An integrative scoring system for ranking SNPs by their
total deleterious effects
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ABSTRACT

Motivation: Identifying single nucleotide polymorphisms (SNPs) that underlie common and complex human diseases, such as cancer, is of major interest in current molecular epidemiology. Nevertheless, the tremendous number of SNPs on the human genome requires computational methods for prioritizing SNPs according to their potentially deleterious effects to human health, and as such, for expediting genotyping and analysis. As of yet, little has been done to quantitatively assess the possible deleterious effects of SNPs for effective association studies.

Results: We propose a new integrative scoring system for prioritizing SNPs based on their possible deleterious effects within a probabilistic framework. We applied our system to 580 disease-susceptibility genes obtained from the OMIM (Online Mendelian Inheritance in Man) database, which is one of the most widely-used databases of human genes and genetic disorders. The scoring results clearly show that the distribution of the functional significance (FS) scores for already known disease-related SNPs is significantly different from that of neutral SNPs. In addition, we summarize distinct features of potentially deleterious SNPs based on their FS score, such as functional genomic regions where they occur or bio-molecular functions that they mainly affect. We also demonstrate, through a comparative study, that our system improves upon other functional-assessment systems for SNPs, by assigning significantly higher FS scores to already known disease-related SNPs than to neutral SNPs.


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1 INTRODUCTION

Much effort in current epidemiology, medicine, and pharma
genomics is focused on the identification of genetic variations that are involved in common and complex diseases. In particular, single nucleotide polymorphisms (SNPs), which are substitutions of single nucleotides at specific positions on the genome occurring in more than 1% of the human population, are at the forefront of such studies, as they are the most common form of genetic variations on the genome. Knowledge of disease-causing SNPs is expected to enable early diagnosis, effective treatment, and ultimately, prevention of target disease.

Typically, the first step toward identifying causal SNPs for common and complex human diseases, involves association studies. However, due to the tremendous number of SNPs on the human genome, estimated at over ten million (Sherry et al., 2001), there is a clear need to prioritize SNPs based on their potential deleterious functional effects (Bhatti et al., 2006). For instance, SNPs occurring in functional genomic regions such as protein coding or regulatory regions are more likely to have deleterious effects, and, as such, more likely to underlie disease. By focusing on a small number of these functionally significant SNPs that are likely to be involved in disease, a substantial amount of genotyping and analysis overhead can be reduced.

To pursue this aim, a variety of web services and public databases have been recently introduced to prioritize SNPs by their putative deleterious effects on major bio-molecular functions (for review, see Rebbeck et al. (2004)). These tools examine the functional category of genomic regions where each SNP occurs, such as exons, splice sites, or transcription regulatory sites, and predict the potential corresponding functional effects that the SNP may have, using a variety of machine-learning approaches. These computational methods, along with other tools in molecular genetics and epidemiology, are expected to enhance the identification of SNPs underlying human diseases (Rebbeck et al., 2004).

Yet, such tools and systems, which prioritize functionally significant SNPs, still suffer from two main limitations. First, most systems examine the putative deleterious effects of SNPs with respect to only a single biological function, such as protein coding or splicing regulation (but not both). Thus, to comprehensively analyze the functional significance of SNPs, researchers must spend much time and effort to separately apply multiple tools, and interpret/integrate their (often conflicting) predictions.

Second, while many systems classify SNPs into qualitatively distinct groups (e.g., ‘deleterious’ vs. ‘neutral’), few systems numerically score or rank SNPs according to their functional significance. Budget considerations often force researchers to select a limited number of SNPs on the target genomic region for conducting association studies. When the number of putatively deleterious SNPs presented by current tools is larger than this pre-specified limit, without additional ranking information, selecting only some of them is not straightforward. As a result, researchers must rely on other resources, such as the published literature, to finalize their decision.

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To address these limitations, we propose a new integrative scoring system for ranking SNPs based on their putative deleterious effects. We aim to provide more comprehensive information about the functional significance of SNPs. We thus assess the deleterious effects of SNPs with respect to four major bio-molecular functional categories: splicing, transcription, translation, and post-translational modification. We attempt to overcome the incompleteness and possible false findings of any individual bioinformatics tool by combining the assessment results from multiple independent prediction tools within a probabilistic framework. Most significantly, we assign a specific numerical score to each SNP, representing its putative deleterious effects. Using this score, a limited subset of the most functionally significant SNPs can be ranked and selected.

