**ABSTRACT:** With the advance of sequencing technologies, whole exome sequencing has increasingly been used to identify mutations that cause human diseases, especially rare Mendelian diseases. Among the analysis steps, functional prediction (of being deleterious) plays an important role in filtering or prioritizing nonsynonymous SNP (NS) for further analysis. Unfortunately, different prediction algorithms use different information and each has its own strength and weakness. It has been suggested that investigators should use predictions from multiple algorithms instead of relying on a single one. However, querying predictions from different databases/Web-servers for different algorithms is both tedious and time-consuming, especially when dealing with a huge number of NSs identified by exome sequencing. To facilitate the process, we developed dbNSFP (database for nonsynonymous SNPs’ functional predictions). It compiles prediction scores from four new and popular algorithms (SIFT, Polyphen2, LRT, and MutationTaster), along with a conservation score (PhyloP) and other related information, for every potential NS in the human genome (a total of 75,931,005). It is the first integrated database of functional predictions from multiple algorithms for the comprehensive collection of human NSs. dbNSFP is freely available for download at http://sites.google.com/site/jpopgen/dbNSFP. Hum Mutat 32:894–899, 2011. © 2011 Wiley-Liss, Inc.

**KEY WORDS:** dbNSFP; functional prediction; database; SIFT; Polyphen2; LRT; MutationTaster; PhyloP

**Introduction**

A nonsynonymous SNP (NS) is a single nucleotide variant that causes an amino acid change of its corresponding protein. Because of their direct link to protein changes, NSs are believed to be the major contributor to heritable human diseases among all types of variants in the human genome. As evidence, NSs constitute more than half of the entries of disease-causing genetic changes in the Human Gene Mutation Database (HGMD) [Stenson et al., 2009].

Based on current technologies, it is too costly and time-consuming to investigate the functional effect of every NS experimentally. Fortunately, there are algorithms available to help to predict the potential of a NS being functional or deleterious.

Functional prediction algorithms for NSs have been developed in recent years with the advance of phylogenetics, structure biology, bioinformatics and population genetics (see reviews by [Horner et al., 2010; Karchin, 2009; Ng and Henikoff, 2006; Thubsberg and Vihinen, 2009]). Usually, the algorithms output a score measuring how likely an NS is deleterious, along with its binary prediction. Functional predictions play an important role in sequencing based genotype–phenotype association analyses. Prediction scores have been used to weight different NSs in order to increase the power of detecting genes influencing a trait [Price et al., 2010]. More importantly, functional prediction has become an indispensable step in identifying genes causing rare Mendelian diseases using an exome-sequencing approach [Cooper et al., 2010; Ng et al., 2010a,b,c]. Functional predictions or conservation information are used to filter or prioritize the novel NSs for further analysis or confirmation [Ng et al., 2010]. Because different algorithms use different information and are based on different training data, each has its own strength and weakness. It has been suggested to use the outputs from multiple algorithms to make a more reliable prediction for a NS (e.g., by using consensus prediction or majority vote) [Chun and Fay, 2009].

Because querying predictions from different databases/Web-servers for different algorithms is both tedious and time-consuming, we developed dbNSFP (database for nonsynonymous SNPs’ functional predictions) to facilitate the process. We first compiled a collection of all possible NSs in the human genome (a total of 75,931,005) based on the annotation of the Consensus Coding Sequence (CCDS) project [Pruitt et al., 2009]. We next collected their corresponding prediction scores from four new and popular prediction algorithms (SIFT [Kumar et al., 2009], Polyphen2 [Adzhubei et al., 2010], LRT [Chun and Fay, 2009], and MutationTaster [Schwarz et al., 2010]). We also added other related information including a conservation score (PhyloP) [Siepel et al., 2006], degenerate type, and corresponding codons and genes. The dbNSFP is the first known integrated database of functional predictions from multiple algorithms for the comprehensive collection of human NSs.

**Data Sources and Processing**

The genes and their corresponding codons were determined based on CCDS version 20090327. This is the latest version based on the human reference sequence build hg18. Although the current human reference sequence is build hg19, many important human gene sequences are based on hg18, including current
exome capture chips and the 1,000 genomes pilot data. To facilitate NS queries based on hg19, we used the liftOver tool from the UCSC Genome Browser [Rhead et al., 2010] to convert the coordinates of NSs to hg19. Only 561 NSs out of 75,931,005 (0.0007%) were not converted successfully.

