Cancer Diagnosis with QUIRE: QUadratic Interactions among infoRmative fEatures

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ABSTRACT

Motivation: Responsible for many complex human diseases including cancers, disrupted or abnormal gene interactions can be identified through their expression changes correlating with the progression of a disease. However, the examination of all possible combinatorial interactions between gene features in a genome-wide case-control study is computationally infeasible as the search space is exponential in nature.

Results: In this paper, we propose a novel computational approach, QUIRE, to identify discriminative complex interactions among informative gene features for cancer diagnosis. QUIRE works in two stages, where it first identifies functionally relevant feature groups for the disease and, then explores the search space capturing the combinatorial relationships among the genes from the selected informative groups. Using QUIRE, we explore the differential patterns and the interactions among informative gene features in three different types of cancers, Renal Cell Carcinoma (RCC), Ovarian Cancer (OVC) and Colorectal Cancer (CRC). Our experimental results show that QUIRE identifies gene-gene interactions that can better identify the different cancer stages of samples and can predict CRC recurrence and death from CRC more successfully, as compared to other state-of-the-art feature selection methods. A literature survey shows that many of the interactions identified by QUIRE play important roles in the development of cancer.

1 INTRODUCTION

In this paper, we focus on the task of cancer diagnosis using molecular signatures, such as gene expression measured using microarray experiments or protein expression values measured in blood. In the past decade, advancement in the genome-wide monitoring of gene expression has enabled the scientists to look into the interplay among multiple genes and their products. Differential analysis of gene expression helps identification of individual genes that show altered behavior in the phenotype of interest. Previous systematic analysis of gene expression has enabled identification of single gene markers implicated in different types of cancers such as breast cancer (Perou et al., 2000), lung cancer (Beer et al., 2002), and prostate cancer (Lapointe et al., 2004).

Complex diseases like cancers are the results of multiple genetic and epigenetic factors. Although single gene markers can provide valuable information about the process under study, a major problem with these markers is that they offer limited insight into the complex interplay among the molecular factors responsible for progression of complicated diseases, like cancers. So, recently, research focus in complex diseases shifts towards the identification of multiple genes that interact directly or indirectly in contributing their association to the target disease. Several complex interactive partners from previous studies have been shown to give important insight into the mechanism of breast cancer (Chuang et al., 2007) and colorectal cancer (Chowdhury et al., 2011).

However, due to the combinatorial nature of the problem, the identification of groups of genes that show differential behavior in the manifestation of complex phenotypes is computationally infeasible. For instance, for a set of 30,000 genes, there are about 4500 million possible gene-gene interactions in the search space. Several recent methods propose to reduce the search space using orthogonal prior knowledge about connections amongst the genes, such as interactions collected from protein-protein interaction (PPI) network or grouping information from functional annotations of proteins. One notable computational method named Group Lasso, which is proposed by Yuan and Lin (2006), incorporates such prior interaction or grouping among the genes to detect gene groups that contribute to human disease, by enforcing sparsity at the group level in a supervised regression framework. Group Lasso is extended by Jacob et al. (2009) to a more general setting that incorporates groups whose overlaps are nonempty. Such overlaps in groups is biologically more significant, because many genes participate in multiple pathways and manifest themselves in several biological processes.

Although Group Lasso is very useful in identifying biologically relevant groups of genes and proteins, they cannot capture complex combinatorial relationships among the features within and across the groups. Also, current PPI network data is inherently noisy due to experimental constraints. Algorithmic approaches based solely on the noisy prior information can result in many false positive interactions which are absent in the real genome space.

In this paper, our main goal is to identify the complex combinations of pairwise interactions among the genes that might help us (1) better diagnosis and prognosis of different types of
cancer, and (2) gain novel insights into the mechanistic basis of the diseases. Since the total number of possible pairwise human gene interactions is huge, it is computationally infeasible to examine all possible combinatorial combinations of them when trying to understand their relevance to the phenotype under consideration. Due to this “Curse of Dimensionality” issue, our first target is to reduce the dimensionality of the search space in such a manner that it enables us to identify informative interacting gene partners in a reasonable limit of time and memory space. This reduced search space then enables us to look for combinations of interacting pairs of informative genes in a more practical sparse learning setting.

In this paper, we propose a two-stage solution, named as QUIRE, i.e. to detect QUadratic Interactions among infoRmative fFeatures which show differential behavior for diagnosing a target disease using molecular signatures. In the first stage of our proposed approach, we use Overlapping Group Lasso (Jacob et al., 2009) to identify biologically relevant informative feature groups and physical gene interaction groups that exhibit differential patterns for the studied disease. Then in the second stage, we search exhaustively on this reduced feature space by examining all possible pairs of interacting features to identify the combination of markers and complex patterns of feature interactions that are informative about the phenotypes in a sparse learning framework. Systematic experimental results have been obtained on protein and gene expression data from three different types of cancers, Renal Cell Carcinoma (RCC), Ovarian Cancer (OVC) and Colorectal Cancer (CRC). Compared with other state-of-the-art feature selection methods, the results show that QUIRE can discover complementary sets of markers and pairwise interactions that can better classify the diseases. Since the total number of possible pairwise human events, like cancer recurrence and death from cancer with higher samples from different stages of cancer and predict post-cancerous sets of markers and pairwise interactions that can better classify the diseases under study.

