Genome analysis
A web server for inferring the human N-acetyltransferase-2 (NAT2) enzymatic phenotype from NAT2 genotype

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1 INTRODUCTION
N-acetyltransferase-2 (NAT2) is an important enzyme that catalyzes the acetylation of aromatic and heterocyclic amine carcinogens. Individuals in human populations are divided into three NAT2 acetylator phenotypes: slow, rapid and intermediate. NAT2PRED is a web server that implements a supervised pattern recognition method to infer NAT2 phenotype from SNPs found in NAT2 gene positions 282, 341, 481, 590, 803 and 857. The web server can be used for a fast determination of NAT2 phenotypes in genetic screens.

Availability: Freely available at http://nat2pred.itl.albany.edu
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Supplementary information: Supplementary data are available at Bioinformatics online.

ABSTRACT
Summary: N-acetyltransferase-2 (NAT2) is an important enzyme that catalyzes the acetylation of aromatic and heterocyclic amine carcinogens (Blum et al., 1990). Based on the level of NAT2 acetylator activity, individuals in human populations are divided into three enzymatic phenotypes: rapid (normal activity), intermediate and slow (reduced activity) (Hein et al., 2000). Single nucleotide polymorphisms (SNPs) within NAT2 determine the NAT2 acetylator phenotype. A consensus has been reached on association between gene variants and acetylator phenotypes to the risk of several malignant and pre-malignant conditions (Brockton et al., 2004). However, methods for statistical determination of phase are computer intensive and require specific data formatting steps. The goal of the present work was to develop a web server that implements a supervised pattern recognition approach to infer NAT2 acetylator phenotype (slow, intermediate or rapid) directly from the observed combinations of NAT2 SNPs, without taking the extra step of determining the haplotypes for each individual.

2 METHODS
The dataset used in this work was obtained from the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial of the National Cancer Institute (see Moslehi et al., 2006 for details). Genotyping for six NAT2 SNPs (C282T, T341C, C481T, G590A, A803G and G857A) was performed using the TaqMan® (Applied Biosystems Inc., Carlsbad, CA, USA) kit. The acetylator phenotypes were assigned in our previous study based on the haplotypes determined from SNP genotyping data for each subject (Moslehi et al., 2006). The dataset consists of 1377 subjects (see Supplementary Table 1 for details and ethnic makeup). Prediction of the acetylator phenotype from combinations of SNPs, as defined here, is a three-class classification problem that can be addressed using a supervised pattern recognition method. We used Support Vector Machine (SVM) as a method of choice (Vapnik, 1998). We constructed a three-class SVM predictor using the one-against-one approach which was shown to perform better than other approaches in multi-class SVMs (Hsu and Lin, 2002). We used SVM implemented in the LIBSVM package (Chang and Lin, 2003) with the linear kernel. Each NAT2 SNP was encoded using a set of three mutually orthogonal binary vectors: homozygote for the most frequent allele (1,0,0), heterozygote for the most frequent allele (0,1,0) and homozygote for the least frequent allele (0,0,1). For a given subject corresponding vectors describing each of the six observed SNPs were concatenated together, resulting in a final binary feature vector of dimension 18. Thus, the SNP combination of each subject was described by 18 binary variables. We used a 7-fold cross-validation to test the SVM predictor of the acetylator phenotype. In this approach, the dataset is randomly partitioned into seven groups, each containing 1/7 of the dataset. At each cross-validation run, one group is removed and the predictor is trained on the remaining observations and tested on the removed group. The process is repeated seven times, so that each group is used for testing once. In order to assess different aspects of classification quality, we used the following performance measures: overall accuracy (ACC), sensitivity (SN...
After the genotype is selected, the user can click 'Submit' button.

Table 1. The performance of NAT2PRED server

<table>
<thead>
<tr>
<th>NAT2 phenotype</th>
<th>Sensitivity (SN)</th>
<th>Specificity (SP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid</td>
<td>99.6%</td>
<td>100%</td>
</tr>
<tr>
<td>n = 84</td>
<td>(93.4%)</td>
<td>(90.1%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>100%</td>
<td>99.7%</td>
</tr>
<tr>
<td>n = 503</td>
<td>(94.0%)</td>
<td>(95.4%)</td>
</tr>
<tr>
<td>Slow</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>n = 790</td>
<td>(92.5%)</td>
<td>(93.1%)</td>
</tr>
</tbody>
</table>

The total number of cases for a given phenotype is shown in a corresponding row.

There is also an option for a batch submission of genotypes for multiple individuals. Detailed instructions and information about the methodology and output format can be found by clicking the corresponding help hyperlink located on the input page. To the best of the authors’ knowledge, NAT2PRED is the only existing web server for inferring NAT2 acetylator phenotypes from genotyping data. NAT2PRED was developed on a dataset where majority of subjects are Caucasian (94%). However, the prediction model utilizes generally observed linkage disequilibrium between the six NAT2 SNPs and can be applied to individuals from any ethnicity. The web server is publicly available at http://natspred.rutgers.edu.

3 RESULTS

The results of the cross-validation are shown in Table 1. If all six SNPs are used, the predictor of the NAT2 acetylator phenotype achieves a nearly perfect accuracy of 99.9% (Equation 1) and nearly perfect class-specific sensitivities and specificities (Equation 2) between 99.6 and 100%. Such a well-balanced performance is observed despite the highly unbalanced nature of the dataset, meaning that the number of subjects with the slow phenotype is almost an order of magnitude larger than that of subjects with the rapid phenotype. Importantly, individuals with the slow phenotype, who are at increased risk of developing tumors, are identified with 100% SN, meaning that no at-risk individuals are missed. If data for two synonymous SNPs (C282T and C481T) are removed and only non-synonymous SNPs are used, the accuracy of the prediction drops from 99.9 to 93.2%, with similar declines in SN and SP (Table 1). We therefore conclude that all six SNPs used in the present study are required to reliably assign the acetylator phenotype.

The web server implementation of the SVM predictor of the NAT2 acetylator phenotype was trained using the data on all 1377 subjects. It has a simple intuitive user interface. The user is asked to select a genotype for each of the six SNP loci using radio buttons (Supplementary Fig. 2). There are three possible genotypes for each SNP locus, which corresponds to three radio buttons per locus.

The output page displays the selected genotype and the probabilities of each of the three acetylator phenotypes (slow, intermediate and rapid) for these genotypes (Supplementary Fig. 3). The final prediction is the phenotype with the highest probability. There is also an option for a batch submission of genotypes for multiple individuals. Detailed instructions and information about the methodology and output format can be found by clicking the corresponding help hyperlink located on the input page. To the best of the authors’ knowledge, NAT2PRED is the only existing web server for inferring NAT2 acetylator phenotypes from genotyping data. NAT2PRED was developed on a dataset where majority of subjects are Caucasian (94%). However, the prediction model utilizes generally observed linkage disequilibrium between the six NAT2 SNPs and can be applied to individuals from any ethnicity. The web server is publicly available at http://natspred.rutgers.edu.

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Conflict of Interest: none declared.

REFERENCES