We applied our system to 112,949 SNPs located on 580 disease-susceptibility genes obtained from the OMIM database. Consistent with previous findings (Xu et al., 2005), our results show that splice sites and exonic regions are most enriched for potentially deleterious SNPs. We further demonstrate the utility of our scoring system by showing that the functional significance score of known disease-related SNPs from OMIM is significantly higher than the score assigned to randomly selected neutral SNPs. We also show the improved performance of our system through a comparative study based on two evaluation measures. Finally, we discuss the impact of our work, and outline directions for future research.

2 PROBLEM DEFINITION

We aim to quantitatively measure the potential deleterious effects of SNPs on the bio-molecular function of their genomic region. For simplicity, we refer to the assessed score as the functional significance (FS) score of each SNP.

To formally define a scoring function for calculating the FS score, we first introduce basic notations. Suppose that we are given \( p \) SNPs on the target genomic region. Each SNP can be represented as a discrete random variable, \( X_i \) \((i = 1, \ldots, p)\), whose possible values are the 4 nucleotides, \{a, c, g, t\}. The true (and unknown) functional category of SNP \( X_i \) is then represented by another discrete random variable \( Y_i \), whose value is 1 when SNP \( X_i \) is deleterious and 0 otherwise. We note that we do not know the true functional category \( Y_i \) of SNP \( X_i \) in most cases. We thus estimate it using \( q \) bioinformatics tools that predict, for each SNP \( X_i \), the functional label (i.e., "deleterious" or "neutral") along four major bio-molecular functions: protein coding, splicing regulation, transcriptional regulation, or post-translational modification.

For each of the \( p \) SNPs and \( q \) tools, we define two random variables, \( \delta_{ij} \) and \( S_{ij} \) \((i = 1, \ldots, p; \ j = 1, \ldots, q)\). The variable \( \delta_{ij} \) denotes the label assigned to the \( i^{th} \) SNP by the \( j^{th} \) tool, that is, \( \delta_{ij} = 1 \) when the \( j^{th} \) tool predicts SNP \( X_i \) to be deleterious, and 0 otherwise. The variable \( S_{ij} \) represents the tool’s own confidence score with respect to the assigned label. The higher the value of \( S_{ij} \), the more strongly the tool supports its own prediction, \( \delta_{ij} \). As different tools use different confidence scales, we define another random variable, \( S_{ij} \), representing a normalized confidence score. The normalization procedure is explained in Section 3.

We also define a random variable, \( F_{jk} \), to indicate the bio-molecular functions that each tool examines. We define the set \( F \) = \{ "protein coding", "splicing regulation", "transcriptional regulation", "post-translational modification" \} consisting of the four bio-molecular functions with which we are concerned. For each of the \( q \) tools and four bio-molecular functions in \( F \), a random variable \( F_{jk} \) \((j = 1, \ldots, q; \ k \in F)\) is defined such that its value is 1 when the \( j^{th} \) tool examines the deleterious effects of SNPs on function \( k \), and 0 otherwise.

Last, for each tool, we define a continuous random variable \( TR_{ij} \) \((j = 1, \ldots, q)\), corresponding to the tool reliability (TR) score for the \( j^{th} \) tool.

2.1. Compute Tool Reliability Scores based on the Agreement of Each Tool with Other Tools

\[ TR_{ij} = \frac{1}{q} \sum_{k \in F} F_{jk} \cdot TR_{ij} \]

2.2. Normalize Confidence Scores according to the Predicted Functional Labels and Conserveness

\[ S_i = \frac{\max_{j=1}^q F_{ij} \cdot TR_{ij} \cdot (\delta_{ij} \cdot S_{ij})}{\sum_{j=1}^q F_{ij} \cdot TR_{ij}} \]

That is, for each bio-molecular functional category \( k \), we compute the weighted average of the confidence of each prediction tool with respect to the deleterious\(^{1}\) effect of the SNP, where the weight is the reliability score of each tool. Note that although summation is done over all the tools (i.e., \( j = 1 \) to \( q \)), where \( q \) is the total number of tools, regardless of the bio-molecular functional categories that they examine), \( F_{jk} \) allows only the ones that examine the specific bio-molecular functional category \( k \) to be considered. The maximum score, over all examined bio-molecular functions, is then assigned as the FS score for the SNP.

