

ORIGINAL ARTICLE

Molecular docking predictions of fragrance binding to human leukocyte antigen molecules

Ryan J. Schutte¹  | Xiaojuan Zhang¹ | Nan An²  | David A. Ostrov¹  |
Stanislav Vukmanović²

¹Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida

²Division of Cosmetics, Office of Cosmetics and Colors, CFSAN, FDA, College Park, Maryland

Correspondence

Dr David A. Ostrov, Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL.
Email: ostroda@pathology.ufl.edu

Funding information

This work was supported by UT-Battelle LLC/Department of Energy subcontract 6400014703 (Ostrov).

Abstract

Background: Over 4000 small chemicals have been identified as allergens capable of inducing skin sensitization. Many sensitizers are hypothesized to act as haptens producing novel antigens, which can be presented to T cells by human leukocyte antigens (HLAs). Recent studies suggest that some chemical allergens use hapten-independent mechanisms.

Objective: To determine whether molecular docking can identify HLA molecules that bind skin-sensitizing chemical allergens.

Methods: Structural models of HLA molecules were used as the basis for molecular docking of 22 chemical allergens. Allergens predicted to bind HLA-B*57:01 were tested for their ability to stimulate T cells by the use of proliferation and interferon-gamma enzyme-linked immunospot assays.

Results: Chemical allergens that did not satisfy the criteria for hapten activity in vitro were predicted to bind more strongly to common HLA isoforms than those with known hapten activity. HLA-B*57:01, which is an HLA allele required for drug hypersensitivity reactions, was predicted to bind several allergens, including benzyl benzoate, benzyl cinnamate, and benzyl salicylate. In in vitro T cell stimulation assays, benzyl salicylate and benzyl cinnamate were found to stimulate T cell responses from HLA-B*57:01 carriers.

Conclusions: These data suggest that small-molecule skin sensitizers have the potential to interact with HLA, and show that T cell-based in vitro assays may be used to evaluate the immunogenicity of skin-sensitizing chemicals.

KEYWORDS

fragrances, HLA, human leukocyte antigen, molecular docking, skin sensitization, T cell, RRID: SCR_001905

1 | INTRODUCTION

Skin sensitization reactions are most frequently reflected by the development of allergic contact dermatitis (ACD). ACD is a common environmental and occupational health problem; its average prevalence in western Europe and North America is estimated to be

~20%.¹ In the United States, contact dermatitis affects 72.3 million people and imposes a burden of \$2.191 billion in direct and indirect costs per year.² The most frequent causes of allergic reactions to cosmetics are metals, fragrances, and preservatives.³ Between 1.8% and 4.2% of the general adult population are allergic to fragrances.⁴ Therefore, evaluation of the sensitization potential of cosmetic

ingredients is important for the safety assessments of cosmetic products.

In the past, data for evaluating sensitization have been obtained by the use of laboratory animals and human subjects.⁵ Examples of tests in animals have included the guinea-pig maximization test and the local lymph node assay. Sensitization in humans has been evaluated with the clinical patch test, the human maximization test, and the human repeat insult patch test.⁶ However, the use of human subjects is fraught with scientific and ethical considerations, and the use of animals for ingredient testing is either banned, as in the EU,⁷ or discouraged by agreements that it be reduced and/or replaced.⁸ Consequently, there is an urgent need for alternative (in vitro, in chemico, and in silico) assays that would address the need to evaluate the safety of cosmetic products for sensitization potential.

Reliable alternative tests should be based on known mechanism(s) of sensitization induction. Skin-sensitizing chemicals are small molecules (<500 Da) that are considered to be too small for direct recognition by the immune system and may therefore function as haptens.⁹ Haptens form covalent adducts with self-proteins (acting as carriers), leading to the formation of neoantigens (hapten-carrier complex) that are recognized by the immune system. Because small-molecule drugs (eg, sulfamethoxazole¹⁰) have been shown to elicit T cell responses by acting as haptens, skin-sensitizing chemicals have been proposed to also serve as haptens by binding covalently to keratinocyte proteins. However, T cells may respond to small-molecule drugs in a hapten-independent manner, by non-covalent binding of the drugs to human leukocyte antigen (HLA) molecules or T cell antigen receptors. For example, abacavir is a small-molecule drug that induces potentially lethal hypersensitivity reactions in susceptible individuals expressing HLA-B*57:01.^{11,12} The mechanism of stimulation of adverse T cell responses is non-covalent binding of the drug to the antigen-binding cleft of HLA-B*57:01, which has been shown to change the repertoire of presented peptides and trigger a systemic hypersensitivity reaction.^{13,14} Because current methods for measuring the potential of skin-sensitizing chemicals focus on hapten-mediated responses, there is a need for assays to detect small molecules that elicit ACD by such alternative mechanisms.

In this study, we investigated whether in silico screening combined with in vitro testing could be utilized to detect skin-sensitizing chemicals that induce ACD by non-hapten mechanisms. We estimated the potential for 22 skin-sensitizing chemicals to interact directly with HLA molecules by using molecular docking. Because HLA alleles are highly polymorphic, we docked each skin-sensitizing chemical to 77 class I and class II HLA molecules (on the basis of HLA class I and class II crystal structures, or homology models for class I and class II alleles with no crystal structure available). The most common HLA-A, HLA-B and HLA-DR allotypes were selected for molecular docking. Skin-sensitizing chemicals predicted to bind specific HLA molecules (eg, HLA-B*57:01) were tested for their abilities to stimulate T cell responsiveness in healthy donors. Skin-sensitizing non-haptens were, on average, predicted to bind specific HLA molecules preferentially and with different affinities than those of skin-sensitizing haptens.

