)} = \frac{h_0(t_i)e^{\theta_i}}{\sum_{j: t_j \geq t_i} h_0(t_i)e^{\theta_j}} = \frac{e^{\theta_i}}{\sum_{j: t_j \geq t_i} e^{\theta_j}} \tag{2.4}
\]

which is independent of \( h_0(t) \).

The objective function used in Cox-PH is the partial log-likelihood over all observed events, assuming independence between the events.

\[
PL(\beta) = \log \prod_{i: C(i) = 1} l_i(\beta) = \sum_{i: C(i) = 1} (\theta_i - \log \sum_{j: t_j \geq t_i} e^{\theta_j}) \tag{2.5}
\]

It is called ”partial” because each \( l_i \) is conditioned on observing exactly one death at time \( t_i \). Following the maximum likelihood estimation, we can maximize the partial log-likelihood
to estimate the parameter $\beta$.

As a final remark, the objective $PL(\beta)$ is essentially a multi-class cross entropy loss with softmax over the logits $\theta$'s. On the other hand, $\theta$ is a linear function of the input $x$. Hence, Cox-PH is essentially a logistic regression model. Cox-boost modifies Cox-PH by applying ”gradient boosting” in training [2]. This is a standard way of using boosting to improve logistic regression model, which we do not discuss in detail.

2.2.2 Cox-nnet

In Cox-PH, the logits $\theta_i$ is linear in the input $x_i$, which implies that different features contribute to the hazard ratio independently. This naive assumption is not desirable as co-occurrence of two specific features may result in a much larger impact on survival, for example, expression of two functionally related genes. Cox-PH can be too simple to model complicated patterns on gene level. Cox-nnet replaces the linear predictor with a multi-layer perceptron (MLP) of 1 hidden layer. [3] Specifically, they compute $\theta_i$ as

$$\theta_i = G(Wx_i + b)^\top \beta$$

(2.6)

Here $W$ is the transforming matrix from input to hidden layer, $b$ is the bias on hidden layer, and $G$ is the non-linear activation function applied coordinate-wise. In their paper, the tanh function is used for $G$. Similarly, Cox-nnet maximize the partial log-likelihood to estimate parameters $W$, $b$, $\beta$.

Although Cox-nnet is advantageous in model capacity compared with Cox-PH, overfitting becomes a big problem as the artificial neural network, or MLP, introduces too many parameters. Let $m$ be the input dimension (the number of input features), $n$ be the number of patients in the dataset. Usually, $n \approx 10^3 \sim 10^4$. Following the authors’ design, they set the hidden layer size to be the square root of the input size. Thus the total number of parameters in Cox-nnet is $O(m^{3/2})$. Typically $m \approx 10^4$, which results in millions of parameters. Hence, we expect that modifying the network structure by reducing the number of parameters would substantially improve the performance.

Besides that, the Cox-nnet is incapable of incorporating prior biological knowledge on genes. Since the size of survival dataset is usually small, incorporating knowledge from outside resources can potentially benefit the performance as a type of data augmentation.
2.3 GRAPH NEURAL NETWORKS

In this section, we review several graph neural networks that are potentially useful to prognosis prediction. Before that, we first look at how to reformulate the Cox model to include a graph neural network.

2.3.1 General framework

We consider the protein-protein interaction (PPI) network as our outside knowledge. The PPI network represents the relationship between proteins, and equivalently between genes. To incorporate this graph structure into the prediction model, we can modify the network structure that outputs $\theta_i$. In general, we define an attributed graph $(G, X)$, where $G = (V, E)$ is the unweighted PPI network whose nodes are genes, and the $k-$th row of matrix, denoted by $x_k$, is the feature vector on gene $k$. Suppose we have information on $N$ genes and there are $D$ features for each gene, then $X \in \mathbb{R}^{N \times D}$. We compute $\theta$ through a graph neural network (GNN).

$$\theta = (\text{GNN}(G, X))^\top \beta$$ (2.7)

Notice that $G$ is fixed across patients since we use the same PPI network for all patients. This is typically represented as an adjacency matrix $A \in \mathbb{R}^{N \times N}$. The output $\theta$ only depends on the input feature $X$ which is distinct for each patient. Compared with the vectorized input used in Cox-PH and Cox-nnet, here we reorganize the same input into a matrix.