3 METHODS

Our system conducts three main steps to calculate the FS score of SNPs, as outlined in Figure 1. In step I, the functional labels for the SNPs (i.e., 'deleterious' or 'neutral'), predicted by \( q \) bioinformatics tools, are retrieved. Confidence scores associated with these predictions are also retrieved, when available. In step II-1, the reliability score of each tool is computed based on its tendency to agree with other tools’ predictions. In step II-2, the confidence scores, obtained in step I, are normalized to a value between 0 and 1, as explained below. In step III, the FS score of SNPs is computed as shown in Definition 2.1. We further describe each step below.

\(^{1}\) We note that by multiplying by \( \delta_{ij} \), the confidence score of each tool is counted only when the tool predicts the SNP to be deleterious.
STEP 1. Retrieving Predicted Labels and Scores

Given a set of $p$ SNPs, $\{X_1, \ldots, X_p\}$, we first retrieve their predicted functional labels (i.e., ‘deleterious’ or ‘neutral’) and corresponding confidence scores from 16 publicly available web services and databases, as illustrated in Figure 2. We obtain the genomic location of each SNP (e.g., exon, intron, splice site, 5’/3’ un-translated regions of a gene, or directly upstream or downstream from a gene) from the dbSNP database (build 126) (Sherry et al., 2001). According to the genomic location, each SNP is examined for its possible deleterious effects with respect to the corresponding bio-molecular functional category as follows:

- **Protein Coding**: SNPs in exonic regions may alter protein structure and/or function by creating a new start or stop codon (i.e., nonsense SNPs) or a deleterious amino acid substitution (i.e., missense SNPs);
- **Splicing Regulation**: SNPs in (canonical) splice sites may disrupt splicing regulation, resulting in exon skipping or intron retention. SNPs in exonic splice sites may interfere with alternative splicing regulation by changing exonic splicing enhancers or silencers;
- **Transcriptional Regulation**: SNPs in transcription regulatory regions (e.g., transcription factor binding sites, CpG islands, microRNAs, etc.) can alter binding sites, and thus disrupt proper gene regulation;
- **Post-Translational Modification**: SNPs in protein coding regions may alter post-translational modification sites, interfering with proper post-translational modification.

As shown in Figure 2, the confidence scores for the SNPs that create a new start or stop codon (i.e., nonsense SNPs) or the SNPs that occur in the first two or in the last two bases of intronic splice sites (i.e., canonical splice sites) are set to one. This is because their deleterious effects to either Protein Coding or Splicing Regulation is unequivocal. Nonsense SNPs lead to a premature termination of amino acid peptides, often resulting in loss of protein function (Yamaguchi-Kabata et al., 2008). The change to the canonical splice sites is also known to be detrimental as suggested by the high selection pressure on the splice sites among mammalian genomes (Busset et al., 2000). We note that other SNP prioritization studies (Bhattacharyya et al., 2006; Yuan et al., 2006; Xu et al., 2005) assign the highest rank or score of functional impact to these two kinds of SNPs, as well. For the remaining cases, the confidence scores are obtained from the tools that are used to assess the corresponding bio-molecular functions.

**STEP II-1. Computing Tool Reliability**

The tool reliability score, $TR_j$, denotes how likely the $j^{th}$ tool is to correctly predict deleterious SNPs ($j = 1, \ldots, q$). We express the tool reliability score using the conditional probability as defined below:

$$ TR_j \triangleq Pr(Y_j = 1 \mid \delta_{ij} = 1). $$

That is, for each tool $j$, we calculate the conditional probability of any SNP $X_i$ to actually be deleterious ($Y_j = 1$) when the tool predicts so ($\delta_{ij} = 1$). If the true labels of the SNPs, $Y_1, \ldots, Y_p$, are known, this score can be statistically estimated. For example, using a maximum likelihood approach, $TR_j$ can be estimated as the ratio between the number of correctly predicted deleterious SNPs and the total number of SNPs predicted to be deleterious by the tool. However, in most cases we do not know the true functional categories of SNPs. We thus estimate the probability $Pr(Y_j = 1 \mid \delta_{ij} = 1)$ using the theoretical work proposed by Long and his colleagues on classification (Long et al., 2005). When class labels are unknown, they propose to estimate the prediction accuracy of a classifier based on the extent to which the classifier tends to agree with other classifiers. They prove that the conditional probability $Pr(\delta_{ij} = 1 \mid Y_j = 1)$ can be calculated in this context as follows:

$$ Pr(\delta_{ij} = 1 \mid Y_j = 1) = 
\left(1 - Pr(Y_j = 1)\right) \frac{Pr(\delta_{ij} = 1) I_{\delta_{ij} \neq \delta_m} - Pr(\delta_{ij} = 1) I_{\delta_{ij} = \delta_m}}{Pr(Y_j = 1) - Pr(\delta_{ij} = 1) I_{\delta_{ij} \neq \delta_m} - Pr(\delta_{ij} = 1) I_{\delta_{ij} = \delta_m}}. $$

(1)

where $m$ and $n$ represent the indices of any two distinct tools ($m \neq n \neq j$), $u_{jm} \triangleq Pr(\delta_{ij} = 1, \delta_{ij} = 1)$, and $u_{jn} \triangleq Pr(\delta_{ij} = 1)$. For the detailed proof of Equation (1), we refer to the work by Long et al. (2005). Using Bayes’ rule and Equation (1), we compute the tool reliability score of the $j^{th}$ tool, $TR_j$, as follows:
STEP II-2. Normalizing Confidence Scores

To account for the fact that different tools use different scales to report their confidence scores, we normalize the obtained confidence scores \( S_{ij} \) to be between 0 and 1 as follows:

\[
S_{ij} = \frac{1}{2} \left( \delta_{ij} + (1 - C_{ij}) \cdot \frac{(S_{ij} - \min S_{ij})}{\max S_{ij} - \min S_{ij}} \right)
\]

where

\[
\begin{align*}
C_{ij} &= 1 \quad \text{if } X_i \text{ resides on a nonconserved regulatory site;} \\
C_{ij} &= 0 \quad \text{otherwise},
\end{align*}
\]

and \((1 \leq i \leq p; 1 \leq j \leq q)\). In principle, when SNP \( X_i \) is predicted to be deleterious (i.e., \( \delta_{ij} = 1 \)), the confidence score \( S_{ij} \) is converted to a value between 0.5 and 1; otherwise (i.e., \( \delta_{ij} = 0 \)), \( S_{ij} \) is converted to a value between 0 and 0.5. We note that, for the SNPs occurring in regulatory regions, we examine whether the SNP’s region is conserved across multiple species (i.e., chimpanzee, dog, mouse, rat, chicken, zebrafish, and fugu) – information that is obtained from GoldenPath (Kuhn et al., 2007) – to reduce the effects of possible false-positive predictions. As is widely known, there is a high rate of false positive findings of regulatory sites by in silico prediction tools due to their relatively short length of DNA sequences (typically 6 to 10-mers) (Zhang et al., 2003). Thus, when the predicted regulatory sites are not within a conserved region, the confidence score for the SNPs in the region is set to 0.5, reflecting our uncertainty regarding the functionality of the region, and the consequent, lack of confidence about potential deleterious effects of SNPs on the function.

We also note that some prediction tools, such as SNPeff (Reumers et al., 2005) or LS-SNP (Karchin et al., 2005), do not provide confidence scores. For these systems, we impute the confidence scores using the confidence scores for the same SNP obtained from other tools. Suppose that the \( j^{th} \) tool, which examines the possible effects of SNP \( X_i \) on the bio-molecular functional category \( k \), does not provide a confidence score on its prediction. Among the other tools that provide the confidence scores for the same function \( k \), let us denote the index of the tool whose tool reliability score is highest as \( t \). The imputed value is calculated as:

\[
S_{ij} = \max \left( \frac{TR_j}{TR_t}, S_{it}, 1 \right).
\]

That is, when the \( j^{th} \) tool is more reliable than the \( t^{th} \) tool (i.e., \( TR_j > TR_t \)), its confidence score would be imputed to be higher than that of the \( t^{th} \) tool, but not greater than one. Otherwise (i.e., \( TR_j \leq TR_t \)), the confidence score would stay the same or be reduced proportionally to the ratio of the respective tool reliabilities.

