# A Vaccine Strategy for Plant Allergy by RNA Interference – An *in Silico* Approach

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**Abstract.** Worldwide population affected by allergic rhinitis and asthma are estimated to 400 million and 300 million respectively, and the medical costs for treatment are estimated to exceed that of tuberculosis and AIDS allied. The main objective of this research is to propose a vaccine design strategy for the management of allergy through siRNA vaccination in silencing IgE VH region. The allergen *Che a 3* was chosen to demonstrate our approach. Docking interactions between *Che a 3* and modeled structures of heavy chain variable region of 31 Immunoglobulin E clones were analyzed in AutoDock. Concurrently, small interference RNA sequences targeting the Immunoglobulin E clone with least binding energy were designed in siDRM.

**Keywords:** Allergy, Asthma, Immunoglobulin E, Vaccine, Immunotherapy, Small interference RNA, AutoDock, Bioinformatics, Docking, *In silico*.

## 1 Introduction

Allergy is defined as the acute immune reaction induced by allergic compounds. The main objective of this study is to mediate the molecular mechanisms behind allergy and propose a novel strategy to reduce this adverse reaction. It has been demonstrated by Zhang *et al.*[1] that bioinformatics can serve as a catalyst to drive the wet lab experiments into a cost-effective and time-effective paradigm to formulate epitope-based vaccines. Various remedies for allergy in current practice are reviewed by Holgate and Polosa [2]. Traditional treatments include Corticosteroids, $\beta$2- adrenoceptor agonists, Mediator antagonists and synthesis inhibitors and phosphodiesterase inhibitors. However, they fail to eradicate natural history of the disease [3],[4], virus-induced exacerbations [30] and ineffective in smoking asthma patients. And their side effects like anaphylaxis [6], central nervous system incitement and cerebral appraisals of sleep and early morning behavior [7] were inevitable. This led to the discovery of cetirizine, levocetirizine, loratadine and desloratadine [8]. The side effects and non-uniform effectiveness among patients instigated evolvement of Allergen-specific immunotherapy (SIT).

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Where T regulatory (T REG) cells boost protective immunotolerance against allergens and maintain balance between TH1 and TH2 cells populations to subdue allergic reactions [9]. SIT entails injection of allergic protein(s) in incremental doses to suppress immune responses mediated by mast cells, basophils and eosinophils and allergen-specific immunoglobulin A (IgA) and immunoglobulin G4 (IgG4) antibodies on sensitization. Vaccines based on allergen extracts, allergoids, peptides, recombinant allergens, epitope modified allergens or allergen-CpG fusion molecules [10],[11],[12] and RNA interference (RNAi) mediated therapies are currently being widely researched. Functionally, mRNA molecules are translated into a protein. So, targeting mRNA than a protein is potentially an efficient approach [12]. It has been shown that RNAi is highly competent in suppression of specific genes when compared to the traditional antisense approaches [13],[14],[15] .

Suzuki *et al* [16] have established that siRNA dependent silencing of CD40 mediated immune responses [17],[18] can be effective therapy against allergy. Amidst various allergic reactions that can be suppressed by siRNA, IgE seems to be an efficient candidate as it was evolved in mammals as the first line of defense against pathogens [19]. Furthermore, therapies arresting IgE mediated responses have been highly successful. Antibody specific to low-affinity IgE receptor Fc$\xi$RII, Lumiliximab has passed Phase 1 trial against asthma [20] and the IgE-specific antibody, Omalizumab (Xolair; Novartis Pharmaceuticals Ltd) has been effective in treatment of asthma and other allergic diseases [21].

IgE is associated with many other mechanisms apart from aggravating allergic responses like restraining malaria parasites [22], helminthes infection [23], *Trichinella spiralis* infection [24] and ovarian tumor cells [25] making it vulnerable to block expression of complete IgE molecule to combat allergy. Alternatively, gene expression of heavy chain variable (VH) region of IgE specific to allergies can be silenced. Steering towards VH region is quiet convincing as a potent IgE is assembled only on successful rearrangement of VH region by V(D)J recombination [26],[27] . Also, it is the variable domain H3 [28] (heavy chain region) that determine specificity of antigen binding sites in an Ig [28],[29] . The abovementioned discussions affirm an effective therapy for allergy by targeting VH region of IgE while preserving its role in other immune reactions.