2 | METHODS

2.1 | Chemical allergens

The 22 fragrances selected for molecular docking are shown in Table 1. These were among the 26 cosmetic ingredients recognized as fragrance allergens by the Scientific Committee on Cosmetics and Non-Food Products in the EU.¹⁸ Two fragrance allergens (oak and tree moss extracts; *Evernia prunastri* and *Evernia furfuracea*, respectively) were disregarded because, as extracts, they are complex mixtures of multiple compounds, and two others (amylcinnamyl alcohol and anisyl alcohol) were not evaluated because there were no in vitro/in chemico data regarding their potential for hapten binding. The hapten activity was evaluated on the basis of published results obtained with the direct peptide reactivity assay, one of the ARE/Nrf2-based assays (Keratinosens or LuSens), and one of the two assays that measure accessory (dendritic and myeloid) cell activation (human cell line activation test or myeloid U937 skin sensitization test). Three sets of data were considered,¹⁵⁻¹⁷ and a weight of evidence (WoE) approach was taken in each case of conflicting results, whereby the results of two concordant out of three performed tests were considered for consensus determination. According to these criteria, nine chemicals were designated WoE-negative (WoE⁻), that is, having insufficient data for hapten activity, whereas 13 chemicals that showed reactivity in at least two assays were designated WoE-positive (WoE⁺) and were thus considered to be haptens (Table 1). Simplified molecular-input line entry system (SMILES) strings¹⁹ for each molecule were used to generate three-dimensional structural models with the National Cancer Institute Computer-Assisted Design group SMILES Translator and Structure File Generator.²⁰

2.2 | HLA selection and modelling

In this study, the most frequently expressed HLA-A, HLA-B and HLA-DRB alleles, that is, those expressed at a frequency of >0.01 within the world population,²¹ were selected as potential targets for molecular docking (Table 2). Solved crystal structures from the Protein Data Bank (PDB), or models generated by SWISS-MODEL²² (based on HLA sequences available from IMGT/HLA²³), were used as the basis for molecular docking. HLA-A and HLA-B structures were superimposed onto the solved crystal structure of HLA-B*57:01 (PDB 3UPR) as a reference. HLA-DR molecules were superimposed onto the solved structure of HLA-DR1 (HLA-DRA*01:01/HLA-DRB*01:01) (PDB 1FYT). The program ssm (Secondary-Structure Matching) in coot²⁴ was used to superimpose crystal structures and homology models.

2.3 | Molecular docking

Initial molecular docking of the chemical allergen library to the tested HLA structures was performed with MtiOpenScreen on the Mobyle bioinformatics framework, Ressource Parisienne en Bioinformatique

TABLE 1 Summary of in vitro hapten-probing data on 26 fragrance allergens

Fragrance	CAS no.	In chemico/in vitro test results ^a			Consensus determination ^b
		D/K/C ¹⁵	D/K/U ¹⁶	D/L/U ¹⁷	
Amyl cinnamal	122-40-7	0 (0/?/1)	0 (0/0/1)	NA	WoE [−]
Amylcinnamyl alcohol	101-85-9	NA	NA	NA	Not selected
Anisyl alcohol	105-13-5	NA	NA	NA	Not selected
Benzyl alcohol	100-51-6	0 (0/0/1)	NA	NA	WoE [−]
Benzyl benzoate	120-51-4	0 (0/1/0)	0 (0/1/0)	NA	WoE [−]
Benzyl cinnamate	103-41-3	0 (0/1/0)	NA	NA	WoE [−]
Benzyl salicylate	118-58-1	0 (0/1/0)	NA	NA	WoE [−]
Cinnamyl alcohol	104-54-1	1 (1/1/1)	1 (1/1/1)	1 (1/1/0)	WoE ⁺
Cinnamal	104-55-2	1 (1/1/1)	1 (1/1/1)	NA	WoE ⁺
Citral	5392-40-5	1 (1/1/1)	1 (1/1/1)	1 (1/1/0)	WoE ⁺
Citronellol	106-22-9	1 (1/0/1)	NA	NA	WoE ⁺
Coumarin	91-64-5	0 (0/1/0)	0 (0/1/0)	NA	WoE [−]
Eugenol	97-53-0	1 (1/?/1)	1 (1/0/1)	1 (1/1/1)	WoE ⁺
Farnesol	4602-84-0	1 (0/1/1)	NA	NA	WoE ⁺
Geraniol	106-24-1	1 (0/1/1)	1 (0/1/1)	NA	WoE ⁺
Hexyl cinnamal	101-86-0	0 (0/?/?)	1 (0/1/1)	0 (0/1/0)	WoE [−]
Hydroxycitronellal	107-75-5	1 (1/1/1)	1 (1/1/1)	1 (1/1/1)	WoE ⁺
Hydroxyisohexyl 3-cyclohexene carboxaldehyde, Lyrall	31906-04-4	1 (1/1/1)	1 (1/1/1)	NA	WoE ⁺
Isoeugenol	97-54-1	1 (1/1/0)	1 (1/1/1)	1 (1/1/1)	WoE ⁺
Lilial	80-54-6	1 (1/0/1)	1 (1/0/1)	NA	WoE ⁺
o-Limonene	5989-27-5	1 (1/0/1)	NA	NA	WoE ⁺
Linalool	78-70-6	0 (0/0/1)	0 (0/0/1)	NA	WoE [−]
Methyl 2-octynoate	111-12-6	1 (1/1/1)	NA	NA	WoE ⁺
γ-Methylionone	127-51-5	0 (0/0/1)	NA	NA	WoE [−]
<i>Evernia prunastri</i> (oak moss extract)	90028-68-5	NA	NA	NA	Not selected
<i>Evernia furfuracea</i> (tree moss extract)	90028-67-4	NA	NA	NA	Not selected

Abbreviations: C, human cell line activation test; D, direct peptide reactivity assay; K, Keratinosens; L, LuSens; NA, not available; U, U937 skin sensitization test; WoE, weight of evidence.

^aWoE values are shown.^{15–17} Results from individual assays are shown in parentheses. “1” indicates positive results, “0” indicates negative results, and “?” indicates borderline or inconclusive results.

^bOn the basis of available in vitro data, compounds were selected (or not) for molecular docking and were assigned an overall WoE status.

Structurale.²⁵ Molecular docking of the top-scoring chemical allergens with specific HLA isoforms was performed with AUTODOCK VINA.²⁶ Docking results were visualized and images were generated with PYMOL (PyMOL Molecular Graphics System, Version 1.2; Schrödinger, New York, New York).

2.4 | Proliferation assay

Frozen peripheral blood mononuclear cells (PBMCs) were purchased from Cellular Technology (CTL; Shaker Heights, Ohio), and were cultured in RPMI-1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1% penicillin/streptomycin, and 25 µg/mL transferrin. Cells (1×10^5) were placed in a 96-well plate in

triplicate. Graded concentrations of compounds and controls were added at the beginning of the culture of each set of cells. On day 6 and day 9, 20 International Units of interleukin-2 were added to each well. On day 14, Cell counting kit 8 solution (Dojindo Molecular Technologies, Rockville, Maryland) was added to each well, and incubated was performed for an additional 4 hours. Absorbance at 450 nm was measured by use of a microplate reader (Molecular Devices, San Jose, California).

