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Structure based selection of Human metabolite binding P4 pocket of *DRB1*15:01* and *DRB1*15:03*, with implications for multiple sclerosis

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Abstract

Binding of small molecules in the human leukocyte antigen (HLA) peptide-binding groove may result in conformational changes of bound peptide and an altered immune response, but previous studies have not considered a potential role for endogenous metabolites. We performed virtual screening of the complete Human Metabolite Database (HMDB) for docking to the multiple sclerosis (MS) susceptible *DRB1*15:01* allele and compared the results to the closely related yet non-susceptible *DRB1*15:03* allele; and assessed the potential impact on binding of human myelin basic peptide (MBP). We observed higher energy scores for metabolite binding to *DRB1*15:01* than *DRB1*15:03*. Structural comparison of docked metabolites with *DRB1*15:01* and *DRB1*15:03* complexed with MBP revealed that Phenylalanine^{MBP92} allows binding of metabolites in the P4 pocket of *DRB1*15:01* but Valine^{MBP89} abrogates metabolite binding in the P1 pocket. We observed differences in the energy scores for binding of metabolites in the P4 pockets of *DRB1*15:01* vs. *DRB1*15:03* suggesting stronger binding to *DRB1*15:01*. Our study confirmed that specific, disease-associated human metabolites bind effectively with the most polymorphic P4 pocket of *DRB1*15:01*, the primary MS susceptible allele in most populations. Our results suggest that endogenous human metabolites bound in specific pockets of HLA may be immunomodulatory and implicated in autoimmune disease.

Keywords

*DRB1*15:01*; *DRB1*15:03*; myelin basic protein; multiple sclerosis; metabolites

Introduction

Multiple Sclerosis (MS) (OMIM: 126200, MIM: 142857) is a chronic inflammatory and demyelinating disorder of the central nervous system (CNS). Demyelinated lesions throughout the CNS, involving both the white and gray matter, are responsible for progressive neurological deficits, although extensive heterogeneity in disability exists

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between individuals. Genes within the human leukocyte antigen (HLA) region account for the largest component of the genetic risk for MS. The primary signal in the region maps to the *DRB1* gene, and specifically to the *DRB1*15:01* allele, in the class II segment of this locus[1]. This strong association is observed across multiple populations, including virtually all European-ancestry population examined to-date. Interestingly, *DRB1*15:01* is also observed to be the primary predisposing allele in African American MS patients, despite the fact that the closely related *DRB1*15:03* is much more common in this population[2]. Although *DRB1*15:03* differs from *DRB1*15:01* at only a single amino acid position 30 (His >Tyr), our recent work has demonstrated that *DRB1*15:03* does not play a role in susceptibility to MS in African Americans (Damotte et al, in prep).

The prevailing view of HLA-mediated autoimmune pathogenesis involves presentation of self-derived cellular proteins by HLA molecules to T cells, with resulting activation and immune responsiveness against the self-antigen[3]. In MS, several myelin-derived antigens have been proposed in disease pathogenesis. Both myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) have been shown to bind to *DRB1*15:01*[4, 5], and initiate an immune response against myelin. The ligands of HLA class II molecules are produced mainly from exogenous protein sources[6]. Subsequently, they are digested by proteases and loaded onto class II HLA molecules after internalization in a procedure catalyzed by an endosomal and lysosomal chaperone *HLA-DM*[7]. The processing and loading occurs in a devoted endosomal compartment[8]. The peptide-loaded HLA are subsequently transported to the cell surface and when recognized by CD4+ T cells, stimulate the Ag-specific immune response[9]. The peptide-binding groove of the class II HLA molecule consists of nine different structural pockets (P) from P1 to P9, which accommodate the antigen peptide side chains.

A peptide from the middle region of human MBP consisting of amino acids from position 84 to 102 has been shown to be immunodominant for human MBP-specific T cells in individual carriers of the HLA-DR2 (which includes *DRB1*15:01*) haplotype[10, 11]. Further, it has been suggested that MBP (residue 85-99)-specific T cell clones from individuals with the DR2 haplotype are restricted to DRB1*15:01 and the MBP (85-99) peptide was also discovered to bind with purified *DRB1*15:01*[12, 13]. Subsequently, two residues of MBP (85-99), valine (Val) and phenylalanine (Phe) at position 89 and 90, respectively, were recognized as P1 (Val^{89}) and P4 (Phe^{92}) anchor residues for the binding in *DRB1*15:01*[13]. *DRB1*15:01* is distinguished by the presence of a large, predominantly hydrophobic P4 pocket, due to the presence of alanine (Ala) in the polymorphic DR β-chain at amino acid position 71 (DRβ71)[14]. Thus the large phenylalanine residue of the MBP peptide at position 92 in P4 functions as a major anchor residue for *HLA-DR2* binding[14]. In contrast, the presence of valine at amino acid position 86 in the DR β-chain at the base of the P1 pocket gives rise to a smaller pocket, obligating residence of a smaller amino acid residue[14].

Recent work suggests that natural, synthetic or environmental small molecule compounds may efficiently occupy the pockets of *HLA-DR*[9, 15, 16]. An examination of HLA alleles important in type-2 diabetes mellitus (T2DM) showed that small molecules influence the immune response in an allele-specific way by transiently occupying a dimorphic pocket on the HLA-DR molecule, preventing the rapid inactivation of the heterodimer so that free

peptides can reach the vacant peptide binding site[9, 15]. These studies suggest that small molecule binding within the HLA peptide binding groove can result in profound alterations in peptide binding and potentially immune responsiveness[9, 15, 16]. This notion is bolstered by robust models of *HLA* mediated drug hypersensitivity demonstrating that the binding of small molecules in the peptide binding groove results in either changes in conformation or causes a register shift of the bound peptide, leading to an aberrant immune response[17, 18]. It is important to consider that accommodation of a register shift is possible only for HLA class II, as the binding groove of the class I HLA molecule is restricted at both ends, with the N and C-terminal residues of the peptide acting as anchors[19]. Peptides cross-presented by different class I alleles must bind in the same register, though not necessarily in the same conformation[20].