In the rest of this section, we will explore the possibility of the "GNN" by reviewing the recently developed graph neural networks. The general idea of graph neural network is to treat the input graph as a computation graph. At each time step, the node state is updated by aggregating information from its neighbors. Figure 2.1 shows the information flow in neighborhood aggregation. $^1$

We simplify the message passing framework suggested in [6] to divide a graph neural network into two phases: a message passing phase and a readout phase. The message passing phase runs for $T$ steps. At each step $t$, the hidden state $h^t_v$ of each node $v$ is updated by the message from neighbors:

$$h^{t+1}_v = f_t(h^t_v, \{(h^t_w, e_{vw}) : w \in N(v)\})$$ (2.8)

The readout phase compute an output vector using the final hidden states:

$^1$Picture modified from [5]
\[ s = R(\{h^T_v | v \in G\}) \]  

\( e_{vw} \) is the edge between \( v \) and \( w \). Since we do not have edge attributes, this can be omitted. \( N(v) \) denotes the neighbors of \( v \) in \( G \). Usually 1-neighbor is used for \( N(v) \), but one can define multi-hop neighbors. \( f_t \) is the updating rule at time \( t \), which can be different across time. \( R \) is the readout function. \( f_t \) and \( R \) need to be learned from data. The initial hidden state is set to be the input feature on \( v \). (i.e. \( h^0_v = x_v \)).

It is worth pointing out that instead of iterating over all nodes in the graph, sometimes all the hidden states at one time step are written as a matrix \( H^t \) such that each row represents the hidden state of one node. This formulation results in easy implementation with sparse matrix multiplication in some cases.

![Figure 2.1: neighborhood aggregation in graph neural network](image)

2.3.2 Graph convolutional network

The graph convolutional network [7] updates the hidden states by the following layer-wise propagation rule:

\[ H^{(l+1)} = \sigma(\tilde{D}^{-\frac{1}{2}}\tilde{A}\tilde{D}^{-\frac{1}{2}}H^{(l)}W^{(l)}) \]  

(2.10)

Here \( \tilde{A} = A + I_N \) is the adjacency matrix with self connections. \( I_N \) is the identity matrix. \( \tilde{D} \) is the diagonal matrix such that \( \tilde{D}_{ii} = \sum_j \tilde{A}_{ij} \). \( W^{(l)} \) is the trainable weight matrix. \( \sigma(\cdot) \) is the nonlinear activation function such as ReLU. In the original paper the final aggregation for readout is unnecessary because the method is tested with node classification. For our purpose, we may use sum, mean or a fully connected layer on concatenation of hidden states for the readout function \( R \).
One problem with this method is that it treats the node \(v\) and its neighbors equally. It is more desirable that the centroid can be distinguished from the neighbors.

2.3.3 GraphSAGE

GraphSAGE [5] adopts the following forward propagation rule:

\[
h_t^{N(v)} = \text{AGG}_t(\{h_{u}^{t-1} : u \in N(v)\}) \tag{2.11}
\]

\[
h_v^t = \sigma(W^t(h_v^{t-1}, h_{N(v)}^t)) \tag{2.12}
\]

\[
h_v^t = h_v^t/||h_v^t|| \tag{2.13}
\]

\(\sigma(\cdot)\) is the non-linear activation function. \(W^t\) is the weight matrix. \((h_v^{t-1}, h_{N(v)}^t)\) is the concatenation of the two vectors. \(\text{AGG}_t\) is the aggregation function. The authors provide three options for the aggregation function, including mean aggregator, LSTM aggregator and (max) pooling aggregator.

GraphSAGE also uses sampling to reduce the computational complexity. Instead of enumerating all the neighbors of a node, it randomly sample a fixed number of neighbors to form \(N(v)\) at each iteration.

2.3.4 Gated graph neural network

The gated graph neural network (GG-NN) [8] works for graphs with different types of edges. In the message passing phase, it computes

\[
a_v^{t+1} = \sum_{w \in N(v)} A_{vw} h_w^t \tag{2.14}
\]

\[
h_v^{t+1} = \text{GRU}(h_v^t, a_v^{t+1}), \tag{2.15}
\]

where \(A_{vw}\) is a learned matrix that is shared across edges of the same type, and GRU is the Gated Recurrent Unit. Parameters in GRU are shared across time steps and across nodes. Horizontally (one time step over the whole graph), GG-NN performs convolution over the graph. Vertically (one node over all time steps), GG-NN is a recurrent neural network receiving inputs from neighbors over the time.

Finally, the graph level output is defined as
\[ s = \tanh \left( \sum_{v \in V} \sigma(i(h_v^T, x_v)) \odot \tanh(j(h_v^T, x_v)) \right) \]  

(2.16)

where \( \sigma \) is the logistic sigmoid function, \( \odot \) is coordinate-wise multiplication, \( i(\cdot), j(\cdot) \) are neural networks. The \( \sigma(i(h_v^T, x_v)) \) part serves as an attention mechanism to decide which node more relevant to the graph level task.

