Decoding the Relationship between Cow Milk Proteins and Type 1 Diabetes Mellitus Development

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Short Report

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Abstract

Introduction: Introduction: Type 1 diabetes mellitus (T1DM) is an autoimmune disease that develops due to the destruction of insulin-producing beta-cells in the pancreas by the immune system. Cow milk is one of the dietary factors associated with the development of T1DM, as it contains proteins that may trigger the autoimmune response.

Objective: To analyze *in silico* the evidence of molecular mimicry between Glutamic Acid Decarboxylase-65 (GAD-65)/ Human insulin/Zinc Transporter 8 (ZnT8) and bovine serum albumin (BSA) and beta-lactoglobulin (BLG) as a potential trigger for T1DM.

Method: The *in silico*analysis was performed using bioinformatics tools to compare the amino acid sequences of cow milk proteins (BSA and BLG) and human beta-cell autoantigens (GAD-65, Human insulin and ZnT8). The structural and functional characteristics of the proteins were analyzed to identify potential molecular mimicry mechanisms.

Results: The results of the *in silico* analysis showed significant sequence similarity between BSA, BLG, and GAD-65, Human insulin and ZnT8 ranging from 19.64% to 27.27%. The cow's milk proteins evaluated shared structural features with the beta cell antigens selected for comparison, indicating the potential for molecular mimicry between these proteins. **Conclusion**: The findings of this study provide further evidence for the potential role of cow milk proteins in the triggering of T1DM. The *in silico* analysis suggests that molecular mimicry mechanisms between cow milk proteins and human beta-cell antigens may contribute to the autoimmune response that leads to T1DM. This study highlights the importance of dietary factors in the development of T1DM and the need for further research to understand the mechanisms involved.

INTRODUCTION

Molecular mimicry refers to the structural similarity of two or more molecules that may result in the human immune system mistaking a harmless substance for a pathogen. This is a common mechanism in the development of autoimmune diseases, where the immune system attacks healthy tissues and organs, leading to chronic inflammation and tissue damage (1).

Type 1 diabetes mellitus (T1DM) is an autoimmune disease caused by the destruction of pancreatic beta cells resulting in insufficient production of insulin. The exact pathogenesis of T1DM remains unknown, but several factors have been implicated, including genetic predisposition, environmental triggers, and host immune factors (2). Recent studies have suggested a molecular mimicry mechanism as a potential environmental trigger for T1DM development. Molecular mimicry refers to the ability of foreign antigens to resemble self-antigens, leading to cross-reactive autoimmune responses (3).

Cow's milk proteins have been identified as potential trigger antigens in molecular mimicry with Glutamic Acid Decarboxylase-65 (GAD-65), human insulin and Zinc Transporter 8 (ZnT8) in the development of

T1DM (4). Cow milk is a complex mixture of proteins, lipids, and carbohydrates that serve as a source of nutrition for newborn calves. However, some of the proteins in cow milk have structural similarities to human proteins, which could trigger an autoimmune response in susceptible individuals. High levels of antibodies to cow's milk proteins such as bovine serum albumin (BSA) and beta-lactoglobulin (BLG) have been associated to T1DM (5). Improved humoral immune response IgG and IgA antibodies to protein fragments in cow's milk have been described in individuals with a recent diagnosis T1DM (6).

Thus, the molecular mimicry among GAD-65/Human insulin/ZnT8 and cow's milk proteins is a well-documented phenomenon that may contribute to the pathogenesis of T1DM by inducing cross-reactive immune responses against beta cell antigens. These observations highlight the importance of dietary interventions and antigen-specific therapies in the prevention and treatment of autoimmune diseases. In this paper, we analyze *in silico* the evidence of molecular mimicry among GAD-65/ Human insulin/ZnT8 and cow's milk proteins as a potential trigger for T1DM.

METHODS

We analyzed the potential link among GAD-65/Human insulin/ZnT8 and cow's milk proteins as a trigger for T1DM, using the Protein Data Bank (PDB) database to obtain the structural information of the proteins involved, and the Uniprot website to evaluate the similarity between proteins.

The first step was to obtain the crystal structure data of GAD-65, human insulin, Znt8 and cow's milk proteins (BSA and BLG) from the PDB database. Subsequently, the JSmol tool was used to visualize the structures of these proteins. The next step was to analyze the potential molecular similarities between these proteins using Pairwise Structure Alignment.