The PhyloP scores were extracted from the placental subset of the precomputed phyloP4way scores [Pollard et al., 2010] provided by the UCSC Genome Browser (see details at http://hgdownload. cse.ucsc.edu/goldenPath/hg18/phyloP4way/). The original score is presented as phyloP_{ori} = -\log_{10} P \text{ if the site is more}

conserved than neutral (phyloP_{ori} < 0) or less conserved than neutral (phyloP_{ori} > 0), respectively. P is a two-sided P-value based on a likelihood ratio test. To make it easier to compare with the prediction scores, we rescaled it to a new score as phyloP_{new} = 1 - 0.5 \times 10^{-\text{phyloP}_{ori}} if phyloP_{ori} > 0 or phyloP_{new} = 0.5 \times 10^{\text{phyloP}_{ori}} if phyloP_{ori} < 0 to mimic a one-sided P-value. The new score ranges from 0 to 1 and a larger score signifies higher conservation. We used phyloP_{new} > 0.95 as a rule to predict conserved site [Siepel et al., 2006]. That is, the prediction is "C(onserved)" if phyloP_{new} > 0.95; otherwise, the prediction is "N(on-conserved).

Original SIFT scores were provided by ANNOVAR [Wang et al., 2010], which were originally from a local database format of SIFT 4.0.3. The original SIFT scores (SIFT_{ori}) range from 0 to 1. If a score is smaller than 0.05 the corresponding NS is predicted as "D(amaging)"; otherwise it is predicted as "T(olerated)." We defined a new score SIFT_{new} = 1 - SIFT_{ori}. The new score still ranges from 0 to 1 and a larger score means more likely to be deleterious. Correspondingly, if a new score is larger than 0.95, the prediction is "D"; otherwise, it is "T".

Original LRT scores (LRT_{ori}) were downloaded from the LRT Webserver (http://www.genetics.wustl.edu/jflab/lrt_query.html). LRT_{ori} is a two-sided P-value of the likelihood ratio test of codon constraint. Each LRT_{ori} is associated with an estimated nonsynonymous-to-synonymous-rate ratio (\omega) and an amino acid alignment of 31 species at the test codon. If the codon is more constrained than neutral, \omega < 1; otherwise, \omega \geq 1. We defined our LRT score as LRT_{new} = 1 - LRT_{ori} \times 0.5 if \omega < 1, or LRT_{new} = LRT_{ori} \times 0.5 if \omega \geq 1 to mimic a one-sided P-value. The score ranges from 0 to 1 and a larger score signifies that the codon is more constrained or a NS is more likely to be deleterious. LRT predictions were derived using the rules summarized in Supp. Figure S1, which is similar to those of Chun and Fay [2009]. In short, (1) a predicted D(eleterious) NS needs to fulfill three requirements: (i) from a codon defined by LRT as significantly constrained (LRT_{ori} < 0.001 and \omega < 1), (ii) from a site with \geq 10 eutherian mammals alignments, and (iii) the alternative AA is not presented in any of the eutherian mammals; (2) a predicted N(eutral) NS needs to fulfill either of the two requirements: (i) the alternative AA is presented in at least one of the eutherian mammals, or (ii) from a codon defined by LRT as not significantly constrained (LRT_{ori} \geq 0.001 or \omega \geq 1) and with \geq 10 eutherian mammals alignments; (3) otherwise, the NS is reported as U(nknown). More details of the LRT predictions can be found in the Discussion section.

Polyphen2 scores were manually queried and downloaded as ~500 batches from its batch query server (http://genetics.bwh. harvard.edu/pph2/bgi.shtml). The default query settings (Classifier model: HumDiv, Transcripts: Canonical, Annotations: Missense) were used except with genome assembly NCBI36/hg18 instead of GRCh37/hg19. We used ph2_prob as our Polyphen2 score, which is the classifier probability of the alternative allele being deleterious. The score ranges from 0 to 1, and the corresponding prediction is "probably damaging" (coded as "D") if it is larger than 0.85; "possibly damaging" (coded as "P") if it is between 0.85 and 0.15 and "benign" (coded as "B") if it is smaller than 0.15. Throughout this article, we regard "D" as its category for deleterious NSs.