The main objective of this study is to design siRNA based vaccination against allergy *in silico*. siRNA sequences are designed using the online tool, siDRM [30] which is available at `http://sirecords.umn.edu/siDRM/` pertaining to its high Positive Predictive value compared to other tools [30] . The vaccine is involved in silencing gene expression of IgE VH region. siRNA is designed to target the IgE specific to a particular allergen. Furthermore, Allergen *Che a 3* and IgE VH regions were docked in AutoDock [31],[32],[33] to identify the genetic variant capable of recognizing the epitopes in the allergen where binding sites of IgE are predicted in the online prediction tool, Q-SiteFinder (`http://www.modelling.leeds.ac.uk/qsitefinder/`) [34] prior to docking analysis.

## 2 Methods

### 2.1 IgE Sequence Retrieval from NCBI

Coker et al [35] have published 112 protein sequences for IgE VH region which were retrieved from NCBI. Only 31 sequences among them could be successfully modeled in Swiss-Model Workspace.

### 2.2 Protein Modeling

Protein structures of IgE were modeled in Swiss-Model Workspace (URL: http://swissmodel.expasy.org/workspace/) using the Protocol devised by Bordoli et al. [36] which consists of the following steps (See Fig 1): Step 1: The target sequence (IgE VH region mRNA) was first examined by submitting its FASTA format or UniProt Accession Code in Sequence Features Scan session (with default settings), found under Tools. Step 2: Suitable template (s) for building homology model(s) was identified in Template Identification session (with default options) under Tools. Step 3: Most identical template spanning one or more domains of the target with least e-value was selected from the resulting hit list (a condensed graphical overview of template coverage with respect to domain boundaries with underlying target-template alignment and SWISS-MODEL template library (SMTL)). Step 4: Target-template sequence identity was considered for choice of modeling modes. (Automated mode : >50% identity, Alignment mode: 50%-30% identity, Project mode: <30% identity)

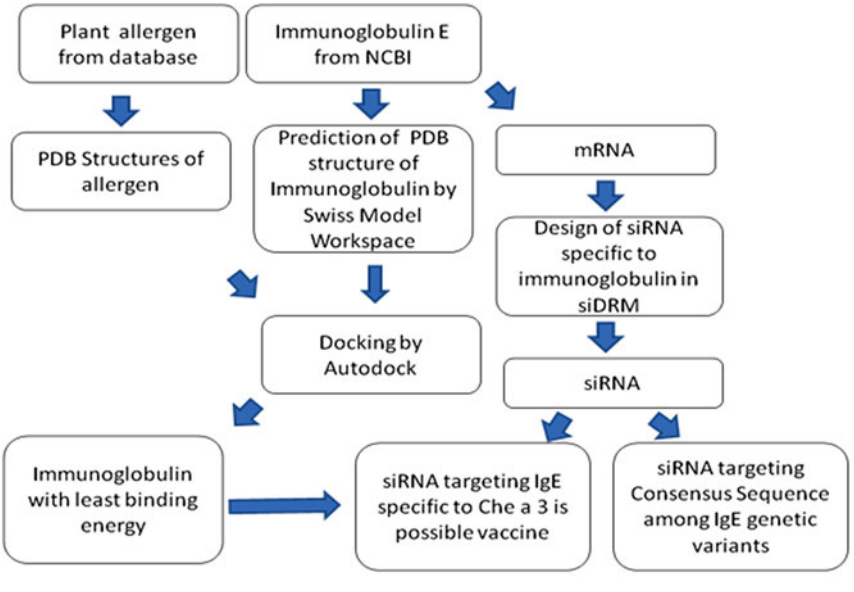


**Fig. 1.** The chronology of methods is summarized in this figure

Step 5: Modeling was done in the respective mode and model coordinates were output in PDB file format. Step 6: Quality of the modeled structure was estimated in 'Structure Assessment session' under 'Tools,' to identify incorrect regions.