Despite decades of research and development of multiple lines of disease modifying therapies for MS, no drugs have been successfully developed that directly inhibit the pathogenic T cells via inhibition of *HLA-DRB1* mediated presentation of neuroantigens. However, recent studies suggest promise in structure-based selection and identification of small molecules for the alteration and inhibition of allele specific *HLA* class II antigen presentation[15, 16]. At the same time, it goes to follow that if exogenous molecules are capable of altering immune-responsiveness, naturally occurring endogenous small molecules may function to trigger the aberrant response observed in autoimmune diseases. Human metabolites are abundant, naturally occurring small molecules produced endogenously in the course of normal metabolic processes. Additionally, scores of small molecules have been shown to be derived from the human microbiome; each of the major metabolite classes have been observed and participate in a range of biological activities, including immune modulation and antibiosis[21].

In this study, we hypothesized that naturally occurring small molecules may bind the MS susceptibility allele *DRB1*15:01*, resulting in conformational changes or causing a register shift to the bound peptide and thus altering T-cell recognition and responsiveness, initiating an autoimmune response. We performed virtual screening of the complete Human Metabolite Database (HMDB) for docking to the susceptible *DRB1*15:01* allele and compared the results to the closely related yet non-susceptible *DRB1*15:03* allele; and assessed the potential impact on binding of human myelin basic peptide (MBP).

Results

Structural analysis and identification of pockets

Structural analysis of the MBP bound *DRB1*15:01* and *DRB1*15:03* protein structure shows a total of six chains: chain A, B, D and E represent the β-chain of *DRB1* protein and chain C and F belongs to MBP (Figure 1A). The Chain A and D each consist of 180 amino acid residues individually, while each of chain B and E contains 191 amino acid residues. C and F chains each comprise 15 amino acid residues.

We identified a total of 9 pockets in the MBP peptide of *DRB1*15:01* and *DRB1*15:03* protein structures. Val 89 and Phe 92 of MBP peptide were recognized as P1 and P4 anchor residues for *DRB1*15:01* and *DRB1*15:03* protein structures, as previously reported[14]. The

surface hydrophobicity of P1, P4 and P9 pockets are shown in Figure 1B, illustrating the large hydrophobic P4 pocket that is specific to *DRB1*1501*.

Docking of human metabolites as MHC Loading Enhancers for DRB1 alleles

We provided energy score, van der Waals component, electrostatic component, polar solvation component and apolar solvation component, molecular weight, partition coefficient (xlogP), molecular charge, heavy atoms, polar contacts, nonpolar contacts, and number of conformations for the docked metabolites binding with *DRB1*15:01* and *DRB1*15:03* in Supplementary Tables S1, S2 and S3.

We selected the top 200 significant metabolites from the docking results in the P4 pocket for both *DRB1*15:01* and *DRB1*15:03* and ranked them on the basis of their energy score. We found that 78 and 81 metabolites bind individually with *DRB1*15:01* (Supplementary Table S1) and *DRB1*15:03* (Supplementary Table S2) respectively, whereas 79 metabolites bind commonly to both *DRB1*15:01* and *DRB1*15:03* (Supplementary Table S3). We observed overall higher energy scores for the binding of human metabolites with *DRB1*15:01* than *DRB1*15:03* (Figure 2), suggesting generally stronger binding of metabolites to *DRB1*15:01*.

Determination of optimal pockets of the DRB1*15 alleles for binding to human metabolites

Structural comparison of the docked *DRB1*15:01* and *DRB1*15:03* metabolite complexes with *DRB1*15:01* and *DRB1*15:03* MBP complexes revealed that Phenylalanine^{MBP92} allows the binding of metabolites in the P4 pocket of *DRB1*15:01* but Valine^{MBP89} in the P1 pockets does not (Table 1). We observed differences in the energy score even for the binding of the same metabolites in the P4 pockets of *DRB1*15:01* and *DRB1*15:03* (Table 1) or different metabolites in the P4 pockets of *DRB1*15:01* and *DRB1*15:03* (Table 1).

Mapping Human Metabolites on the MS phenotype

We performed data mining using metabolites we identified that bind either *DRB1*15:01* or both *DRB1*15:01* and *DRB1*15:03* as input in ingenuity pathway analysis (IPA) to screen for human metabolites that have been previously associated with MS. We identified several human metabolites previously associated with MS and/or Neurodegeneration (Table 2).

Discussion

HLA-DRB1 is the primary susceptibility locus in MS[1]. It is likely that the crucial role of this molecule is mediated by presentation of peptide antigens to myelin-reactive T cells, which are subsequently responsible for the stimulation, proliferation, and production of pathogenic cytokines. Recent studies have identified small molecules that bind to the peptide-binding groove of *DR1* and *DR3*, and act as a catalyst to enhance peptide loading[9, 15, 16]. We used an *in silico* molecular docking algorithm to virtually screen the complete Human Metabolite Database (HMDB) for docking to the MS predisposing *DRB1*15:01* allele and compared the results to the closely related yet non-susceptible *DRB1*15:03* allele; and evaluated the potential impact on binding to MBP peptide. Although small molecules have been evaluated with respect to antigen presentation by the HLA class II molecule (*DR1* and *DR3)*, the

present study is the first to screen a large database of naturally occurring human metabolites using a molecular docking algorithm to predict interaction with the peptide-binding groove of *HLA-DR2*.