Pairwise Structural Alignment is a method of comparing two proteins by aligning their amino acid sequences and identifying similarities and differences in their structures. In the analysis of GAD-65/Human insulin/Znt8, BSA, and BLG, a pairwise alignment was performed between the amino acid sequences of each protein to determine their degree of structural similarity.

Once the sequences were obtained, Pairwise Structural Alignment compared each amino acid in the two sequences to find matches and gaps in the sequence, and then calculated the optimal alignment based on the highest number of matching amino acids.

The degree of similarity between the proteins was quantified based on the number and location of similar features in the structures. Thus, this method of analysis using Pairwise Structure Alignment allowed for a detailed comparison between two proteins and can provide insight into the similarities and differences in their structure and function.

To establish structural mimicry we used align Uniprot which is based on a simple approach, maximizing the TM score and the quantitative root-mean-square evaluation deviation, and the server is available for free https://www.uniprot.org/align.

RESULTS

GAD-65, Human insulin and Znt8, are two autoantigens associated with T1DM. The cow's milk proteins BSA and BLG share structural similarities with GAD-65, Human insulin and Znt8, respectively. By performing the protein with Pairwise Structural Alignment analysis between GAD-65, insulin, Znt8 and cow's milk proteins, we check the extent of molecular mimicry between them.

The Pairwise Structure Alignment analysis shows that there is sequence similarity between GAD-65 and BSA, with a 19.64% identity and 20.54% similarity (Figure 1). While the structural similarity between GAD65 and BLG were of 18.25% identity and 19.65% similarity respectively (Figure 2).

The Pairwise Structure Alignment analysis shows that there is significant sequence similarity between the Human insulin and BLG share 26.43% sequence identity and 27.27% sequence similarity (Figure 3). While the structural similarity between Human insulin and BSA was of 18.25% identity and 21.70% similarity respectively (Figure 4). Human insulin and BSA share structural similarity in their tertiary structures, as both proteins have alpha-helices and beta-sheets. Additionally, both proteins have a similar fold in their binding domains. Overall, they have a RMSD value of 2.00 Å, indicating good similarity.

The Pairwise Structure Alignment analysis shows that there is significant sequence similarity between the ZnT8 and BLG share 26.43% sequence identity and 27.27% sequence similarity (Figure 5). While the structural similarity between Human insulin and BSA was of 18.29% identity and 21.80% similarity respectively (Figure 6).

DISCUSSION

The molecular mimicry mechanism between cow's milk proteins and human insulin and GAD-65 provides a potential environmental trigger for T1DM development in genetically predisposed individuals. The present study was performed to investigate the possibility of cow’s milk proteins BSA and BLG share homology with GAD-65, Human insulin and ZnT8 to cause the T1DM.

One of the environmental factors that have been implicated in the development of T1DM is cow's milk. Cow's milk contains several proteins that may trigger an autoimmune response in susceptible individuals. Two of these proteins are BLG and BSA (7).

Several studies have investigated the role of molecular mimicry between cow's milk proteins and GAD-65/human insulin/ZnT in the development of T1DM. Some studies have reported that cow's milk consumption during infancy is associated with an increased risk of developing T1DM later in life, while others have failed to find a significant association. One of the limitations of these studies is the difficulty in establishing an accurate measure of cow's milk consumption. Cow's milk is often used as an ingredient in many processed foods, making it difficult to accurately assess the amount of cow's milk consumed by an individual.

One of the earliest studies on molecular mimicry in DM1 was conducted by Elliott et al. (8). The authors used monoclonal antibodies to examine the cross-reactivity between GAD65 and several proteins, including BSA and BLG, the two most abundant proteins in cow milk. The study found that some of the monoclonal antibodies created against BSA and BLG recognized GAD-65, indicating a structural similarity between the two proteins. However, the authors did not investigate the clinical implications of this observation.

Another study provided evidence for the role of molecular mimicry in the development of DM1. The authors demonstrated that feeding cow milk formula to infants who carried the HLA-DQB1*0201 allele, a genetic risk factor for DM1, increased the risk of developing islet autoantibodies. They also showed that the autoantibodies recognized an epitope in BSA that shared structural similarity with GAD-65. The authors concluded that cow milk proteins could induce the production of autoantibodies against GAD-65 through molecular mimicry, which could contribute to the development of DM1 (9).