MutationTaster scores were queried from its Webserver (http://www.mutationtaster.org/) using its batch query Perl scripts for each of the 75,931,005 NSs. The input file needs an ENSEMBL transcript ID and a sequence snippet (the immediate upstream and downstream sequence from the query site) for each SNP. We first used ANNOVAR to annotate the NSs using ENSEMBL database to get the corresponding ENSEMBL transcript ID for each NS. If there is more than one transcript ID, initially the first ID was used to build an input file for the first round query. If it turned out that transcript ID is not in the MutationTaster’s database (as reported in its output file), we then used the second ID to build an input file for the second round of query. Snippets were built using the FASTA files of the human reference sequence downloaded from the UCSC Genome Browser. For the first round query, we used 12 upstream and 12 downstream nucleotides from the query site to build the snippet. If the output file reported that the snippet is not unique, then 100 upstream and 100 downstream nucleotides from the query site were used to build a new snippet for a second round query. MutationTaster has four types of predictions: "disease_causing_automatic," "disease_causing," "polymorphism," and "polymorphism_automatic," which we coded as "A," "D," "N," and "P" respectively. Among them, "D" and "N" are determined by the prediction algorithm, whereas "A" and "P" are determined by external information (see the Discussion section for details). We regard "A" and "D" as categories for deleterious NSs. MutationTaster also reports a P-value for each prediction, which represents the probability that the prediction is true. We defined our MutationTaster score using the following rule: if the prediction is "disease_causing" or "disease_causing_automatic," score = P-value; if the prediction is "polymorphism" or "polymorphism_automatic," score = 1 - P-value. The resulting score ranges from 0 to 1 and a larger score means more likely to be deleterious.

For various reasons, an algorithm may not output a prediction or score for a NS, thus regarded as missing data and reported it as "NA." In dbNSFP, we reported imputed scores for missing scores (see Missing Data and Imputation). Therefore, a prediction of "NA" can be used as an indicator to determine whether the corresponding score is imputed or not. Rarely, probably due to different gene annotations for the same NS, an algorithm may report a different reference AA or alternative AA as to those defined by CCDS. In that case, we considered the predictions and scores (if reported) as missing data. On the other hand, for a small number of NSs, CCDS may define more than one reference AA or alternative AA for the same nucleotide site change, due to different annotations for the same gene. We treated those definitions as different NSs. In another words, we defined a NS as a unique combination of chromosome, position, reference nucleotide allele, alternative nucleotide allele, reference AA allele, and alternative AA allele.

**Database Contents**

In the current version, each NS links to the following entries: chromosome number, physical position on the chromosome as to hg18 (1-based coordinate), reference nucleotide allele (as on the + strand), alternative nucleotide allele (as on the + strand), reference AA, alternative AA, physical position on the chromosome as to hg19 (1-based coordinate), gene name, gene Entrez ID, CCDS ID, reference codon, position on the codon (1, 2, or 3),
degenerate type (0, 2, or 3), AA position as to the protein, coding sequence (CDS) strand (1 or 0), estimated nonsynonymous-to-synonymous-rate ratio (, reported by LRT), PhyloP score, PhyloP prediction, SIFT score, SIFT prediction, Polyphen2 score, Polyphen2 prediction, LRT score, LRT prediction, MutationTaster score, MutationTaster prediction. If any of the PhyloP, SIFT, Polyphen2, LRT, and MutationTaster scores were missing, their imputed scores (see Missing Data and Imputation) were reported (but their corresponding predictions will be “NA”). There are a total of 75,931,005 entries for 64,646,969 unique NSs, each of which is defined as a unique combination of physical position and alternative allele. This is due to the fact that the same NS may be annotated by CCDS multiple times for different forms of the same gene. The numbers of nonmissing entries of NS, hg19 position, PhyloP score, SIFT score, Polyphen2 score, LRT score, and MutationTaster score per chromosome are summarized in Table 1.

