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RESEARCH PAPER

Predicting Epitopes for Alzheimer's Disease Using Bioinformatics Tools

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ABSTRACT

Alzheimer's disease (AD) is an old age neurodegenerative disease and still needs to find a cure. Pathogenic proteins involved in AD disease progression are most promising targets to treat Alzheimer's disease. Epitopes of these pathogenic proteins can be targeted with exogenous antibodies, or passive immunotherapy to control the AD. Therefore, study of novel epitopes regions in the pathogenic protein helps in developing new therapies and accurate diagnosis. Here, we use bioinformatic approaches to identify novel epitopes regions in pathogenic proteins of AD. Finally, we will validate the newly identified epitope regions (peptides), which generate a potential peptide region to be used as drug targets and biomarkers for AD diagnosis.

Keywords: Alzheimer's Disease, Bioinformatics Epitopes, Apolipoprotein E

Alzheimer's disease is a progressive neurological disorder that primarily affects the elderly population[1]. Named after Dr. Alois Alzheimer, who first identified the disease in 1906, it is the most common form of dementia[1]. The hallmark of Alzheimer's disease is the accumulation of abnormal protein deposits called beta-amyloid plaques in the brain[1]. Another characteristic feature is the presence of neurofibrillary tangles, which are twisted fibers within nerve cells[1]. Alzheimer's disease leads to a gradual decline in cognitive function, affecting memory, thinking, and reasoning abilities[1]. Early symptoms often include forgetfulness, confusion, and difficulty with familiar tasks[1]. As the disease progresses, individuals may experience language difficulties and changes in behavior and personality[1]. There is currently no cure for Alzheimer's disease, and available treatments focus on managing symptoms and slowing its progression[1]. The exact cause of Alzheimer's is not fully understood, but age, genetics, and environmental factors are believed to play a role[1]. The global prevalence of Alzheimer's disease is expected to rise as the population ages[1]. Diagnosis is often challenging, requiring a combination of clinical evaluation, medical history, and cognitive assessments[1]. Ongoing research aims to find innovative approaches for early detection and effective treatment of Alzheimer's[1]. Supportive care, education, and awareness campaigns are crucial in promoting understanding and compassion for those affected by Alzheimer's disease[1].

Amyloid Beta (Aβ)

Amyloid Beta (Aβ) is a peptide derived from the larger amyloid precursor protein (APP), which is processed in the brain[2]. In Alzheimer's disease, Aβ peptides abnormally aggregate and form insoluble

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plaques in the extracellular spaces of the brain[2]. These amyloid plaques, also known as neuritic plaques, are a hallmark pathology of Alzheimer's disease[2].

Aβ exists in various forms, with Aβ40 and Aβ42 being the most prevalent. Aβ42 is more prone to aggregation and is particularly implicated in Alzheimer's pathology[2]. Aggregated Aβ is associated with neurotoxicity, leading to damage and dysfunction of neurons in the brain. Aβ interacts with tau protein, contributing to the formation of neurofibrillary tangles, another characteristic feature of Alzheimer's[2]. The amyloid cascade hypothesis posits that the accumulation of Aβ initiates a series of events leading to neurodegeneration in Alzheimer's disease[2]. Mutations in genes such as APP, PSEN1, and PSEN2 are associated with early-onset familial Alzheimer's and increased production of Aβ[2]. In a healthy brain, mechanisms exist to clear Aβ, but in Alzheimer's, this clearance is impaired, leading to Aβ accumulation[2]. The presence of Aβ can trigger an inflammatory response in the brain, contributing to disease progression[2]. Aβ and tau proteins may act synergistically, exacerbating neurodegeneration in Alzheimer's disease[2]. Elevated levels of Aβ42 or an increased Aβ42/Aβ40 ratio in cerebrospinal fluid are considered potential biomarkers for Alzheimer's disease[2].

Research focuses on developing therapeutics that target Aβ, aiming to either prevent its formation or enhance its clearance as a potential treatment for Alzheimer's disease[2].

Apolipoprotein E (apoE)

Apolipoprotein E (APOE) is a gene that provides instructions for making a protein called apolipoprotein E[3]. There are three main variants of the apoE gene: APOE2, APOE3, and APOE4. APOE4 is associated with an increased risk of developing Alzheimer's disease[3]. Possessing one copy of the APOE4 allele increases the risk of Alzheimer's and having two copies (homozygous APOE4) significantly raises the risk[3].

APOE4 carriers may have an increased susceptibility to neuroinflammation, which is implicated in Alzheimer's disease progression. APOE4 has been shown to interact with tau protein, another protein associated with Alzheimer's[3]. This interaction may contribute to the development of neurofibrillary tangles[3]. APOE is involved in maintaining synaptic function, which is critical for communication between neurons[3]. Disruptions in synaptic function are a common feature in Alzheimer's disease[3]. APOE has an impact on cerebral blood flow. APOE4 carriers may have altered blood flow in the brain, which can contribute to cognitive decline[3].

The association between APOE4 and Alzheimer's risk is more pronounced in late-onset cases[3]. APOE4 has a stronger impact as a risk factor for Alzheimer's in older individuals[3]. APOE4's influence on Alzheimer's risk can vary between genders[3]. Some studies suggest that APOE4 may have a more significant impact on women than men[3]. Genetic testing for APOE4 is sometimes used in research and clinical settings as a potential diagnostic marker to assess the risk of developing Alzheimer's disease[3]. Researchers are exploring APOE as a potential therapeutic target for Alzheimer's disease[3]. Developing interventions to modify the effects of APOE4 may offer new avenues for treatment[3].

Epitopes/peptide immunogenic regions important for the immunotherapies[4]. Epitope is peptide region in the protein that is recognized by the immune system, importantly by B cells, T cells or antibodies[4]. In this regard's antibodies play important role in immunotherapies, Antibodies are combined proteins that target and bind to epitope regions in the pathogenic proteins[4]. The epitopes are regions in the protein (Amyloid Beta Aβ or APOE) which so high specificity binding antibodies which act as biomarkers or disease targets, which makes them good candidates for disease diagnose and treatment[4,5,6]. For example, anti-APOE antibody or Amyloid Beta Aβ antibody can be used for to detect alzhmer' disease andcan be used for AD treatment[5]. The new epitopes identified can be used to develop antibody humanization and novel therapeutics for AD[5]. To control and prevent the AD, the development of novel therapies is important issue[4]. In addition, bioinformatic tools that can measure/monitor T-cell and B-cell responses to know how our immune system is responding to pathogenic proteins of AD (Amyloid Beta Aβ or APOE)[4,5]. However, little information is currently available about the T-cell or b-cell epitopes of Amyloid Beta Aβ or APOE proteins[5,6].