2.5 | T cell stimulation assay

The enzyme-linked immunospot (ELISPOT) assay was carried out according to the human interferon (IFN)-gamma ELISPOT protocol (Catalogue no. 887386; Invitrogen, Carlsbad, California). In brief, the

TABLE 2 Common human leukocyte antigen (HLA)-A, HLA-B and HLA-DRB alleles selected as targets for molecular docking

HLA	Alleles (overall frequency)				
A	24:02 (0.18816)	02:01 (0.15284)	11:01 (0.11661)	01:01 (0.04843)	03:01 (0.04272)
	31:01 (0.04093)	33:03 (0.04081)	02:06 (0.03469)	26:01 (0.03354)	30:01 (0.02505)
	23:01 (0.02335)	68:01 (0.02299)	02:07 (0.02127)	29:02 (0.01626)	34:01 (0.01618)
	02:03 (0.01510)	30:02 (0.01489)	32:01 (0.01434)	68:02 (0.01323)	33:01 (0.01153)
B	35:01 (0.05466)	51:01 (0.05222)	40:01 (0.05120)	44:03 (0.04473)	40:02 (0.04175)
	07:02 (0.04105)	15:01 (0.03431)	07:04 (0.03122)	08:01 (0.02960)	58:01 (0.02891)
	13:01 (0.02570)	37:01 (0.02498)	46:01 (0.02358)	52:01 (0.2331)	18:01 (0.02299)
	44:02 (0.02162)	39:01 (0.02149)	40:06 (0.01799)	48:01 (0.01764)	53:01 (0.01622)
	13:02 (0.01418)	56:01 (0.01416)	54:01 (0.01386)	15:02 (0.01319)	35:07 (0.01281)
	27:05 (0.01236)	14:02 (0.01234)	15:03 (0.01184)	42:01 (0.01045)	57:01 (0.01018)
	55:02 (0.01000)				
DRB	15:01 (0.07864)	07:01 (0.06986)	03:01 (0.06760)	11:01 (0.05945)	09:01 (0.05450)
	04:05 (0.04776)	15:02 (0.04507)	01:01 (0.04123)	08:03 (0.03864)	13:02 (0.03746)
	14:01 (0.03218)	13:01 (0.03152)	04:01 (0.02896)	12:02 (0.02896)	16:02 (0.02850)
	12:01 (0.02712)	04:03 (0.02659)	08:02 (0.02104)	04:04 (0.01795)	11:04 (0.01780)
	16:01 (0.01656)	14:02 (0.01649)	04:07 (0.01536)	10:01 (0.01284)	04:06 (0.01194)
	01:02 (0.01161)				

plate (MAIPS4510; Thermo Fisher, Waltham, Massachusetts) was coated with coating antibody (1:250) at 4°C overnight, and blocking was then performed with complete RPMI-1640 medium for 1 hour at room temperature. Then, 4×10^5 PBMCs were incubated with compounds at different concentrations (each dissolved in dimethylsulfoxide [DMSO]) and controls (10 µg/mL phytohaemagglutinin-A [PHA], 2 µg/mL CEF-positive control peptides, culture medium control, DMSO control, and no cell negative control) at 37°C for 48 hours with 5% CO₂. The CEF control peptides are 8–12 amino acids in length, with sequences derived from human cytomegalovirus, Epstein-Barr virus, and influenza virus.²⁷ After washing, the detection antibody (1:250) and then avidin-horseradish peroxidase reagent were added to the plate and incubated at room temperature for 2 hours and 45 minutes, respectively. Distilled water was added to the plate to stop the colour development with 3-amino-9-ethylcarbazole substrate for 30 minutes. The plate was read and analysed with the ImmunoSpot analyser from CTL.

2.6 | Statistical analysis

Statistical analyses were carried out in the R statistical environment (www.r-project.org; RRID:SCR_001905) for data regarding molecular docking of 22 skin sensitizers into HLA-A, HLA-B and HLA-DRB1 isoform antigen-binding clefts.²⁸ Binding energies were compared between WoE[−] and WoE⁺ chemical allergens within the HLA isoforms, as well as within all combined. The contributions of alleles were weighted according to their frequencies (Table 2). Weighted unpaired *t* tests with Welch's correction were performed with the "weight" R package.²⁹ Weighted box-and-whisker plots were generated with the "ENmisc" package.³⁰

3 | RESULTS

3.1 | Binding of WoE⁺ and WoE[−] chemical allergens to HLA molecules

We used molecular docking to predict the potential binding of each of 22 chemical compounds within the antigen-binding clefts of frequently expressed (>1% population frequency)²¹ HLA-A, HLA-B and HLA-DR molecules. The chemical allergens were then separated on the basis of consensus determinations regarding their hapten activity in in vitro/in chemico sensitization assays (Table 1): Thirteen were considered to have hapten activity (WoE⁺), whereas nine were considered to be without hapten activity (WoE[−]). Chemical allergens classified as WoE[−] were predicted to bind to common HLA molecules with higher affinity (based on prediction of ΔG) than those classified as WoE⁺ (Figure 1).

Evaluation of the lowest-energy (ΔG) docking conformations identified 14 chemical allergens predicted to bind to HLA molecules (−7.0 to −9.0 kcal/mol; Figure 2). There were proportionally more WoE[−] chemicals (7/9) than WoE⁺ chemicals (7/13). In addition, benzyl cinnamate, benzyl salicylate and benzyl benzoate (WoE[−]) were predicted to bind with higher affinities and with more HLA molecules than the other chemical allergens screened. Each of these three compounds is predicted to bind to four to eight times more HLA molecules than the fourth-ranking coumarin. The chemical allergens predicted to have one or more interactions with HLA molecules are summarized in Table 3.