### 2.3 Collection of Allergen Sequences

mRNA sequences of plant food and air borne allergens were retrieved from the public database Food Allergy Research and Resource Program (FARRP) (`http://www.allergenonline.org/`) [37] (See Supplementary1) by filtering based on the text terms Aero plant and Food plant for type of allergens. PDB structures were obtained from Swiss-Prot [38] (See Fig. 2; Supplementary2).

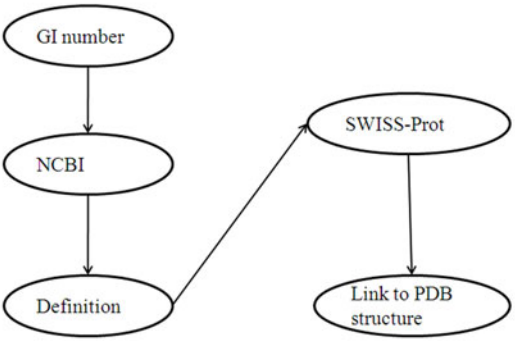


**Fig. 2.** Steps involved in fetching structural information of allergens

### 2.4 Docking in AutoDock

The PDB structures of the ligand (*Che a 3*) and receptor (IgE VH region) proteins were used after the addition of polar hydrogen atoms. The ligand protein of *Che a 3* is composed of 4 chains (See Fig. 3) with 2068 non polar hydrogen bonds, 1594 rotatable bonds and a torsion degree of freedom of 1224. The protein was split into individual chains and each chain was docked to each receptor of interest. Each chain was made rigid by making all rotatable bonds non-rotatable. A binding site detection program, QSiteFinder was used to identify the binding sites in receptor protein which were ranked based on binding energy. Residues in the top ranked binding site were made flexible in receptor. Mass-centered grid maps were generated with 0.75Å spacing [39] in AutoGrid program for the whole protein receptor. The best fitted conformation of the ligand was identified in AutoDock [31],[32],[33] where each chain of *Che a 3* (ligand) was docked with a total of 31 immunoglobulins (receptors) to find the IgE specific to that allergen. Each docking was performed in 50 trials (runs) (See Table 2 and Fig. 4).

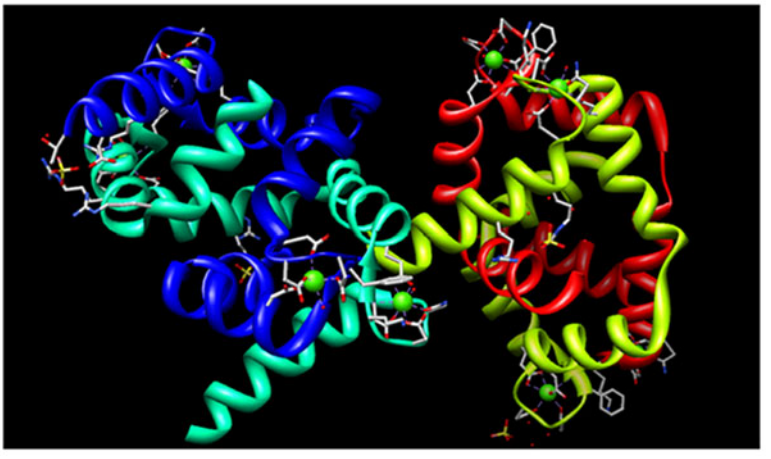

**Fig. 3.** *Che a 3* with each of its chain depicted in different colors: Chain A-Blue, Chain B- Cyan, Chain C- Green, Chain D-Red