B-cell epitopes

B-cell epitopes play key role in humoral immune response[7,10]. Discovering B-cell epitopes is a fundamental step for development of noveltherapies and diagnostic tools for AD[7,10]. A particular region in the protein the regnosed by antibodies are called B-cell epitopes[4,7]. To further describe B-cell epitopes, a small cluster of amino acids recognized by B-cell receptors which helps in generate cellular or humoral immune response[7,10]. Recent advancement of bioinformatics, epitope mapping technologies using computational methods will help discovering novel epitopes for AD which can enhance immune response by antibody, B-cell, T-cell[7,10]. Thus, it is crucial to predict epitopes of APOE or Amyloid Beta Aβ to develop new therapeutic or diagnostic approaches for AD[7,10]. Last decade, methods for epitope mapping by using structural and functional approach are cost intensive, laborious and time consuming[7,10]. In contrast, last decade advance discoveries in bioinformatics lead path forward to computational tools to identify epitopes which are cost effective and time saving[7,10].

T Cell Epitope

In immune system, T-cell paly critical role in immune response for pathogenic proteins. T cells recognize epitopes presented by major histocompatibility complex (MHC) molecules to generate the immune response[8,9,11,12]. The MHC complex is in two classes: class I and class II[5,8,9]. The class I is expressed on surfaces of all nucleated cells and class II present on surface of antigen-presenting cells (APCs)[8,9,11,12]. In addition, we predict CD4+T cell epitopes, which critical to play a key role immunity for AD[8,9,11,12]. Therefore, we predicted and identified novel CD4+T cell epitopes for Amyloid Beta Aβ or APOE proteins[8,9,11,12].

METHODS

Sequence retrieval of structural proteins of Amyloid Beta Aβ or APOE proteins

Protein sequences of Amyloid Beta Aβ or APOE proteins sequences were searched in NCBI protein data base at following link https://www.ncbi.nlm.nih.gov/protein/. The sequences which retrieved were copied to the results section.

Sequence alignment

Alignment of protein sequences (Amyloid Beta Aβ or APOE proteins) was performed on the protein blast using (BlstP) https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins.

Protein 3D structure

Protein 3D was developed using following webserver: SWISS-MODEL at https://swissmodel.expasy.org/. NCBI protein sequences of Amyloid Beta Aβ or APOE protein were supplied into 3D model creation at https://swissmodel.expasy.org/.

Linear B-cell epitope prediction

http://tools.iedb.org/bcell/result web servers were employed for B-cell epitope forecast. The length of linear B-cell epitopes normally varies from 5 to 30 residues. In this study, we used the default window length of 16 to obtain the maximum accuracy of prediction. Predicted epitopes were given in figure form in results and highlighted which are high score.

T-cell epitope prediction

In this study, we used the online service provided by IEDB, http://tools.iedb.org to forecast T-cell epitopes. A relatively small pool of HLA alleles covering the majority of the population, over 97 and 99% for class I and class II respectively, were chosen in the analysis. The sequences were given in plain format and the top 100% scoring peptides were retained for further analysis. Detection of antigen specific CD4+ T cells epitopes also searched using IEDB to forecast.

Profiling and evaluation of predicted epitopes

Immunogenicity of predicted peptides where assed highest scored epitopes will be recommended for therapeutic use.

RESULTS AND ANALYSIS

Sequence retrieval of APOE4 protein

APOE protein sequence is generated using NCBI protein data base, APOE contains is 317 amino acids (Fig. 1).

MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGR
FWDYLRWVQTLSEQVQEELLSSQVTQELRALMDETMKELKAYKSELEEQL
TPVAEETRARLSKELQAAQARLGADMEDVCGRLVQYRGEVQAMLGQSTEE
LRVRLASHLRKLRKRLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLG
PLVEQGRVRAATVGSLAGQPLQERAQAWGERLRARMEEMGSRTRDRLDEV
KEQVAEVRAKLEEQAQQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEK
VQAAVGTSAAPVPSDNH

Fig. 1: APOE 4 protein sequence

1. BLASTP analysis of the APOE 4 protein

The protein sequence is analyzed using BLAST P program from NCBI, APOE 4 protein have common protein sequence among different homo sapiens scores have 100% match with the apolipoprotein E [Homo sapiens] (Fig. 2).

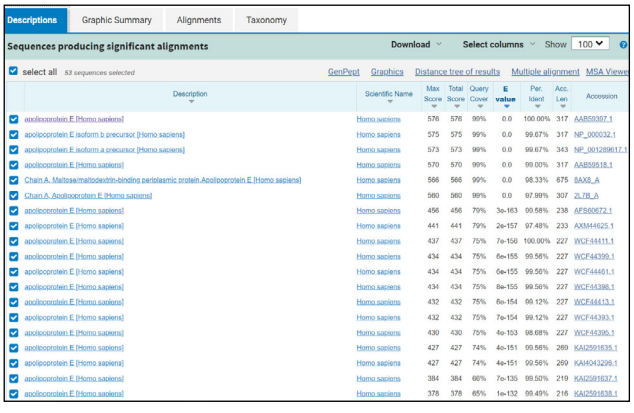


Fig. 2: BLAST P analysis of APOE 4 protein

3. Protein structure analysis of APOE 4 protein

The protein 3D structure is predicted using Swiss-Model, SWISS-MODEL at https://swissmodel.expasy.org/. Fig. 3 shows the APOE 4 protein 3D structure.