We found considerably higher energy scores for the binding of human metabolites with *DRB1*15:01* than *DRB1*15:03* (Figure 2), suggesting stronger binding of metabolites with *DRB1*15:01 (DR2)* the primary MS associated allele. Further, our results confirm that phenylalanine^{MBP92} allows the binding of metabolites in the P4 pocket of *DRB1*15:01* (DR2) but valine^{MBP89} prohibits metabolite binding in P1 pockets. The most commonly accepted explanation for this is that the predominantly hydrophobic P4 pocket (Figure 1B), occupied by a phenylalanine^{MBP92} of the MBP peptide, is the critical feature of the *DR2* peptide-binding site and the existence of an alanine residue at the polymorphic DRβ71 position generates the essential space for the binding of an aromatic side chain in the P4 pocket[13, 22]. The presence of two aromatic residues of the P4 pocket of *DR2*, β26 Phe and β78 Tyr, of which β26 is polymorphic, also facilitate the binding of aromatic side chains by the P4 pocket[14]. The occurrence of alanine at DRβ71 is exceptional for *DRB1* alleles and has been observed distinctively for some *DR2* alleles including *DRB1*15:01* and *DRB1*15:03;* other *DRB1* alleles encode arginine, glutamic acid, lysine, threonine, glycine and serine at this position (IMGT/HLA- database version 3.27.0). *DRB1*15:01* is the most frequently observed *DR2* allele in individuals with European ancestry, who also have augmented risk for the development of MS. Large-scale GWAS confirms that the main susceptibility signal maps to the *DRB1* gene in the HLA class II segment, and elucidates up to 10.5% of the genetic variance causal for MS susceptibility[23]. *DRB1*15:01* has the greatest effect with an average odds ratios (OR) of 3.08, and all additional *DRB1* associations emerge to account for less than 2% of the residual variance[23]. There is significant variation in the distribution of *DRB1*15* haplotypes across different worldwide populations, and these tend to correlate with the incidence of MS[24]. It would be interesting to examine whether metabolomic profiles differ similarly between world populations, and consider their relationship to *HLA* class II frequency distributions.

Previous studies reported the binding of adamantyl derivatives and plant alkaloid Cepharanthine in the P1 pockets of *DRB1*01:01* (*DR1*)[9], and *DRB1*03:01* (*DR3*)[16]. *HLA-DR1* (*HLA-DRA*, *DRB1*0101*) and DR4 (*HLA-DRA*, *DRB1*0401*) have large P1 pockets due to the presence of Gly at DRβ86 position, while the arginine residue at position DRβ74 is responsible for the larger P1 size for *DR3* (*HLA-DRA*, *DRB1*03:01);* thus, the P1 pockets may accommodate metabolite and peptide binding in *DR1*, *DR3* and *DR4*. Meanwhile, the P1 pocket of *DR2* (*HLA-DRB, DRB1*15:01*) is too small (Val at DRβ86) to accommodate small molecules such as human metabolites, whereas the greater size of the P4 pocket due to alanine at DRβ71 allows the binding of metabolites in the P4 pockets of both *DRB1*15:01* and *DRB1*15:03*. The polymorphism at position 71 in *DR2* (alanine) emerges to be most significant in terms of producing the accessible space for the P4 aromatic side chain (Phe) of the MBP peptide[14]. The *HLA-DR2* P4 pocket has an inclination for aromatic as well as aliphatic residues[14]. The P4 pocket of *HLA-DR2* binding is detected with analogues of the MBP peptide, which contains a substitution by histidine, arginine, lysine, glutamine or asparagine at P4; substitution by aspartic acid is not tolerated[13]. Human metabolites appear to bind more strongly with P4 pockets of

*DRB1*15:01* than *DRB1*15:03* due to the presence of a tyrosine residue at DRβ30 position, the only amino acid difference between the two alleles. The tyrosine side chains have a neutral charge, in contrast to the positively charged side chains of histidine at position 30 of *DRB1*15:03*, which may facilitate the stronger binding of metabolites.

Numerous studies have reported the association of the P4 pocket, the most polymorphic pocket of the *HLA-DR* binding site, in augmented risk to other autoimmune diseases[25-28], which further strengthens our observation. For example, *DRB1*04:02*, which has been associated with predisposition to pemphigus vulgaris[26, 27], contrasts from the rheumatoid arthritis linked *DRB1*04:04* allele at only three positions: DRβ67, - DRβ70, and -DRβ71[25, 28]. In *DRB1*04:02*, DRβ70 and -71 of the P4 pocket carry negative charges due to residues aspartic acid and glutamic acid respectively[26, 27]. In contrast, glutamine and lysine/arginine residues are present respectively at DRβ70 and -71 positions in the rheumatoid arthritis correlated *DRB1*04:01* and *DRB1*04:04* alleles.