2 METHODS

Mathematically, the identification of single gene markers in a genome-wide study is an ill-posed problem. This is due to the fact that the number of genes in human cells are much more in numbers than the number of samples that are available for these kind of studies. For such problems, Lasso, proposed by Tibshirani (1996) is very popular for selecting a small number of features relevant to the problem under study. When a set of features are highly correlated to each other, Lasso selects one from that set randomly, ignoring others. So, in our current setting, there is a possibility that Lasso leaves out biologically relevant genes from its set of selected informative features. In this paper, we consider a linear regression setting. Suppose we have a data set \( D \) containing \( n \) observations \((x^{(i)}, y^{(i)})\) with response variable \( y \in R \) and feature vector \( x \in R^n \), where \( i \in \{1, \ldots, n\} \), and we assume that features are standardized with zero mean and unit standard deviation (we will prove the effects of feature standardization on linear classifiers later in this section) and the \( y \)s are centered in \( D \). The Lasso approach optimizes the following objective function,

\[
el(w) = \sum_{i=1}^{n} (y_i - \sum_{j=1}^{p} w_j x_j^{(i)})^2,\]

\[
el_{\text{lasso}}(w) = \ell(w) + \lambda \sum_{j=1}^{p} |w_j|, \tag{1}
\]

where \( \ell(w) \) is the loss function of linear regression, and \( w \) is the weight parameter. The \( \ell_1 \) norm penalty in lasso induces sparsity in the weight space for selecting features. It is obvious that the sum of the least squared errors and the \( \ell_1 \) norm are convex functions with respect to the weights \( w \), thus, we have the following lemma.

**Lemma 1.** Lasso-penalized linear regression has global optimum for any fixed penalty coefficient \( \lambda \).

According to Lemma 1, Lasso has global optimum, which can be found by any convex optimization technique. The coordinate descent approach proposed in Friedman et al. (2010) sets the gradient of the loss function \( \ell_{\text{lasso}}(w) \) to 0 to solve each weight \( w_j \) iteratively, and it is one of the most computationally efficient methods.

\[
w_j = S(\frac{1}{n} \sum_{i=1}^{n} x_j^{(i)} (y^{(i)} - \sum_{k \neq j} w_k x_k^{(i)}), \lambda)_+, \tag{2}
\]

where \( S(z, \lambda)_+ \) is a soft-thresholding operator. The value of \( S(z, \lambda)_+ \) is \( z - \lambda I \text{ if } z > 0 \) and \( \lambda < |z|, z + \lambda \text{ if } z < 0 \) and \( \lambda < |z|, 0 \text{ if } |z| \geq \lambda \).

In spite of the computational efficiency and the popularity of Lasso for feature selection, its formulation prevents it from capturing any prior information on possible group structures among the features. Group Lasso (Yuan and Lin, 2006) proposed using \( \ell_2,1 \) penalty to select groups of input features which are partitioned into non-overlapping groups. The group penalty is the sum of the \( \ell_2 \) norm on the features belonging to the same group. Overlapping Group Lasso (Jacob et al., 2009) extends Group Lasso to handle groups of features with overlapping group members by duplicating input features belonging to multiple groups in the design matrix. Because a lot of real applications involve overlapping feature groupings, Overlapping Group Lasso is a more natural choice than Group Lasso. Suppose that we partition \( p \) features in data set \( D \) into \( q \) overlapping groups \( \{g_1, g_2, \ldots, g_q\} \), the following objective function is minimized in Jacob et al. (2009) and Qi,

\[
\ell_{\text{oglasso}}(w) = \ell(w) + \lambda \sum_{g \in D} ||w_g||_2, \tag{3}
\]

where \( \lambda \) is the regularization parameter. \( w_g \) denotes the set of weights associated with features in group \( g \), and \( || \cdot ||_2 \) is the Euclidean norm. The above optimization problem is separable, so we can use block coordinate descent to optimize the weights associated with each group \( g \) separately. The subgradient of the optimization takes the following form,

\[
- \sum_{i=1}^{n} x_g^{(i)} T (y^{(i)} - \sum_{g' \neq g} w_{g'} x_g^{(i)}) + \lambda w_g \in \mathbb{R}^|g|; \tag{4}
\]