Because all scores (PhyloP, SIFT, Polyphen2, LRT, and MutationTaster) were scaled to [0, 1] with a larger score corresponding to higher conservation (PhyloP and LRT) or more likely to be deleterious (SIFT, Polyphen2, and MutationTaster), we can easily compare their distributions. Figure 1 shows the histograms of their total scores in different score ranges. An interesting observation is that all five methods show some form of bimodal distribution, with the majority of the scores clustered around 1 and a small proportion of the scores clustered around 0. The predictions from Polyphen2 and MutationTaster are more balanced, which have about 30% of the scores in range [0, 0.1), whereas the remaining three only have less than 7% scores in that range. Correspondingly, SIFT, LRT, and MutationTaster all predict more than half of the total NSs being deleterious, whereas Polyphen2 predicts that slightly less than half of the NSs are deleterious (Table 2). The proportion of pairwise agreement between methods on whether a NS is deleterious is around 60–70%, with the highest agreement between the LRT and MutationTaster (77.19%) (Table 3). The Spearman’s rank correlation coefficients (RCCs) between scores of different

| Chromosome | NS   | hg19 | PhyloP | SIFT  | Polyphen2 | LRT   | MutationTaster |
|------------|------|------|--------|-------|-----------|-------|----------------|
| 1          | 8329939 | 8329939 | 8329796 | 7611044 | 7279405 | 7644552 | 7959066 |
| 2          | 5115071 | 5115063 | 5114264 | 4376414 | 4428042 | 4765076 | 4858487 |
| 3          | 4280786 | 4280782 | 4280786 | 3804983 | 3756644 | 4063236 | 4108445 |
| 4          | 2868872 | 2868872 | 2868872 | 2532905 | 2554593 | 2752943 | 2766053 |
| 5          | 3484907 | 3484907 | 3484907 | 3134219 | 3043592 | 3207583 | 3347045 |
| 6          | 4075171 | 4075036 | 4075171 | 3710396 | 3586226 | 384055 | 3803359 |
| 7          | 3268295 | 3268295 | 3267997 | 2819288 | 2856409 | 2989039 | 3132425 |
| 8          | 2597009 | 2597009 | 2597009 | 2272632 | 2207857 | 2454441 | 2539459 |
| 9          | 3164462 | 3164462 | 3164462 | 2839322 | 2842191 | 2902636 | 2906881 |
| 10         | 3273027 | 3273027 | 3273027 | 2924577 | 2922389 | 3093339 | 3092560 |
| 11         | 4253046 | 4253046 | 4253046 | 3793927 | 3795848 | 4004784 | 4098786 |
| 12         | 3852960 | 3852960 | 3852960 | 3412527 | 3332085 | 3698888 | 3723502 |
| 13         | 1420096 | 1420096 | 1420096 | 1278533 | 1235036 | 1378650 | 1374240 |
| 14         | 2494135 | 2494135 | 2494135 | 2262099 | 2265265 | 2383704 | 2432185 |
| 15         | 2470042 | 2470042 | 2470042 | 2206987 | 2219513 | 2317477 | 2335630 |
| 16         | 3273027 | 3273027 | 3273027 | 2924577 | 2922389 | 3093339 | 3092560 |
| 17         | 4372234 | 4372295 | 4371639 | 3943103 | 3822645 | 4019068 | 4228681 |
| 18         | 1178377 | 1178377 | 1178377 | 1031129 | 1039874 | 1090517 | 1131942 |
| 19         | 4415600 | 4415600 | 4415600 | 3916565 | 3779650 | 3368085 | 3783007 |
| 20         | 2103098 | 2103098 | 2103098 | 1950303 | 1881731 | 1998568 | 1932748 |
| 21         | 989231  | 989231  | 989231  | 843131  | 842902  | 890113  | 925335  |
| 22         | 1553703 | 1553703 | 1553703 | 1388173 | 1338949 | 1440475 | 1461233 |
| X          | 3176068 | 3176068 | 3176068 | 2941495 | 2719583 | 2834074 | 2998558 |
| Y          | 224235  | 224235  | 224235  | 205265  | 195144  | 138312  | 146802  |
| Total      | 75931005 | 75930444 | 75927740 | 67833759 | 66462785 | 70052541 | 71969712 |
| Total (unique)* | 6464696 | 64646408 | 64643479 | 5741955 | 58620320 | 59415946 | 61277012 |

*NSs with the same position and alternative allele were counted only once.
methods show low to moderate positive correlations, with the highest RCC between LRT and MutationTaster being 0.62 (Table 3). We also calculated the Pearson’s correlation coefficients (data not shown) for each pair of the methods. They are all smaller than their corresponding RCCs, which suggests stronger nonlinear correlation between those scores.

