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ORIGINAL ARTICLE

Evaluation of different generic in silico methods for predicting HLA class I binding peptide vaccine candidates using a reverse approach

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Abstract Since $CD8^+$ T cell response is crucial to combat intracellular infections and cancer, identification of class I HLA binding peptides is of immense clinical value. The experimental identification of such peptides is protracted and laborious. Exploiting in silico tools to discover such peptides is an attractive alternative. However, this approach needs a thorough assessment before its elaborate application. We have adopted a reverse approach to evaluate the reliability of eight different servers (inclusive of 55 predictors) by exploiting experimentally proven data. A comprehensive data set of more than 960 peptides was employed to test the efficacy of the programs. We have validated commonly used strategies to predict peptides that bind to seven most prevalent HLA class I alleles. We conclude that four of the eight servers are more adept in predictions. Although the overall predictions for class I MHC binders were superior to class II MHC binders, individual predictors for different alleles belonging to the same program were highly variable in their efficiencies. We have also addressed whether a consensus approach can yield better prediction efficiency. We observed that combining the results from different in silico programs could not increase the efficiency significantly.

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Keywords HLA · In silico methods · Promiscuous peptides

Abbreviations

HLA	Human leukocyte antigen
MHC	Major histocompatibility complex
SVM	Support vector machine
ANN	Artificial neural networks

Introduction

The $CD8^+$ cytotoxic T lymphocytes (CTLs) are very crucial in mediating protection against viruses, intracellular bacterial infections, cancer, etc. The CTLs respond to peptides of 8–13 amino acids (epitopes) in length (Burrows et al. 2006). T cells recognize peptides only in context with self molecules encoded by the genes of major histocompatibility complex (MHC) (Garcia et al. 1999; Zinkernagel and Doherty 1974). The MHC in humans is called as human leukocyte antigen (HLA). HLA molecules are mainly of two types: class I and class II. CTLs recognize peptide presented along with HLA class I molecules, where as $CD4^+$ T cells recognize peptides in the context of class II HLA molecules. The binding chemistry between the two different types of MHCs and peptides differs greatly (Stern and Wiley 1994). The class I molecules (HLA-A, HLA-B, HLA-C) are present on the surface of all nucleated cells. Identification of CTL epitopes can be very important in developing vaccines. The usage of peptides, instead of whole protein, that evokes a $CD8^+$ T cell response offers the advantage of influencing the immune system specifically. There are many such peptide vaccines that are

currently in clinical trials (Parmiani et al. 2007; Purcell et al. 2007). Conventionally, such CTL epitopes have been identified through complex immunological and biochemical assays. These assays, although accurate, are laborious and expensive. Further, HLA is one of the most polymorphic antigens. More than 3,000 variants of HLA have been identified worldwide (IMGT database, www.ebi.ac.uk/imgt/hla/stats.html). The extensive polymorphism of the HLA molecules is a major challenge in developing peptide vaccines for global immunization (Gowthaman and Agrewala 2008, 2009; Robinson et al. 2009; Reche and Reinherz 2003). This can be overcome by identifying promiscuous peptides that bind to many HLA alleles and hence will eventually work in genetically diverse human population. Conventional identification of epitopes for different antigens (and their variants) and a very large number of diverse HLA variants is a gargantuan task. A number of computational methods have been developed to facilitate the identification of MHC binding peptides (Tong et al. 2006; Gowthaman and Agrewala 2009). Thus, in silico tools offer an attractive alternative method that is quick and less expensive. There are many reports that advocate the utility of these methods; however, some suggest that in silico methods are not adept in identifying T cell epitopes (Gowthaman and Agrewala 2009). CTL epitopes have direct relevance in clinical immunology as they can be exploited in viral, bacterial and cancer immunotherapy. This necessitates a high-throughput evaluation of the efficacy of in silico tools to predict class I MHC binding peptides.

Recently, we demonstrated that in silico methods for the prediction of HLA class II binding peptides may not be completely relied (Gowthaman and Agrewala 2008). The reasons like well-defined anchor residues, limited length of peptides, etc. are claimed to make prediction of peptides binding to HLA class I more facile than HLA class II molecules. Nevertheless, as it still warrants an insightful evaluation, using a reverse approach, we have attempted to validate the efficiency of popular methods for the prediction of HLA class I binding peptides with the existing experimental data. We studied the in silico binding of bona fide HLA-binding peptides from different antigens to seven prevalent HLA class I alleles. Earlier studies had used less than 200 peptides from limited number of antigens to compare the efficiencies (Lin et al. 2008). We have validated a total of 960 peptides covering many different antigens of HIV, influenza and cancer. We conclude that these methods are more adept compared to class II MHC–peptide prediction methods; however, the prediction efficacies are not balanced for all the alleles. Hence it may be difficult to identify promiscuous peptides. Furthermore, we have also evaluated the consensus approach of identifying class I HLA binding peptides. We conclude that this approach may not significantly improve the performance of prediction.