BSA has been shown to share structural similarities with GAD-65 (10). The molecular mimicry between cow's milk proteins and GAD-65 has been suggested as another potential trigger of T1DM. GAD-65 is an enzyme in pancreatic beta cells that catalyzes the conversion of glutamate to gamma-aminobutyric acid. GAD-65 is a major autoantigen in T1DM, and autoantibodies to GAD-65 are detected in 60–70% of newly diagnosed with T1DM individuals, suggesting that the destruction of beta-cells may be mediated by the immune response against GAD-65 (11).

Despite their different functions and structures, BSA and GAD-65 share a certain degree of amino acid similarity. Several studies have reported sequence alignments and comparisons between BSA and GAD-65 using bioinformatics tools. Study using multiple sequence alignment to compare the amino acid sequences of BSA and GAD-65, found that the two proteins presented 29% sequence identity and 48% sequence similarity, with most of the similar residues located in the flexible loops and turns of the proteins (12). Our study, using pairwise structure alignment analysis, compared the amino acid sequences of BSA and GAD-65. The evaluation between these two proteins showed a lower percentage than the studies presented in the literature, being 19.64% sequence identity and 20.54% sequence similarity, with most of the similar residues located in the flexible loops and bends of the proteins. In addition, we used homology modeling to generate a 3D structure of BSA and compared it to the crystal structure of GAD-65. Our results showed that BSA shares an overall fold similar to that of GAD-65, which has not been described in previous studies.

We found no studies in the literature evaluating the similarity between GAD-65 and BLG. In our study, we compared the amino acid sequences of BLG and GAD-65, which showed 18.25% sequence identity and 19.15% sequence similarity between these two proteins.

Insulin and BLG, despite their distinct functions, these two molecules share a resemblance in their amino acid sequences. Several studies have suggested that the amino acid sequence of BLG contains regions that resemble the amino acid sequence of human insulin, thereby leading to molecular mimicry.

Molecular mimicry occurs when a foreign antigen (in this case, BLG) shares structural similarities with a self-antigen (in this case, human insulin), leading to cross-reactivity of T cells and autoimmunity (13).

Several scientific papers have evaluated molecular mimicry between insulin and cow's milk proteins in *in silico* studies on the triggering of T1DM. These studies aimed to investigate the structural and immunological similarities between the two proteins, and their potential role in the development of autoimmune reactions leading to diabetes. Previous studies have shown that antibody cross-reactivity between human insulin and BLG is possible, indicating that molecular mimicry may be a contributing factor (14), however we found no *in silico* studies in the literature comparing the similarity between BLG and human insulin. Our study using Pairwise Structure Alignment analysis compared the amino acid sequences of BGL and human insulin, the evaluation between these two proteins showed 26.43% sequence identity and 27.27% sequence similarity, and structurally showed a required minimum distributions indicative of good similarity.

Reviewing the medical literature, we found no studies in which the similarity between human insulin and BSA was assessed. In our study, we compared the amino acid sequences of human insulin and BSA, which showed a sequence identity of 18.25% and a sequence similarity of 19.15% between these two proteins.

Analysis focused on discovering beta-cell-specific proteins associated with the regulatory pathway of secretion recently identified ZnT8 as a novel autoantigen (15). ZnT8 is found in the membrane of insulin-containing secretory granules and is responsible for transporting zinc ions from the cytosol into the vesicles (16). ZnT8 has emerged as a potential target for humoral autoimmunity, making it highly relevant for the early detection of T1DM. The presence of ZnT8-specific CD8 + T cells can be observed in the majority of T1DM patients, and these cells play a crucial role in the development of T1DM. As a target for immunotherapy, ZnT8 offers the potential to ameliorate beta cell dysfunction in T1DM, presenting new avenues for the treatment of this condition (17).

It has been reported that anti-ZnT8 antibodies which target homologous membrane extension sequences exhibit cross-reactivity and have the ability to elicit robust immune responses, thus raising the possibility of a molecular mimicry mechanism that triggers T1DM (18). Literature suggests that infection with Mycobacterium avium subspecies paratuberculosis is linked to T1DM by production of anti-ZnT8 antibodies via molecular homology (19). However, there are no studies available which are related to molecular mimicry between cow’s milk proteins and ZnT8. Our study compared the amino acid sequences of BSA and ZnT8. The evaluation between these two proteins showed 18.25% sequence identity and 21.70% sequence similarity. When comparing the amino acid sequences of ZnT8 and BSL, we found a sequence identity of 26.43% and a sequence similarity of 27.27% between these two proteins.