**Missing Data and Imputation**

The numbers of missing data of PhyloP, SIFT, Polyphen2, LRT, and MutationTaster per chromosome can be derived from Table 1. Among them, PhyloP has the lowest missing data rate (0.004%). As for the four prediction methods, MutationTaster has the lowest missing data rate (5.2%), followed by LRT (7.7%), SIFT (10.7%), and Polyphen2 (12.5%). This is partially because SIFT and Polyphen2 do not provide prediction scores for stop-gain (a mutation that changes an AA codon to a stop codon) or stop-loss (a mutation that changes a stop codon to an AA codon) and LRT does not provide prediction scores for stop-loss.

An advantage of having multiple prediction scores for the same NS is that it facilitates better imputation for missing scores. Some statistical methods for detecting association between (groups) of rare variants and phenotypes (e.g., VT test) [Price et al., 2010] use prediction scores to weight NSs to increase their detecting power. If scores are missing, typically they are imputed using the average of the nonmissing scores (of the same algorithm) in the sample. As we observed in Figure 1, the prediction scores are bimodal distributed. Therefore, using the average score to impute may not be a good choice. Although there are only low to moderate correlations between different types of scores, it is still possible to make full use of those relationships to obtain better imputation scores.

We chose the program BPCAfill [Oba et al., 2003] to impute the missing scores in dbNSFP, because of its higher accuracy and faster computation comparing to other methods [Aittokallio, 2010]. Although BPCAfill is originally designed for imputing missing expression data for microarray analyses, it can be applied to any data set with correlated columns or rows. To evaluate the performance, (1) we compiled a subset of dbNSFP, in which all five scores are NOT missing; (2) for each missing pattern observed in dbNSFP, we randomly picked the same number of NSs as those with the missing pattern in dbNSFP, and marked their corresponding scores as missing (e.g., dbNSFP has 334,421 NSs with LRT and MutationTaster scores missing while all other three scores are present; we randomly picked 334,421 NSs from the subset and marked their LRT and MutationTaster scores as missing); and (3) after BPCAfill imputed the missing scores, we compared the imputed scores with the corresponding original scores. Figure 2 shows the histograms of the error (imputed score minus original score) distribution from two different imputation strategies: imputing with BPCAfill or with the average scores of the same algorithm. Imputation errors for each algorithm with different missing patterns are summarized in Supp. Table S1. Comparing the imputation accuracies for different prediction scores over all missing patterns, LRT has the smallest mean squared error (MSE) of 0.035, followed by SIFT (0.054) and PhyloP (0.066), whereas MutationTaster and Polyphen2 have the largest MSEs of 0.133 and 0.142, respectively (Supp. Table S1). A t-test of the difference between mean error and 0 shows that imputations with both BPCAfill and the average scores were unbiased (data not shown), with average errors equal to $-6.25 \times 10^{-6}$ (BPCAfill) and $-3.6 \times 10^{-5}$ (average score), respectively. However, the distribution of the errors using BPCAfill had a significantly smaller standard deviation than the other strategy (0.303 comparing to 0.341, F-test, one tailed). Therefore, we concluded that BPCAfill can produce significantly better imputation scores than simply imputing with the average score.