Materials and methods

Alleles selected in the study

Predominantly occurring HLA class I alleles (A*0101, A*0201, A*0301, A*1101, A*2402, B*0702, B*0801) were selected for the study.

Peptides used in the study

A total of 960 experimentally validated peptides derived from AntiJen database were chosen for the study (http://www.jenner.ac.uk/antijen/) (Toseland et al. 2005). The selection of the peptides to validate the tools was primarily based on the fact that they were derived antigens of cancer, virus and intracellular pathogens. The peptides were classified as binders ($IC_{50} < 1{,}000$ nM), weak binders (IC_{50} values that were between 1,000 and 5,000 nM) and non-binders ($IC_{50} > 5{,}000$ nM). Selected peptides for each allele, based on experimental IC_{50} values, included $\sim 50\%$ non-binders. This ensured a balanced evaluation of all the alleles.

Programs used in the study

Eight different programs/servers were employed consisting of 55 individual predictors in total. The servers could be classified into four major generic methods: the matrix-based methods RANKPEP (Reche et al. 2002, 2004), ARB (Bui et al. 2005); ANN based programs NetMHC-3.0 (Lundegaard et al. 2008), NetMHCPan 2.1 (Hoof et al. 2009), IEDB-ANN (Zhang et al. 2008; Peters et al. 2005);

Table 1 Best three predictors for each allele (based on MCC)

	A*0101	A*0201	A*0301	A*1101	A*2402	B*0702	B*0801
1	IEDB	NetMHC	IEDB	NetMHCPan	ARB	NetMHCPan	IEDB
2	ARB	NetMHCPan	NetMHC	IEDB	IEDB	NetMHC	ARB
3	SVMHC (Syf)	IEDB	NetMHCPan	NetMHC	NetMHC	IEDB	SVMHC (syf)

SVM-based SVMHC (Syfpeithi), SVMHC (MHCPep) (Donnes and Kohlbacher 2006; Rammensee et al. 1999); and hybrid method ComPred (ANN along with matrix-based technique) (Bhasin and Raghava 2007). The predictions were done with default threshold values of the programs to avoid variation.

Consensus method

Consensus binding and non-binding were computed based on combining results of all the eight servers, for which a consensus positive or negative was arrived if a minimum of four programs suggested positivity or negativity, or by combining the best three predictors, for which a consensus positive or negative was arrived if two programs suggested positivity or negativity.

The peptides examined in the current study were experimentally proven binders/non-binders. Based on the predictions by the chosen programs, the results were classified as true positives (binders predicted as binders), false positives (nonbinders predicted as binders), true negatives (nonbinders predicted as nonbinders) and false negatives (binders predicted as nonbinders). Taking these four values into consideration, statistical parameters were used to analyze the performances of the programs to obtain sensitivity, specificity, accuracy and other statistical indices (Gowthaman and Agrewala 2009; Baldi et al. 2000).

Results

We evaluated the prediction efficiency of the in silico programs with the experimentally proven data. The binding of seven mainly occurring HLA class I alleles was analyzed using the dataset peptides selected from diverse antigens. The overall performances of the programs were analyzed by combining the results of the individual predictors (for the alleles tested). IEDB (ANN) had the best overall performance measures as evidenced by high and balanced sensitivity, specificity and accuracy values. Further, its correlation co-efficient was 0.59 suggesting that the correlation between prediction and experimental datasets were close to 80% agreement (Fig. 1a, b). Other programs that showed good overall efficiency were NetMHC (0.54), NetMHC Pan (0.51) and ARB matrix (0.49) (Fig. 1b). The highest sensitivity (89.4%) and specificity (88.4%) were of the programs ARB matrix and NetMHC, respectively.

We then analyzed the results obtained for individual alleles using different programs. For the allele A*0101, different programs showed variable results and the most efficient predictor among the programs tested was IEDB (ANN). It showed very high and balanced accuracy, specificity and sensitivity values. Further its MCC values showed close to 85% linearity with experimental data (Figs. 2a, 3a) (MCC values of −1, 0, +1 shows 0, 50, 100% linearity with experimental data, respectively). Interestingly, Rankpep showed very low sensitivity (1%) and correlation (−0.01). Of the 93 authentic binders used to test this allele, only one was identified by Rankpep, which could explain its poor performance. It is noteworthy that the overall performance of a server (pooled results) did not strictly reflect the efficiency of the individual predictors (for each allele) (Figs. 1b, 3, S1). In other words, the programs showed very heterogeneous efficiency in their individual predictors for the alleles tested. For the allele A*0201, a vast amount of knowledge on experimental binders is available. Contrary to our expectations, many of the predictors used were not very efficient in their prediction for this allele. Among the programs used, NetMHC, NetMHCpan, IEDB showed better efficiency than the rest. NetMHC showed highest overall accuracy and MCC (Figs. 2b, 3b). When it came to A*0301, IEDB, closely followed by NetMHC, outperformed the rest of the programs (Figs. 2c, 3c). For, the next tested allele A*1101, none of the programs showed very efficient prediction, as compared to their overall performances (Fig. 2d). The programs IEDB and NetMHCpan showed the highest MCC of 0.39 each (Fig. 3d). It is worth mentioning that none of the programs for this allele had high and balanced sensitivity and specificity. SVMHC (syf) showed the poorest sensitivity of 4% among all the programs. For A*2402, ARB matrix showed highest sensitivity (88.5%), specificity (92.3%) and accuracy (90.1%) (Fig. 2e). This was reflected in its MCC score (0.80), suggestive of 90% correlation between experimental and predicted data (Fig. 3e). For the allele B*0702, very high efficiency of prediction was not observed for any of the programs (Figs. 2f, 3f). The highest overall correlation (MCC) was observed for NetMHCPan predictor for this allele. Although IEDB showed the highest accuracy, its MCC was compromised due to its lower specificity compared to NetMHCPan (Figs. 2f, 3f). For the next tested allele, B*0801, both IEDB and ARB matrix showed the greatest efficiency. Both the programs showed identical accuracy and MCC of 94.1% and 0.87, respectively (Figs. 2g, 3g). In general, IEDB, NetMHC, ARBmatrix and NetMHCpan were more adept than others in prediction efficiencies. The propensity of predicted binders/non-binders to be true could be calculated based on PPV and NPV. The PPV and NPV values for these programs also reflected their superior efficiency compared to other programs (data not shown).