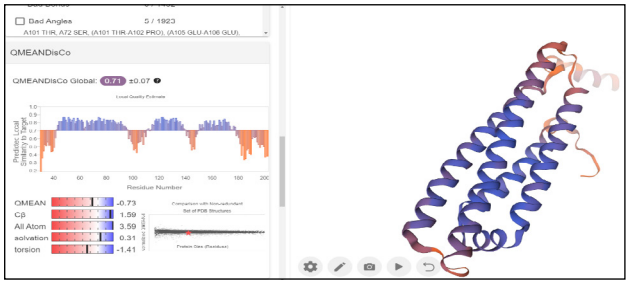


Fig. 3: 3D structure of APOE 4 protein

4. General epitope prediction of APOE 4 protein

The general protein epitope predication was carried out, the predicted peptide regions that likely to have immunologic properties. The analysis is carried using sysbio server (http://sysbio.unl.edu/SVMTriP/result.php?jobid=65bbd928de3145.87905992, https://fuxmanlab.shinyapps.io/Epitope-Evaluator/.). The Table 1 indicated epitope regions score 1.000 indicate the best possible epitope region, which is located amino acid locations from 233 – 252. These predications do not give information about T-cell or b-cell recognition, to get more strong epitope regions we need further analysis using T-cell and B-cell epitope evaluations.

Table 1: General Epitope predictions of APOE 4 protein

Rank	Location	Epitope	Score	Recommend*
1	233 - 252	RARMEEMGSR TRDRLDEVKE	1.000	
2	112 - 131	SKELQAAQARL GADMEDVCG	0.397	
3	41 - 60	GQRWELALGR FWDYLRWVQT	0.250	
4	297 - 316	LVEKVQAAVG TSAAPVPSDN	0.241	

5. Linear B-cell epitope prediction

The B-cell epitopes were predicated using IEDB server, http://tools.iedb.org/bcell/result/ b.cellpreditcor These APOE 4 protein epitopes recognized by B-cells. The IEDB server analysis is sequence hit to this database with identity ≥80% and length ≥8 is considered a B cell epitope. Fig. 4 A and B shows the predicated epitope sequences, 21-49 region of the APOE 4 protein is chosen for as B- cell epitope region.

The region of 21-49 is better in immunogenicity, while comparing the results of t-cell epitope regions. The sequence of the B-cell epitope for AD therapies is EQAVETEPEPELRQQTEWQSGQRWELALG.

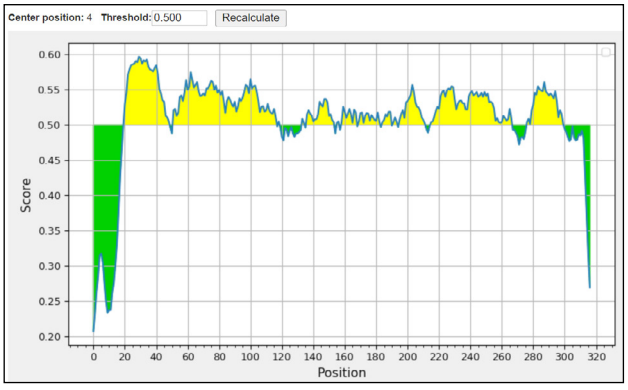


Fig. 4A: B-cell epitope analysis of the APOE 4 protein

Predicted peptides:

No.	Start	End	Peptide	Length
1	21	49	EQAVETEPEPELRQQTEWQSGQRWELALG	29
2	52	117	WDYLRWVQTLSEQVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQA	66
3	119	119	Q	1
4	134	135	VQ	2
5	137	154	RGEVQAMLGQSTEELRVR	18
6	156	157	AS	2
7	159	168	LRKLRKRLLR	10
8	170	183	ADDLQKRLAVYQAG	14
9	185	190	REGAER	6
10	192	194	LSA	3
11	196	212	RERLGPLVEQGRVRAAT	17
12	216	267	LAGQPLQERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQ	52
13	277	300	ARLKSWFEPLVEDMQRQWAGLVEK	24

Fig. 4B: B-cell epitope regions for possibility of immunogenicity

6. T-cell epitope prediction

❍ Prediction of MHC I-binding epitopes

The Epitopes that can be recognized by the T-cell receptors after a particular APOE 4 protein has been intracellularly processed, bound to MHC I molecule. The MCH I binding epitopes were analyzed using IEDB server. Fig. 5 A and B shows the predicted epitope regions, the score 100 are strogest epitopes, the region 24-37 was chosen as epitope region based on b-cell epitopes, which can generate both T-cell and B-cell reponce. The epitope region, chosen is QKRLAVYQAGAREG, 24-37, which can be a novel epitope region for the therpeutic development for AD.

