

# TYPE 1 DIABETES AND VIRAL INFECTIONS

• *similarities among human glutamic acid decarboxylase-65 (gad65), human insulin and H1N1 influenza a virus* •

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## Abstract

Background: Exposure to viral antigens that share amino acid (AA) sequence similar with self-antigens might trigger autoimmune diseases in genetically predisposed individuals, and the molecular mimicry theory suggests that epitope mimicry between the virus and human proteins can activate autoimmune diseases like type 1 diabetes (T1DM). Objective: The purpose of this study is to explore the possible similarity between the AA sequences of human glutamic acid decarboxylase - 65 kDa isoform (GAD65) human insulin, and proteins of H1N1 influenza (strain (A/California/7/2009(H1N1))), using databanks of proteins and immunogenic peptides to explain the development of T1DM. Methods: AA sequences of the A/California/7/2009(H1N1) strain, GAD65 and human insulin, available in the NCBI (National Center for Biotechnology Information) database were compared using the Basic Local Alignment Search Tool (BLAST) software. Results: Similarities were found among the A/California/7/2009(H1N1) strain, GAD and the human insulin. The similarities between Influenza A virus (A/California/7/2009(H1N1)) and the GAD65 ranged from 15.0 % to 56.0%, with statistical significance (P 0.006 and P 0.017). The similarities between the Influenza A virus (A/California/7/2009(H1N1)) and insulin ranged from 38.0 % to 45.0%, but without statistical significance. Conclusion: Bioinformatics data suggest a possible pathogenic link between A/California/7/2009(H1N1) and T1DM. Through molecular mimicry is has been observed that sequences similarity between viral Polyprotein and self-proteins could induce a crossover immune response to self-antigens, with a breakdown of self-tolerance, resulting in autoimmune disease.

**Keywords:** H1N1 influenza A virus; Type 1 diabetes; Sequence alignment; Similarity; Bioinformatics.

## INTRODUCTION

Exposure to viral antigens that share amino acid (AA) sequences similar with self-antigens might trigger autoimmune diseases in genetically predisposed individuals, and the molecular mimicry theory suggests that epitope mimicry between the virus and human proteins can activate autoimmune disease.

Type 1 diabetes (T1DM) is a heterogeneous disorder characterized by destruction of pancreatic beta cells, resulting in the loss of insulin secretion. The pathogenesis of T1DM is predicated on two factors: genetic predisposition and the presence of autoantibodies against islet cells, insulin, tyrosine phosphatase, and glutamic acid decarboxylase-65 kDa isoform (GAD65).<sup>(1)</sup> In the past decades, the incidence of T1DM has increased of 2-5% annually worldwide. Genetic factors are important components for the development of T1DM, but environmental factors are thought to play an important role in the expression of the disease, since genetic factors alone can hardly explain the rapid "increase in incidence". Viruses are one of the environmental factors implicated in the pathogenesis of T1DM in susceptible individuals.<sup>(2)</sup> Studies in humans and animal models indicate that various viruses are clearly able to modulate the development of T1DM by different mechanisms, including direct beta-cell lysis, bystander activation of autoreactive T cells, loss of regulatory T cells and molecular mimicry.<sup>(3)</sup> Recent studies suggest an association between T1D and viral infections including enteroviruses, rubella, mumps, rotavirus, parvovirus, cytomegalovirus and H1N1 influenza.<sup>(2,3)</sup> Influenza A virus causes systemic and most commonly non-systemic infection, and it can replicate only in the presence of trypsin-like enzymes.<sup>(4)</sup>

Bioinformatics programs currently available to run structural analyzes of H1N1 influenza virus components provide an essential framework for virion assembly and understanding of their molecular mechanisms. Similarly, molecular modeling has facilitated the understanding of molecular mimicry as a result of a cross-immune

response to similar bacterial or viral antigens with GAD65 and human insulin antigens. The necessary condition for successful similarity modeling is a sufficient similarity among the protein sequences.

The purpose of this study is to explore the possible similarity between the AA sequences of GAD65 and human insulin antigens, and proteins of H1N1 influenza (strain A/California/7/2009(H1N1)), using databanks of proteins and immunogenic peptides to explain T1DM.

## RESEARCH DESIGN AND METHODS

AA sequences of the strain A/California/7/2009(H1N1)'s protein, GAD65 and human insulin antigens, available in the database of NCBI (National Center for Biotechnology Information) were compared through the Basic Local Alignment Search Tool (BLAST).<sup>(5)</sup>

We used BLAST program for performing statistical significance. The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. It decreases exponentially as the Score (S) of the match increases. The lower the E-value, or the closer it is to zero, the more "significant" the match is. However, identical short alignments have relatively high E values. This is because the calculation of the E-value takes into account the length of the query sequence. These high E values make sense because shorter sequences have a higher probability of occurring in the database purely by chance. The Expect value can also be used as a convenient way to create a significance threshold for reporting results.