We then analyzed the efficiency of the programs to predict weak binders. Weak binders were chosen based on experimental IC_{50} values that were between 1,000 and

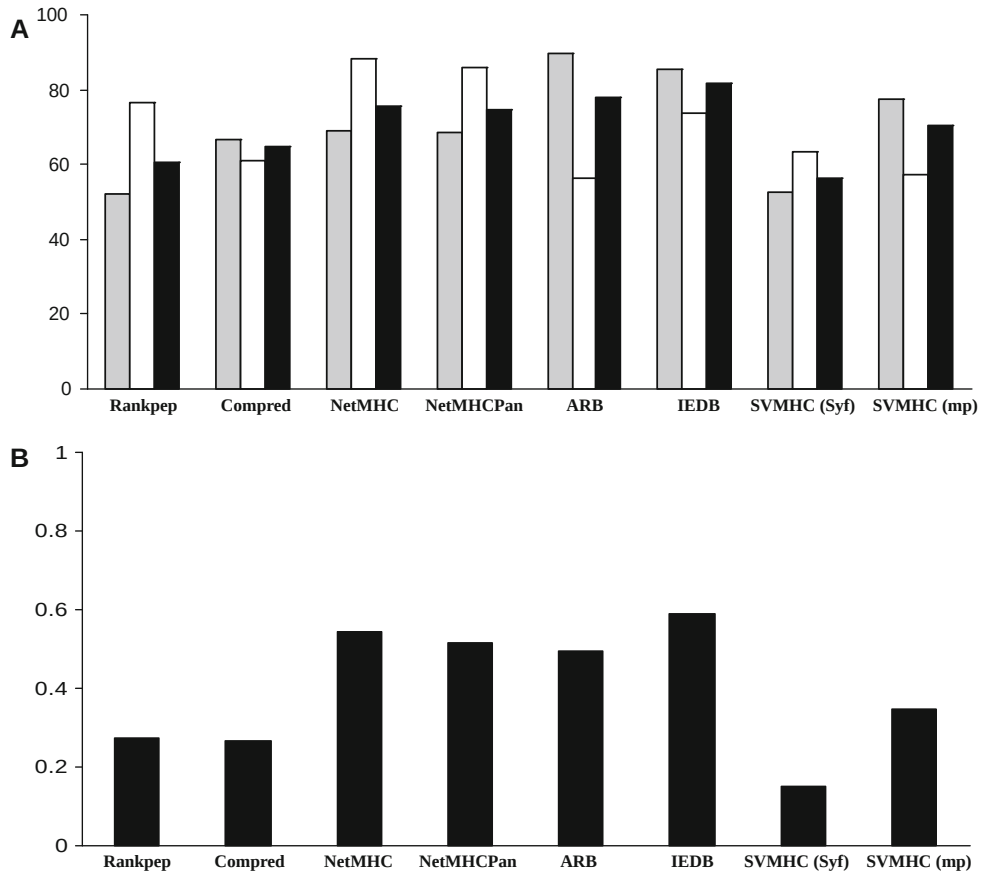


Fig. 1 Comparative analysis of the performance of in silico programs with the experimental data illustrating varied efficiencies. Predominantly occurring HLA class I alleles (A*0101, A*0201, A*0301, A*1101, A*2402, B*0702, B*0801) were selected. A total of 960 peptides from different antigens that are experimentally verified in the literature as bona fide binders/non-binders were selected to validate the efficiency of various in silico programs. The results derived after validation were subjected to statistical analysis to evaluate the sensitivity, specificity and accuracy of the in silico programs. **a** Performance measures of the programs using pooled data correspond to percentage. *Gray*, *white*, and *black bars* symbolize sensitivity, specificity and accuracy, respectively. **b** Matthew's correlation coefficient (matching coefficient between predicted and experimental data) for the overall performance plotted against the programs used

5,000 nM (Harrison et al. 1997; Topalian et al. 1996; Wang et al. 2008). Although they are classified as weak binders based on the in vitro assay, they may be immunologically relevant. In comparison with the total sensitivity, NetMHC, NetMHCPan, IEDB programs had 20–25% lower sensitivity in identifying weak binders as compared to other programs. ARB matrix, compared to, SVMHC (syf) showed marginal reduction in sensitivity (Fig. 4). ARBmatrix was more sensitive than the other three in identifying weak binders as well. But it is to be noted that it had higher incidence of false positives as compared to the other three programs.