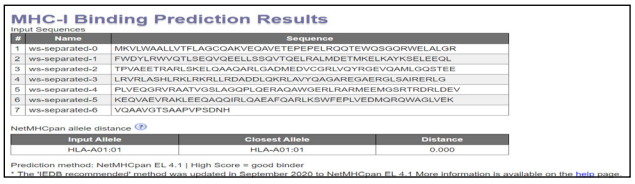


Fig. 5A: MHC-I bindiing epitopes

HLA-A*01:01	3	29	39	11	VCGRLVQYRGE	VCGRLVQYE	VCGRLVQYRGE	2e-06	99
HLA-A*01:01	3	12	25	14	SKELQAAQARLGAD	SAAQARLGA	SKELQAAQARLGA	2e-06	99
HLA-A*01:01	1	16	29	14	CQAKVEQAVETEPE	CQAKETEPE	CQAKVEQAVETEPE	2e-06	99
HLA-A*01:01	1	5	16	12	WAALLVTFLAGC	WAALLVTFC	WAALLVTFLAGC	2e-06	99
HLA-A*01:01	1	4	15	12	LWAALLVTFLAG	LWAVTFLAG	LWAALLVTFLAG	2e-06	99
HLA-A*01:01	5	28	41	14	WGERLRARMEEMGS	WARMEEMGS	WGERLRARMEEMGS	1e-06	100
HLA-A*01:01	5	27	40	14	AWGERLRARMEEMG	ALRARMEEM	AWGERLRARMEEM	1e-06	100
HLA-A*01:01	5	1	14	14	PLVEQGRVRAATVG	PLVEQGTVG	PLVEQGRVRAATVG	1e-06	100
HLA-A*01:01	4	24	37	14	QKRLAVYQAGAREG	QVQAGAREG	QKRLAVYQAGAREG	1e-06	100
HLA-A*01:01	4	14	22	9	KRLLRDADD	KRLLRDADD	KRLLRDADD	1e-06	100
HLA-A*01:01	4	13	21	9	RKRLLRDAD	RKRLLRDAD	RKRLLRDAD	1e-06	100
HLA-A*01:01	4	12	22	11	LRKRLLRDADD	LRKRLLRDA	LRKRLLRDA	1e-06	100
HLA-A*01:01	4	10	21	12	RKLRKRLLRDAD	RKLRKRLLA	RKLRKRLLRDA	1e-06	100
HLA-A*01:01	4	9	21	13	LRKLRKRLLRDAD	LRKLLLRDA	LRKLRKRLLRDA	1e-06	100
HLA-A*01:01	4	8	21	14	HLRKLRKRLLRDAD	HLRKLKRLL	HLRKLRKRLL	1e-06	100
HLA-A*01:01	3	15	28	14	LQAAQARLGADMED	LQAGADMED	LQAAQARLGADMED	1e-06	100
HLA-A*01:01	1	28	41	14	PEPELRQQTEWQSG	PEPEQQTEW	PEPELRQQTEW	1e-06	100
HLA-A*01:01	1	8	21	14	LLVTFLAGCQAKVE	LLVTQAKVE	LLVTFLAGCQAKVE	1e-06	100
HLA-A*01:01	1	4	17	14	LWAALLVTFLAGCQ	LVTFLAGCQ	LWAALLVTFLAGCQ	1e-06	100
HLA-A*01:01	1	4	16	13	LWAALLVTFLAGC	LWAALLVTC	LWAALLVTFLAGC	1e-06	100
HLA-A*01:01	1	3	16	14	VLWAALLVTFLAGC	VLWAALLGC	VLWAALLVTFLAGC	1e-06	100
HLA-A*01:01	1	2	15	14	KVLWAALLVTFLAG	KVLWAALLG	KVLWAALLVTFLAG	1e-06	100
HLA-A*01:01	4	13	22	10	RKRLLRDADD	RKRLLRDAD	RKRLLRDADD	0.0	100
HLA-A*01:01	4	11	22	12	KLRKRLLRDADD	KLRKRLLRD	KLRKRLLRDADD	0.0	100

Fig. 5B: MHC-1 binding epitopes with strength scores, 100 score is strongest epitopes

❍ Prediction of MHC II -binding epitopes

The Epitopes that can be recognized by the T-cell receptors after a particular APOE 4 protein has been intracellularly processed, bound to MHC II molecule. The MCH II binding epitopes were analyzed using IEDB server. Fig. 6A and 6B shows the predicted epitope regions, the score 100 are strogest epitopes, the region 25-39 was chosen as epitope region based on b-cell epitopes, which can generate both T-cell and B-cell reponce. The epitope region, chosen is ETEPEPELRQQTEWQ, 25-39, which can be a novel epitope region for the therpeutic development for AD.

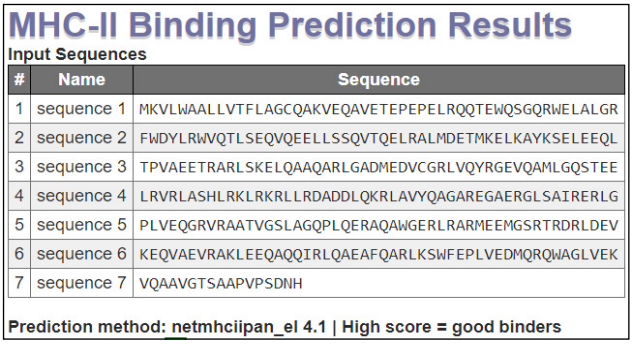


Fig. 6A: MHC-I bindiing epitopes

HLA-DRB1*01:01	3	26	40	15	CGRLVQYRG	MEDVCGRLVQYRGEV	0.0012	84
HLA-DRB1*01:01	3	25	39	15	CGRLVQYRG	DMEDVCGRLVQYRGE	0.0012	84
HLA-DRB1*01:01	4	3	17	15	LASHLRKLR	VRLASHLRKLRKRLL	0.0012	84
HLA-DRB1*01:01	1	23	37	15	VETEPEPEL	AVETEPEPELRQQTE	0.0012	84
HLA-DRB1*01:01	5	23	37	15	RAQAWGERL	ERAQAWGERLRARME	0.0011	85
HLA-DRB1*01:01	4	15	29	15	LLRDADDLQ	RLLRDADDLQKRLAV	0.0011	85
HLA-DRB1*01:01	4	30	44	15	GAREGAERG	YQAGAREGAERGLSA	0.0010	86
HLA-DRB1*01:01	1	3	17	15	WAALLVTFL	VLWAALLVTFLAGCQ	0.0009	87
HLA-DRB1*01:01	5	27	41	15	LRARMEEMG	AWGERLRARMEEMGS	0.0010	87
HLA-DRB1*01:01	3	18	32	15	LGADMEDVC	AQARLGADMEDVCGR	0.0008	89
HLA-DRB1*01:01	4	17	31	15	DDLQKRLAV	LRDADDLQKRLAVYQ	0.0008	90
HLA-DRB1*01:01	5	26	40	15	GERLRARME	QAWGERLRARMEEMG	0.0007	91
HLA-DRB1*01:01	3	16	30	15	QARLGADME	QAAQARLGADMEDVC	0.0007	91
HLA-DRB1*01:01	5	24	38	15	WGERLRARM	RAQAWGERLRARMEE	0.0006	93
HLA-DRB1*01:01	5	25	39	15	WGERLRARM	AQAWGERLRARMEEM	0.0006	94
HLA-DRB1*01:01	3	19	33	15	LGADMEDVC	QARLGADMEDVCGRL	0.0006	94
HLA-DRB1*01:01	5	35	49	15	MEEMGSRTR	RMEEMGSRTRDRLDE	0.0005	95
HLA-DRB1*01:01	3	17	31	15	RLGADMEDV	AAQARLGADMEDVCG	0.0005	95
HLA-DRB1*01:01	5	36	50	15	MGSRTRDRL	MEEMGSRTRDRLDEV	0.0004	96
HLA-DRB1*01:01	3	20	34	15	MEDVCGRLV	ARLGADMEDVCGRLV	0.0004	96
HLA-DRB1*01:01	4	16	30	15	DDLQKRLAV	LLRDADDLQKRLAVY	0.0005	96
HLA-DRB1*01:01	1	24	38	15	TEPEPELRQ	VETEPEPELRQQTEW	0.0002	99
HLA-DRB1*01:01	1	25	39	15	PEPELRQQT	ETEPEPELRQQTEWQ	0.0001	100

Fig. 6B: MHC-II binding epitopes with strength scores, 100 score is strongest epitopes

Selection of CD4⁺ T cell epitopes of APOE 4 protein by bioinformatic prediction

T cell (CD4) reaction and the key to the development and design of candidate therapeutics and biomarkers. The CD4⁺ T cell epitopes were analyzed using IEDB server. The study is to analyze and predict CD4⁺ recognizing epitopes, which are following around region 6-20. We are proposing the 6-20 region, sequence ASHLRKLRKRLLRDA, as biomarker for AD diagnosis (See Fig. 7).