Therefore, if \( || \sum_{i=1}^{n} x_g^{(i)} T (y^{(i)} - \sum_{g \neq g'} w_{g'} x_g^{(i)}) || < \lambda \), then \( w_g = 0 \); otherwise, \( w_g \) can be obtained by solving several one-dimensional optimization problems based on coordinate descent. In details, let \( Z_g^{(i)} = e_g^{(i)} = [(Z_1^{(i)}, \ldots, Z_{|g|}^{(i)}), w_g = \theta = (\theta_1, \ldots, \theta_{|g|}) \text{, and residual } x^{(i)} = y^{(i)} - \sum_{g' \neq g} w_{g'} x_g^{(i)} \text{, then } \theta_j \text{ of } w_g \text{ can be solved by minimizing the following objective function},

\[
\frac{1}{2} \sum_{i=1}^{n} (y^{(i)} - \sum_{j=1}^{p} Z_j^{(i)} \theta_j)^2 + \lambda ||\theta||_2. \tag{5}
\]
The final solution of the overlapping group lasso is obtained by iterating the above optimization procedure over each feature group $g$ until convergence.

The underlying idea of QUIRE is to incorporate all possible complementary biological knowledge into the above infeasible optimization problem to reduce search space. By restricting discriminative gene interactions to happen only between genes in some informative gene groups, we can use existing functional annotations of input genes to identify these groups thereby to throw away a lot of interaction terms during the optimization. In addition, available physical interactions between the protein products of input genes can also be used to cut the search space, although discriminative gene feature interactions for prediction do not always necessarily correspond to physical interactions. The general working model of QUIRE is shown in Figure 1. In details, QUIRE takes the expression profile of $n$ samples over $p$ genes (proteins), the physical interactions among the genes products (i.e. protein protein interaction network) and the disease status of these samples as input, and it outputs a (small) set of discriminative genes and gene interactions with corresponding learned weights for predicting the disease status of any incoming test sample. The step by step working model of QUIRE is given below:

1. Functional group generation:
   a. QUIRE groups the $p$ input gene features into $q$ overlapping functional categories according to the existing Gene Ontology (GO) based functional annotations, such as Cellular Colocalization (CC), Molecular Function (MF), and Biological Process (BP).
   b. QUIRE clusters the given interaction network (i.e. PPI) into subsets of overlapping gene products based on GO functional annotations, CC, MF and BP.

2. Informative genes and functional interactions selection:
   a. Given the GO functional grouping of input gene features, Overlapping Group Lasso is run to select $m$ top discriminative genes for disease status prediction according to the absolute values of the learned weights of gene features.
   b. Overlapping group lasso is run on the clustered interaction network to select informative groups of protein-protein interactions. In this case, each cluster is considered as a group and quadratic interactions (discussed later) among the interacting proteins in a group are used as expression.

3. Selection of most informative interactions and genes: QUIRE first enumerates all possible quadratic feature interactions among the informative genes selected at step 2(a). Then it takes these quadratic interactions, single informative gene features and the informative functional interactions identified at step 2(b) as input and it outputs the final selected gene interactions and single genes as biomarkers.

In order to identify the discriminative combinations of single gene features and quadratic interactions among pairwise informative genes, we define our proposed objective function for Lasso as follows,

$$
\ell(w, U) = \sum_{i=1}^{n} (y^{(i)} - \sum_{j=1}^{p} w_j x_j^{(i)})^2 - \sum_{j=1}^{p-1} \sum_{k=j+1}^{p} U_{jk} x_j x_k
+ \lambda_1 \sum_{j=1}^{p} |w_j| + \lambda_2 \sum_{j=1}^{p-1} \sum_{k=j+1}^{p} |U_{jk}|.
$$

(6)

However, the above model has $O(p^2)$ features and is not applicable to genome-wide biomarker discovery studies. Provided that the training data is often very limited, it is almost impossible to identify the discriminative single or quadratic interaction features by solving the above optimization problem. We propose QUIRE (QUadratic Interactions among infoRmative iFeatures) to address these challenges, which is based on Overlapping Group Lasso and Lasso. And it takes advantage of both of these feature selection methods.
in a genome-wide case control study, QUIRE reduces the search space by using the features that are selected by Overlapping Group Lasso as the informative ones, and then it relies on Lasso with $\ell_1$ penalties to identify the discriminative combination of informative individual features and gene interaction features, which provides an approximation to the problem of searching an exponential number of all possible combinations of single features and pairwise interaction features.

As we mention earlier in this paper, we perform feature standardization before running Lasso or Group Lasso. Instead of using the original quadratic interactions $x_j x_k$ between pairwise variables $x_j$ and $x_k$, we standardize $x_j x_k$ by $g(x_j x_k)$ as input feature, where $g(x) = \frac{x - \mu}{\sigma}$, and $\mu$ and $\sigma$ are respectively the mean and standard deviation of feature $x$. As shown in Lemma 2 and Lemma 3, we see that feature standardization has nice properties when running Lasso, and quadratic feature interactions calculated by $g(x_j x_k)$ is more sensible than $g(x_j)g(x_k)$ for biomarker discovery because it does not have weight sharing constraints involving both gene interaction features and single gene features. Moreover, $g(x_j)g(x_k)$ can result in inaccurate calculations because the product of two large negative values for normalized features is a large positive value, which is not desirable in most applications. The advantage of $g(x_j x_k)$ over $g(x_j)g(x_k)$ is supported by our experimental results in this paper.