There are reports that suggest that taking a consensus of results of different programs can lead to better identification of binders (Toseland et al. 2007). But a few others have suggested that it may not improve predictions significantly (Gowthaman and Agrewala 2009). Since this approach has not been tried much for class I binders, we attempted to arrive at a consensus, qualitatively. We considered a consensus positive or negative peptide if a minimum of four programs suggested positivity or negativity. We then calculated correlation coefficients of consensus binders/non-binders and compared it with the MCC of the best individual predictor (based on Fig. 3) for each allele. We found that the consensus method could marginally increase the overall prediction efficiency for alleles A*0301 and A*1101. But for the other alleles, the best individual predictors outperformed consensus method (Fig. 5). Since individual predictors vary greatly in their efficiency (Fig. S1), we reasoned that the poor predictors ameliorate the efficiency of consensus method. Hence, we selected only the top three predictors for each allele (Table 1), based on their MCC values, and calculated consensus among them. We considered a peptide consensus positive or negative, if two of the three chosen predictors suggested

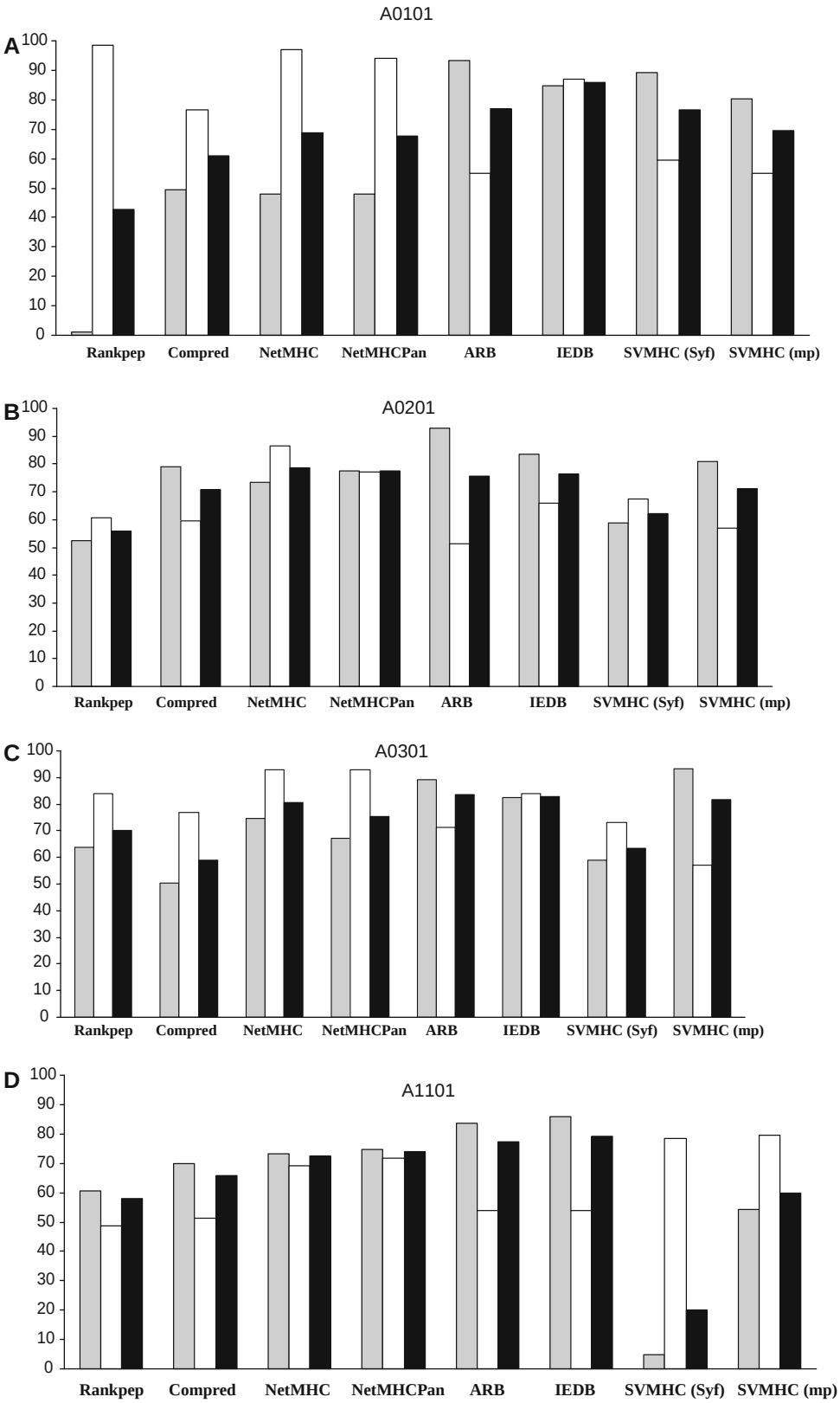
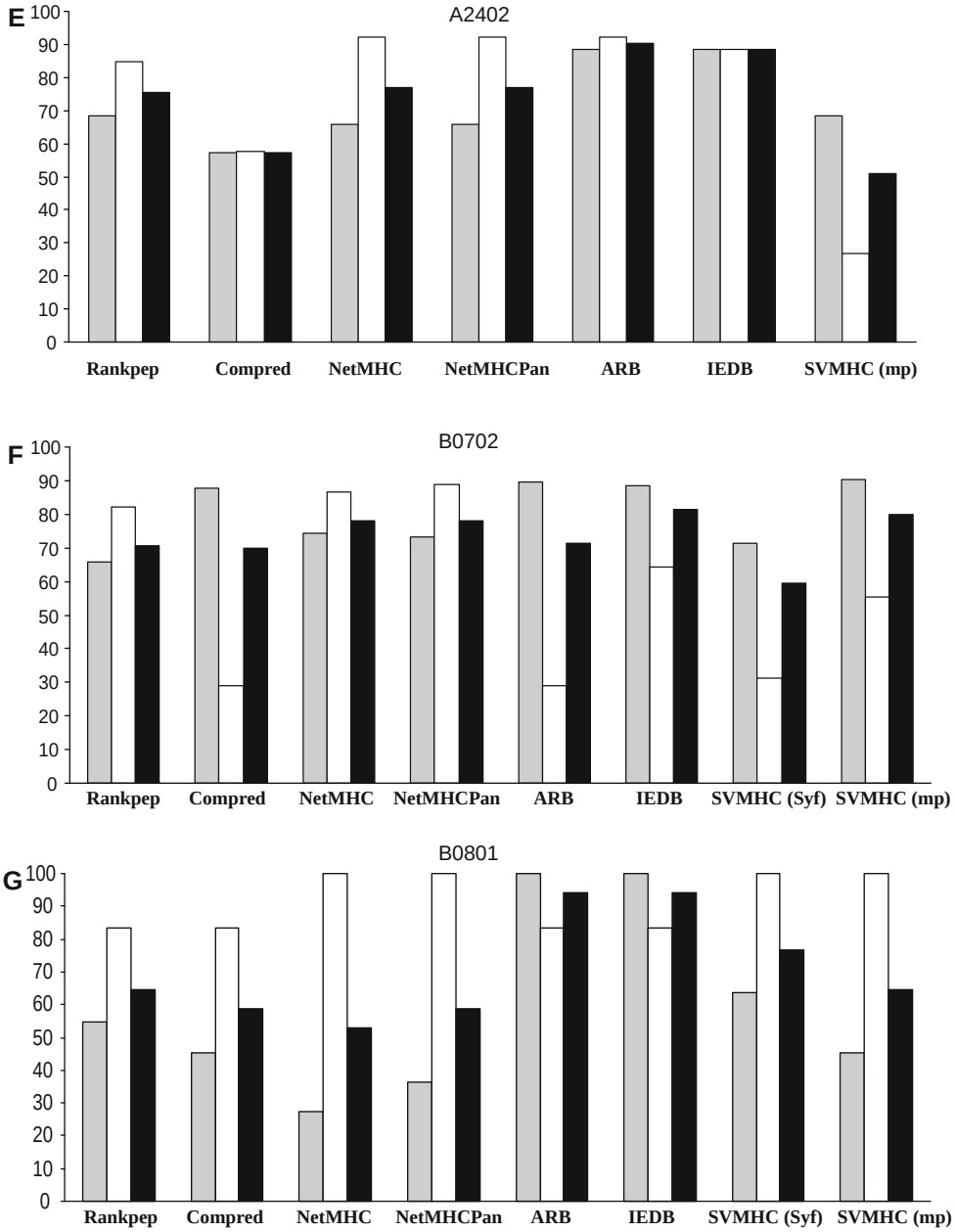


Fig. 2 Sensitivity, specificity and accuracy values of different predictors. The proficiency of individual predictors (of servers) for each allele tested is represented as percentage for A*0101 (**a**), A*0201 (**b**), A*0301 (**c**), A*1101 (**d**), A*2402 (**e**), B*0702 (**f**) and B*0801 (**g**). *Gray*, *white*, and *black bars* symbolize sensitivity, specificity and accuracy, respectively

Fig. 2 continued



positivity or negativity. Although this analysis improved the efficiency compared to consensus of all the programs, efficacy did not greatly increase as compared to the best predictor for a particular allele (Fig. 5). Efficiency marginally increased for A*0101 and A*1101, but for the rest it either remained equivalent or marginally decreased.