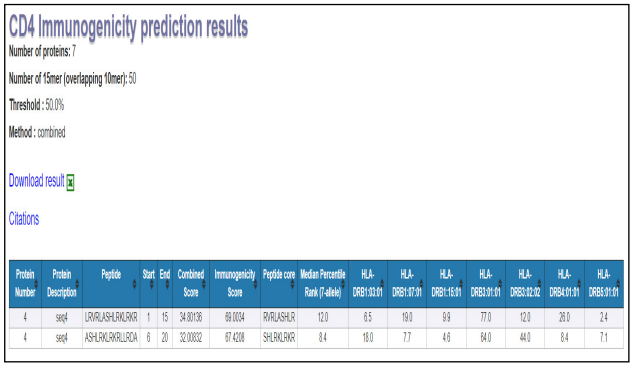


Fig. 7: CD4 Immunogenicity prediction results

7. Sequence retrieval of Beta Amyloid protein

Beta Amyloid protein sequence is generated using NCBI protein data base, Beta Amyloid contains is 317 amino acids **(**Fig. 8).

1 mtelpaplsyfqnaqmsednhlsntvrsqndnrerq
ehndrrslghpeplsngrpqgnsr

61 qvveqdeeedeeltlkygakhvimlfvpvtlcmvvvvatiksv
sfytrkdgqliytpfte

121 dtetvgqralhsilnaaimisvivvmtillvvlykyrcykvihaw
liisslllfffsfi

181 ylgevfktynvavdyitvalliwnfgvvgmisihwkgplrlqqay
limisalmalvfiky

241 lpewtawlilavisvydlvavlcpkgplrmlvetaqernetlfpali
ysstmvwlvnmae

301 gdpeaqrrvsknskynaesteresqdtvaenddggfseeweaqr
dshlgphrstpesraa

361 vqelsssilagedpeergvklglgdfifysvlvgkasatasgdw
nttiacfvailiglcl

421 lllaifkkalpalpisitfglvfyfatdylvqpfmdqlafhqfyi

Fig. 8: Beta Amyloid protein sequence

8. BLAST P analysis of the Beta Amyloid protein

The protein sequence is analyzed using BLAST P program from NCBI, Beta Amyloid protein have common protein sequence among different homo sapiens scores have 100% match with the apolipoprotein E [Homo sapiens] (Fig. 9).

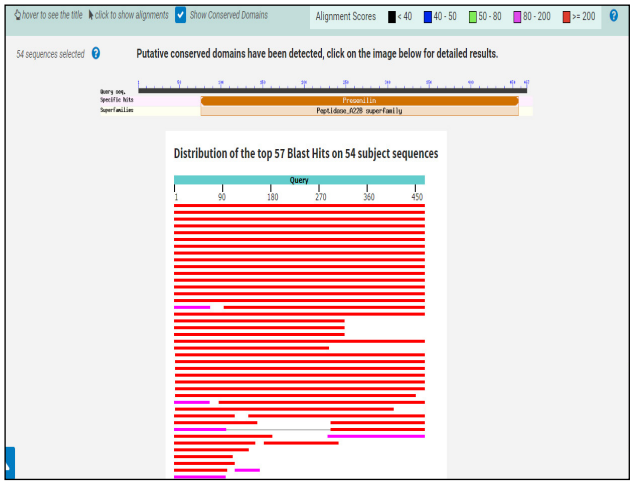


Fig. 9: BLAST P analysis of Beta Amyloid protein

9. Protein structure analysis of Beta Amyloid protein

The protein 3D structure is predicted using Swiss-Model,SWISS-MODEL at https://swissmodel.expasy.org/. Fig. 10 shows the Beta Amyloid protein 3D structure.

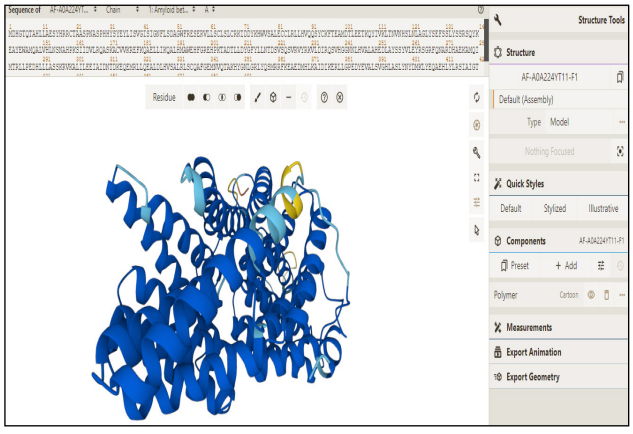


Fig. 10: 3D structure of Beta Amyloid protein

10. General epitope prediction of BETA AMYLOID 4 protein

The general protein epitope predication was carried out, the predicted peptide regions that likely to have immunologic properties. The analysis is carried using sysbio server (http://sysbio.unl.edu/SVMTriP/result.php?jobid=65bbd928de3145.87905992,https://fuxmanlab.shinyapps.io/Epitope-Evaluator/.). The Table 2 indicated epitope regions score 1.000 indicate the best possible epitope region, which is located amino acid locations from 284 – 303 and 76-95. These predications do not give information about T-cell or b-cell recognition, to get more strong epitope regions we need further analysis using T-cell and B-cell epitope evaluations.