**Lemma 2.** The solution of Lasso-penalized linear regression on standardized input features with one fixed penalty coefficient $\lambda$ is equivalent to the solution of a Lasso problem on original input features with adaptive penalty coefficients for different weights being $\lambda$ weighted by the standard deviations of different corresponding original features.

**Proof.** Suppose that the optimal solution of lasso-penalized linear regression on standardized features takes the following linear form, $f(x; w) = \sum_j w_j^* g(x_j) = \sum_j \frac{w_j}{\sigma_j} x_j - \sum_j \frac{\mu_j}{\sigma_j}$, in which $w$ minimizes $\ell(w) = \sum_j |y^{(i)} - f(g(x^{(i)}); w)|^2 + \lambda \sum_j |w_j|$. Let $w_j^* = \frac{w_j}{\sigma_j}$, we can show that $w^*$ is the global optimal of $\ell'(w') = \sum_j |y^{(i)} - \sum_j w_j^* x_j| + \lambda \sum_j |w_j|$. Because $x_j = \sigma_j g(x_j) + \mu_j$, $\ell'(w') = \sum_j |y^{(i)} - \sum_j \frac{w_j}{\sigma_j} (\sigma_j g(x_j) + \mu_j)| + \lambda \sum_j \frac{w_j}{\sigma_j} = \sum_j |y^{(i)} - f(g(x_j); w)|^2 + \lambda \sum_j |w_j| = \ell(w)$. Therefore, if $w$ is a global optimum of $\ell(w)$, then $w^*$ must be a global optimum of $\ell'(w')$, which proves the lemma.

**Lemma 3.** In the setting of Lasso-penalized linear regression, our proposed quadratic feature interaction $g(x_j x_k)$ has different effect compared to $g(x_j)g(x_k)$. $g(x_j x_k)$ only constrains original feature interactions $x_j x_k$ while $g(x_j)g(x_k)$ results in weight sharing constraints involving both interaction features and single features.

**Proof.** First, if $x_j x_k$ correlates with a feature $x_l$, $g(x_j x_k)$ does not change the correlation coefficient, because $corr(x_j x_k; x_l) = E[x_j x_k - \mu_j x_k - \mu_k] x_l - \mu_l = E[g(x_j x_k) g(x_l)] = corr(g(x_j x_k), x_l)$. However, $corr(g(x_j) g(x_k), x_l)$ is different from $corr(x_j, x_k; x_l)$. Second, $g(x_j x_k) = x_j x_k - \mu_j x_k - \mu_k$ includes both a quadratic interaction term and linear terms, but $g(x_j x_k) = x_j x_k - \mu_j x_k - \mu_k$ only involves a quadratic interaction term. Therefore, linear classifier $\sum_{jk} w_{jk} g(x_j g(x_k)) + b$ puts a lot of constraints on weight sharing between quadratic interaction features and single features. Moreover, according to Lemma 2, the solution to Lasso on features $g(x_j x_k)$ is equivalent to the solution to the lasso on features $x_j x_k$s with adaptive penalty coefficients for different weights.

### 3 RESULTS AND DISCUSSION

In this section, we perform comprehensive classification experiments to evaluate the performance of our proposed method, QUIRE, in classifying samples across different stages of Renal Cell Carcinoma (RCC), Ovarian Cancer (OVC) and in predicting cancer recurrence and death due to cancer in Colorectal Cancer (CRC). We compare the performance of QUIRE with state-of-the-art feature selection techniques, Lasso, Overlapping Group Lasso and SVM. We then perform a literature survey and enrichment analysis of the informative interactions selected by QUIRE and show that they are relevant to the progression of the disease.

### 3.1 Datasets

We use three datasets with samples from RCC, OVC and CRC for classification experiments. For RCC and OVC datasets, Blood samples are collected and Somamer (aptamer) based proteomic technology [Gold et al. 2010] is used to measure the concentration of a selected set of marker proteins. The CRC samples belong to a publicly available microarray dataset collected from gene expression omnibus (GEO), and referenced by accession number GSE157563 [Smith et al. 2010]. We give detailed description of the datasets below:

1. **RCC Dataset:** This dataset contains 212 RCC samples from Benign and 4 different stages of tumor. Expression levels of 1092 proteins are collected in this dataset. The number of Benign, Stage 1, Stage 2, Stage 3 and Stage 4 tumor samples are 40, 101, 17, 24 and 31 respectively.