To sum up, four of the eight programs tested were fairly efficient at predicting class I HLA binders. But the individual predictors within a program greatly varied in their prediction efficiencies. This could occlude efficient identification of promiscuous peptides for which the individual predictors must have balanced efficiency. Thorough standardization is required with known datasets before employing these programs. As a single program may not be very efficient for all the alleles, users have to select the better predictors from individual programs to get the best result. Consensus method does not seem to significantly improve the prediction efficacies.

Discussion

Employing peptide vaccines offers several advantages including the circumvention of antigen processing, selecting epitopes that can elicit optimum immune response and eliminating the regions involved in autoimmunity and immunosuppression. In principle, in silico methods could identify CTL epitopes that bind to diverse class I HLA

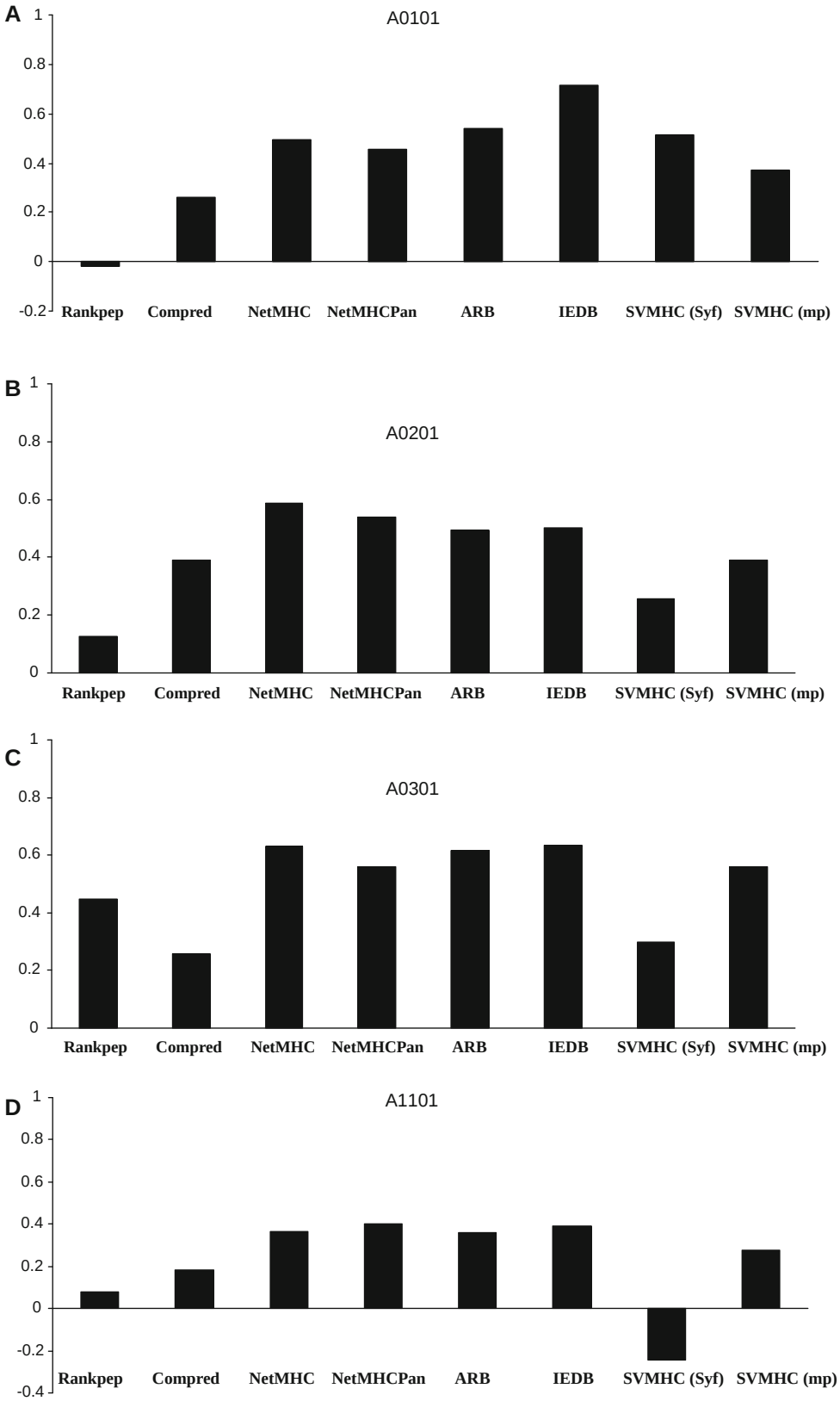
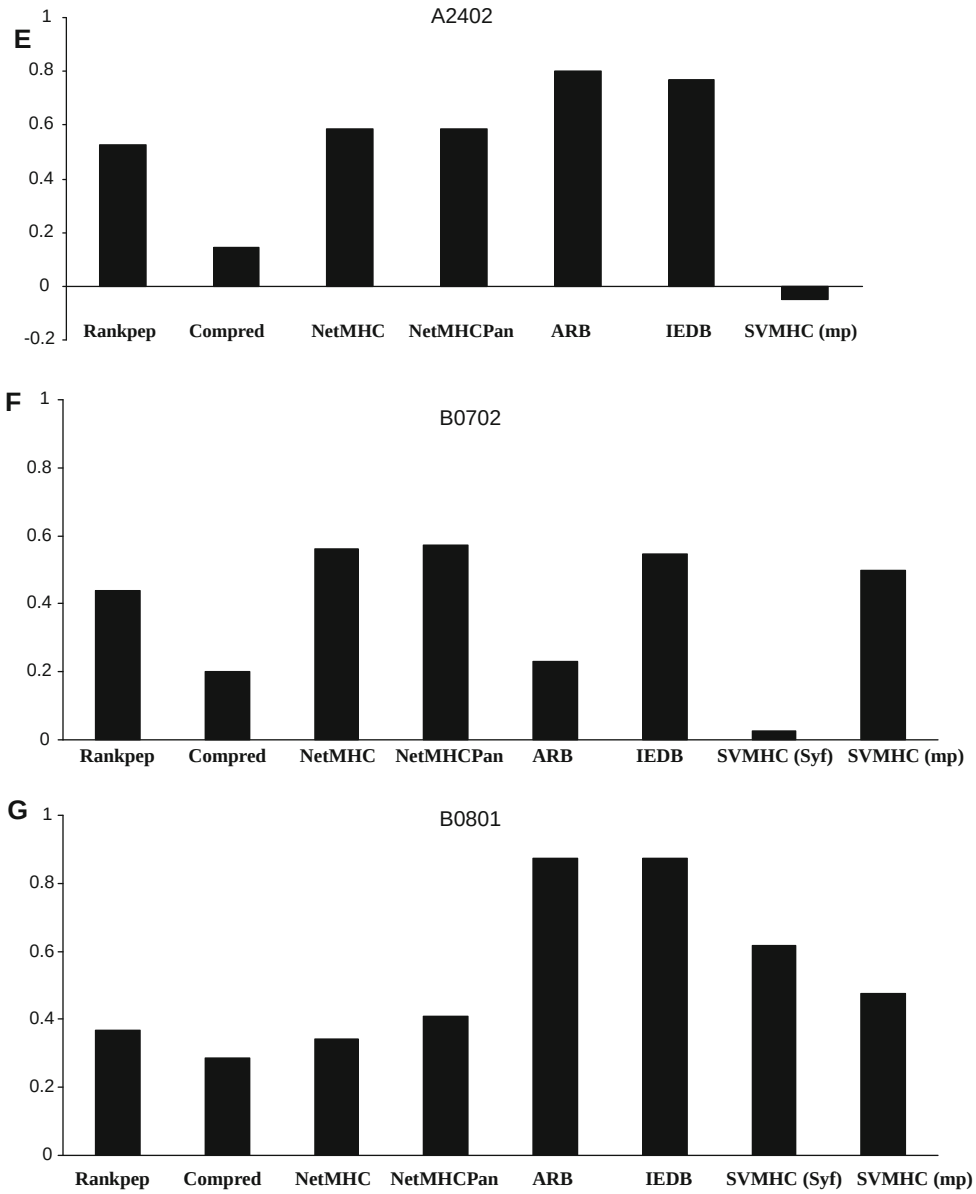