Table 2: General Epitope predictions of Beta Amyloid protein

Rank	Location	Epitope	Score	Recommend*
1	284 - 303	PALIYSSTMVW LVNMAEGDP	1.000	
2	76 - 95	KYGAKHVIMLF VPVTLCMVV	0.994	
3	101 - 120	KSVSFYTRKDG QLIYTPFTE	0.696	
4	236 - 255	VFIKYLPEWTA WLILAVISV	0.650	
5	389 - 408	YSVLVGKASAT ASGDWNTTI	0.475	
6	322 - 341	RESQDTVAEND DGGFSEEWE	0.451	

11. Linear B-cell epitope prediction

The B-cell epitopes were predicated using IEDB server, http://tools.iedb.org/bcell/result/b.cellpreditcor These Beta Amyloid protein epitopes recognized by B-cells. The IEDB server analysis is sequence hit to this database with identity ≥ 80% and length ≥ 8 is considered a B cell epitope. Fig. 11 shows the predicated epitope sequences, 5-77 region of the Beta Amyloid protein is chosen for as B- cell epitope region. The region of 21-49 is better in immunogenicity, while comparing the results of t-cell epitope regions.

Predicted peptides:

No.	Start	End	Peptide	Length
1	5	77	PAPLSYFQNAQNSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQVVEQDEEEDEELTLKY	73
2	104	111	SFYTRKDG	8
3	121	127	DTETVGQ	7
4	190	192	NVA	3
5	276	281	QERNET	6
6	294	376	WLVNMAEGDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGPHRSTPESRAAVQELSSSILAGEDPEE	83
7	399	403	TASGD	5
8	433	433	P	1

Fig. 11: B-cell epitope regions for possibility of immunogenicity

12. T-cell epitope prediction

Prediction of MHC I-binding epitopes

The Epitopes that can be recognized by the T-cell receptors after a particular Beta Amyloid protein has been intracellularly processed, bound to MHC I molecule. The MCH I binding epitopes were analyzed using IEDB server. Fig. 12 A and B shows the predicted epitope regions, the score 100 are strogest epitopes, the region 18-31 was chosen as epitope region based on b-cell epitopes, which can generate both T-cell and B-cell reponce.The epitope region, chosen is EDNHLSNTVRSQNDN, 18-31, which can be a novel epitope region for the therpeutic development for AD.

Prediction of MHC II -binding epitopes

The Epitopes that can be recognized by the T-cell receptors after a particular Beta Amyloid protein has been intracellularly processed, bound to MHC II molecule. The MCH II binding epitopes were analyzed using IEDB server.

MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	ws-separated-0	MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPL
2	ws-separated-1	SNGRPQGNSRQVVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATI
3	ws-separated-2	KSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTILL
4	ws-separated-3	VVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVAL
5	ws-separated-4	LIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLIL
6	ws-separated-5	AVISVYDLVAVLCPKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAE
7	ws-separated-6	GDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGP
8	ws-separated-7	HRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATA
9	ws-separated-8	SGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATD
10	ws-separated-9	YLVQPFMDQLAFHQFYI

NetMHCpan allele distance

Input Allele	Closest Allele	Distance
HLA-A01:01	HLA-A01:01	0.000

Prediction method: NetMHCpan EL 4.1 | High Score = good binder

Fig. 12-A: MHC-I bindiing epitopes for Beta Amyloid protein

HLA-A*01:01	9	40	50	11	TFGLVFYFATD	TLVFYFATD	TFGLVFYFATD	1e-06	100
HLA-A*01:01	9	18	31	14	LCLTLLLLAIFKKA	LLLAIFKKA	LCLTLLLLAIFKKA	1e-06	100
HLA-A*01:01	9	8	21	14	IACFVAILIGLCLT	IACFGLCLT	IACFVAILIGLCLT	1e-06	100
HLA-A*01:01	9	4	17	14	WNTTIACFVAILIG	NTTFVAILI	NTTIACFVAILI	1e-06	100
HLA-A*01:01	8	31	44	14	LGLGDFIFYSVLVG	LGLGDFIFY	LGLGDFIFY	1e-06	100
HLA-A*01:01	7	33	46	14	DGGFSEEWEAQRDS	DSEEWEAQR	DGGFSEEWEAQR	1e-06	100
HLA-A*01:01	7	25	33	9	QDTVAENDD	QDTVAENDD	QDTVAENDD	1e-06	100
HLA-A*01:01	7	21	34	14	ERESQDTVAENDDG	ERESQDTVG	ERESQDTVAENDDG	1e-06	100
HLA-A*01:01	7	13	26	14	SKYNAESTERESQD	SSTERESQD	SKYNAESTERESQD	1e-06	100
HLA-A*01:01	6	17	30	14	PLRMLVETAQERNE	PLRMLVETE	PLRMLVETAQERNE	1e-06	100
HLA-A*01:01	6	14	27	14	PKGPLRMLVETAQE	PMLVETAQE	PKGPLRMLVETAQE	1e-06	100
HLA-A*01:01	6	14	26	13	PKGPLRMLVETAQ	PKGPLRMLQ	PKGPLRMLVETAQ	1e-06	100
HLA-A*01:01	4	32	44	13	LGEVFKTYNVAVD	LFKTYNVAV	LGEVFKTYNVAV	1e-06	100
HLA-A*01:01	4	21	34	14	LLLLFFFSFIYLGE	LLFFFSFIY	LLLLFFFSFIY	1e-06	100
HLA-A*01:01	2	38	49	12	PVTLCMVVVVAT	PVMVVVVAT	PVTLCMVVVVAT	1e-06	100
HLA-A*01:01	2	5	17	13	PQGNSRQVVEQDE	PQGNSRQVE	PQGNSRQVVEQDE	1e-06	100
HLA-A*01:01	2	5	16	12	PQGNSRQVVEQD	PSRQVVEQD	PQGNSRQVVEQD	1e-06	100
HLA-A*01:01	2	3	16	14	GRPQGNSRQVVEQD	GSRQVVEQD	GRPQGNSRQVVEQD	1e-06	100
HLA-A*01:01	1	19	32	14	DNHLSNTVRSQNDN	DTVRSQNDN	DNHLSNTVRSQNDN	1e-06	100
HLA-A*01:01	1	19	31	13	DNHLSNTVRSQND	DSNTVRSQN	DNHLSNTVRSQN	1e-06	100
HLA-A*01:01	1	18	31	14	EDNHLSNTVRSQND	EDNHLSNTD	EDNHLSNTVRSQND	1e-06	100
HLA-A*01:01	1	7	19	13	PLSYFQNAQMSED	PSYFQNAQM	PLSYFQNAQM	1e-06	100
HLA-A*01:01	7	21	33	13	ERESQDTVAENDD	ERESQDTVD	ERESQDTVAENDD	0.0	100
HLA-A*01:01	2	7	20	14	GNSRQVVEQDEEED	GVEQDEEED	GNSRQVVEQDEEED	0.0	100
HLA-A*01:01	2	5	18	14	PQGNSRQVVEQDEE	PQGNSRQEE	PQGNSRQVVEQDEE	0.0	100
HLA-A*01:01	1	7	20	14	PLSYFQNAQMSEDN	PLSYFQNAN	PLSYFQNAQMSEDN	0.0	100