In general, the skin-sensitizing chemical allergens were predicted to bind with higher affinities to HLA class I molecules than the class II HLA-DR molecules, on the basis of the observed ΔG scores. The HLA class I alleles with the most predicted binding interactions were HLA-B*57:01, HLA-A*24:02, and HLA-A*23:01. The HLA class II isoform

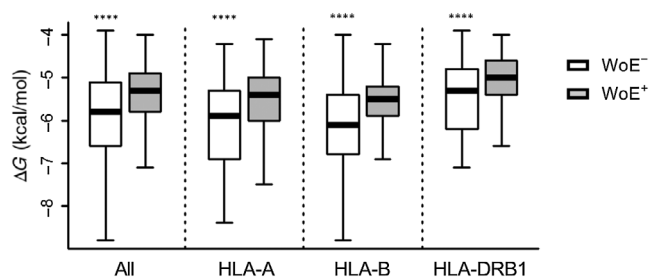


FIGURE 1 Non-hapten chemical allergens were predicted to bind common human leukocyte antigen (HLA) isoforms more strongly than those with identified hapten activity. Molecular docking by 22 skin sensitizers into the HLA-A, HLA-B and HLA-DRB1 isoform antigen-binding clefts predicts that allergens identified by in vitro sensitization assays as non-hapten forming (WoE⁻) form more stable interactions with frequently expressed HLA isoforms than those with demonstrated hapten activity (WoE⁺). More stable allergen-HLA interactions are predicted for frequently expressed HLA-B isoforms. Box-and-whisker plots are shown that provide median, minimum and maximum predicted change in free energy (ΔG ; kcal/mol) upon drug binding. Unpaired *t* tests were performed with Welch's, correction and *t* tests and box plots were weighted by allele frequencies. *****P* < 0.0001. WoE, weight of evidence

predicted to bind the most chemical allergens was HLA-DRB1*03:01, which was predicted to interact with six chemical allergens. The HLA molecules predicted to bind skin-sensitizing chemicals are summarized in Table 4.

The strongest predicted interactions were between HLA-B*57:01 and benzyl cinnamate (-8.9 kcal/mol), benzyl benzoate (-8.8 kcal/mol), and benzyl salicylate (-8.5 kcal/mol) (Figure 3). These allergens are structurally similar: as compared with benzyl benzoate, benzyl cinnamate has two additional carbons, and benzyl salicylate has two additional carbons and an extra hydroxyl group. On the basis of molecular docking, the most likely site of interaction between these chemical allergens and HLA-B*57:01 was in the F pocket accommodating the P9 position of bound peptide, previously identified as the abacavir-binding site (Figure 3).^{13,14} To determine whether chemical allergens have the capacity to bind HLA molecules in the presence of peptide (as is the case with abacavir and HLA-B*57:01 in crystal structures), we generated models of peptide-HLA complexes (using peptide HSITYLLPV from 3UPR). The chemical allergens predicted to bind HLA molecules with the highest affinities in the absence of peptide were predicted to bind more strongly in the presence of this peptide: benzyl cinnamate (-10.5 kcal/mol), benzyl salicylate (-10.0 kcal/mol), and benzyl benzoate (-9.9 kcal/mol). These data suggest that these chemical allergens, like abacavir, have the capacity to bind within the HLA antigen-binding cleft, forming contacts with the peptide and the HLA molecule.

3.2 | Benzyl salicylate-stimulated T cells from an individual carrying HLA-B*57:01

Because molecular docking suggested that WoE⁻ skin-sensitizing chemicals bind specific HLA molecules, we tested the ability of the

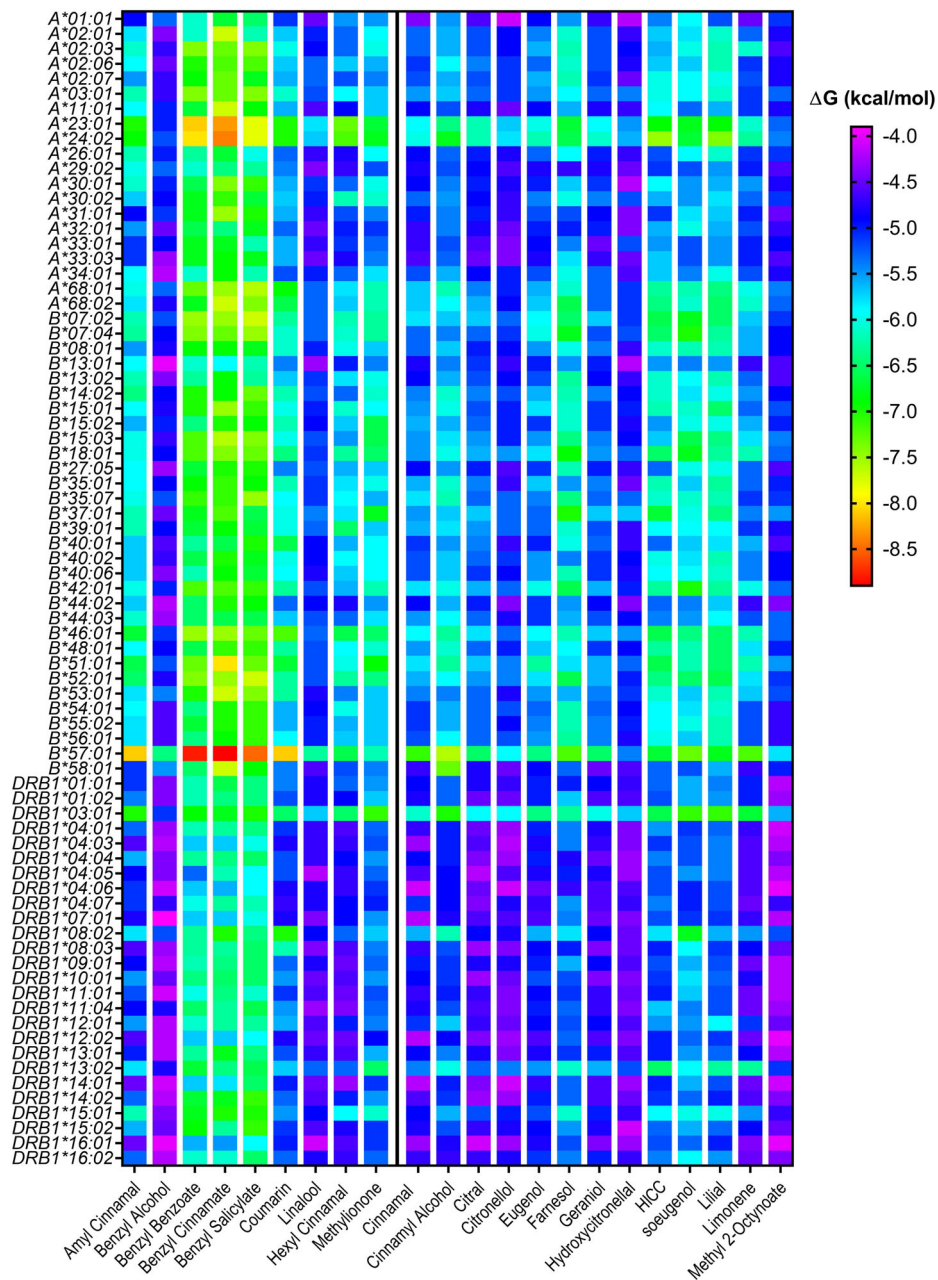
three chemicals with the best binding scores to stimulate T cells in PBMC samples from two HLA-B*57:01-carrying donors. T cell proliferation and IFN-gamma ELISPOT assays were performed with benzyl cinnamate, benzyl salicylate, and benzyl benzoate. Positive controls included PHA and CEF peptide pools. Stimulation of T cell proliferation was observed in samples from both HLA-B*57:01 individuals. PBMCs from an HLA-B*57:01-homozygous individual responded to lower concentrations of benzyl cinnamate and benzyl salicylate, whereas PBMCs from the heterozygous HLA-B*57:01 donor responded to a broader range of concentrations of benzyl salicylate (Figure 4A; Table 5). No significant responses to benzyl benzoate or abacavir were observed in PBMC samples from either donor.