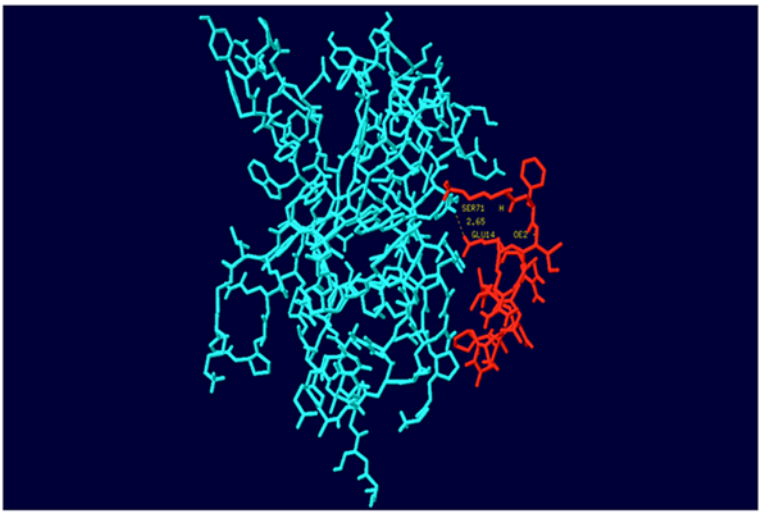


**Fig. 4.** The IgE, RH (represented in Cyan) bound to Che a 3 A chain (represented in Red) with a hydrogen bond of length 2.65Å between Serine 71 and Glutamine 14 respectively

### 2.5 siRNA Design Specific to IgE

Each immunoglobulin mRNA sequence was input into the online siRNA designing tool, siDRM [30] (URL: `http://sirecords.umn.edu/siDRM/`) with all default parameters to design a siRNA specific to each immunoglobulin. siRNA for all the genetic variants of IgE as a whole was designed based on the protocol devised by Birmingham *et al.*[40].

## 3 Results

### 3.1 Allergen Database

Complete record of 432 aero and 335 food allergens were fetched from FARRP (Food Allergy Research and Resource Program) database dated 4 November 2008. Each record is defined by the fields of species name and common name of the plant source, name of the allergen assigned by International Union of Immunological Societies (IUIS), Gene Identification Number and sequence length. The PDB structures thus obtained were validated for their accuracy as suggested by Kosloff and Kolodny [41] that only the structures with >70% sequence identity are similar. There were a total of 24 aero and 38 food allergens meeting this requirement (See Supplementary2).

### 3.2 Immunoglobulin E heavy Chain Variable Region

Search for IgE heavy chain variable region in Map Viewer [42] database of NCBI shed light on its respective chromosomal regions in Human genome. The region of interest was centered at Chromosomes 14q, 16p and 21p.

### 3.3 Modeling Protein Structures

The structures of IgE VH region were modeled using SWISS-MODEL workspace [43] as specified by Bordoli *et al.* [36]. The result from Sequence Feature annotation session was contributed by three individual tools, InterPro, domain scan tool, PsiPred, secondary structure prediction tool and DisoPred, disorder (Flexible, dynamic regions that can be partially or completely extended in solution) prediction tool to analyze features of the target sequence (See Supplementary3). The results of InterPro domain scan revealed that all the proteins were composed of immunoglobulin-like Domain and immunoglobulin V-set domain. And, the results of DisoPred indicated prevalence of disorderliness in 4-10 residues in the protein segment ranging from 8th to 15th position (See Supplemntary4). Also, the results from PsiPred reveals overrepresentation of extended $\beta$-sheets among secondary structures in 14 IgEs but 15 of them had an equal contribution from coil and sheet structures. And, four IgEs were more coiled in structure. Template Identification session facilitated selection of suitable templates for protein structure modeling (See Supplementary5).

The template with highest identity and least e-value was chosen for modeling. The template identities varied from 60-65% and Automated mode was chosen for modeling. The result page of SWISS-Model workspace [36],[43],[44] include energy profiles from ANOLEA statistical potential [45], GROMOS force field [46] along with percentage sequence identity between target and template (to build the model). ANOLEA statistical potential is to analyze the packing quality of predicted models which is graphically presented with y-axis representing energy for each amino acid in the protein chain (See Supplementary6). The green spikes (negative values) represent favorable energy environment whereas the red spikes

(positive values) are unfavorable energy environment for a given amino acid. While, GROMOS is used for analysis of conformations obtained by computer simulations and the results are similar to ANOLEA with green spikes (negative values) represent favorable energy environment and the red spikes (positive values) represent unfavorable energy environment for a given amino acid.