We used the strain A/California/7/2009 of H1N1 influenza due to the fact that the vaccine with strain A/California/7/2009 has been included in the influenza vaccine recommended for the Southern hemisphere from 2010 on. GAD 65 was used because autoantibodies to GAD65 precede

the development of T<sub>1</sub>DM and are clinical markers of that and of some other neural autoimmune diseases.<sup>(6)</sup>

## SIMILARITY SEARCHES - PROTEIN DATABASE SEARCH AND ANALYSIS

A protein-protein sequence alignment method, the BLAST<sub>2p</sub> program, was used to search for similarities between the A/California/7/2009(H1N1) strain, GAD65 and human insulin antigens. This method is limited to the searching for linear epitope similarities, which will miss three-dimensional conformational similarities and possible cross-reactivity between protein and non-protein epitopes. Since most (though certainly not all) molecular mimicry is likely to involve T-cell mediation, and T cells generally recognize linear peptides 8–20 AA in length, these limitations seemed acceptable for the present study

### H1N1 INFLUENZA EXAMINED

We have evaluated the following H<sub>1</sub>N<sub>1</sub> influenza virus strain A/California/7/2009(H1N1), with the respective NCBI sequence identification number: Influenza A virus (A/California/7/2009(H1N1)) GenBank: AGK63060.1.<sup>(7)</sup>

### PANCREAS GLAND PROTEINS EXAMINED

We have studied the following proteins of pancreatic tissue: glutamic acid decarboxylase (65 kDa isoform; GAD65) [*Homo sapiens*] and insulin [*Homo sapiens*].

### EXPOSED PROTEIN DATABASES

Influenza A virus (A/California/7/2009(H1N1)):

The strain A/California/7/2009(H1N1) GenBank: AGK63060.1 study allowed us to obtain an ID that contained 757 AA protein sequences.<sup>(7)</sup>

Glutamic acid decarboxylase (65 kDa isoform; GAD65) [*Homo sapiens*]:

Regarding GAD65=autoantigen glutamic acid decarboxylase, partial [*Homo sapiens*] GenBank: AAB28987.1 the ID contained 341 AA protein sequences.<sup>(8)</sup> In the case of RecName: Full=Glutamate decarboxylase 2, AltName: Full=65 kDa glutamic acid decarboxylase, Short=GAD-65, AltName: Full=Glutamate decarboxylase 65 kDa isoform [*Homo sapiens*] UniProtKB/Swiss-Prot: Q05329.1 the ID contained 585 AA protein sequences.<sup>(9)</sup> The Glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa) [*Homo sapiens*] GenBank: AAI26330.1 study allowed us to obtain an ID that comprised 585 AA protein sequences.<sup>(10)</sup>

Insulin [*Homo sapiens*]:

Regarding insulin [*Homo sapiens*] GenBank: AAA59172.1, the ID contained 110 AA protein sequences.<sup>(11)</sup> The insulin [*Homo sapiens*] GenBank: AAA59179.1 contained 107 AA protein sequences.<sup>(12)</sup> The insulin [*Homo sapiens*] GenBank: AAN39451.1 contained 110 AA protein sequences.<sup>(13)</sup> In the case of insulin [*Homo sapiens*] GenBank: AAA59173.1, the ID had 110 AA protein sequences.<sup>(14)</sup> The Insulin [*Homo sapiens*] GenBank: AAH05255.1 contained 110 AA protein sequences.<sup>(14)</sup> The insulin [*Homo sapiens*] GenBank: AAP35454.1 contained 110 AA protein sequences.<sup>(10)</sup>

## RESULTS

Similarities between Influenza A virus (A/California/7/2009(H1N1)), GAD65 [*Homo sapiens*] and insulin [*Homo sapiens*] were found.

The similarities between Influenza A virus (A/California/7/2009(H1N1)) and the GAD65 [*Homo sapiens*] ranged from 15.0 % (6 identical residues out of 40 AA in the sequence) to 50.0% (5 identical residues out of 10 AA in the sequence). Statistical significance occurred between influenza A virus A / California / 7/2009 (H1N1)) GenBank AGK63060.1 and RecName: Full=Glutamate decarboxylase 2;

AltName: Full=65 kDa glutamic acid decarboxylase; Short=GAD-65; AltName: Full=Glutamate decarboxylase 65 kDa isoform [Homo sapiens] UniProtKB/Swiss-Prot: Q05329.1 (P 0.006) and between Influenza virus A / California / 7 / 2009 (H1N1) GenBank DNA AGK63060.1 Glutamate

decarboxylase 2 (pancreatic islets and brain, 65kDa) [Homo sapiens] GenBank: AA126330.1 (P 0.009). The sequences similarities are shown in Table 1. AA appears in standard single letter code. The symbol + indicates conserved or semi-conserved substitutions.