ELISPOT-based IFN-gamma secretion assays were utilized to further characterize T cell responses to the tested chemical allergens (Figure 4B; Table 5). Direct readout of IFN-gamma secretion 48 hours after drug exposure showed T cell responses to 100 μ M benzyl salicylate in the heterozygous donor. Cells from the heterozygous donor were also stimulated by PHA and the CEF peptide pool, but not by abacavir. IFN-gamma secretion was not detected from homozygous donor cells after treatment with chemical allergens or abacavir, but homozygous donor cells were stimulated by PHA and the CEF peptide pool.

4 | DISCUSSION

In this study, we investigated whether in silico prediction combined with in vitro validation may provide an assay with which to detect ensuing T cell responses to skin-sensitizing chemical allergens. We used molecular docking to predict binding of fragrance compounds to common HLA molecules that differ in the structures of their antigen-binding clefts. Many chemical allergens were predicted to bind with different affinities to different HLA molecules, because of structural differences in the cleft. Importantly, the group of fragrance allergens with insufficient in vitro data for hapten activity (WoE⁻) was predicted to bind to HLA molecules significantly better than the group with demonstrable hapten activity (WoE⁺). The chemical allergens predicted to bind with the highest affinities were benzyl benzoate, benzyl cinnamate, and benzyl salicylate. This is, to our knowledge, the first indication that skin sensitizers might bind directly to HLA molecules, suggesting that additional hapten-independent mechanisms of skin sensitization may be operational. This notion has recently been suggested³¹ on the basis of existing evidence of hapten-independent mechanisms of allergies to drugs.³² It is important to note that binding to HLA molecules is not the only hapten-independent mechanism. Therefore, not all WoE⁻ compounds are expected to bind to HLA molecules, as they may promote skin sensitization by other hapten-independent mechanisms (eg, p-i or cryptic epitope mechanisms). At the same time, there is no reason to exclude the possibility that a single chemical can promote sensitization using more than one mechanism.³¹ Hence, individual

FIGURE 2 Molecular modelling predicted binding of chemical allergens to specific human leukocyte antigen (HLA) isoforms. A heatmap presentation of predicted ΔG (kcal/mol) upon binding of 22 identified skin sensitizers to frequently expressed HLA isoforms is shown. The solid black line separates WoE^- (left) and WoE^+ (right) allergens. Benzyl cinnamate, benzyl salicylate and benzyl benzoate are predicted to be more allergenic, with the strongest interactions with these compounds being predicted for HLA-B*57:01. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde. WoE , weight of evidence



WoE^+ compounds may be able to bind HLA molecules and act as haptens.

Initially inert or weakly reactive chemicals may acquire or enhance hapten-like abilities because of abiotic and/or biotic activation, and are referred to as prehaptens and prohaptens, respectively.³³ Most frequently, abiotic activation is caused by autoxidation resulting from exposure to air. Similar chemical effects (oxidation of weakly reactive chemicals) can be induced by cytochrome P450, although the activity of these enzymes in human skin is not very high.³⁴ Of the fragrance compounds studied here, citral,

α -limonene and linalool were found to be prehaptens, eugenol and isoeugenol are prohaptens, and cinnamyl alcohol and geraniol are both prehaptens and prohaptens.^{33,35,36} In agreement with these observations, recent experiments showed that benzyl benzoate, benzyl salicylate and benzyl cinnamate remained chemically stable when exposed to forced degradation by oxidation, and remained non-reactive to cysteine.³⁷ It would therefore appear that the sensitization potential of benzyl benzoate, benzyl cinnamate or benzyl salicylate is unlikely to be attributable to biotic or abiotic activation.

TABLE 3 Chemical allergens predicted to interact with human leukocyte antigen molecules

Compound	Moderate-affinity interactions
Benzyl cinnamate	37
Benzyl salicylate	32
Benzyl benzoate	19
Coumarin	5
Isoeugenol	4
Amyl cinnamal	3
Cinnamyl alcohol	3
Farnesol	2
Hexyl cinnamaldehyde	2
Lilial	2
Limonene	1
Cinnamal	1
Methylionone	1
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	1

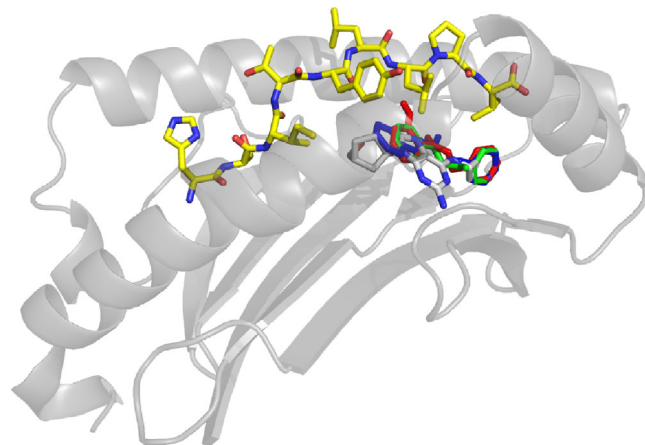
Moderate-affinity interactions are defined as those with predicted ΔG or between -9 and -7 kcal/mol. Weight of evidence-negative chemical allergens are in bold. Benzyl cinnamate and benzyl salicylate are predicted to be the most broadly allergenic.