Fig. 12-B: MHC-1 binding epitopes for Beta Amyloid protein with strength scores, 100 score is strongest epitopes

Fig. 13 A and B shows the predicted epitope regions, the score 100 are strogest epitopes, the region 18-32 was chosen as epitope region based on b-cell epitopes, which can generate both T-cell and B-cell reponce. The epitope region, chosen is ISSLLLLFFFSFIYL, 18-32, which can be a novel epitope region for the therpeutic development for AD.

MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	sequence 1	MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPL
2	sequence 2	SNGRPQGNSRQVVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVVATI
3	sequence 3	KSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTILL
4	sequence 4	VVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVAL
5	sequence 5	LIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLIL
6	sequence 6	AVISVYDLVAVLCPKGPLRMLVETAQERNETLFPALIYSSTMVWLVNMAE
7	sequence 7	GDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGP
8	sequence 8	HRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASATA
9	sequence 9	SGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATD
10	sequence 10	YLVQPFMDQLAFHQFYI

Prediction method: netmhciipan_el 4.1 | High score = good binders

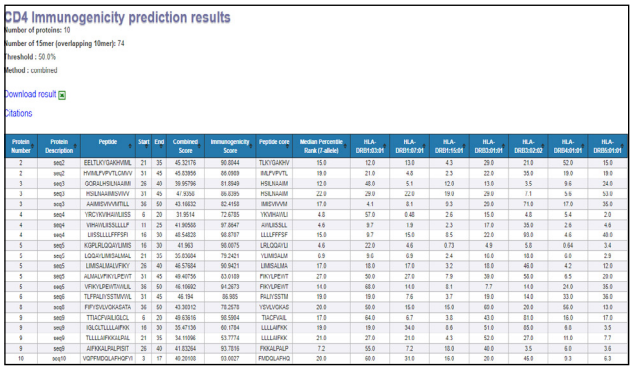
Fig. 13-A: MHC-II bindiing epitopes for Beta Amyloid protein

HLA-DRB1*01:01	1	33	47	15	QEHNDRRSL	RERQEHNDRRSLGHP	0.0003	98
HLA-DRB1*01:01	4	22	36	15	FFSFIYLGE	LLLFFFSFIYLGEVF	0.0003	98
HLA-DRB1*01:01	2	13	27	15	EEEDEELTL	VEQDEEEDEELTLKY	0.0003	98
HLA-DRB1*01:01	6	10	24	15	LCPKGPLRM	AVLCPKGPLRMLVET	0.0003	98
HLA-DRB1*01:01	1	30	44	15	ERQEHNDRR	NDNRERQEHNDRRSL	0.0003	98
HLA-DRB1*01:01	7	29	43	15	DDGGFSEEW	AENDDGGFSEEWEAQ	0.0003	98
HLA-DRB1*01:01	9	10	24	15	VAILIGLCL	CFVAILIGLCLTLLL	0.0002	99
HLA-DRB1*01:01	7	28	42	15	DDGGFSEEW	VAENDDGGFSEEWEA	0.0002	99
HLA-DRB1*01:01	1	34	48	15	DRRSLGHPE	ERQEHNDRRSLGHPE	0.0003	99
HLA-DRB1*01:01	9	35	49	15	ITFGLVFYF	LPISITFGLVFYFAT	0.0003	99
HLA-DRB1*01:01	1	28	42	15	DNRERQEHN	SQNDNRERQEHNDRR	0.0003	99
HLA-DRB1*01:01	1	29	43	15	ERQEHNDRR	QNDNRERQEHNDRRS	0.0003	99
HLA-DRB1*01:01	7	27	41	15	DDGGFSEEW	TVAENDDGGFSEEWE	0.0003	99
HLA-DRB1*01:01	4	18	32	15	LLFFFSFIY	ISSLLLLFFFSFIYL	0.0000	100
HLA-DRB1*01:01	4	16	30	15	ISSLLLLFF	LIISSLLLLFFFSFI	0.0000	100
HLA-DRB1*01:01	4	17	31	15	LLFFFSFIY	IISSLLLLFFFSFIY	0.0000	100
HLA-DRB1*01:01	4	19	33	15	LLFFFSFIY	SSLLLLFFFSFIYLG	0.0001	100
HLA-DRB1*01:01	9	14	28	15	IGLCLTLLL	ILIGLCLTLLLLAIF	0.0001	100
HLA-DRB1*01:01	4	15	29	15	WLIISSLLL	WLIISSLLLLFFFSF	0.0001	100
HLA-DRB1*01:01	9	13	27	15	IGLCLTLLL	AILIGLCLTLLLLAI	0.0001	100
HLA-DRB1*01:01	9	15	29	15	CLTLLLLAI	LIGLCLTLLLLAIFK	0.0001	100
HLA-DRB1*01:01	1	26	40	15	DNRERQEHN	VRSQNDNRERQEHND	0.0001	100
HLA-DRB1*01:01	9	12	26	15	LIGLCLTLL	VAILIGLCLTLLLLA	0.0002	100
HLA-DRB1*01:01	9	11	25	15	ILIGLCLTL	FVAILIGLCLTLLLL	0.0002	100
HLA-DRB1*01:01	4	20	34	15	FFSFIYLGE	SLLLLFFFSFIYLGE	0.0002	100
HLA-DRB1*01:01	1	27	41	15	DNRERQEHN	RSQNDNRERQEHNDR	0.0002	100
HLA-DRB1*01:01	8	19	33	15	DPEERGVKL	LAGEDPEERGVKLGL	0.0002	100

Fig. 13-B: MHC-II binding epitopesforBeta Amyloid protein with strength scores, 100 score is strongest epitopes

Selection of CD4⁺T cell epitopes of Beta Amyloid protein by bioinformatic prediction

T cell (CD4) reaction and the key to the development and design of candidate therapeutics and biomarkers. The CD4⁺ T cell epitopes were analyzed using IEDB server. The study is to analyze and predict CD4⁺ recognizing epitopes, which are following around region 21-35. We are proposing the 21-35 region, sequence TLKYGAKHV, as biomarker for AD diagnosis.