### 3.4 AutoDock

Each chain of the aero plant allergen *Che a 3* was docked to each of 31 immunoglobulins of interest. It is clear from the results that the IgE clone RH has least binding energy when docked to chain A of *Che a 3* (See Table 1; Supplementary7).

**Table 1.** Immunoglobulin clones with least six binding energies

| Ligand Protein (Chain) | Receptor protein | Overall Binding Energy (Kcal/mol) | Ref R.M.S (Å) | Running time (Hours) |
|---|---|---|---|---|
| A | Homo sapiens clone BG immunoglobulin E variable region | 8.77 | 109.77 | 6hrs 25min 15.43 sec |
| A | Homo sapiens clone PO immunoglobulin E variable region | 8.76 | 127.74 | 7hrs 03min 05.04sec |
| A | Homo sapiens clone PT immunoglobulin E variable region | 3.97 | 43.65 | 8hrs 23min 17.76sec |
| A | Homo sapiens clone RC immunoglobulin E variable region | 5.9 | 77.72 | 9hrs 33min 49.39sec |
| A | Homo sapiens clone RD immunoglobulin E variable region | 6.32 | 122.35 | 15hrs 46min 41.87sec |
| A | Homo sapiens clone RH immunoglobulin E variable region | 0.14 | 42.21 | 10hrs 35min 52.77sec |

**Table 2.** siRNAs designed (in siDRM [12]) to target IgE heavy chain variable region

| Immunoglobulin | siRNA | GC content | Location | Off targets |
|---|---|---|---|---|
| Homo sapiens Clone RH immunoglobulin E variable region | CCGGUUCACCAUCUCCAGA | 57% | 198-216 | Yes |
| Consensus Sequence among variable regions of Immunoglobulin E clones | CCGAUUCACCAUCUCCAGA | 52% | 197-215 | No |

### 3.5 siRNA Specific to Immunoglobulins

siRNA against the immunoglobulin specific to *Che a 3* was designed with the tool siDRM [30]. (See Table 2). Possibility for inhibition of non targeted transcripts by the designed siRNA was monitored by checking the homology of the respective siRNA with other transcripts. For each siRNA being designed, it was checked for the following possibilities in siDRM [30] : a) Homology to the whole transcript (5'UTR or CDS or 3'UTR), b) Homology of its subsequence by excluding last two positions to the 3'UTR region of another transcript, c) Homology of position 2-8 (seed region) to the 3'UTR region of another transcript, or d) Homology of position 2-8 (seed region) to the 3'UTR region of another transcript and the homologous region is followed by four consecutive mismatches.

## 4 Discussion

The variable region in IgE is most often specific for an allergen and is different in each repertoire produced by B cells as proved by Xu and Davis [47] in their experiments on transgenic mouse with diverse variable region in heavy chain and restricted lambda light chains, which concluded that most of the antibody specificity was generated by the molecular diversity specified by heavy chain.

This study focuses on the approach of designing a siRNA based vaccine (targeting IgE heavy chain variable region) *in silico*. Though, siRNA based vaccines in allergen immunotherapy are prevalent [16],[48] it will be a new strategy to focus on IgE heavy chain variable region as a vaccine target. A very recent research about development of IgE-based and allergen specific gene vaccine for food allergy pioneered by Behnecke *et al.* [49] further supports this viewpoint. siRNA is designed for the IgE clone specific to a particular allergen after docking analysis. In this study, the IgE specific for aero allergen from *Chenopodium album, Che a 3,* is analyzed based on their least binding energy obtained by docking. *Che a 3* is a tetramer with 86 residues in each chain (See Fig. 3), 2068 non-polar hydrogen bonds, 163 aromatic bonds, 1594 rotatable bonds and 1224 torsion degrees of freedom. But since AutoDock [31],[32],[33] docking tool is optimized only for 2048 molecules, there was a need to perform chain-wise docking to IgE.