The binding of human metabolites in the P4 pockets of *DRB1*15:01* potentially have profound effects on T cell proliferation and antigen presentation. One possible scenario for altered T-cell recognition related to bound metabolites in *DRB1*15:01* is shown in Figure 3A. In this model the bound metabolite causes a register shift in the 9mer segment of MBP peptide from the normal bound position (Figure 3B). HLA class II molecules consists of an open binding groove, which permits higher flexibility and bound peptides may be up to 14-20 amino acids[29]. The peptide-binding segment of the HLA class II molecule binds specific 9mer residues, which is recognized as the peptide binding register[29]. This 9mer segment is located within the binding groove of the HLA class II molecule, while the residues of the remaining peptide are present exterior to the groove. As the peptide occupies the binding groove, the anchor residues at locations P1 or P4 of the peptide register act as primary *HLA* contacts, interacting with residues in the pockets of the HLA molecule. The binding pockets differ among HLA molecules, which are encoded by distinctive alleles and are accountable for binding the peptide within the groove. The pocket residues are mainly obscured deep inside the binding groove. In a register shift, the MBP peptide and human metabolite complex may transiently occupy a pocket in the *HLA* peptide-binding groove and shift forward the next residues, sliding along the groove until the trimolecular complex of HLA-Peptide-Metabolite is stabilized. Alternatively, the presence of one of the high scoring metabolites in the P4 pocket of *DRB1*15:01* may enhance the risk of MS by augmenting antigen loading. This possibility is supported by previous reports suggesting that a transient occupation of *HLA-DR* pocket by an organic compound stabilizes the peptide-receptive conformation allowing rapid antigen loading[9].

Interestingly, our IPA analysis revealed that specific metabolites that bind the P4 pocket of *DRB1*15:01*, L-tryptophan, glutamic acid, D-sphingosine, sphingosine-1-phosphate, Cysteinyl-Glycine and NAD+ have been previously associated with MS and Neurodegeneration (Table 2). Similarly, the metabolomic signatures of tryptophan and glutamic acid have been suggested to be associated with disease severity in multiple sclerosis[30]. The sphingolipids are a class of biologically active lipids; sphingosine 1-phosphate receptors 1 and 3 have been shown to be upregulated in multiple sclerosis lesions[31]. A homocysteinemia regulator cysteinylglycine has been linked with physiological

and pathological conditions in cerebro-vascular and multiple sclerosis patients[32]. More recently, human metabolites NAD(+), Myo-Inositol hexakisphosphate and glutathione have been correlated respectively with neurodegeneration[33], regulation of morphology and synapse formation of cerebellar purkinje cells[34], and metabolism impairment in MS[35].

Recently, a study conducted in autoimmune Goodpasture disease has shown that HLA-DR15-$\alpha_{3135–145}$ tetramer^{+} T cells in *HLA-DR15* transgenic mice display a conventional T-cell phenotype, which secretes pro-inflammatory cytokines and may be associated with augmented disease risk[36]. Meanwhile, HLADR1-$\alpha 3_{135–145}$ tetramer^{+} T cells in *HLA-DR1* and *HLA-DR15/DR1* transgenic mice are mainly CD4^{+}Foxp3^{+} regulatory T cells (Treg cells) expressing tolerogenic cytokines, which is linked with disease protection[36]. This study provides a mechanistic model for the relative abundance of self-epitope specific Treg cells, which leads to either protection or risk of autoimmunity. The same concept may be applicable to the transient occupation of certain human metabolites in the P4 pocket *HLA-DRB1*15:01*, altering peptide presentation and potentially resulting in a change of the balance of Treg vs. conventional T-cell phenotype.

Our analysis of *HLA* interactions with human metabolites could pave the way for similar investigations in clinical allo-transplantations and other autoimmune diseases, such as rheumatoid arthritis (RA), celiac disease (CD), systematic lupus erythmatosis (SLE), ankylosing spondylitis, dermatomyositis and type I diabetes mellitus (T1DM). Our approach might be helpful to weight or group together various *HLA* genes and alleles that are involved in susceptibility to different diseases. The findings of this study could also be useful in resolving the conflicts regarding the association of *HLA* polymorphisms with RA susceptibility association with anti-cyclic citrullinated peptide (ACPP) autoantibody. In some studies, ACCP antibodies are associated with the presence of *HLA* class II alleles; especially the *DRB1* shared epitope (SE) alleles in European-ancestry RA patients[37, 38], whereas subsequent studies have failed to replicate this association[39]. The identification of specific pathogenic metabolites in RA could help to explain some of these discrepancies. Similarly, Lee and colleagues have recently suggested that the genetic contribution to prognosis in Crohn's disease is largely independent of the contribution to disease susceptibility[40]. The detection of highly immunogenic metabolite-HLA loads in individuals could provide insight regarding prognosis as well as new therapeutic opportunities. Finally, donor-recipient matching for immunogenic metabolites that occupy specific pockets in HLA molecules could be a strategy employed in *HLA* mismatched donor-recipient pairs to improved transplant outcome and avoid graft rejections, providing a potential applicability of our study in terms of clinical allo-transplantation. Although finding the exact match of donor-recipient pairs for immunogenic metabolites could be challenging, donor-recipient pairs with low metabolite mismatch loads or without highly immunogenic metabolite mismatches could be considered.

In summary, we have shown that naturally occurring human metabolites bind to *DRB1*15:01*, the primary MS susceptible allele in most populations, and may potentially impact MS risk. An improved understanding of the kinetics of HLA binding of human metabolites may allow development of small molecule based immunomodulatory drugs antagonistic to metabolites that enhance antigen loading or alter presentation. Identification

of our high scoring metabolites in metabolomics studies in MS may also be informative in identification of suitable MS biomarkers. A limitation of our study is that we did not test the docked DRB1-Metabolite complex in the laboratory. This is an outstanding point of uncertainty and will need to be addressed in future studies to fully elucidate the role of the metabolites identified *in silico* in T-cell proliferation and antigen presentation. A structure-based strategy could determine structural features at the peptide/HLA interface and recognize molecules that may stimulate or suppress TCR signaling in response to particular peptide antigens. The present study suggests that the combination of structure directed virtual screening and the notion that human metabolites targeted to certain HLA pockets may be immunomodulatory contains extensive applicability to adaptive immunity and autoimmune conditions.