CD4 Immunogenicity prediction results

Number of proteins: 10

Number of 15mer (overlapping 10mer): 74

Threshold : 50.0%

Method : combined

Download result

Citations

Protein Number	Protein Description	Peptide	Start	End	Combined Score	Immunogenicity Score	Peptide core	Median Percentile Rank (7-alleles)	HLA-DRB1*03:01	HLA-DRB1*07:01	HLA-DRB1*15:01	HLA-DRB3,01:01	HLA-DRB3:02:02	HLA-DRB4:01:01	HLA-DRB5:01:01
2	seq2	EELTLKYGAHVMIL	21	35	45.32176	90.8044	TLKYGAHVM	15.0	12.0	13.0	4.3	29.0	21.0	52.0	15.0
2	seq2	HVMILPVPVTLGMVV	31	45	45.03856	86.0869	IMLPVPVTL	19.0	21.0	4.0	2.3	22.0	35.0	19.0	19.0
3	seq3	DGSKLHSLNAAIMI	26	40	39.95796	81.8949	HSLNAAIMI	12.0	46.0	5.1	12.0	13.0	3.5	9.6	24.0
3	seq3	HSLNAAIMISVIVV	31	45	47.9356	86.8395	HSLNAAIMI	22.0	29.0	22.0	19.0	29.0	7.1	5.6	53.0
3	seq3	AAIMISVIVVMTLL	36	50	43.16832	82.4158	IMISVIVVM	17.0	4.1	8.1	9.3	20.0	71.0	17.0	36.0
4	seq4	VRCYKVWAVRLISS	6	20	31.9514	72.6785	YKVWAVRLI	4.6	57.0	0.48	2.6	15.0	4.8	5.4	2.0
4	seq4	VYKVRLISSLLLLF	11	25	41.90503	97.8647	AVRLISSLL	4.6	9.7	1.9	2.3	17.0	25.9	2.6	4.6
4	seq4	LRSSLLLLFPFSFI	16	30	48.54828	98.8787	LLLLFPFSF	15.0	9.7	15.0	8.5	22.0	93.0	4.6	48.0
5	seq5	KGPLRLQGAVLIMS	16	30	41.963	96.0075	LRLQGAVLI	4.6	22.0	4.6	0.73	4.9	5.8	0.64	3.4
5	seq5	LQGAVLIMSALMAL	21	35	35.63004	79.2421	YLIMSALMA	6.9	9.6	0.8	2.4	16.0	18.0	6.8	2.9
5	seq5	LIMSALMALVFKY	26	40	48.57684	90.9421	LIMSALMAL	17.0	18.0	17.0	3.2	18.0	46.0	4.2	12.0
5	seq5	ALMALVFKYLPEVIT	31	45	49.40756	83.0169	FKYLPEVIT	27.0	50.0	27.0	7.9	39.0	56.0	9.5	28.0
5	seq5	VFKYLPEVITVIVLL	36	50	46.16662	84.2673	FKYLPEVIT	14.0	68.0	14.0	8.1	7.7	14.0	24.0	36.0
6	seq6	TLFRALIYSTMVVL	31	45	48.194	89.985	FALIYSTMV	19.0	19.0	7.6	3.7	19.0	14.0	33.0	36.0
8	seq8	FIFVSVLVCKASATA	36	50	43.30312	78.2578	YSVLVCKAS	20.0	58.0	16.0	16.0	60.0	20.0	56.0	13.0
9	seq9	TTIACFVALIGLCL	6	20	49.63616	98.5984	TIACFVALI	17.0	64.0	6.7	3.8	43.0	81.0	16.0	17.0
9	seq9	IGLCLTLLLLAIFKK	16	30	35.47139	66.1784	LLLLAIFKK	19.0	19.0	34.0	8.6	51.0	65.0	8.8	3.5
9	seq9	TLLLLAIFKKALPAL	21	35	34.11696	53.7774	LLLLAIFKK	21.0	27.0	21.0	4.3	52.0	27.0	11.0	7.7
9	seq9	AIFKKALPALPLPST	26	40	41.83264	93.7816	FKKALPALP	7.2	55.0	7.2	10.0	40.0	3.5	6.0	3.6
10	seq10	VGPTMDGLAFHQFI	3	17	49.26908	93.0027	FMDGLAFHQ	29.0	68.0	31.0	16.0	20.0	46.0	9.3	6.3

Fig. 14: CD4 T cell epitopes prediction for Beta Amyloid protein

DISCUSSION

The APOE and Amyloid Beta Aβ protein could become fatal causative of AD. Effective and economic preventive approaches are in need urgently to control AD. Compared to traditional treatment development, potent epitopes can be predicted via bioinformatics analysis, which makes the therapeutic design straightforward and fast. The latest bioinformatic tools could be an ideal to search for B-cell epitopes or T-cell epitopes.

APOE and Amyloid Beta Aβ protein have been shown to major players in causing AD. Thus, we applied bioinformatics to predict epitopes to target APOE and Amyloid Beta Aβ protein. Some of the epitopes showed 100% score, which we expect that an antibody recognizing the epitope could have expanded therapeutic function. The predicated epitopes can have potential to initiate protective humoral and cellular immune response against APOE and Amyloid Beta Aβ proteins in AD.

CONCLUSION

B-cell epitopes and T-cell epitopes in the Amyloid Beta Aβ or APOE proteins were predicted and analyzed in the current study. Several linear B-cell epitopes on Amyloid Beta Aβ or APOE protein were forecasted by IEDB server. IEDB server was used for T-cell epitopes prediction, which gave rise to several epitopes with binding capability to class-I, class-II molecule and CD4 respectively. Three B-cell epitopes: with high score were chosen and further recommended for therapeutic and diagnostic. The T-cell epitopes predicated in this study could bind a wide spectrum of both HLA-1 and HLA-2 molecules. The epitopes predicted consists of T-cell and B-cell epitopes that potentially protect individuals against AD inducing both humoral and cellular immune response, this study results can help scientist to further validate within both *in vitro* and *in vivo* models.

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