**Table 1** - The AA sequences similarity between Influenza A virus (A/California/7/2009(H1N1)) and GAD65 [*Homo sapiens*].

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RecName: Full=Glutamate decarboxylase 2; AltName: Full=65 kDa glutamic acid decarboxylase; isoform [Homo sapiens]  
 Sequence ID: [sp|Q05329.1|DCE2\\_HUMAN](#)

Score	Expect	Method	Identities	Positives	Gaps
25.4 bits(54)	0.009	Compositional matrix adjust.	13/40(33%)	19/40(47%)	0/40(0%)

Query 547 ALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWDQTQSK 586  
 A LF +D Y GD +Q R ++ KLW ++K  
 Sbjct 423 ASYLFQQDKHYDLSYDTGDKALQCGRHVDVFKLWLMWRAK 462

GAD65=autoantigen glutamic acid decarboxylase [human, pancreatic islets, Peptide Partial, 341 aa]  
 Sequence ID: [gb|AAB28987.1|](#) Length: 341 Number of Matches: 4

Score	Expect	Method	Identities	Positives	Gaps
25.0 bits(53)	0.006	Compositional matrix adjust.	13/40(33%)	19/40(47%)	0/40(0%)

Query 547 ALQLFIKDYRYTYRCHRGDTQIQTRRSFELKKLWDQTQSK 586  
 A LF +D Y GD +Q R ++ KLW ++K  
 Sbjct 179 ASYLFQQDKHYDLSYDTGDKALQCGRHVDVFKLWLMWRAK 218

**Query:** A/California/7/2009(H1N1). **Sbjct:** GAD65 [*Homo sapiens*]. +: conservative substitution.

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The similarities between the Influenza A virus (A/California/7/2009(H1N1)) and insulin [*Homo sapiens*] ranged from 38.0 % (5 identical residues out of 13 AA in the sequence) to 45.0% (5 identical residues out of 11 AA in the sequence), but with

no statistical significance present. The sequences similarities are shown in Table 2. AA appears in standard single letter code. The symbol + indicates conserved or semi-conserved substitutions.

**Table 2** - The AA sequences similarity between Influenza A virus (A/California/7/2009(H1N1)) and insulin [*Homo sapiens*]

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insulin [*Homo sapiens*]  
Sequence ID: [gb|AAA59179.1](#) Length: 107 Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
14.6 bits(26)	2.3	Composition-based stats.	5/13(38%)	9/13(69%)	0/13(0%)
Query	584	QSKVGLLVSDGGP	596		
		+++VG + GGP			
Sbjct	57	EAEVGQVELGGP	69		

insulin [*Homo sapiens*]  
Sequence ID: [gb|AEG19452.1](#) Length: 94 Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
13.9 bits(24)	4.4	Composition-based stats.	5/11(45%)	7/11(63%)	0/11(0%)
Query	586	KVGLLVSDGGP	596		
		+VG + GGP			
Sbjct	46	QVGQVELGGP	56		

**Query:** A/California/7/2009(H1N1). **Sbjct:** insulin [*Homo sapiens*]. +: conservative substitution.

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## DISCUSSION

It is suspected that the H1N1 swine flu virus caused the pandemic of 2009, and is still circulating, could be a particularly good trigger for the development of T1DM in young children. Studies have reported many newly diagnosed cases of T1DM in people who recently had a flu, and an upsurge in T1DM after the 2009 pandemic<sup>(2)</sup>. This study suggests the possible role of molecular mimicry between the AA sequence of Influenza A virus (A/California/7/2009(H1N1)), GAD65 [*Homo sapiens*] and insulin [*Homo sapiens*] in T1DM development. Calculations of similarity between GAD 65 and insulin protein Influenza A virus (A/California/7/2009(H1N1)) version were carried and showed an important protein similarity. We used the protein of strain A/California/7/2009 of H1N1 influenza due to the fact vaccine with strain A/California/7/2009 has been included in the influenza vaccine recommended for the Southern hemisphere from 2010. The GAD 65 was used because it provides many insights into the molecular determinants of antigenicity in the development of T1DM.<sup>(15)</sup>

Childhood vaccinations have been introduced around the world over a period corresponding to the rise of T1DM. Therefore it has been suggested that vaccinations might increase the risk of T1DM in selected groups, and attempts have been made to link the introduction of new vaccines with changes in the incidence of T1DM. The hypothesis that foreign antigens of viral proteins, sharing similarity with islet beta cells proteins, might activate autoimmunity and contribute to the development of T1DM, via a molecular mimicry mechanism, has been already suggested.<sup>(16)</sup>