Methods

Selection and Preparation of DRB1 Structure

The X-ray crystal structure for the *DRB1*15:01* complex with bound peptide from human myelin basic protein (MBP) with a resolution of 2.6 Å was obtained from the protein data bank (PDB) under accession number 1BX2[14]. We prepared the target (*DRB1*15:01* and *DRB1*15:03*) and binding site residue with N-acetyl-D-glucosamine (NAG) for docking with human metabolites using the UCSF Chimera software package (version 1.11.2)[41]. *DRB1*15:01* exon-2 sequence differs from *DRB1*15:03* at amino acid position 30 from tyrosine (Tyr) to histidine (His). The *in silico DRB1*15:03* structure was obtained from the known structure of *DRB1*15:01* by mutating the residue Tyr^{30} > His^{30}.

Virtual Screening and molecular docking of DRB1*15:01, DRB1*15:03 and Human Metabolites

The Human Metabolite database (HMDB) version 3.6 consists of 41,993 metabolites derived from various sources such as exogenous, endogenous, food, microbial, toxic and drug metabolites. We screened the complete HMDB library (version 3.6) for docking with *DRB1*15:01* and *DRB1*15:03*. The schematic representation of the overall study design and molecular docking pipeline is shown in Figure 4.

Virtual screening was performed using the UCSF Chimera version 1.11.2[41], Clipper version 1.6.0[42], and Dockblaster version 1.6.0 softwares[43] as previously described[41-43]. UCSF Chimera 1.11.2 was used for the selection, visualization and preparation of target and ligand and the visualization and analysis of DRB1-metabolite complexes. Virtual Screening for *DRB1*15:01* and *DRB1*15:03* was conducted on a structure obtained in molecular dynamics (MD) simulations of the complex with a bound peptide from MBP. Clipper 1.6.0 was used for the identification and analysis of different peptide binding pockets in *DRB1*15:01* and *DRB1*15:03*.

Subsequently, we conducted independent screenings to probe each pocket from P1 to P9 of both *DRB1*15:01* and *DRB1*15:03* with HMDB (version 3.6) using Dockblaster 1.6.0[43], including the calculation of energy score, van der Waals component, electrostatic component, polar solvation component, molecular charge, heavy atoms, polar contacts (3.3

A), nonpolar contacts (3.3-4.5 A), and the number of conformations for docked *DRB1* and metabolite complexes. The Dockblaster 1.6.0 results were used to select the top ranked metabolites and investigate the distribution of their scores in assessing the point beyond which their scores become indistinguishable. On this basis we selected metabolites from the docking results in each pocket and ranked them on the basis of their energy score. We used the following criteria to compute the energy score of docked metabolite with *DRB1*15:03* and *DRB1*15:03* in kcal/mol:

$$\text{Energy Score} = \text{Energy of Electrostatic interaction (ES)} + \text{Energy of van der Waals interaction (VdW)} + \text{Desolvation energy}$$

Desolvation energy was calculated by combining the energy of polar solvation component and apolar solvation component.

Sequence alignment

In order to understand whether the human metabolites are binding in the P1 or P4 pockets, we performed sequence alignment of both *DRB1*15:01*-metabolite complex and *DRB1*15:03*-metabolite complex individually with the crystal structure of *DRB1*15:01* and *DRB1*15:03* with UCSF Chimera version 1.11.2[41]. This comparison of each protein structure prior to and after the metabolite binding through sequence alignments allowed assessment of changes in the dynamics of *DRB1*15:01* and *DRB1*15:03* protein structures attributable to metabolite binding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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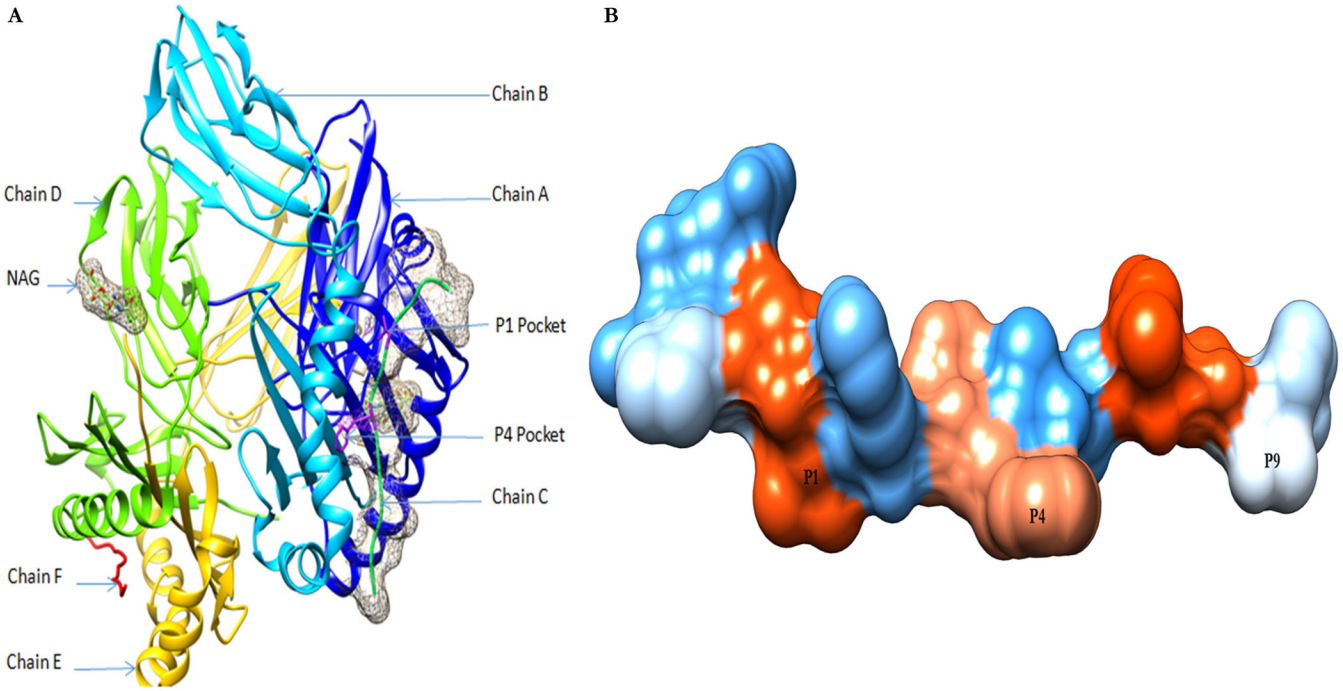