STEP III. Computing Functional Significance

Given the prediction results obtained in step I and the tool reliability and normalized confidence scores computed in step II, the functional significance (FS) score of SNP \( X_i \) is computed as stated earlier in Definition 2.1.

4 EXPERIMENTS AND RESULTS

We applied our method to 112,949 SNPs located on 580 disease-susceptible genes for which the OMIM database references the biomedical literature that report the existence of SNPs on these genes that are either disease-causing or associated with common disorders. The list of SNPs linked to the 580 genes, along with their primary information (e.g., genomic location), were downloaded from the dbSNP database (build 126) (Sherry et al., 2001). The number of known disease-causing or disease-associated SNPs on these 580 genes is 1,399 (the list was obtained from ftp://ftp.ncbi.nih.gov/snp/database/organism_data/human_9606/OMIM-VarLocusdSNP/ftp.pgp.gz). The remaining 111,550 SNPs are not yet identified to be related to disease. For simplicity, we refer to the former set (of 1,399 SNPs) as disease-related SNPs, and the latter set (of 111,550 SNPs) as neutral SNPs. We note, however, that currently known disease-related SNPs can explain only a fraction of the genetic basis of human disease, and as such, the latter set of SNPs that are temporarily classified as neutral may include functionally significant SNPs with deleterious effects that are not yet identified.

In Section 4.1, we summarize the scoring results by our system for all 112,949 SNPs, and show the distinguishing features of disease-related SNPs compared to neutral SNPs. In Section 4.2, we further validate that our integrative scoring system improves upon the state-of-the-art when applied to the same set of SNPs.

4.1 Review of the Scoring Results

First, we examine the scores that our system assigns to disease-related SNPs compared to neutral SNPs. Figure 3 shows the distribution of the FS score for disease-related SNPs (shown on the left) along with that of neutral SNPs (shown on the right). The figure clearly shows that the distribution of the FS scores for disease-related SNPs is significantly different from that of neutral SNPs.

\[\text{5 The difference is also statistically significant, with a p-value of practically 0, according to the Kolmogorov-Smirnov two-side test with } \alpha = 0.05\]
The distribution of low FS scoring vs. high FS scoring SNPs based on functional genomic locations. The X-axis denotes 6 types of genomic regions that are used in the decision procedure (shown in Figure 2), while the Y-axis shows the percentage of SNPs whose FS scores are at least 0.5 (black bars) vs. the percentage of SNPs whose scores are lower than 0.5 (gray bars) on each region type.

As is clear from the data shown above, most disease-related SNPs are located within exons (94.21%). This is indeed expected, as most association studies that validated these SNPs to be disease-related, have focused on protein coding SNPs, whose functional effects are relatively easy to pinpoint due to their direct impact on protein products. Aside for the outstanding proportion of exonic SNPs, disease-related SNPs show a similar scoring pattern to that of neutral SNPs. Most SNPs on intronic, 5’/3’ UTR, and up/downstream regions are assigned an FS score lower than 0.5, but more than half of SNPs in exonic regions (53.04%) and all SNPs in canonical splice sites and to 46.07% of the SNPs in coding regions. This scoring pattern is consistent with previous findings that mutations in splice sites and coding regions are likely to have direct impact on gene function (Yue et al., 2006; Cartegni et al., 2003; Reumers et al., 2005).

Meanwhile, Figure 4(b) shows the FS score distribution for disease-related SNPs as a function of their genomic regions. In contrast to the case for neutral SNPs, shown above, most disease-related SNPs are located within exons (94.21%). This is indeed expected, as most association studies that validated these SNPs to be disease-related, have focused on protein coding SNPs, whose functional effects are relatively easy to pinpoint due to their direct impact on protein products. Aside for the outstanding proportion of exonic SNPs, disease-related SNPs show a similar scoring pattern to that of neutral SNPs. Most SNPs on intronic, 5’/3’ UTR, and up/downstream regions are assigned an FS score lower than 0.5, but more than half of SNPs in exonic regions (53.04%) and all SNPs in canonical splice sites and to 46.07% of the SNPs in coding regions. This scoring pattern is consistent with previous findings that mutations in splice sites and coding regions are likely to have direct impact on gene function (Yue et al., 2006; Cartegni et al., 2003; Reumers et al., 2005).