TABLE 4 Frequently expressed human leukocyte antigen (HLA) alleles predicted to interact with the tested chemical allergens with moderate affinity

Allele	Moderate-affinity interactions
B*57:01	10
A*24:02	7
A*23:01, DRB1*03:01	6
B*07:04, B*42:01, B*46:01	4
A*02:03, A*02:06, A*03:01, A*68:01, B*07:02, B*15:01, B*15:03, B*18:01, B*35:07, B*51:01, B*52:01, B*53:01	3
A*30:01, A*31:01, A*68:02, B*14:02, B*27:05, B*37:01, B*48:01, B*54:01, B*55:02, B*58:01, DRB1*08:02, DRB1*15:01	2
A*02:01, A*02:07, A*11:01, A*30:02, B*15:02, B*35:01, B*40:01, B*40:02, B*44:02, B*56:01, DRB1*14:02, DRB1*15:02	1

Moderate-affinity interactions are defined as those with predicted ΔG at or between -9 and -7 kcal/mol. HLA-A, HLA-B and HLA-DRB1 haplotypes containing A*24:02 or A*23:01, B*57:01, and DRB1*03:01 are predicted to have more interactions with the 22 tested allergens

Compounds with a benzene ring (as well as other aromatic compounds) were found to be efficient as peptide exchangers for HLA molecules.³⁸⁻⁴⁰ Peptide exchangers are defined as chemicals that can displace existing peptides from HLA grooves, and thus enable the binding of other peptides present in the milieu. These data, therefore, independently suggest that benzyl benzoate, benzyl

**FIGURE 3** Molecular modelling of three chemical allergens with HLA-B*57:01. Molecular docking of benzyl benzoate (green), benzyl cinnamate (blue) and benzyl salicylate (red) in the antigen binding cleft of HLA-B*57:01 in the presence of the peptide HSITYLLPV (Protein Data Bank 3UPR) is shown. Crystallized abacavir (white with nitrogens in blue) is included to show overlap of the ring structures, namely the abacavir cyclopropyl group, with hydrocarbon rings of the three chemical allergens

salicylate and benzyl cinnamate are able to interact with HLA molecules. It is worth noting that benzyl alcohol is an exception, in that it is not predicted to bind to HLA molecules (Figure 2). Benzyl alcohol, but not benzyl benzoate, benzyl salicylate, and benzyl cinnamate, was found to degrade and acquire hapten-like activities following autoxidation.³⁷ Benzyl alcohol is therefore probably a prehapten.

The possibility that some WoE⁻ compounds may bind HLA molecules is important from the aspect of choosing appropriate in vitro assays for predicting sensitization potential. Currently, the adverse outcome pathway (AOP) of skin sensitization⁴¹ describes four key events that serve as focus points for the development of alternative tests: (a) covalent modification of epidermal proteins; (b) keratinocyte signalling and activation; (c) dendritic cell activation, migration, and antigen processing; and (d) antigen recognition and an ensuing T cell response. This mechanistic understanding of the AOP has enabled the development of alternative assays that test the impacts of chemical compounds on defined key points of the AOP. Successful assays could replace animal testing, or at least serve as first-tier assays designed to significantly reduce the amount of testing performed in animals. If hapten-independent mechanisms of skin sensitization operate, additional in vitro assays will be needed. It should be noted that hapten-independent mechanisms of some drug allergies have already been proven,³² and some drugs induce sensitization through binding to HLA, despite being capable of forming hapten-carrier complexes.¹⁰

To determine whether carriers of HLA-B*57:01 were sensitive to benzyl benzoate, benzyl cinnamate, or benzyl salicylate, in vitro T cell