The interaction between two proteins typically involves binding between specific domains [50]. Hence, the domain based docking can give a greater insight into the interaction between allergen and immunoglobulin. Since each domain is enclosed by individual chains, *Che a 3* was split into distinct chains in SWISS PDB viewer [51]. While proteins can be docked by any of the two approaches, blind and focused dockings. In blind docking, the receptor protein is docked without knowledge of its binding site with Mass-centered grid box. In focused docking, grid box covering the predicted ligand binding sites on receptor protein is defined. Comparing both, blind docking has greater efficacy than focused [52],[53].

In this research, the protein is made rigid except for the residues in top ranking binding site predicted by QSiteFinder [30] and mass-centered grid box. The ligand protein was made rigid and receptor protein was docked in AutoDock

[31],[32],[33] docking tool. As stated earlier, each of the 4 chains of allergen were docked with individual immunoglobulin. From the docking results, (See Table 1; Supplementary6) Chain A and RH IgE VH region exhibited least binding energy of 0.14 Kcal/mol. In RH-Chain A complex, residue GLU 14 in *Che a 3* and SER 71 in IgE form a hydrogen bond between them with the latter as donor and former as acceptor of electrons (See Fig. 4). To explain this phenomenon, we explore the modeled structure of IgE, RH clone. The disorderliness of RH was the least (See Suplementary3). The disordered regions in a protein, are capable of binding their partners with both high specificity and low affinity [54], hence, though the interactions are specific, they are prone to be unstable, which explains the increase in binding energies with increase in disorderliness. On the other hand, the immunoglobulin RM exhibited the third least disorderliness after RG and RH but requires highest energy for binding.

This annotates contribution of other factors apart from disorderliness for increase in binding energy. Structural and energetic analysis of changes during binding process construes that factors like small perturbations on protein structure, hydrogen bonding, buried surface area; shape complementarity and cooperativity between proteins, each have an effect on binding [55]. Adequacy of binding is also determined by protein dynamics, solvation potential, amino acid composition, conservation, electrostatics and hydrophobicity [56]. Residue SER 71 of the IgE RH lies within the sequence containing heterodimer interface (See Supplementary8). While, on the other hand, residue GLU 14 in *Che a 3* was significant too, since, it was nearly conserved among other Polcalcin containing plant allergens and occasionally subjected to homologous exchange with aspartic acid [57]. Hence, it is a good site to induce cross reactivity among Polcalcins. As deduced earlier, the IgE clone RH can be specific to *Che a 3* and siRNA designed against it can be formulated as a vaccine. According to the protocol [40], a siRNA is efficient only if its GC content lies within the optimal range of 30-64%.

Fortunately, both the siRNA targeting RH and consensus sequence of IgE repository had an optimal GC content of 57% and 52% respectively. Also, siRNA candidates must lack the motif GTCCTTCAA correlated with Interferon induction leading to non specificity [58]. There were no siRNAs with more than 6 consecutive Gs or Cs which might have rendered them ineffective [39] (See Supplementary9). siRNAs must be unconserved across multiple organisms. Hence were cross-checked among 62 seed siRNA regions identified by Lewis et al [59].

The resulting siRNA targeting RH heavy chain variable region is predicted to exhibit Off-targeting in siDRM [30]. Furthermore, siRNAs complementary to each of 31 genetic variants were designed (See Supplementary9). Surprisingly, 18 out of 31 variants exhibited Off-targeting while 3 had no siRNA complementary to them. Unique siRNAs and those capable of Off-targeting were examined using YMF [60],[61],[62] and FindExplanators [63] (URL: http://bio.cs.washington .edu/software.html) for motif discovery and selection of significant motifs respectively. No significant motifs were deduced from Off-targeting siRNAs while unique siRNAs were overrepresented with the motif, CGAUUCAC (Z-Score:

268.95, Position: 2-10) which may be significant for their uniqueness. Moreover, a consensus sequence,

[GAGGTGCAGCTGCTGGAGTCTGGGGGAGGCGTGGTGAAGCCTGGGG
GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTT
ATTACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGG
GTGG-AGTTATTATTAATAATGGTAGTAGTAAAAACTACGCCGACTCCG
TGAAGGGCCGATTCACCATCTCCAGAGACAACTCCAAGAACACCCTGT
ACCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTCTATTAC
TGTGCGAGAGGAGG-GGTGG-GATGCTTTTGACTACTGC-ACTAGGGC
GGCGGCGGCGGCGTCGC]

was derived from 294 different clones [64],[65],[66],[67],[68],[69] of IgE VH region with the help of the alignment tool CINEMA [70] and a targeting siRNA was designed (See Table 2) which is found to be unique. The gene sequences were retrieved from NCBI with key words Immunoglobulin E variable region and Immunoglobulin heavy chain variable region and the duplicates were removed.

## 5 Conclusion

The siRNA can be injected in B-Cells to validate their silencing activity prior development of allergy vaccines for maximized vigor. In silico screening strategy preceding laboratory investigations can lead to great breakthroughs and is an economical method to accelerate the pace of researches in developing effectual immunotherapies for allergy. The siRNA, CCG**A**UUCACCAUCUCCAGA targets the consensus sequence at a region with 51-89% conservation among the genetic variants. Furthermore, it has no off-targeting and can serve as a vaccine for any allergy irrespective of the source of allergen. On the contrast, the siRNA, CCG**G**UUCACCAUCUCCAGA complimentary to the IgE (Clone RH) specific to *Che a 3* exhibits 'Off-targeting'. Both of the RNAs are complimentary to similar positions in their target sequences (See Table 2) and differ only by a nucleotide at their 4th position. Thus, proves the reliability of the siRNA against IgE clone RH as a possible vaccine.

### Supplementary Documents

Supplementary 1: Databases with Information about allergens:
`https://docs.google.com/document/edit?id=1Fbq3B-luz1YbrxZ8lxd_RWfzjjNWvx55WRSmK7kVhC4\&hl=en#`

Supplementary 2: List of Allergens with PDB Structures:
`https://docs.google.com/document/edit?id=11s1vQ-SwMru52sc--3IqcZ9WHA8_mWMhWJ3rC_o4zHE\&hl=en#`

Supplementary 3: Sequence Feature annotation in SWISS-MODEL workspace:
`https://docs.google.com/present/edit?id=0AeEKk700XoYVZGNrYnRtM3JfMTY2ZGdtYnpnZzI\&hl=en`

Supplementary 4: Disorderliness of predicted Model given by DisoPred:
`https://docs.google.com/document/edit?id=1JhGF86z2xG15b-pmze6BqXtXB62PvNKuQxYNxi5UnF0\&hl=en#`

Supplementary 5: Results of Template identification session SWISS-Model Workspace:
`https://docs.google.com/document/edit?id=1K84rljE2fhIYT6YMOVvo31sbzLzkAtLZ4wxnKOWqCbo\&hl=en#`

Supplementary 6: ANOLEA statistical potential:
`https://docs.google.com/present/edit?id=0AeEKk700XoYVZGNrYnRtM3JfMTYwY2dmdDNxamM\&hl=en`

Supplementary 7: Autodock results:
`https://docs.google.com/document/edit?id=1gEJWL5MjRdnMEqQmmVvpZSGP_3tQj8mqix6okmTfXF0\&hl=en#`

Supplementary 8: Result page of Conserved Domain Search in NCBI:
`https://docs.google.com/present/edit?id=0AeEKk700XoYVZGNrYnRtM3JfMTYzZzU2dmdtY3o\&hl=en`

Supplementary 9: siRNA targeting IgE heavy chain variable region:
`https://docs.google.com/document/edit?id=1PycmdhiX_195sKf-wuO-P6WU7n-_iHfK83KATT3z6Ww\&hl=en#`

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