As shown in Figure 4(a), the majority of neutral SNPs are located within intronic regions, and the FS score for most intronic SNPs is lower than 0.5 (80.67%). A similar tendency is noted in 5’/3’ untranslated regions (UTR), in regions that are upstream or downstream from genes, and in currently unspecified regions. In contrast, despite the relatively smaller number of SNPs on splice sites and on coding regions, these regions are enriched for high-scoring putatively deleterious SNPs. That is, an FS score of at least 0.5 is assigned to all SNPs in canonical splice sites and to 46.07% of the SNPs in coding regions. This scoring pattern is consistent with previous findings that mutations in splice sites and coding regions are likely to have direct impact on gene function (Yue et al., 2006; Cartegni et al., 2003; Reumers et al., 2005).

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exonic regions may affect either Protein Coding, (exonic) Splicing Regulation, or Post-Translational Modification (as summarized in Figure 2), and we assign the maximum score over the three functional categories to each exonic SNP as its final FS score (as stated in Def. 2.1). We thus examine the proportion of the three biologic-molecular functions that are used to assign the final FS scores for disease-related exonic SNPs and for neutral exonic SNPs. Figure 6 summarizes the results. In the case of disease-related SNPs, more than half of the exonic SNPs affect Protein Coding, and about 37% of the SNPs affect exonic Splicing Regulation. Conversely, only 8.48% of neutral exonic SNPs affect Protein Coding, while more than two thirds of them affect (exonic) Splicing Regulation. In either case, Post-Translational Modification seems to be a minor cause for potential deleterious effects of SNPs.

4.2 A Comparative Study

To validate that our scoring system improves upon the state-of-the-art, we compare our system with two public web-services that numerically score deleterious effects of SNPs: SNPselector (Xu et al., 2005) and FastSNP (Yuan et al., 2006). SNPselector provides a numeric score for each SNP, called function score, which designates the possible effects of SNPs on gene transcript structure or on protein product. The score is a real number between 0.6 and 1.0; the higher the score is, the more deleterious the effects of the SNPs are expected to be. FastSNP is another web-service for SNP function analysis and prioritization. It assigns to each SNP an integer score between 0 and 5, called confidence-score, as well as the reliability of each tool (through tool-reliability-scores).

To compare the three scoring schemes to ours, we generated test datasets using the following sampling procedure. For each disease-related SNP $X_i$, one neutral SNP is selected uniformly at random in the same functional region on the same gene as $X_i$. This selection is done for all disease-related SNPs. As a result, a dataset of 1,399 SNP-pairs, one disease-related and one randomly selected neutral, is generated. We repeat this procedure $M$ times, generating $M$ test datasets (here, $M=100$). We note that, by limiting the random selection to the same functional region on the same gene, we reduce the bias that may arise due to the differences in the functional or chromosomal regions.

Using the test datasets, we examine how well each system distinguishes disease-related SNPs from neutral SNPs. Intuitively, a better scoring system would assign a higher functional score to disease-related SNPs than to neutral SNPs. First, we measure this tendency by directly computing the percentage of disease-related SNPs that are assigned a higher functional significance score than their paired, randomly selected neutral SNPs, averaged over $M$ test datasets. We refer to this measure as Higher Score ($\%$). Second, using the paired t-test, we examine whether the disease-related SNPs and the neutral SNPs in each dataset share the FS score distributions with the same mean. We separately conduct the paired t-test on each of the $M$ datasets, and compute the proportion of the rejected tests along with their average $p$-value. The rejection implies that the FS score distribution of disease-related SNPs and that of likely neutral SNPs are distinctive. Therefore, scoring schemes with a high proportion of rejected t-tests are preferred. We refer to this second measure as Paired T-Test.