**Companion Search Program**

A companion search program written in Java (search_dbNSFP) is freely available for download along with dbNSFP. It can run crossplatform on a wide range of computers, as long as a proper Java Runtime Environment is installed. It enables a simple search for a NS, a chromosome position or a gene. It runs on a command line with a basic format “java search_dbNSFP -i infile -o outfile,” where infile and outfile are the user designated input file name and output file name, respectively. The input file contains one or more lines, with each line representing a query. The query formats for NS, chromosome position and gene are presented in Table 4. The output file contains all NSs that match the query information. For example, the output of a gene query will contain all NSs in that gene, and that of a genome position query will contain all NSs on that position. If there is more than one result for a NS query due to different annotations (see Data Sources and Processing), all

![Figure 2](image-url)
results will be reported. By default, the program searches all chromosomes with the positions according to the human genome reference sequence hg18. Users can specify both the chromosomes and the reference sequence version by using the format 
```
java search_dbNSFP [-v reference_version] [-c chromosome_list] -i infile -o outfile,
```
where the contents in [ ] are optional. reference_version can be either hg18 or hg19. chromosome_list is a list of chromosome number (1, 2, ..., X, Y) separated by commas without space, for example "1,3,22,X." If there are some queries that do not have matched NSs, those failed queries will be listed in the system output (command line environment).

### Discussion

dbNSFP is a database for all potential NSs (as to the reference sequence) in the human genome and their functional predictions. It was developed for two main purposes. The first is to facilitate the SNP filtering/prioritizing step in studies of mapping rare Mendelian disease genes using the exome-sequencing approach. The second is to facilitate the imputation of missing scores that are required in the weighted sum test (e.g., VT test) [Price et al., 2010] for detecting association between phenotypes and groups of rare variants. A companion search program is provided for fast local queries. For the current version, we attempted to keep the database simple and lightweight to ensure storage and running efficiency. Except for five prediction/conservation scores (PhyloP, SIFT, Polyphen2, LRT, and MutationTaster) for each NS, the database only contains information of its corresponding genomic position, gene, codon, among others. Future development may include expanding the collection of NSs from other genome databases, incorporating the results from other prediction algorithms that do not provide genome position searches, such as PHD-SNP [Capriotti et al., 2006] and PANTHER [Thomas et al., 2003], and building a fully functional Webserver for on-line database query. We plan to keep updating the database to have newer CCDS versions, whenever most of the public available human genome sequence data shift to a new human genome reference sequence.

Some cautious are needed when compiling and interpreting the search results from dbNSFP. First, the NSs collected in dbNSFP were based on the annotations of CCDS, which defines consensus CDSs from multiple genome databases. Therefore, CCDS by no means includes the complete set of human CDSs, but rather includes a set of well-annotated CDSs. It is quite possible that some NSs defined by other databases are not contained in dbNSFP. Second, the NSs in dbNSFP are defined as the alternative alleles against the reference alleles of the human reference sequence. However, the reference allele is neither necessary the “wild-type” allele, nor the common allele in all human populations. Some other information about the alternative allele, for example, its frequency in populations or sampled individuals, may help to determine its potential to be deleterious. Third, for various reasons, some scores are missing for some NSs and the imputed scores may not be reliable. For example, scores for stop-gain NSs are not available from SIFT and Polyphen2. Although imputed scores for stop-gain NSs are provided, some researchers may prefer to assign a score based on their knowledge of the protein. For instance, a stop-gain near the beginning of the protein is more likely to be deleterious than one near the end of the protein. Fourth, there may be multiple entries in dbNSFP that match the same NS query. This is primarily due to the fact that CCDS contains different annotations for different forms of the same gene.

Our rules for LRT predictions (Supp. Fig. S1) are similar to those of Chun and Fay [2009], but be aware that our predictions may not be the same as those reported by the LRT Webserver (http://www.genetics.wustl.edu/ijlab/lrt_query.html). The major difference is that the Webserver predicts a NS as either D(eleterious) or N(eutral) according to LRT_{wt} and o, regardless of the quality of the underlying amino acid alignment. Chun and Fay [2009] suggested that if the amino acid alignment has less than 10 eutherian mammals, the prediction power is low. Therefore, we add a new prediction category U(nknown) specifically for the low-power situation. As a result, our LRT predictions for D(eleterious) and N(eutral) are more stringent than those provided by the Webserver.