2.3.5 Graph attention network

Graph attention network (GAT) [9] introduces attention mechanism to decide the importance level among all the neighbors. Compared with the graph convolutional network in section 2.3.2 which uses the normalized adjacency matrix to determine weights of neighboring nodes, GAT directly computes the weight using self attention on the hidden states. Given the centroid \( v \), for \( w \in N(v) \), the attention coefficient is computed as

\[ e_{vw}^t = \text{LeakyReLU}(\langle a^t, W^t h_v^t | | W^t h_w^t \rangle) \]  

(2.17)

and normalized by softmax over all the neighbors:

\[ \alpha_{vw}^t = \frac{\exp(e_{vw}^t)}{\sum_{u \in N(v)} \exp(e_{vu}^t)}. \]  

(2.18)

Here \( \langle \cdot, \cdot \rangle \) denotes inner product. \( | | \) denotes concatenation of vectors. \( a^t \) and \( W^t \) are parameters for step \( t \). The next hidden state is then computed using the attention coefficient and a non-linear activation \( \sigma \):

\[ h_v^{t+1} = \sigma \left( \sum_{w \in N(v)} \alpha_{vw}^t W^t h_w^t \right). \]  

(2.19)

To stabilize self attention learning, the authors also propose to use multiple attention channels. That is to compute \( K \) different \( h_v^{t+1} \) with independent parameters and concatenate them together.

Similar to GG-NN in section 2.3.4, GAT controls the rate of update in neighborhood aggregation. The difference is that GG-NN applies the same update rate for all the neighbors, while GAT computes update rate for individual neighboring node by self attention.
2.3.6 Hierarchical differentiable pooling

Most graph neural networks, such as those mentioned above, are mostly designed for node level classification/embedding. Although these methods can be used for graph-level prediction, they generally take an naive approach in the readout phase, such as mean or average of node vectors. [10] introduces a hierarchical graph network that is specific for graph level prediction. The intuition is that we can shrink the graph step by step and finally the original graph is concentrated onto one node, which represents the whole graph. See figure 2.2 for an illustration. 

![Figure 2.2: Hierarchically contract the graph to a single node](image)

To contract the graph $G$ into a smaller one $G'$, they model $G$ and $G'$ together as a matching graph and learn the node embeddings and adjacency matrix of $G'$ from $G$. Two separate graph neural networks are run on $G$ to obtain an embedding matrix and an assignment matrix. Embedding matrix contains the node embeddings of vertices in $G$, while the assignment matrix measures the edge weight between vertices in $G$ and vertices in $G'$. These edges "softly assign" vertices in $G$ to vertices in $G'$. The adjacency matrix and node embeddings of $G'$ are calculated based on this soft assignment. Such contraction may repeat for several steps such that the final contracted graph contains only one node.

The method is highly flexible in the way that the graph neural network used to compute the embedding matrix and assignment matrix can be any one mentioned before in this section. More importantly, the graph level representation is obtained through a hierarchy of graphs rather than a naive sum over nodes. However, this methods is computationally more expensive since it uses two graph neural networks at each contraction step and typically 3-5 steps are needed for large graphs.

2.3.7 Challenges for GNN-based survival prediction

Although graph neural network is a promising approach to overcome the weakness of existing methods under the Cox model, there remain two challenges. First, there are around

\[ \text{Picture from [10]} \]
20,000 human genes which constitute a large interaction graph. With the high computational complexity of graph neural network, evaluating the partial log-likelihood can be very expensive as it requires running over the whole batch. Secondly, data for cancer patients cannot cover all the genes as covered in a PPI network. We either need to filling the missing information on part of the genes or prune the graph to fit the survival data. Effectiveness for both approaches heavily rely on expert knowledge about the relative importance of each gene. It is important to develop a learning algorithm that is both computationally and statistically efficient for cancer prognostic prediction.

2.4 MESSAGE PASSING NEURAL NETWORK

2.4.1 Model architecture

As described in the previous section, most GNN architectures fall into the general framework of message passing neural network. Hence, we propose our model with a message passing phase followed by a readout phase.