In view of the above, we must take into account that *in silico* studies to determine the triggering of T2DM by molecular mimicry between human pancreatic beta-cell antigens and cow's milk proteins show several limitations must be acknowledged. Firstly, the molecular mimicry mechanism is not the only environmental trigger for T1DM development. Other factors such as viral infections, gut microbiota, and

dietary factors may also contribute to T1DM pathogenesis (20). Secondly, the evidence for the molecular mimicry between cow's milk proteins and human insulin, GAD-65 and ZnT8 are mainly based on in vitro studies, and the clinical significance of these findings is still unclear.

Conclusions

The findings of this study provide further evidence for the role potential of cow's milk proteins in the triggering of T1DM. The *in silico* analysis suggests that molecular mimicry mechanisms between cow milk proteins and beta-cells of the pancreas human antigens may contribute to the autoimmune response that leads to T1DM. This study highlights the importance of dietary factors in the development of T1DM and the need for further research to understand the mechanisms involved and establish the causality of this association.

Declarations

Competing interests: The authors declare no competing interests.

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Figures

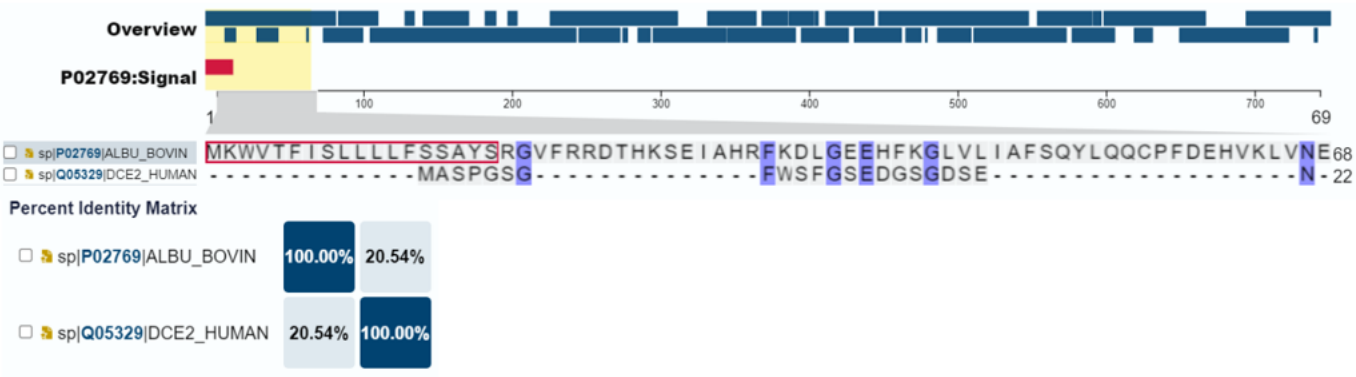

Figure 1

GAD-65 (Q05329 · DCE2_HUMAN) x bovine serum albumin (P02769 · ALBU_BOVIN)

Source: Research result.

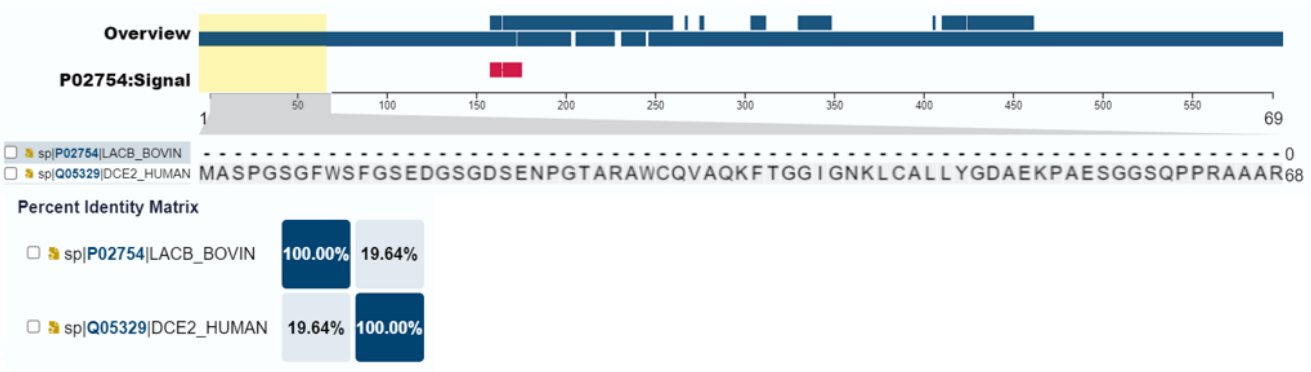

Figure 2

GAD-65 (Q05329 · DCE2_HUMAN) x beta-lactoglobulin (P02754 · LACB_BOVIN)

Source: Research result.