Figure 1. A: Protein structure of DRB1 with bound peptide from MBP

Chain A and D have a length of 180 amino acid residues shown in blue and forest green respectively; the length of chain B and E is 191 amino acid residues, represented in cyan and yellow respectively; a shorter chain of 15 amino acid residues of MBP belongs to chain C and F and is shown in green and red respectively.

B: Hydrophobicity of the DRB1 protein surface. Blue patches are hydrophilic in nature whereas the orange patches are hydrophobic in nature.

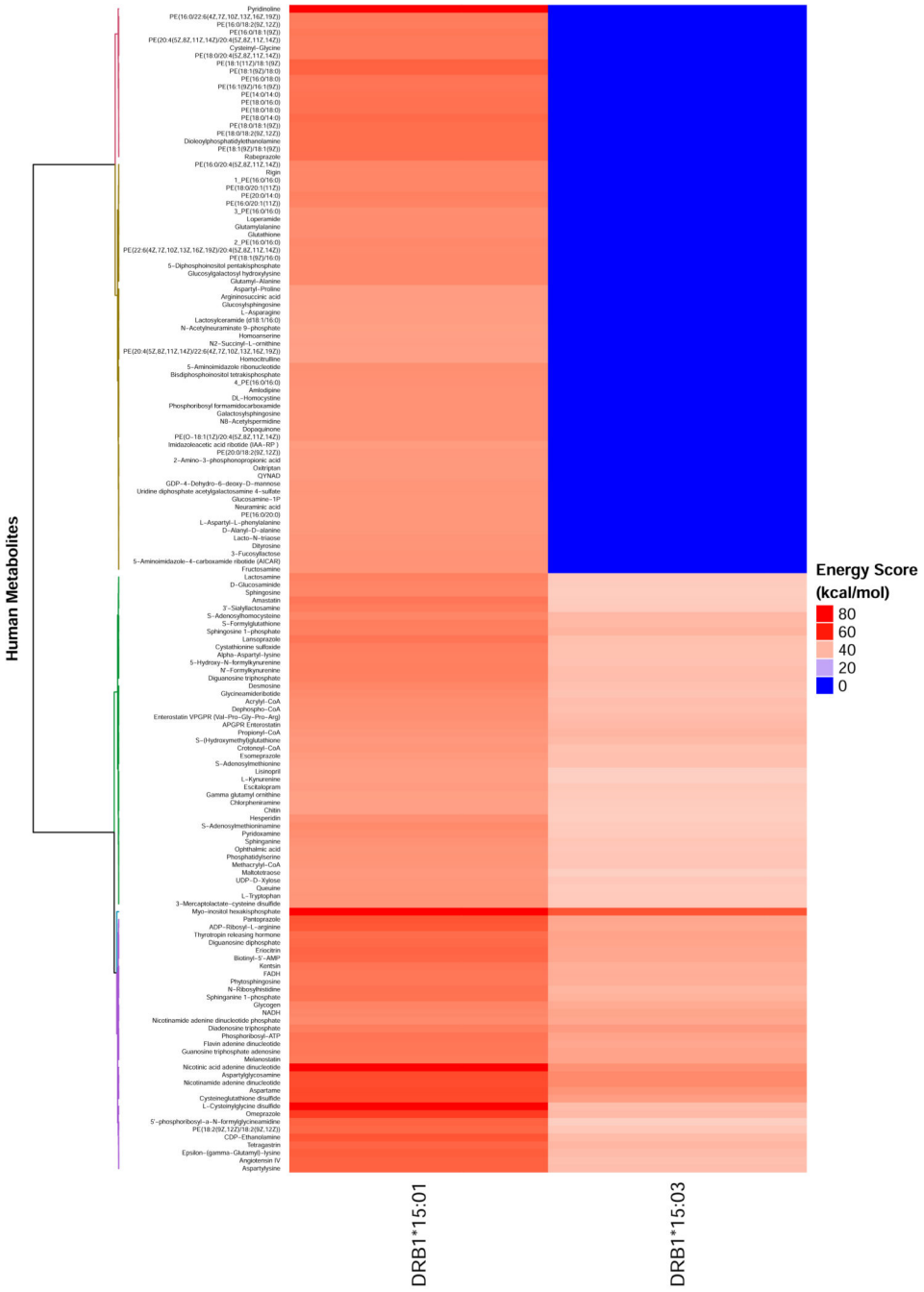

Figure 2. Heat Map of Binding energy Scores of Human Metabolites with either *DRB1*15:01* or both *DRB1*15:01* and *DRB1*15:03*

The 157 metabolites are listed on the y-axis and binding energy score of metabolites with *DRB1*15:01* or both *DRB1*15:01* and *DRB1*15:03* are shown on the x-axis. Each cell represents the binding energy score in kcal/mol on a heatmap color scale.