Table 1 summarizes the results of our comparative study. Overall, our system improves upon all the compared systems with respect to both evaluation measures. In the case of the Higher Score measure, our system assigns higher functional significance scores to about 64% of known disease-related SNPs than to neutral SNPs. FastSNP comes second, and SNPselector and Simple Majority Vote follow. The score difference between our system and the compared systems is also statistically significant ($p$-values are 6.96e-038, 4.82e-105, and 5.26e-174 for FastSNP, SNPselector, and Simple Majority Vote, respectively, using the paired t-test, $\alpha = 0.05$). It is notable that our system greatly outperforms Simple Majority Vote, which demonstrates the utility of the confidence and the tool reliability scores, integrated into our scoring scheme.

In the case of the Paired T-Test measure, the first three systems, namely, our system, FastSNP, and SNPselector perform the same; all of the paired t-tests were rejected with a significance level 0.05. However, the average $p$-value of the rejected tests is smallest (i.e., asymptotically zero) for our system among the three, which means that the score distribution of disease-related SNPs and that of neutral SNPs are most disparate when their FS scores are assigned by our system. In the case of Simple Majority Vote, only 93% of the paired t-tests were rejected. The average $p$-value for the rejected tests is also the largest among all the compared systems.

5 DISCUSSION

We have presented a new scoring system for assessing the putative deleterious effects of SNPs. Our integrative scoring method combines assessments from multiple independent computational tools, using a probabilistic framework that takes into account the certainty of each prediction as well as the reliability of different tools. An empirical study over 580 disease-associated genes taken from the OMIM database shows that our system provides distinct scoring patterns that are consistent with well-established findings about functional SNPs. A comparative study based on two evaluation measures also shows that our scoring system improves upon other SNP scoring systems in terms of distinguishing known disease-related SNPs from likely neutral SNPs.

Two main features distinguish our system from others. First, we integrate multiple tools to overcome the incompleteness or erroneous predictions of individual prediction tools. While a single
tool may fail to capture the deleterious effects of many SNPs, a combination of multiple independent tools, which are based on different resources and algorithms, are less likely to all make the same error. Thus the tools are likely to complement each other, and as we have demonstrated in our results, typically compensate for each other’s errors. As a result, the effect of possible false-negative or false-positive predictions in any single tool is reduced when computing the combined FS score.

Second, unlike other scoring systems, we take into account the reliability of different tools as well as the certainty of each prediction made by the tools. To the best of our knowledge, this is the first SNP prioritization approach to measure the reliability of individual tools and to use this information along with the confidence scores obtained from each tool.

We note, though, that the FS score assigned by our system to about 45% of disease-related SNPs is still below 0.5. There are two possible explanations for this seemingly inappropriate FS score. First, even though some SNPs, obtained from the OMIM database, show a positive statistical correlation with common disorders in some association studies, they may not all be actual disease-causing mutations. Some of these SNPs may represent false positive findings, or may simply be correlated with actual disease-causing mutations. Our future study will focus on investigating the actual disease-causing mutations that could be located near SNPs known to be disease-related with low FS scores.

Second, while the disease-related SNPs may indeed be disease-causing mutations, our current scoring scheme may not capture them properly. For example, in addition to the bio-molecular functions that we currently examine, there could be other genetic mechanisms that have a profound impact on human pathogenesis. We thus plan to update our system through combining other epidemiological resources, such as literature information, as well as integrating more prediction tools for each bio-molecular function.

We note that our integrative scoring system is primarily intended for candidate-gene based association studies, where there is already a region in which some SNPs are likely to have deleterious functional effects. In the case of genome-wide association studies, our system can be used to prioritize a subset of SNPs that needs further investigation after indication for disease-association has been detected for a genomic region.

We currently provide the calculated FS score of 112,949 SNPs through our public web-based database service, F-SNP (available at http://compbio.cs.queensu.ca/F-SNP). We will continue assigning FS scores to other SNPs, and update the scoring results. In addition, we plan to integrate our scoring system with our earlier tagging SNP prioritization approach (Lee and Shatkay, 2006) for association studies. By combining the two most representative selection approaches for SNPs, we expect to provide a comprehensive SNP prioritization system for facilitating effective association studies on common and complex genetic disorders.

ACKNOWLEDGMENTS

This work was supported by HS’s NSERC Discovery grant 298292-04 and CFI New Opportunities Award 10437.

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