2. **OVC Dataset:** This dataset contains 845 proteins’ expressions for 248 samples across Benign and 3 different stages of ovarian cancer. The number of Benign, Stage 1, Stage 2 and Stage 3 tumor samples are 134, 45, 8 and 61 respectively.

3. **CRC Dataset (GSE157563):** This microarray dataset contains 177 samples from 4 different stages (Stage 1 to Stage 4) of CRC. Expression levels of 20125 genes are collected. Besides stage information, this dataset also has records for each patient, the binary valued information of “Cancer Recurrence” and “Death from Cancer”. Out of 177 patients, 55 had recurrence of cancer and 68 died from cancer.

### 3.2 Grouping of Features

In order to group the genes using gene ontology terms, we use the web based tool “Database for Annotation, Visualization, and Integrated Discovery” (DAVID, http://david.abcc.ncifcrf.gov/) [Dennis Jr et al., 2003]. There are a set of parameters that can be adjusted in DAVID based on which the functional classification is done. This whole set of parameters is controlled by a higher level parameter “Classification Stringency”, which determines how tight the resulting groups are in terms of association of the genes in each group. In general, a “High” stringency setting generates less number of functional groups with the member genes tightly associated and more genes will be treated as irrelevant ones into an unclustered group. We set the stringency level to “Medium” which results in balanced functional groups where the association of the genes are moderately tight. For the CRC dataset, functional classification using BP, CC and MF results in total 2434, 520 and 1146 groups respectively. The total number of groups for BP, CC and MF annotations on RCC and OVC datasets are 890, 56, 155 and 321, 23, 56 respectively.
3.3 Protein Protein Interactions

Besides using it for selecting informative single gene features, we use Overlapping Group Lasso to select the informative protein protein interactions. We download the binary protein protein interactions (PPI) data from HPRD(http://www.hprd.org/). For each group $G_i$ in a particular functional grouping of the genes (i.e. BP, CC or MF), we identify the pairs of member genes of $G_i$ whose products interact directly with each other in the PPI network. The set of all such pairs where both interacting partners are members of $G_i$ forms a group. For a pair of interacting genes $x_j$ and $x_k$ in a group, we use their quadratic interaction term $g(x_j, x_k)$ as their expression level. Usage of the quadratic interaction formulation in Overlapping Group Lasso helps us to integrate the resulting informative protein protein interactions into the formulation of QUIRE directly without any transformation. Thus the total number of groups is same in the case of interactions and single gene features. But the cardinality of each group and the expression levels of the members are different.

3.4 Experimental Design

In order to systematically evaluate the classification performance of QUIRE, we perform the following classification experiments:

1. Classification experiments using RCC samples: We perform three stage-wise binary classification experiments using RCC samples:
   a. Classification of Benign samples from Stage 1 – 4 samples.
   b. Classification of Benign and Stage 1 samples from Stage 2 – 4 samples.
   c. Classification of Benign, Stage 1, 2 samples from Stage 3, 4 samples.

2. Classification experiments using OVC samples with intermediate levels of CA125 as test set. CA125 is a well-known marker in ovarian cancer [Suh et al. 2010]. Concentration of CA125 is used to measure the progression of the disease. The suspicious cutoff level of CA125 is 40, meaning that concentration level above 40 of this marker might be indicative of OVC. But CA125 is not a good indicator of early detection of the disease onset, especially when the concentration of this biomarker is between 40 and 100. So we use samples with CA125 concentration level between 40 and 100 as our test set in this experiment. The remaining samples, with concentration of CA125 below 40 and above 100 are used as training set. We perform the following experiments:
   a. Classification of Benign samples from Stage 1 – 3 samples.
   b. Classification of Benign, Stage 1 samples from Stage 2, 3 samples.
   c. Classification of Benign, Stage 1, 2 samples from Stage 3 samples.

3. Classification experiments using CRC samples: We perform two classification experiments using the samples with CRC:
   a. Prediction of cancer recurrence: we build binary classifier to predict whether there is disease free survival in the follow-up time or not.
   b. Prediction of death from colorectal cancer: we train binary classifier to predict if there is death from CRC across all pathological stages of the disease.

3.5 Classification performance of QUIRE

In this section, we report systematic experimental results on classifying samples from different stages of RCC and OVC and in predicting CRC recurrence and death from CRC. In the first stage of QUIRE, we use Overlapping Group Lasso to identify the biologically relevant groups of features and pairwise protein interactions, which in turn, is used in the subsequent stage to explore the set of informative markers and quadratic interactions. However, for the RCC and OVC datasets, we do not use protein protein interactions for prediction purpose. This is because, these datasets include only selected marker proteins distributed sparsely across the protein interaction network and thus most of them do not interact with each other directly.