Because the predictions of LRT and MutationTaster are not entirely dependent on the prediction scores, discrepancy between a score and its corresponding prediction may occur. As to LRT, the discrepancies come from N(eutral) predictions with high scores (i.e., the codon is highly constrained or a NS is likely to be deleterious). This is because of one prediction rule: if the alternative AA allele is observed in at least one eutherian mammal, the NS is predicted as N(eutral), regardless of the score it associated with (Supp. Fig. S1). The discrepancy cases of MutationTaster come from the two “automatic” predictions, that is, "disease_causing_automatic" ("A") and "polymorphism_automatic" ("P"). An NS will be predicted to be "A" if it causes nonsense-mediated decay (i.e., a stop-gain); and an NS will be predicted to be "P" if all three genotypes of the reference/alternative alleles are observed in the HapMap data [The International HapMap Consortium, 2010]. Both types of prediction are not dependent on the prediction scores at all, although the scores are still reported.

As to the missing data imputation, our results clearly showed that BPCAfill can obtain more accurate imputation compared to simply using the average score of the same algorithm (column-average). This is easy to understand because BPCAfill borrows information from the correlated scores of different algorithm while column-average imputation does not use such information. To further evaluate the performance of BPCAfill, we also compared its results to the simple imputation using the average of the nonmissing scores of the other algorithms for the same NS (row-average). For example, if an NS misses a SIFT score while all other four scores (PhyloP, Polyphen2, LRT, and MutationTaster) are available, we compute the SIFT score using the average of the other four scores. The results showed that although the row-average imputation’s mean absolute error is smaller than that of the column-average, it is still larger than that of BPCAfill (data not shown). It is also shown that the row-average imputation is significantly biased and has a significantly larger variance than BPCAfill (data not shown). The superior performance of BPCAfill compared to the row-average can be explained by the nonlinear correlation between the scores of different algorithms and the fact that BPCAfill also borrows information from the correlation between NSs while row-average imputation does not.

### Table 4. Format of Queries for Input File

| Query type | Format* | Example |
|------------|---------|---------|
| NS         | chr pos ref alt | Y 140855 A C |
| Genome position | chr pos ref alt refAA altAA | Y 140855 A C M L |
| Gene       | gene_name     | PLCXD1 |
|            | gene_id       | 53344 |
|            | CCDS_id       | CCDS14103.1 |

*Separated by tab or space. chr: chromosome number; pos: position on chromosome; ref: reference allele; alt: alternative allele; refAA: reference amino acid; altAA: alternative amino acid; gene_name: gene name; gene_id: gene Entrez ID; CCDS_id: CCDS ID.
Collecting predictions and scores from multiple algorithms has many advantages. Besides providing better imputation for missing data, as we mentioned in the introduction, it also helps to get more accurate functional predictions for NS. A simple way to incorporate multiple predictions is to rank each NS according to the number of algorithms that predict it as deleterious. The larger the number, the higher the confidence that it is truly deleterious. A better solution is to develop a unified score (or a score of scores) based on training data sets and infer predictions from it. Besides NS, other types of variants may also contribute to diseases. However, the functional prediction methods of those variants are typically less mature than those of NS, but never the less they still provide valuable information of those variants. For example, a recent study [Waldman et al., 2010] showed that codon bias is significantly correlated with gene expression levels in human. Therefore, some synonymous changes are more likely to have strong impact on gene expression and lead to phenotypic effects. Other information that can be used to prioritize variants including the prediction of promoter regions, linkage disequilibrium with known genome-wide association study (GWAS) hits, gene expression profile of disease related tissues, known biochemical pathways, known or predicted gene interaction networks, among others. In recent years, some Web servers have been developed to integrate searches or results from multiple bioinformatics tools, for example, the SeattleSeq Annotation server, F-SNP [Lee and Shat'kay, 2008], SNPLogic [Pico et al., 2009], SNPIt [Shen et al., 2009], and pSNP [Wang et al., 2011]. As the field moves from whole exome sequencing to whole genome sequencing, more efforts are needed in these nonprotein coding domains.

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References

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. Nat Methods 7:248–249.

Aittokallio T. 2010. Dealing with missing values in large-scale studies: microarray data imputation and beyond. Brief Bioinform 11:253–264.

Capiotti E, Calabrese R, Casadio R. 2006. Predicting the insurgence of human Aittokallio T. 2010. Dealing with missing values in large-scale studies: microarray data imputation and beyond. Brief Bioinform 11:253–264.

Chun S, Fay JC. 2009. Identification of deleterious mutations within three human genomes. Genome Res 19:1553–1561.