**Message passing phase**

Let \( h^k(u) \) be the hidden representation of node \( u \) at layer \( k \). We use unweighted graph with a cutting threshold of 0.4 for edge weights, excluding self connection.

\[
 a^k(u) = \sum_{v \in \mathcal{N}(u)} \mu(h^k(v)) \tag{2.20}
\]

\[
 h^{k+1}(u) = \nu([a^k(u), h^k(u)]) \tag{2.21}
\]

Here \([\cdot, \cdot]\) denotes concatenation of two vectors. \( \mu, \nu \) are two-layer ReLU networks (linear, ReLU, linear, ReLU), \( \mu : \mathbb{R}^{d_k} \mapsto \mathbb{R}^{d_k}, \nu : \mathbb{R}^{2d_k} \mapsto \mathbb{R}^{d_k+1} \). Both of them have hidden dimension 4. A batch norm layer with \( d_{k+1} \) channels is added before the final ReLU of \( \nu \).

**Readout phase**

We use \( K \) layers described above. To make the final prediction, we use the DeepSet architecture.

\[
 \theta = f(\sum_{u \in \mathcal{V}} g(h^K(u))) \tag{2.22}
\]

Here \( f \) and \( g \) are two-layer ReLU networks like \( \mu \) and \( \nu \). Here \( \theta \) serves as the hazard ratio in the cox regression. We minimize the partial log likelihood to train the model.
Details

We set $h^0(u) = x(u)$ to be the input feature, which in our case is the RNA expression value and standardized RNA expression value. We use two GCN layers as described in the message passing phase. The hidden dimension $d_k = 32$ for all layers. Hidden dimensions of $f$ and $g$ are $d_k^{1/2}$ and $d_k^{1/4}$ respectively. Output dimension of $g$ is $d_k^{1/2}$. The final output $\theta$ is treated as the log relative hazard (the same $\theta$ as in section 1.2.1).

2.4.2 Loss function

The original partial log likelihood described in section 1.2.1 requires knowing the $\theta$’s from all patients, which means we need to train the model in full batch. Considering the scale of the interaction graph ($\sim 20,000$) and the number of patients ($\sim 1,000$), the memory cost would be infeasible. Hence we propose two memory efficient adaptation of the partial log likelihood loss.

Minibatch partial log likelihood. We compute the partial log likelihood over $B$, a minibatch of individuals, instead of the whole population.

$$PL(\beta, B) = \log \prod_{i \in B:C(i) = 1} l_i(\beta) = \sum_{i \in B:C(i) = 1} (\theta_i - \log \sum_{j \in B:t_j \geq t_i} e^{\theta_j})$$ (2.23)

This is reduced to the original partial log likelihood formula if $B$ is set to be the whole population.

Pairwise contrastive loss. Since the survival regression is essentially a partial ranking problem, we can use the pairwise ranking loss. In each iteration, we take two individuals $i$ and $j$ that are comparable in the cox model, say $i$ lives longer than $j$. Then

$$L_{i,j}(\beta) = \max\{0, \theta_i - \theta_j + \delta\}$$ (2.24)

Here $\delta$ is the constant offset for hinge loss.

The proposed loss functions can be computed over a small minibatch and thus reduce the memory cost. Empirically we find they do not make big difference in terms of accuracy, so we only report the result with minibatch partial likelihood.

2.5 SPECTRAL GRAPH NEURAL NETWORK

Unlike the recent message passing architectures that aggregates local information for prediction, spectral graph neural networks utilize spectral transformation that can be compu-
ationally more efficient for cases where the graph topology is fixed for all inputs. We adapt the architecture from [11] which proposed a fast localized spectral filter. This method has been shown effective for breast cancer type classification [12].

Let $G = (V, E)$ be the interaction graph with node set $V$ and edge set $E$. Let $x_p \in \mathbb{R}^n$ be the graph signal (or gene expression) of patient $p$ over the $n$ nodes (or genes). $L = D - A$ is the graph Laplacian, where $A$ is the weighted adjacency matrix and $D$ is the degree matrix.

The graph convolution of signal $x$ is defined as follows. Let $L = U \Lambda U^\top$ be an eigen decomposition of $L$, and $\lambda_1, \ldots, \lambda_n$ be the eigen values. The graph Fourier transform is defined as $\hat{x} = U^\top x$ and the inverse graph Fourier transform is defined as $x = U \hat{x}$. Then the graph convolution operator $*$ is defined as

$$x * G y = U((U^\top x) \odot (U^\top y))$$

$$= U((U^\top y) \odot (U^\top x))$$

$$= U y(\Lambda)(U^\top x)$$

where $\odot$ is the element-wise product, and $y(\Lambda) = \text{diag}[\hat{y}(\lambda_1), \ldots, \hat{y}(\lambda_n)]$.