After we run Overlapping Group Lasso on the gene groups, we sort the genes based on the weight value assigned to it by the algorithm. We select top $m = 200$ genes as input to QUIRE for enumerating all pairwise quadratic interactions. We report the effect of $m$ on the classification performance of QUIRE later in this section. For classification of CRC samples, Overlapping Group Lasso on average selects 1000 protein protein interactions as informative ones. We use this whole set of selected protein interactions as input to QUIRE to be considered besides the paired quadratic interactions. In this section, we present Overlapping Group Lasso’s and QUIRE’s performance for features grouped using Cellular Colocalization (CC) ontology, as this grouping strategy shows best classification performance among the three we consider.

The predictive performance of the markers and pairwise interactions selected by QUIRE is compared against the markers selected by Lasso, SVM and Overlapping Group Lasso. We use glmnet [Friedman et al. 2010] and Liblinear [Fan et al. 2008] packages for implementation of Lasso and SVM respectively. We use the Group Lasso implementation (with overlapping groups) from Jacob et al. [2009]. The overall performance of the algorithms are shown in Figure 2. In this figure, we report average AUC score for ten runs of five fold cross validation experiments for cancer stage prediction in RCC (Figure 2(A)) and for predicting cancer recurrence and death from cancer in CRC(Figure 2(C)). In five fold cross validation experiments, we divide the samples equally into five disjoint sets or folds. We keep one fold for testing. On the remaining four folds, we use Overlapping Group Lasso to identify the informative set of markers and protein protein interactions (for CRC). We train QUIRE on these four folds using these markers to identify the pairwise interactions and markers and use the set-aside test set for prediction purpose. For each run, this procedure is repeated for each of the five folds and average AUC score is reported for ten such runs. For OVC, we report average AUC score (Figure 2(B)) for predicting the cancer stage of the samples with intermediate levels of CA125 (concentration of CA125 is between 40 and 100) using the remaining samples for training and informative feature selection.
In cancer stage prediction experiments for RCC and OVC, we see from Figure 2 that the combination of informative markers and pairwise interactions identified by QUIRE show better classification performance in every case, as compared to the markers selected by Lasso, SVM and Overlapping Group Lasso. For early detection of the diseases (classification of Benign, Stage 1 vs. rest of the samples), QUIRE achieves average AUC scores of 0.88 and 0.82 for RCC and OVC respectively. Overlapping group lasso shows next best performance with average AUC scores of 0.83 and 0.80 respectively. Lasso and SVM, which do not use any grouping or interaction information amongst the features, show the worst performance in all of the classification tasks apart from one. As QUIRE markers show consistently better performance across all the stages of RCC and OVC, they can be used for improved diagnosis and prognosis of these two different types of cancers. Also QUIRE helps better prediction of OVC progression for samples with intermediate levels of CA125; so it can be used by the physicians for early detection of this disease.

From Figure 2(C), we can see that gene-gene interactions help us better predict both CRC recurrence and death from CRC, as compared to the other feature selection mechanisms. In the events of cancer recurrence and death from cancer, the average AUC values achieved by features selected with QUIRE are 0.79 and 0.81 respectively, while markers identified by Overlapping Group Lasso show the next best performance with average AUC value of 0.71 in both of these categories. Markers identified by Lasso show the worst performance in prediction of both of these events. The performance gap between QUIRE and the other three popular feature selection techniques hint to the fact that QUIRE can identify interactions that might help us better understand the mechanistic basis of CRC.

These experimental results show that QUIRE identifies markers and interactions that complement each other in such a way that they not only help better diagnosis and prognosis of cancer, but also can predict the advanced events of recurrence of cancer and survival after cancer with higher accuracy than other state-of-the-art algorithms. For each of these datasets, identification of informative pairwise interactions using brute force enumerative technique is computationally impractical due to the huge dimensionality of the search space. QUIRE helps reducing this space by a large margin. The total running time of QUIRE is dominated by the Overlapping Group Lasso stage which takes around one hour to identify biologically relevant groups of genes and protein interactions in traditional desktop computers for the types of problems we study. After the dimensionality is reduced, QUIRE exhaustively enumerates all the pairwise interactions and use the protein interactions identified in the previous stage on this low dimensional space in a couple of minutes.
### 3.6 Informative QUIRE markers and interactions associated with cancer

Cancer is a genetic disease, which originates and develops through a process of mutations. Mutations in individual genes not only disrupt its own function, but also affect its interaction patterns with other genes. As complex diseases like cancer is a result of dysregulation in the interactions among the genes, researchers focus on identifying those relevant interactions to gain more insight into the molecular basis of the disease. On the CRC dataset, QUIRE selects about 120 quadratic interactions on average as informative ones for both CRC recurrence and death from CRC. On the other hand, the average number of markers selected by Overlapping Group Lasso and Lasso on the same prediction tasks are about 1100 and 150 respectively.