Fig. 3 Matthew's correlation coefficients of individual predictors for the different alleles tested. The MCC values, which calculate the correlation between experimental data and in silico predictions, from different predictors are plotted for each of the tested alleles; A*0101 (**a**), A*0201 (**b**), A*0301 (**c**), A*1101 (**d**), A*2402 (**e**), B*0702 (**f**) and B*0801 (**g**)

Fig. 3 continued



alleles. This would help to overcome the restriction imposed by the HLA polymorphism for global vaccination against diseases. There are many reports that highlight the usefulness of in silico methods in peptide identification. In contrast, there are studies that contest this claim (Gowthaman and Agrewala 2009). Hence we have attempted to evaluate the predictive power of many different generic in silico approaches, that are routinely employed, with bona fide class I MHC binding peptides. In the present study, we have employed existing generic in silico methods like ANN, SVM, matrix-based and hybrid methods for predicting HLA class I binding peptides and evaluated their prowess with a large data set of experimentally proven peptides. The major findings that have emerged from this study are as follows: (1) no single method is equally consistent for the alleles, (2) prediction efficiency varies between alleles at the same level of stringency, (3) none of the programs may adeptly identify promiscuous binders, (4) methodical standardization is required from the end user to get maximally effective prediction, (5) a consensus of different results could not increase efficiency significantly, and (6) in silico identification of class I MHC binding peptides are more efficient than class II MHC binding peptides.