The Chebyshev polynomial $T_k(x)$ is recursively defined as

$$T_k(x) = 2xT_{k-1}(x) - T_{k-2}(x), \quad (2.28)$$

with $T_0 = 1$, $T_1 = x$. The final graph convolution filter can be approximated as

$$x * G y_{\theta'} = \sum_{k=0}^{K-1} \theta'_k T_k(\tilde{L})(x), \quad (2.29)$$

where $\tilde{L} = 2L/\lambda_{\text{max}} - I_n$. The final result is obtained by an average pooling layer.

Since the spectral graph convolution method requires less memory, it is affordable to run a full batch training. Thus we keep to the original partial log likelihood as our loss function.

2.6 EXPERIMENT

2.6.1 Data

The RNA expression data of breast cancer patients are downloaded from The Cancer Genome Atlas Program (TCGA-BRCA). We use STRING protein-protein interaction network [13] as the gene interaction network. Genes with missing values in expression data are
removed and gene entrez ID from expression data are further mapped to STRING protein ID. Table 1.1 shows the number of overlap genes from above data. Finally, we get 12032 genes on the interaction network with expression data.

<table>
<thead>
<tr>
<th></th>
<th>TCGA</th>
<th>TCGA (no NaN)</th>
<th>map</th>
<th>STRING ppi</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGA</td>
<td>18321</td>
<td></td>
<td>16821</td>
<td>16742</td>
</tr>
<tr>
<td>TCGA (no NaN)</td>
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<td>map</td>
<td></td>
<td></td>
<td>18593</td>
<td>18543</td>
</tr>
<tr>
<td>STRING ppi</td>
<td></td>
<td></td>
<td></td>
<td>19354</td>
</tr>
</tbody>
</table>

Table 2.1: Number of overlap genes

2.6.2 Result

We report the 5-fold cross validation result of C-Index on test set. For each split, we use 15% of training data for validation purpose. Results are compared against two baselines: the linear Cox-PH model and multi-layer perceptron Cox model (Cox-MLP)[3]. In the table below, ”Message passing” stands for message passing graph neural network, and ”Spectral” stands for the spectral convolution neural network. For both proposed methods, we do early stopping according to the C-Index on validation set which is evaluated every 10 epochs. We use Adam optimizer for all models with learning rate $10^{-4}$.

<table>
<thead>
<tr>
<th></th>
<th>C-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cox-PH</td>
<td>59.7</td>
</tr>
<tr>
<td>Cox-MLP</td>
<td>62.5</td>
</tr>
<tr>
<td>Message passing</td>
<td>57.4</td>
</tr>
<tr>
<td>Spectral</td>
<td>66.3</td>
</tr>
</tbody>
</table>

Table 2.2: Performance comparison

2.6.3 Discussion

The performance of message passing is worse than the two baselines. We suspect the reason is that message passing neural networks requires rich topological information during training, but in our experiment, the underlying interaction graph is an invariant across samples, which significantly limited the power of such kind of models. In general, graph neural networks are used in both transductive and inductive scenarios. The survival prediction problem should be considered as inductive learning. However, typically inductive learning on graphs requires
a number of graphs that differ in graph topology. In our case, the graph topology is fixed, and only node attributes change across training samples. Interestingly, when the STRING PPI graph is replaced with a random graph, we get almost the same result, which indicates that graph structure is poorly utilized.

On the other hand, the spectral graph convolution network performs the best among all the models. This confirms us that information from biological interaction network is useful for prognostic prediction in breast cancer, as compared to Cox-MLP and Cox-PH which do not receive such information during training and inference. The success of spectral method over message passing methods also demonstrate the importance of the model being topologically invariant to input graphs, which improves not only the performance, but also the computational efficiency as the topological information can be pre-computed.

2.7 CONCLUSION

We present the statistical model for survival prediction and identify the underlying machine learning problem. Although message passing-based graph neural networks seems a promising approach to overcome the weakness of the classical Cox model, the empirical result is not satisfactory. On the other hand, spectral-based graph neural network achieves strong performance over baselines, demonstrating the power of biological network in cancer prognostic prediction. It is promising to explore other graph neural network architectures for better performance in the future. It is also interesting to develop an algorithm to identify important pathways over the interaction network that are unique to a disease.

From the machine learning perspective, though various message passing style graph neural networks have achieved great success in tasks on social networks, academic graphs and molecular structures, they may fail in other scenarios, as shown in our experiment. Diversity in graph topology is critical for effectively training graph neural networks. On the other hand, spectral based graph learning which is less popular these days turns out to be more effective and more efficient for problems with fixed topology. Apart from our prognostic prediction problem with genetic interaction network, there are other graph learning problems with fixed graph topology such as predicting weather change or population growth across regions. It would be valuable to study the computational methods applied in these domains and adapt to cancer genomic problems.