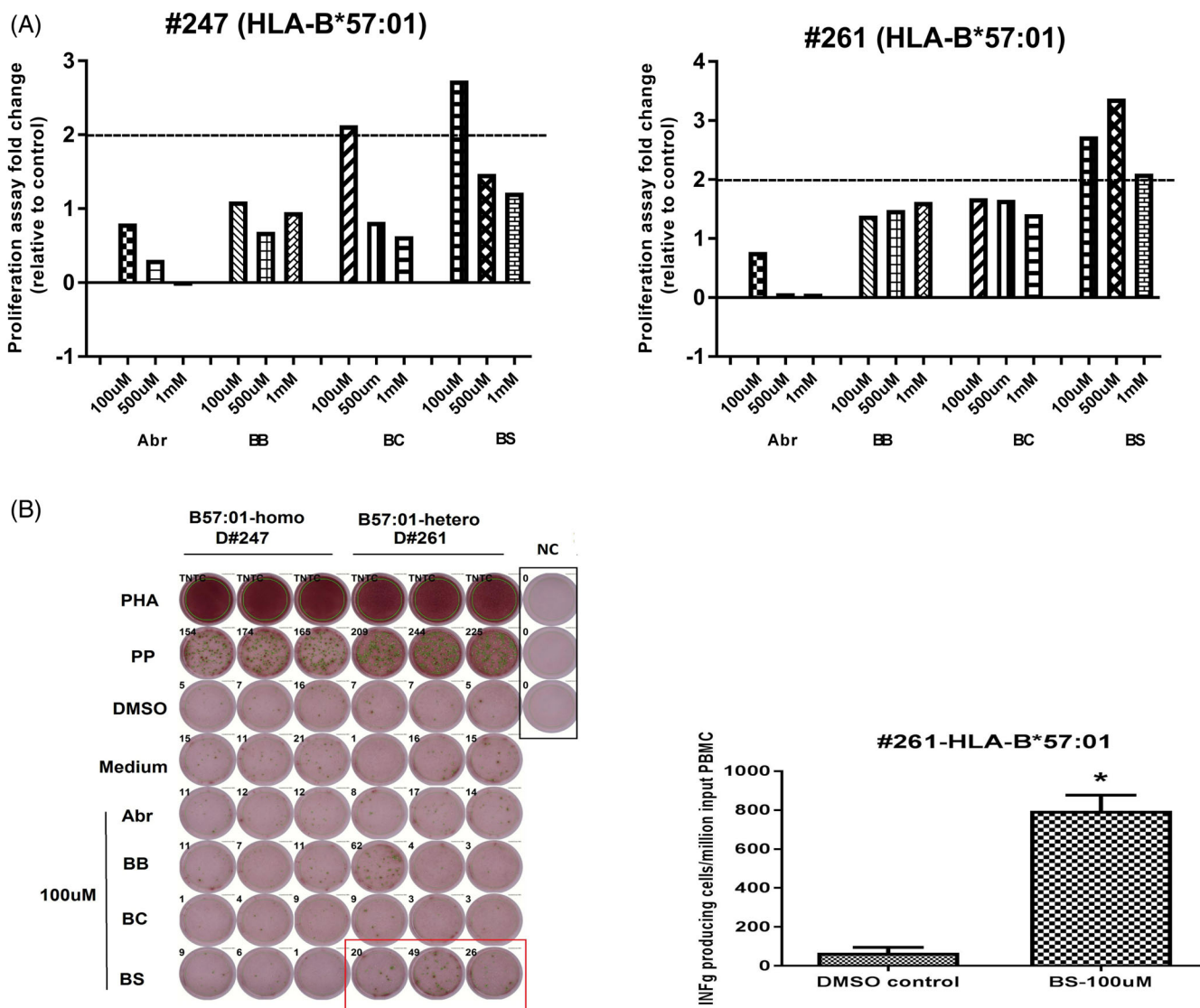


FIGURE 4 In vitro T cell responses upon stimulation with skin sensitizers. Skin sensitizers that had been predicted by molecular docking to have the highest potential to bind HLA-B*57:01 were shown to stimulate T cell responses in peripheral blood mononuclear cells (PBMCs) from HLA-B*57:01 donors. (A), Proliferation assay of PBMCs with compounds of interest. Two-fold differences relative to control were considered to be significant. (B), Enzyme-linked immunospot (ELISPOT)-based interferon (IFN)-gamma secretion assay of PBMCs with compounds of interest. Quantitative data from the ELISPOT assay are presented as mean $\pm$ standard error of the mean; * $P < 0.05$ relative to control. PBMCs from donor 247 were homozygous for HLA-B*57:01; those from donor 261 were heterozygous for HLA-B*57:01. Abbreviations: Abr, abacavir; BB, benzyl benzoate; BC, benzyl cinnamate; BS, benzyl salicylate; DMSO, dimethylsulfoxide; mPHA, phytohaemagglutinin-A, 10 μ g/mL; PP, CEF peptide pools, 2 μ g/mL; HLA, human leukocyte antigen; NC, no cell negative control; TNTC, too numerous to count

stimulation assays were performed. Benzyl salicylate was found to stimulate T cell proliferation in a heterozygous and homozygous carrier of HLA-B*57:01. Interferon-gamma secretion was detected after exposure of cells from an HLA-B*57:01⁺ individual to benzyl salicylate. These data indicate that in silico modelling can serve as a means to identify HLA isoforms at greater risk of interaction with known chemical allergens, and that in vitro T cell-based assays are necessary for verification.

The available data indicate that many small-molecule chemical allergens possess hapten activity. This study suggests that chemical allergens, particularly those without consensus findings of hapten

activity, may interact with HLA directly by binding within the peptide-binding groove. Structure-based prediction combined with in vitro (or ex vivo) T cell assays may be useful to identify the chemical allergens responsible for ACD and to identify those individuals at highest risk.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Susan Spence and Dr Raj Verma for their critical review of this manuscript.

TABLE 5 Summary of in vitro T cell-based assay data from four human leukocyte antigen (HLA)-characterized peripheral blood mononuclear cell (PBMC) donors; benzyl salicylate was observed to stimulate T cell proliferation and interferon-gamma secretion in characterized PBMCs from donors carrying HLA-B*57:01

PBMC donor no. and HLA genotype	Fragrance	Predicted fragrance binding		T cell response	
		Donor HLA allele with the strongest binding	ΔG (kcal/mol)	ELISPOT: 100 μM fragrance (spots per 1 million cells)	Proliferation: 100 μM fragrance (OD value, 450 nm)
49	None ^a			10.8	0.4
HLA-A*0201/*2902	BB	B*0801 and DR*0301	−6.9	5.8	0.29
HLA-B*0801/*4001	BC	A*0201	−7.7	5.8	0.25
HLA-DR*0301/*0701	BS	DR*0301	−7.0	10.0	0.34
50	None ^a			15.8	0.38
HLA-A*0201/*0201	BB	DR*1501	−6.8	11.7	0.38
HLA-B*1302/*1804	BC	A*0201	−7.7	20.8	0.32
HLA-DR*0701/*1501	BS	DR*1501	−7.0	45.0	0.31
247	None ^a			23.3	0.2
HLA-A*0101/*0201	BB	B*5701	−8.8	24.2	0.22
HLA-B*5701/*5701	BC	B*5701	−8.9	11.7	0.43
HLA-DR*1101/*1502	BS	B*5701	−8.5	13.3	0.55
261	None ^a			15.8	0.3
HLA-A*0101/*2902	BB	B*5701	−8.8	8.8	0.42
HLA-B*4403/*5701	BC	B*5701	−8.9	12.5	0.51
HLA-DR*0701/*0701	BS	B*5701	−8.5	79.2	0.82

Abbreviations: BB, benzyl benzoate; BC, benzyl cinnamate; BS, benzyl salicylate; ELISPOT, enzyme-linked immunospot; OD, optical density.

^aThe boxes that read "None" with a refer to negative control wells with no drug. These are the vehicle (DMSO) controls.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

Disclaimer

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, and nor does mention of commercial products or organizations imply endorsement by the US government. This article reflects the current thinking and experience of the authors.

ORCID

Ryan J. Schutte  <https://orcid.org/0000-0003-1578-6205>

Nan An  <https://orcid.org/0000-0001-8962-9320>

David A. Ostrov  <https://orcid.org/0000-0002-4696-875X>

REFERENCES

- Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population—prevalence and main findings. *Contact Dermatitis*. 2007;57:287-299.
- Bickers DR, Lim HW, Margolis D, et al. The burden of skin diseases: 2004 a joint project of the American Academy of Dermatology Association and the Society for Investigative Dermatology. *J Am Acad Dermatol*. 2006;55:490-500.
- Peiser M, Tralau T, Heidler J, et al. Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. Current knowledge assembled at an international workshop at BfR, Germany. *Cell Mol Life Sci*. 2012;69:763-781.
- Svensson Å, Ofenloch RF, Bruze M, et al. Prevalence of skin disease in a population-based sample of adults from five European countries. *Br J Dermatol*. 2018;178:1111-1118.
- Botham PA, Basketter DA, Maurer T, Mueller D, Potokar M, Bontinck WJ. Skin sensitization—a critical review of predictive test methods in animals and man. *Food Chem Toxicol*. 1991;29:275-286.
- Api AM. Sensitization methodology and primary prevention of the Research Institute for Fragrance Materials. *Dermatology*. 2002;205:84-87.
- European Commission. Ban on animal testing; 2017. https://ec.europa.eu/growth/sectors/cosmetics/animal-testing_en. Accessed May 16, 2019.
- USDHHS. Memorandum of understanding on high throughput screening, toxicity pathway profiling, and biological interpretation of findings; 2008. https://www.niehs.nih.gov/about/highlights/assets/docs/memorandum_of_understanding_508.pdf. Accessed May 16, 2019.
- Faulkner L, Meng X, Park BK, Naisbitt DJ. The importance of hapten-protein complex formation in the development of drug allergy. *Curr Opin Allergy Clin Immunol*. 2014;14:293-300.
- Ogese MO, Jenkins RE, Maggs JL, et al. Characterization of peroxidases expressed in human antigen presenting cells and analysis of the