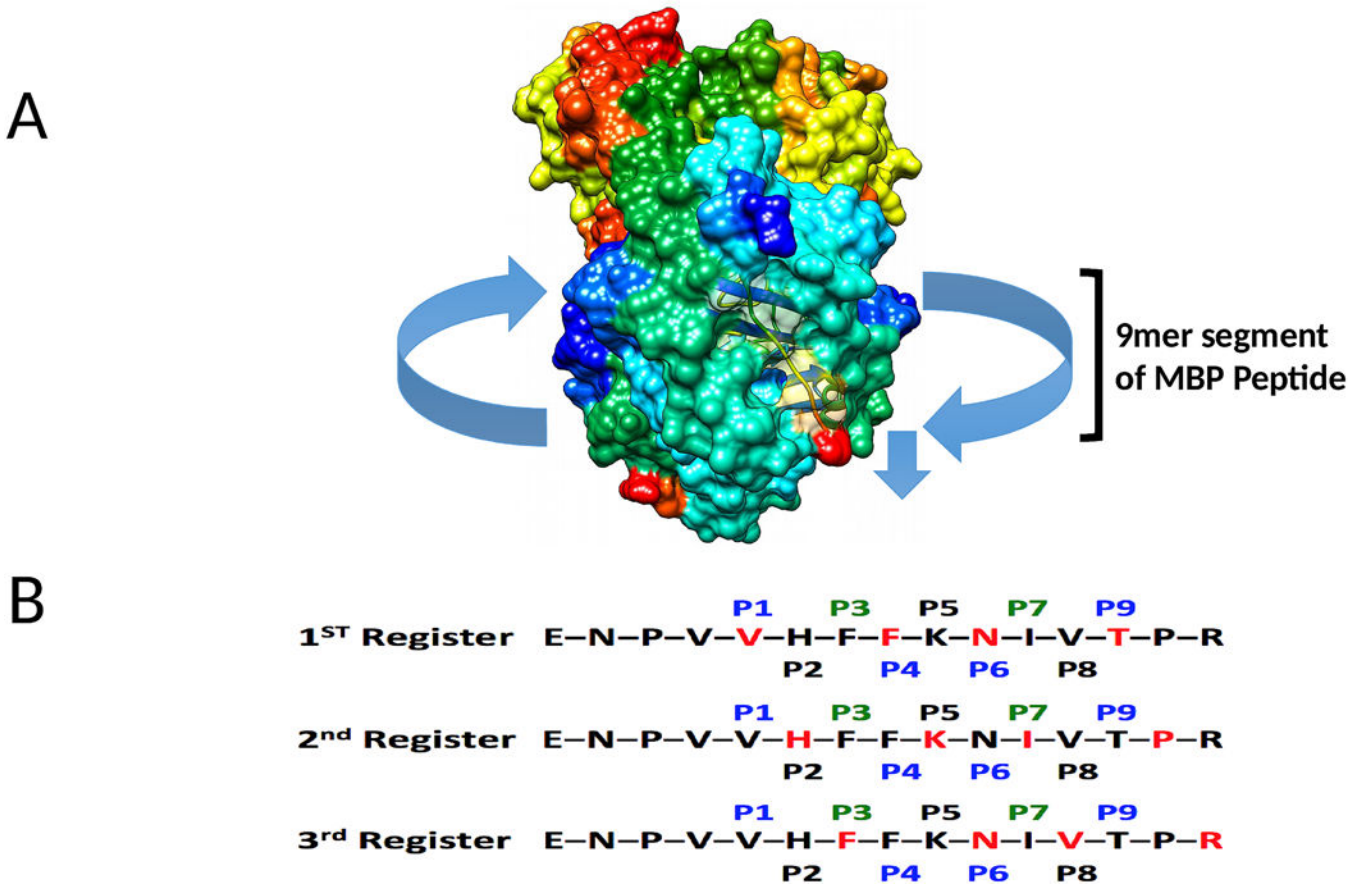

Figure 3. A: Hypothetical Model for the binding of human metabolites with *DRB1*15:01* and *DRB1*15:03* protein structures

In this model, the 9mer segments of MBP are present deep inside the binding grove of *DRB1*15:01* and *DRB1*15:03*, and the remaining residues are present outside the peptide-binding groove.

B: Three different potential registers for Human MBP85-99 binding to *DRB1*15:01*and *DRB1*15:03*. The 9mer residues from position 89 to 97 of MBP85-99 peptide plays a crucial role in metabolite binding as well as T cell recognition and responsiveness. In the 1ST register, the red color represents the *HLA* contact residues of pockets P1, P4, P6 and P9, and the black color shows the TCR contact residues of pockets P2, P3, P5 and P7. The TCR contact residues allow the interaction of the DRB1-metabolite-MBP complex with TCR for presentation to CD4+ T-cells. When a metabolite occupies the binding groove of the DRB1 molecule it may shift the P1 residue and all subsequent residues until the DRB1-metabolite complex is stabilized.