An investigation of the pairwise interactions identified by QUIRE on CRC dataset reveals that many of these interactions are indeed relevant to the progression of cancer in general. Some of such interactions identified for prediction of CRC recurrence include JAK2 - LYN [Samanta et al. 2009]. Transforming growth factor beta (TGF\beta) - SMAD [Grady 2005]. Epidermal growth factor receptor (EGFR) - Caveolin (CAV) [Dittmann et al. 2009], TP53 - TATA binding protein (TBP) [Crighton et al. 2003]. Connective tissue growth factor (CTGF) - Vascular endothelial growth factor (VEGF) [Inoki et al. 2002], Edoglin (ENG) - Transforming growth factor beta receptor (TGF\beta R) [Fonsatti et al. 2003]. Further investigations of the interactions identified by QUIRE might reveal novel gene partners associated with cancer and thus lead to testable hypothesis.

Disruption in pairwise interactions among the genes affects the pathways in which they are located in. Cancer pathways are a set of pathways dysregulations in which have been shown to be associated with initiation and progression of the disease. A pictorial view of the well-known cancer pathways can be found in the KEGG database [http://www.genome.jp/kegg/pathway/hsa05200.html] [Kanehisa et al. 2012]. We perform a pathway enrichment analysis where we test if the set of the markers and interactions identified by QUIRE on the CRC dataset reside in the cancer pathways. As part of this experiment, we first use the partner genes identified by QUIRE as part of the informative interactions while predicting CRC recurrence. We use DAVID to identify the statistically significant pathways that are enriched in these genes. An investigation of the enriched pathways returned by DAVID indicates that many of them are indeed responsible for cancer or related to functions dysregulation in which results in cancer. Some of such KEGG pathways include Apoptosis(p-value 4.7x10^{-4}), Focal adhesion(p-value 3x10^{-3}), Cell adhesion molecules(p-value 9.2x10^{-3}), p53 signaling pathway(p-value 1.3x10^{-2}), Gap junction (p-value 1.3x10^{-2}), MAPK signaling pathway (p-value 4.5x10^{-2}), ErbB signaling pathway (p-value 5.8x10^{-2}), Cell cycle (p-value 6.6x10^{-2}), Pathways in cancer (p-value 7.2x10^{-3}), Colorectal cancer (p-value 10^{-3}). Repeating the same analysis on the interacting partners identified by QUIRE while predicting “Death from CRC” result in identification of similar pathways (data not shown here).

Next we use the informative genes and their associated interactions discovered by QUIRE to identify functional modules that might be associated with pathways known to be dysregulated in cancer. We use the web based tool Gene Mania [www.genemania.org] [Warde-Farley et al. 2010] to identify the statistically significant modules induced by genes and interactions selected by QUIRE. Gene Mania also returns the pathways and functions in which the identified modules are significantly enriched. After investigating these functional modules, we find that many of them are enriched in the well-known cancer pathways. Examples of such pathways include Focal adhesion pathway (p-value 2x10^{-3}), Jak-STAT signaling pathway (p-value 3x10^{-2}), MAPK signaling pathway (p-value 1.4x10^{-3}), NF-kappaB signaling pathway (p-value 4.5x10^{-2}), TGF beta signaling pathway (p-value 2.2x10^{-3}) and Ras protein signaling pathway (p-value 1.3x10^{-3}). Besides, some of the induced modules are functionally enriched in processes disruptions in which are known to be associated with initiation and progression of cancer. Some examples of such functions include Apoptosis (p-value 4.2x10^{-3}), Cell migration (p-value 1.3x10^{-3}), Response to growth factors (p-value 2.5x10^{-2}), Cell cycle checkpoint (p-value 10^{-3}), Cell-cell adhesion (p-value 3.1x10^{-3}) etc. We give examples of some of these modules in Figure 5.

### 3.7 Characteristic Features of QUIRE

#### 3.7.1 Effect of parameter \( m \): In this section, we perform classification experiments to understand the effect of using different number of input genes to QUIRE as identified by Overlapping Group Lasso. QUIRE enumerates all possible pairwise interactions among these input genes and returns the ones selected to most informative. For this experiment, we increase the values of \( m \) from 100 to 400 in steps of 100 and we run QUIRE using these top \( m \) genes. We use the whole set of protein protein interactions identified by Overlapping Group Lasso in all the experiments. We report the average AUC score for ten runs of five fold cross validation score on classifying the CRC samples for prediction of CRC recurrence in Table 1. From this table, QUIRE shows best performance when the value of \( m \) is 200. The performance gets worse as we increase the \( m \). The performance decrease may be attributed to the mechanism by which Overlapping Group Lasso selects the groups. As sparsity is enforced at the group level in Overlapping Group Lasso, some member genes within the selected groups may be less informative about the phenotype and as a result, their interactions result in reduced prediction accuracy of QUIRE.