- covalent binding of nitroso sulfamethoxazole to myeloperoxidase. *Chem Res Toxicol*. 2015;28:144-154.
11. Phillips E, Mallal S. Successful translation of pharmacogenetics into the clinic: the abacavir example. *Mol Diagn Ther*. 2009;13:1-9.
 12. Phillips EJ, Chung WH, Mockenhaupt M, Roujeau JC, Mallal SA. Drug hypersensitivity: pharmacogenetics and clinical syndromes. *J Allergy Clin Immunol*. 2011;127(3 suppl):S60-S66.
 13. Illing PT, Vivian JP, Dudek NL, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature*. 2012;486:554-558.
 14. Ostrov DA, Grant BJ, Pompeu YA, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci U S A*. 2012;109:9959-9964.
 15. Urbisch D, Mehling A, Guth K, et al. Assessing skin sensitization hazard in mice and men using non-animal test methods. *Regul Toxicol Pharmacol*. 2015;71:337-351.
 16. Natsch A, Ryan CA, Foertsch L, et al. A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. *J Appl Toxicol*. 2013;33:1337-1352.
 17. Bauch C, Kolle SN, Ramirez T, et al. Putting the parts together: combining in vitro methods to test for skin sensitizing potentials. *Regul Toxicol Pharmacol*. 2012;63:489-504.
 18. Bennike NH, Zachariae C, Johansen JD. Non-mix fragrances are top sensitizers in consecutive dermatitis patients—a cross-sectional study of the 26 EU-labelled fragrance allergens. *Contact Dermatitis*. 2017;77:270-279.
 19. Siani MA, Weininger D, Blaney JM. CHUCKLES: a method for representing and searching peptide and peptoid sequences on both monomer and atomic levels. *J Chem Inf Comput Sci*. 1994;34:588-593.
 20. NCI CAD group. Online SMILES translator and structure file generator; 2018. <https://cactus.nci.nih.gov/translate/>. Accessed June 10, 2019.
 21. Lancaster AK, Single RM, Solberg OD, Nelson MP, Thomson G. PyPop update—a software pipeline for large-scale multilocus population genomics. *Tissue Antigens*. 2007;69(suppl 1):192-197.
 22. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*. 2006;22:195-201.
 23. Robinson J, Halliwell JA, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA database. *Nucleic Acids Res*. 2013;41(Database issue):D1222-D1227. <https://doi.org/10.1093/nar/gks949>.
 24. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of coot. *Acta Crystallogr D Biol Crystallogr*. 2010;66(Pt 4):486-501.
 25. Labbe CM, Rey J, Lagorce D, et al. MtiOpenScreen: a webserver for structure-based virtual screening. *Nucleic Acids Res*. 2015;43(W1):448-454.
 26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31:455-461.
 27. Moldovan I, Targoni O, Zhang W, Sundararaman S, Lehmann PV. How frequently are predicted peptides actually recognized by CD8 cells? *Cancer Immunol Immunother*. 2016;65:847-855.
 28. R Development Core Team. R: A language and environment for statistical computing; 2010. <http://www.r-project.org>. Accessed June 10, 2019.
 29. Pasek J. Package 'weights'; 2018. <http://www.CRAN.R-project.org/package=weights>. Accessed June 10, 2019.
 30. Neuwirth E. Package 'ENmisc'; 2012. <https://CRAN.R-project.org/package=ENmisc>. Accessed June 10, 2019.
 31. Vukmanovic S, Sadrieh N. Skin sensitizers in cosmetics and beyond: potential multiple mechanisms of action and importance of T-cell assays for in vitro screening. *Crit Rev Toxicol*. 2017;47:415-432.
 32. Pichler WJ, Beeler A, Keller M, et al. Pharmacological interaction of drugs with immune receptors: the p-i concept. *Allergol Int*. 2006;55:17-25.
 33. Karlberg AT, Börje A, Johansen JD, et al. Activation of non-sensitizing or low-sensitizing fragrance substances into potent sensitizers—prehaptens and prohaptens. *Contact Dermatitis*. 2013;69:323-334.
 34. Götz C, Pfeiffer R, Tigges J, et al. Xenobiotic metabolism capacities of human skin in comparison with a 3D epidermis model and keratinocyte-based cell culture as in vitro alternatives for chemical testing: activating enzymes (phase I). *Exp Dermatol*. 2012;21:358-363.
 35. Urbisch D, Becker M, Honarvar N, et al. Assessment of pre- and prohaptens using nonanimal test methods for skin sensitization. *Chem Res Toxicol*. 2016;29:901-913.
 36. Patlewicz G, Casati S, Basketter DA, et al. Can currently available non-animal methods detect pre and pro-haptens relevant for skin sensitization? *Regul Toxicol Pharmacol*. 2016;82:147-155.
 37. Avonto C, Wang M, Chittiboyina AG, Vukmanovic S, Khan IA. Chemical stability and in chemico reactivity of 24 fragrance ingredients of concern for skin sensitization risk assessment. *Toxicol In Vitro*. 2018;46:237-245.
 38. Falk K, Lau JM, Santambrogio L, et al. Ligand exchange of major histocompatibility complex class II proteins is triggered by H-bond donor groups of small molecules. *J Biol Chem*. 2002;277:2709-2715.
 39. Höpner S, Dickhaut K, Hofstätter M, et al. Small organic compounds enhance antigen loading of class II major histocompatibility complex proteins by targeting the polymorphic P1 pocket. *J Biol Chem*. 2006;281:38535-38542.
 40. Call MJ, Xing X, Cuny GD, et al. In vivo enhancement of peptide display by MHC class II molecules with small molecule catalysts of peptide exchange. *J Immunol*. 2009;182:6342-6352.
 41. OECD. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. 2014. www.oecd.org/env/the-adverse-outcome-pathway-for-skin-sensitisation-initiated-by-covalent-binding-to-proteins-9789264221444-en.htm. Accessed May 16, 2019.

How to cite this article: Schutte RJ, Zhang X, An N, Ostrov DA, Vukmanović S. Molecular docking predictions of fragrance binding to human leukocyte antigen molecules. *Contact Dermatitis*. 2019;81:174-183. <https://doi.org/10.1111/cod.13283>