T1DM is a multifactorial immune-mediated disease, characterized by selective destruction of insulin-producing pancreatic beta cells in genetically susceptible individuals. Several viruses have been associated with T1DM either in humans or animal models, such as measles virus, rubella virus, mumps virus, cytomegalovirus, or influenza A and B<sup>(17)</sup>. The pathophysiology of T1D most likely requires the presentation of beta-cell antigens to T cells within lymph nodes, wherein antigen-reactive T cells then migrate to the pancreas where the autoimmune destruction of the beta cells occurs.<sup>(18)</sup>

In late March and early April 2009 the influenza virus A/H1N1 2009 was discovered in Mexico and in the United States in a pandemic that has spread globally.<sup>(19)</sup> In 2010, the World Health Organization declared the A/H1N1 2009pdm-mediated influenza pandemic to be over, however, A/H1N1 2009pdm continues to circulate widely and has replaced seasonal H1N1 as the seasonal virus.<sup>(20)</sup> Since the introduction of H1N1 influenza vaccine in the wake of the 2009 H1N1 pandemic, many serious and non-serious vaccine-related adverse events have been reported. A Swedish study showed that the H1N1 vaccine may affect the development of T1DM in children, because the levels of some autoantibodies associated with T1DM were higher during and after the vaccine was administered, but the proportion of young children with a certain genetic risk diagnosed with T1DM decreased after vaccination. Therefore, the authors state, "it cannot be excluded that the vaccine affected the clinical onset of T1DM".<sup>(21)</sup>

The fact of developing antigen determinants by microorganisms that resemble antigens of the host as a mean of avoiding recognition and elimination by the host was first proposed in 1964 by Damian.<sup>(22)</sup> The molecular mimicry could explain the autoimmune diseases, through cross reactions between epitopes of invading microorganisms and antigens present in the body that promote an adverse autoimmune response.<sup>(23)</sup> In T1DM in which pancreatic beta cells are destroyed by autoimmune phenomena, a linear sequence similarity between a major autoantigen, GAD, and the 2C protein of coxsackie B4 was identified. In addition, a sequence similarity between GAD and the mycobacterial heat shock protein 60 was described and the suggestions were made that molecular mimicry between GAD, coxsackievirus B4-2C protein, and/or heat shock protein 60 may be actively involved in an autoimmune reaction towards the pancreatic beta cells.<sup>(24)</sup>

In this work, we analyzed the similarity between the AA sequences of the Influenza A virus (A/California/7/2009(H1N1)), GAD65 [Homo sapiens], and insulin [Homo sapiens]. We used BLASTp software to search AA sequences available in the database on [www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed). We found that

Influenza A virus (A/California/7/2009(H1N1)) and GAD65 [Homo sapiens] features statistically significant AA sequence similarity, wherein some similar regions contain epitopes of both proteins, which add up to 56.0% of similarity. No similar studies that demonstrate similarities between the proteins evaluated by us have been found yet.

The GAD65 is a major autoantigen in T1DM, and most patients have reactive serum antibodies with conformational epitopes on the GAD65 molecule. The high-resolution crystal structure of GAD65 was determined in 2007, providing many insights into the molecular determinants of antigenicity, as well as an atomic positioning of the epitope-mapping data.<sup>(25)</sup> The study showed that an amino acid segment of GAD65 (AA 247-279) shares sequence similarity with the P2-C protein of Coxsackie B virus,<sup>(24)</sup> which suggests the mechanisms of molecular mimicry to mediate the putative diabetogenic effect. Moreover, seasonal variations in the diagnosis of T1DM have been reported, with peaks during the autumn and winter months and decreases during the summer months,<sup>(26)</sup> and viral infections have been suggested as a potential cause for this seasonality.

The influenza virus has been demonstrated to induce insulinitis and T1DM in transgenic mice expressing hemagglutinin antigen in pancreatic beta cells.<sup>(27)</sup> A study to determine whether A/H1N1-hemagglutinin antibodies are related to insulin autoantibodies in clinical diagnosis of T1DM, before and after Swedish A(H1N1)pdm09 vaccination campaign, showed that before vaccination, 0.6% patients had A/H1N1-hemagglutinin antibodies compared with 40% during and 27% after vaccination, and concluded that it cannot be excluded that the vaccine affected clinical onset of T1DM.<sup>(21)</sup> There are only case reports involving influenza A/California/7/2009(H1N1) at clinical diagnosis of T1DM.<sup>(28)</sup>

In conclusion, bioinformatics data suggest a possible pathogenic link between Influenza A virus (A/California/7/2009(H1N1)) and T1DM. Similarities between viral proteins, GAD65 and human insulin could be a mechanism of crossover induction of immune response by self-antigens, with a breakdown of self-tolerance, resulting in

autoimmunity. As sequence similarity can only be concluded inductively, additional studies are required to elucidate the similarity relationship between peptides of Influenza A virus (A/California/7/2009(H1N1)) and T1DM.

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