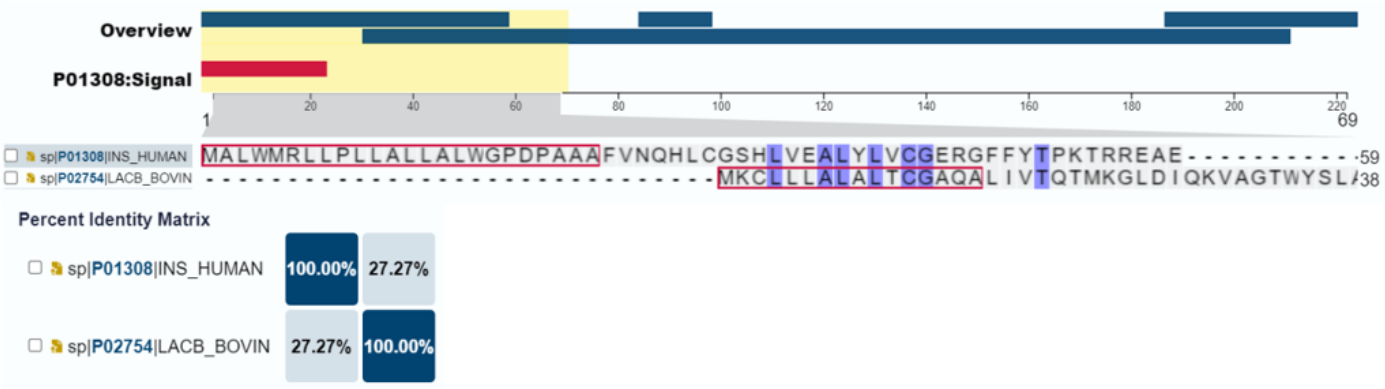

Figure 3

Human insulin hormone (P01308 · INS_HUMAN) x beta-lactoglobulin (P02754 · LACB_BOVIN)

Source: Research result.

Figure 4

Human insulin hormone (P01308 · INS_HUMAN) x bovine serum albumin (P02769 · ALBU_BOVIN)

Source: Research result.

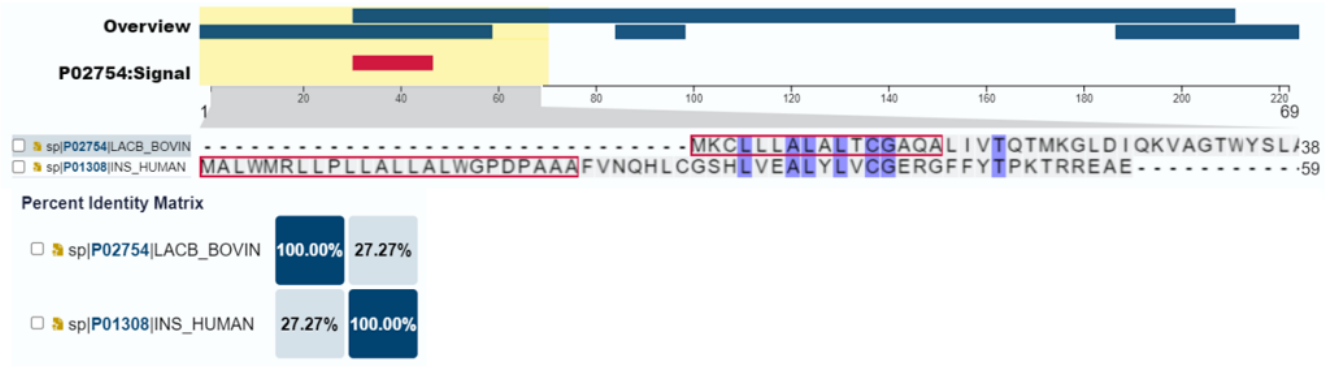

Figure 5

Proton-coupled zinc antiporter SLC30A8 (Q8IWU4 · ZNT8_HUMAN) x beta-lactoglobulin (P02754 · LACB_BOVIN)

Source: Research result.

Figure 6

Proton-coupled zinc antiporter SLC30A8 (Q8IWU4 · ZNT8_HUMAN) x bovine serum albumin (P02769 · ALBU_BOVIN)

Source: Research result.