Although this study has only focused on prognostic prediction of the breast cancer, the philosophy could potentially be extended to many other prediction problems with gene expressions or mutations. Biological interaction networks can always provided additional
information about correlations between genetic covariants. We also suspect that using a problem-specific network, rather than the general purpose network as in our experiment, would potentially benefit the performance. For example, an interaction network constructed from brain cells may be a better fit for predicting neurodegenerative diseases.
CHAPTER 3: GENETIC ASSOCIATION STUDY FOR PARKINSON’S
DISEASE

3.1 INTRODUCTION

The advent of next generation sequencing (NGS) has facilitated the discovery of genetic mutations in human diseases. In contrast to traditional SNP-array approach, NGS is most effective in detecting rare genetics variants or structural genome changes. These genetic mutations are likely to be associated with nervous system disorders, in particular the Parkinson’s disease. The decreasing cost of NGS has provided an abundant resources of data on genetic variants on the scale of whole exome sequencing (WES) or even whole genome sequencing (WGS). However, we are facing the challenge of detecting significance of these rare variants from the large scale data. Traditional statistical methods fail in this case largely due to the sparsity of signals of rare variants.

It is crucial to devise novel methods with sufficient statistical power to reveal the effect of rare variants on neurogenic diseases. Towards this end, several rare variant association tests have been proposed. See [14] for a comprehensive survey over these methods. However, these methods generally aggregate variant by contiguous chunks of loci over the genome, or simply by genes, which potentially limits its power as they only consider rather simple patterns of variant combinations. In the meanwhile, with the extreme sparsity of the data, it is likely that some effective variants are missing (not revealed in experiments) due to limited sample size. Hence, it is important to recover them during the analysis.

In this project, we leverage information from biological gene networks to assist the analysis of rare variants. We adopt a technique called network diffusion to propagate gene-level signals over the interaction graph to get a more comprehensive picture of overall mutation profile of individual patients. By introducing biological networks, we are allowed to extend aggregation patterns from aggregation within genes to across genes. The gene network also makes it possible to determine the effect of genetic mutations that are not experimentally measured. Our assumption is that variant impact comes from not only single genes but also network harbors. In this way, we are able to recover important harbors by locality over the gene network.

It is worth mentioning that the clinical data are used for evaluation only. Our method is based on unsupervised clustering which requires no labels. The feature obtained from random walk over the interaction graph allows us to cluster the patient profiles into groups and we empirically find that these groups are highly correlated to disease phenotypes.
3.2 RELATED WORK

In complement to GenomeWide Association Study (GWAS) for common variants, gene or region based tests for rare variant association study have been recently proposed, most of which use the idea of aggregating single variants with weak signals into larger group of better statistical significance. One important class of these tests are termed as burden tests. They collapse information for various genetic variants into a single score and test it against a given trait. The cohort allelic sums test (CAST) ([15]) assumes equal weight for variants in the same group and variants all have deleterious effects. However, these assumptions are too strong in real cases. The Sequence Kernel Association Test (SKAT) ([16]), uses a variance-component approach to estimate both positive and negative effects. Moreover, SKAT models SNP-SNP interactions by adaptively assigning weight to each variant. SKAT. Although SKAT outperforms burden tests when a large fraction of the variants in a region are noncausal or the effects of causal variants are in different directions, it is inferior in simpler cases when most variants are causal with the same direction of effects. SKAT-O ([17]) combines these two approaches to optimize the performance.

On the other hand, network diffusion has been widely adopted in association studies. Network based stratification (NBS) has successfully applied network diffusion to type tumors by propagating somatic mutation information over tumor-specific interaction networks. ([18],[19]) While neurogenic diseases typically exhibit more complex patterns than cancers, we expect the power of network diffusion can help in our case.

3.3 PROBLEM DEFINITION

Let $M \in \mathbb{R}^{n \times p}$ be the patient-by-gene profile. The value $M_{ij}$ encodes the SNP information of gene $j$ in patient $i$, which can be either a binary value, or a normalized count. In the binary case, $M_{ij} = 1$ if there is at least one variant in the gene region, and $M_{ij} = 0$ otherwise. In case of count, we normalize the number of SNPs over the gene region by background mutations. We also introduce a gene interaction network $G = (V, E)$, whose vertices are all human genes and edges are interactions. We always treat $G$ as a weighted graph. If the network is originally unweighted, we assign 0/1 weights to edges. The interaction network is shared for all patients.