A high degree of variability in the prediction efficacy for different alleles of the same program was observed, in spite of employing the same threshold level (stringency level). This variability is observed irrespective of the server used. The total efficiency of a program is directly proportional to its poorest prediction for one of its alleles; hence this may limit the programs ability to identify promiscuous binders. The reasons for the heterogeneity in

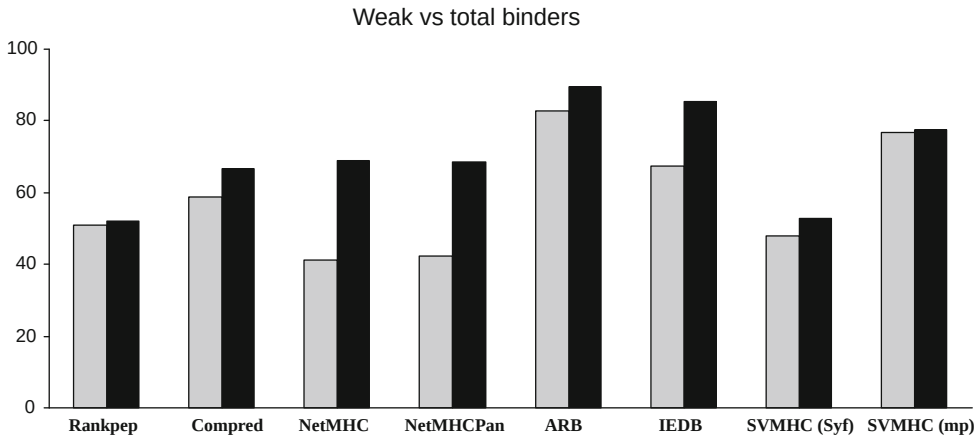


Fig. 4 Sensitivity of identifying weak binders: a total of 104 binders that were $IC_{50} \geq 1{,}000 \leq 5{,}000$ nM were classified as weak binders. The servers were tested for their ability to identify them. The results were computed for sensitivity and was compared with the over all sensitivity (as in Fig. 1a). Values expressed as percentage. *Gray* and *black bars* indicate sensitivity for weak binders and total sensitivity, respectively

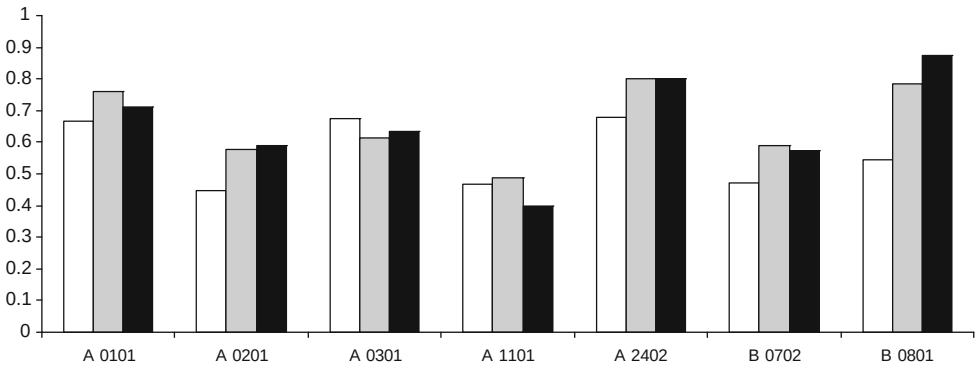


Fig. 5 Consensus approach is not significantly efficient than the best individual predictor. Comparison of Matthew's correlation coefficients of consensus methods and the best predictor for an allele (Table 1). *White bar* denotes consensus from all the eight predictors. *Gray bar* indicates consensus from the best 3 programs (Table 1) for a given allele. *Black bars* indicate the best individual (no consensus) predictor

prediction may be due to biased data sets used at the time of their development. For some alleles, the available published data may be more affluent than others. This may lead to imbalance in the prediction efficiency among the alleles. This could in principle be corrected by a process called "rescaling". But a recent report suggests that this may mask the biological variations and hence lead to inaccurate predictions (MacNamara et al. 2009). The methods may also have their own limitations. For example, SVM and ANN methods completely rely on the training data set and matrix-based method depends on independent contribution of amino acids in the peptides, therefore ignoring their neighboring residues. The hybrid approach which combines two different prediction models is also not competent enough to be equally efficient for all the alleles. In order for better performance of these programs, the data set used for training or developing the matrix must be balanced for all the alleles. Moreover, as current research helps in constant improvement in the existing knowledge, these servers must be cognizant of the latest developments and get periodically updated. We have also valuated whether consensus method is more adept than results from individual predictors. Consensus method could improve prediction efficiency marginally if results from poor predictors were pooled. However, this approach did not seem to considerably improve good performing predictors. This suggests that some of the peptides were commonly missed by majority of the programs thus inflating the false negative values. Indeed we were able to observe some peptides of similar sequence that remained unidentified by most or all predictors. Hence, this shows that there are yet to be identified motifs/sequences in peptides that bind to MHC molecules. Further understanding of peptide–MHC chemistry, computational (structural/sequence based) scrutiny may help resolve this issue.

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Conflict of interest statement The authors declare no conflict of interests.

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