#### 3.7.2 Usage of different objective functions: We test the effect of using different types of objective functions on the performance of QUIRE. In our formulation of QUIRE, we use linear regression setting in the objective function. The other option is to use logistic regression. We perform classification experiments on CRC dataset which show that the linear regression setting results in better performance on prediction of both CRC recurrence and death from CRC, compared to the case where we use logistic regression.

| Value of \( m \) | Avg AUC score |
|---------------|----------------|
| 100           | 0.77           |
| 200           | 0.79           |
| 300           | 0.77           |
| 400           | 0.73           |
formulation in the objective function. We present the results from ten runs of five fold cross validation classification experiments in Table 2 where we show average AUC score both for linear and logistic regression formulation of QUIRE.

**Table 2.** Classification performance of QUIRE with Linear Regression and Logistic Regression formulations

| Classification Experiment | Logistic Reg. | Linear Reg. |
|---------------------------|---------------|-------------|
| CRC Recurrence            | 0.69          | 0.79        |
| Death from CRC            | 0.69          | 0.81        |

3.7.3 Quadratic feature interaction function: Next, we perform classification experiments to show the effect of our proposed quadratic feature interaction $g(x_j, x_k)$ compared to $g(x_j)g(x_k)$. The results from the ten runs of five fold cross validation classification experiments for prediction of CRC recurrence and death from CRC are shown in Table 3. The AUC scores in the experimental results show that our proposed original feature interaction formulation $g(x_j, x_k)$ is more effective in predicting post cancerous events than the alternate weight sharing formulation $g(x_j)g(x_k)$.

**Table 3.** Classification performance of QUIRE with different feature interaction mechanisms.

| Classification Experiment | $g(x_j)g(x_k)$ | $g(x_j, x_k)$ |
|---------------------------|----------------|--------------|
| CRC Recurrence            | 0.71           | 0.79         |
| Death from CRC            | 0.70           | 0.81         |

3.7.4 Performance of single gene features: We now illustrate the effects of using pairwise interaction in combination with the genes identified by Overlapping Group Lasso as informative ones. For this experiment, we use the group of genes discovered by Overlapping Group Lasso as “Single Gene” features in Lasso setting and compare it to the case where we additionally include pairwise interactions among these features and the protein protein interactions, as formulated in the objective function of QUIRE.

We present results from ten runs of five fold cross validation classification experiments on CRC dataset in Table 4. The improved classification performance of the combination of single genes and pairwise interactions show that these interactions indeed provide additional information about the underlying biological phenomenon which cannot be captured by using single gene markers alone.

**Table 4.** Classification experiment to illustrate the effect of using pairwise gene interactions.

| Classification Experiment | Single Gene Features | QUIRE |
|---------------------------|----------------------|-------|
| CRC Recurrence            | 0.70                 | 0.79  |
| Death from CRC            | 0.70                 | 0.81  |

3.7.5 Performance of protein protein interactions: Finally, we perform classification experiments to observe the performance of protein protein interactions on predicting CRC recurrence and death from CRC. For this experiment, we use the single gene markers and the protein protein interactions selected by Overlapping Group Lasso as input to QUIRE and enumeration of the pairwise interactions among the marker genes is avoided. For ten runs of five fold cross validation experiment on this modified feature set, we observe average AUC score of 0.71 for both classification tasks. These results show that besides physical interactions, indirect higher level interactions among the genes must be taken into account to understand the basic mechanism of complex diseases.

4 CONCLUSION

In this paper, we propose a computational approach, QUIRE, to identify combinatorial interactions among the informative genes in complex diseases, like cancer. Our algorithm uses Overlapping Group Lasso to identify functionally relevant gene markers and protein interactions associated with cancer. It then explores the pairwise interactions among these relevant genes within this reduced space exhaustively and the selected pairwise physical protein interactions to discover the combination of individual markers and gene-gene interactions that are informative for prediction of the disease status of interest. The application of QUIRE on three different types of cancer samples collected using two different
techniques shows that our approach performs significantly better than the state-of-the-art feature selection methods such as Lasso and SVM for biomarker discovery while selecting a smaller number of features, and it also shows that our approach can capture discriminative interactions with high relevance to cancer progression. Further investigations show that QUIRE can identify markers and interactions that have been associated previously with pathways associated with cancer. Moreover, the good performance of QUIRE on the CRC dataset suggests that applications of QUIRE on genome-wide microarray experimental data can be used to help prioritize Somamer design for blood-based cancer diagnosis. And QUIRE applied to blood-based experimental data has the great potential to impact the field of practical medical diagnosis.

However, QUIRE performs search for informative interactions on a lower dimensional space, which means that there is potential to miss interesting interactions relevant to the diseases. Extension of QUIRE to explore the whole genome wide interaction space will enable it to identify markers and interactions of more biological relevance. And more detailed investigations of the identified pairwise interactions among the informative genes will shed more light into the dynamics of the complex diseases.

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