We formulate the problem as an unsupervised clustering. Our goal is to cluster all the patients by their mutation profiles. To evaluate the resulting clusters, we use information from clinical diagnosis of progression of Parkinson’s disease. Specifically, we look at the
UPDRS score (Unified Parkinson Disease Rating Scale). The UPDRS score is computed by rating on various Parkinson-related symptoms. For each patient, UPDRS is measured multiple times over the years to reflect the progression of disease. In our study, we discretize the timeline by a bin size of 10 months and take the average of UPDRS scores in the same bin. Consequently, for each cluster of patients we can draw a curve of average UPDRS versus time, which serves as the phenotype. An effective clustering algorithm should separate apart curves from different clusters. In case of binary clustering, the result should correspond to a high risk group and a low risk group.

3.4 METHOD

In this section, we describe our network-based stratification method. As described in the previous section, our input data is a patient-by-gene mutation profile $M$, and an interaction network $G = (V, E)$.

To outline, our method consists of three main steps. First, since the network $G$ may be constructed for general purpose and not for our problem specifically, we need to find a proper subnetwork with some prior domain knowledge (Section 4.1). By doing this we also reduce the computational burden. The next step is to run random walk with restart over the subgraph to get smoothed patient profiles (Section 4.2). Finally, we do clustering over the result from network diffusion (Section 4.3).

3.4.1 Network Filtering

We first filter the network $G$ by a pre-determined gene set $S$, which comes from (1) important genes related to Parkinson’s disease found by previous studies, and (2) genes corresponding to non-zero columns of our data matrix $M$ (i.e. at least one patient has a mutation on this gene). Although the original network $G$ is usually well connected, its subgraph directly from $S$ may be fragmented. There are three criterions for choosing the subgraph $\hat{G}$:

1. $\hat{G}$ should be connected, for the sake of random walk in the next step.

2. $\hat{G}$ should contain as many genes from $S$ as possible.

3. $\hat{G}$ should contain as few genes outside $S$ as possible.
For these three purposes, we first partition the induced subgraph of \( G \) from \( S \) into connected components. We take the largest component \( c_1 \) as the base and grow \( c_1 \) by adding other components. If another component \( c_i \) can be connected to \( c_1 \) via a single vertex \( v \), we attach \( c_i \) and \( v \) to the existing subgraph. Otherwise, distance between \( c_i \) and \( c_1 \) would be larger than 1, and we simply drop \( c_i \). Details of network filtering is in Algorithm 1.

**Algorithm 3.1: Network filtering**

**Input:** Network \( G = (V, E) \), Node Set \( S \)

\[ G^S = G\text{.subgraph}(S); \]

\[ c_1, c_2, \ldots, c_k = \text{ConnectedComponent}(G^S); \]

Let \( c_1 \) be the largest connected component.

\( N = c_1 \) for \( i \leftarrow 2 \) to \( k \) do

\[ \text{if there is } v \in V \text{ such that } c_1 \text{ and } c_i \text{ are connected through } v \text{ then} \]

\[ \quad \text{Add } c_i \text{ to } N; \]

\[ \quad \text{Add } v \text{ to } N; \]

end

end

return \( G\text{.subgraph}(N) \)

### 3.4.2 Random Walk with Restart

We perform random walk with restart over the subnetwork obtained from the previous step. Let \( A \) be the adjacency matrix of the subnetwork. The transition probability matrix \( B \) is defined by normalizing edge weights by out degrees.

\[
B_{ij} = \frac{A_{ij}}{\sum_j A_{ij}} \tag{3.1}
\]

Let \( F_0 = M \) be the initial state, \( F_t \) be the state at time \( t \), and \( \alpha \) be the restart probability. At each iteration, we update \( F_t \) according to the following:

\[
F_{t+1} = (1 - \alpha)F_t B + \alpha F_0 \tag{3.2}
\]

We iteratively update the state until the convergence criterion \( \| F_{t+1} - F_t \|_F \leq \epsilon \) is met. Here \( \| \cdot \|_F \) is Frobenius norm, and \( \epsilon \) is set to \( 10^{-6} \).
3.4.3 Clustering

The output $\bar{F}$ from random walk is an $n \times q$ matrix, where $q$ equals the number of vertices in the subnetwork. We reduce the dimension using singular value decomposition (SVD). Formally,

$$\bar{F} = USV^T \quad (3.3)$$

$$M = U_d S_d^{1/2} \quad (3.4)$$

$U_d, S_d$ denote the first $d$ columns of $U, S$ respectively. We use $d = 50$ and take $M$ as the truncated patient profile. We further compute a cosine distance matrix $D$ of rows in $M_d$.

$$D_{ij} = 1 - \cos(M_i, M_j) \quad (3.5)$$

KMeans++ clustering is applied to matrix $D$ to generate the final clusters.