Cooper GM, Goode DL, Ng SB, Sidow A, Bamshad MJ, Shendure J, Nickerson DA. 2010. Single-nucleotide evolutionary constraint scores highlight disease-causing mutations. Nat Methods 7:250–251.

Horner DS, Pavesi G, Castrignano T, Meso PDD, Liuni S, Sammeth M, Picardi E, Pesole G. 2010. Bioinformatics approaches for genomics and post genomics applications of next-generation sequencing. Brief Bioinform 11:181–197.

Karchin R. 2009. Next generation tools for the annotation of human SNPs. Brief Bioinform 10:35–52.

Kumar P, Henikoff S, Ng PC. 2009. Predicting the effects of coding non-synonymous variants on protein function using the sift algorithm. Nat Proto 4:1073–1081.

Lee PH, Shat’kay H. 2008. F-SNP: computationally predicted functional SNPs for disease association studies. Nucleic Acids Res 36:2820–2824.

Ng PC, Henikoff S. 2006. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet 7:61–80.

Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Melford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshida KL, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J. 2010a. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet 42:790–793.

Pico AR, Smirnov IV, Chang JS, Yeh YF, Wiermels JL, Wienceke JK, Tihan T, Conklin BR, Wrensch M. 2009. SNPLogic: an interactive single nucleotide polymorphism selection, annotation, and prioritization system. Nucleic Acids Res 37:D803–D809.

Pollard KS, Hubisz MJ, Robinson AM, Slepian A. 2010. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res 20:110–112.

Price AL, Kryukov GV, de Bakker PIW, Purcell SM, Staples J, Wei LJ, Sunyaev SR. 2010. Pooled association tests for rare variants in exome-resequencing studies. Am J Hum Genet 86:832–838.

Rajput B, Riddick LD, Snow C, Steward C, Webb D, Weber JA, Wilming L, Wu W, Birney E, Haussler D, Hubbard T, Ostell J, Durbin R, Lipman D. 2009. The consensus coding sequence (CCDS) project: identifying a common protein-coding gene set for the human and mouse genomes. Genome Res 19:1316–1323.

Rhead B, Karolchik D, Kuhn RM, Hinrichs AS, Zweig AS, Fujita PA, Diekhans M, Smith KE, Robinson KR, Raney BJ, Pheasant M, Meyer LR, Learned K, Hsu F, Hillman-Jackson J, Hart A, Girardine B, Dresser TR, Clawson H, Barber GP, Haussler D, Kent WF. 2010. The UCSC genome browser database: update 2010. Nucleic Acids Res 38:D613–D619.

Schwarz JM, Rödelsperger C, Schultke M, Seelow D. 2010. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 7:575–576.

Shen TH, Carlson CS, Tarczy-Hornoch P. 2009. SNPIt: a federated data integration system for the purpose of functional SNP annotation. Comput Methods Programs Biomed 95:181–189.

Siepel A, Pollard K, Haussler D. 2006. New methods for detecting lineage-specific selection. In: Proceedings of the 10th international conference on research in computational molecular biology (RECOMB 2006), p 190–205.

Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas CS, Cooper DN. 2009. The Human Gene Mutation Database: 2008 update. Genome Med 1:133.

The International HapMap Consortium. 2010. Integrating common and rare genetic variation in diverse human populations. Nature 467:52–58.

Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Davenport R, Diemer K, Murphyajun A, Naranchia A. 2003. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res 13:2129–2141.

Thuberg J, Vihinen M. 2009. Pathogenic or not? And if so, then how? Studying the effects of missense mutations using bioinformatics methods. Hum Mutat 30:703–714.

Waldman YY, Tuller T, Shlomi T, Sharan R, Ruppin E. 2010. Translation efficiency in humans: tissue specificity, global optimization and differences between developmental stages. Nucleic Acids Res 38:2964–2974.

Wang X. 2010. Efficient gene and subfamily indexing by function. Genome Res 20:1103–1109.

Wang J, Wang H, Chou C-K, Li C. 2011. psSNP: an integrated potentially functional SNP resource that facilitates hypotheses generation through knowledge-edge syntheses. Hum Mutat 32:19–24.

Wang K, Li M, Hakonarson H. 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164.