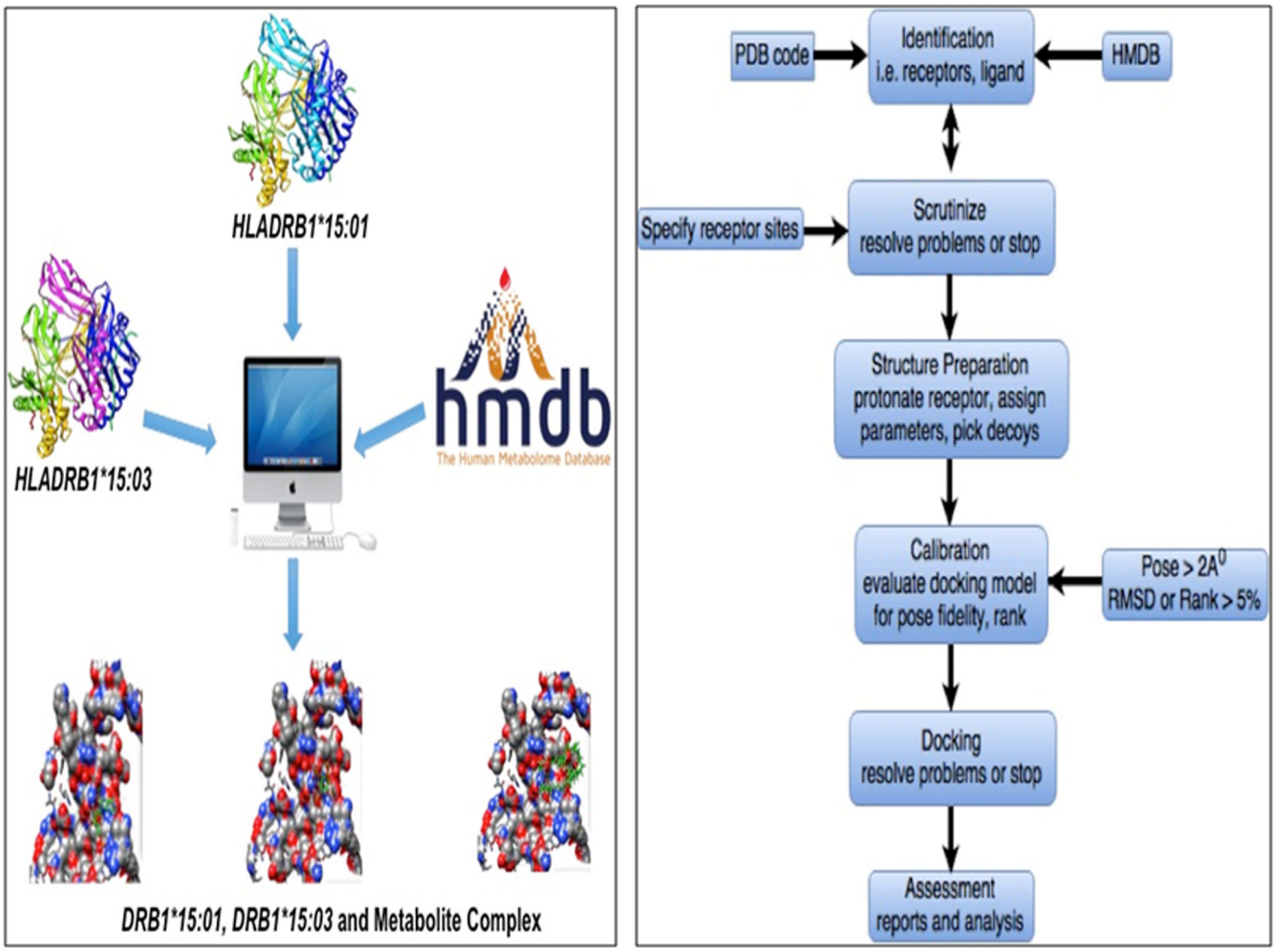

Figure 4. Schematic representation of overall study design (left panel) and docking pipeline (right panel)

Table1

Binding pocket, residue and pattern of binding of top scoring human metabolites with either *DRB1*15:01* or both *DRB1*15:01* and *DRB1*15:03*

Haplotype	Human Metabolites	Energy Score	*DRB1* Binding Pocket		MBP Residue and Binding Position	
			P4 Pocket	P1 Pocket	Phenylalanine^{MBP92}	Valine^{MBP89}
*DRB1*15:01*	Myo-inositol hexakisphosphate	−85.92	Yes	No	Yes	No
*DRB1*15:03*	Myo-inositol hexakisphosphate	−55.03	Yes	No	Yes	No
*DRB1*15:01*	Pyridinoline	−60.69	Yes	No	Yes	No
*DRB1*15:03*	Mesobilirubinogen	−45.32	Yes	No	Yes	No

Table 2

Human metabolite binding either *DRB1*15:01* or both *DRB1*15:01* and *DRB1*15:03* associated Phenotypes

Phenotypes	Human Metabolites	Pockets
Multiple Sclerosis	L-tryptophan, glutamic acid, D-sphingosine, sphingosine-1-phosphate, Cysteinyl-Glycine	P4
Neurodegeneration	D-sphingosine, NAD+, sphingosyl beta-glucoside	P4
Progressive motor neuropathy	D-sphingosine, escitalopram, glutathione, L-tryptophan, loperamide, sphingosine-1-phosphate	P4
Hereditary central nervous system demyelinating disease	glutathione, psychosine	P4
Cell death of central nervous system cells	dityrosine,glutathione, NAD+	P4
Cell death of neuroglia	glutathione, NAD+	P4
Apoptosis of motor neurons	glutathione	P4
Apoptosis of cerebellar macroneurons	sphingosine-1-phosphate	P4
Astrogliogenesis of neural stem cells	AICAR	P4
Discharge of axons	AICAR	P4
Invasion of nervous tissue cell lines	S-adenosylmethionine	P4
Mitogenesis of neurons	sphingosine-1-phosphate	P4
Migration of astrocytes	sphingosine-1-phosphate	P4
Conduction of nerves	glutathione	P4
Neuritogenesis of neurons	sphingosine-1-phosphate	P4
Prevention of Multiple Sclerosis	Escitalopram	P4
Proliferation of neuronal cells	AICAR, hesperidin, nicotinamide adenine dinucleotide phosphate, sphingosine-1-phosphate, sphingosyl beta-glucoside	P4
Morphology and Synapse Formation of Cerebellar Purkinje Cells	Myo-Inositol hexakisphosphate	P4