3.5 EXPERIMENT

We evaluate our method on datasets from Parkinson’s Progression Markers Initiative (PPMI). We extract Single nucleotide variants (SNVs) from both whole exome sequencing data and target gene sequencing data. In this project, we only focus on nonsynonymous exonic variants, which result in amino acid change in corresponding proteins. There totally 402 patients diagnosed with Parkinson’s disease from WES dataset, and 430 from target gene sequencing dataset. Variant information in VCF format is converted to one patient-by-gene matrix with binary values. The STRING protein-protein interaction network [13] is used for random walk propagation. We run our algorithm on the mutation matrix and plot the UPDRS curves of resulting clusters.

On both datasets our method can clearly separate the two curves apart, indicating that the population is stratified into two groups with different risks. As expected, the whole exome sequencing data gives a more comprehensive picture than the target gene sequencing data that only covers a few hundred of genes. This also demonstrates the importance of studying Parkinson’s disease on a whole-genome level to discover a larger set of relevant genes.
3.5.1 Counting-based Variant Matrix

Instead of using binary indicators, we also tried normalized counting matrix as the input to network diffusion. For each patient and each gene, we divide the number of nonsynonymous exonic variants by the number of background variants on the same gene. Background types include intronic, regulatory, and synonymous exonic variants, which we believe has less impact on the disease. Below we present the results.

As one can see, using normalized count does not improve the result. We suspect this is due to the type of variants in use. It is likely that even a single nonsynonymous variant can result in malfunction of the whole protein, and thus the number of variants is not super important.
3.5.2 Rare Variant Filtering

We also considered using rare variants only in whole exome sequencing dataset. For this purpose, we filtered all nonsynonymous variants by Non-Finish European minor MAF in ExAC ([20]) and CADD score ([21]). Specifically, we retain variants with NFE MAF < 0.02, and CADD score > 10. The results are shown below (with binary mutations).

The result shows that common variants may still play an important role in Parkinson’s disease, as the performance significantly decreases without them. It would be interesting to study common variants and rare variants separately and then combine the results, which will be done in further experiments.
3.5.3 Splicing and regulatory effects

In addition to exonic mutations, we turn to variants with splicing and regulatory effects. The whole genome study (WES) dataset is downloaded from PPMI website. Variants are annotated by the Ensembl Variant Effect Predictor (Ensembl VEP) [22] and ANNOVAR [23] for downstream effects. We collected variants with splicing effects and variants with regulatory effects respectively. Results are shown in Figure 2.6 and Figure 2.7.

In contrast to the exonic variants, the splicing and regulatory variants show little discriminative power for disease phenotypes. Although it is believed that regulatory variants play an important role in neurodegenerative diseases, we may need to devise new algorithms to explore their effects.
3.6 CONCLUSION

We proposed a network-based stratification method to study rare variant association in Parkinson’s disease. Compared to the classical single variant tests, our method considers a large number of variants in a joint manner to predict their biological effects. Our method also has an advantage over the class of burden tests in that we leverage domain knowledge about genetic interactions by incorporating an interaction network. On one hand, the burden tests simply condense variants over a particular gene into one number without considering their interactions. On the other hand, statistically it is almost impossible to learn so many second-order parameters of gene-gene correlations from limited patient profiles. The prior knowledge from the summarized interaction network illuminates the relation between genes and is able to guide the model to capture the joint effects of neighboring genes by signal propagation, which overcomes data sparsity. Experiments on PPMI data show our method overcomes the weakness of existing and is able to achieve a strong correlation with clinical diagnosis.

It is worth mentioning that pre-filtration of genetic variants is critical for genetic association study. Should we study exonic variants, rare variants or regulatory variants? How to combine the different results together. This is an interesting problem for future study. For now we have simply condensed different kinds of variants of our concern together, but ideally they should be treated differently. Constructing a large heterogeneous network to incorporate all the type information would be a promising way, but may incur high computational cost due to large number of variants. Efficient training and inference algorithms are required for handling variants of different types.
We also hope to extend the NBS framework to supervised settings and perform pathway detection to discover important genes for Parkinson’s disease that are previously unknown. There have been works on plant genome-wide association studies using multiple linear regressions. See [24] and [25]. Nevertheless, human genomics is more complicated than plants. It is important to study how to deal with the correlations between large number of variants of different types.
REFERENCES


