

## Relationship between vitiligo and Hashimoto's thyroiditis

### Relação entre vitiligo e tireoidite de Hashimoto

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#### Abstract

**Introduction:** vitiligo is a multifactorial acquired depigmenting disorder, characterized by a spontaneous loss of functional melanocytes from the epidermis. Vitiligo and Hashimoto's thyroiditis (HT) often occur in association and seem to be characterized by an autoimmune process. The vitiligo associated with HT suggests genetic homologies between them. **Objective:** to identify protein sequence homology between melanocyte protein (Pmel) and thyroid peroxidase (TPO), using bioinformatics tools, to propose an initial mechanism which could explain the production of cross-reacting autoantibodies to melanocyte and TPO. **Methods:** we performed a comparison between Pmel and TPO amino acids (AA) sequences, available on the National Center for Biotechnology Information (NCBI) database by BLAST (Basic Local Alignment Search Tool) in order to find local homology regions between the AA sequences. **Results:** the homology sequence between the Pmel and TPO ranged from 21.0% (19 identical residues out of 90 AA in the sequence) to 55.0% (6 identical residues out of 11 AA in the sequence). The identical alignments presented relatively high E values due to presence of short alignment. **Conclusion:** bioinformatics data suggest a possible pathological link between Pmel and TPO. Sequence homology between Pmel and TPO may present a molecular mimicry suggesting the possibility of antigen crossover between Pmel and TPO that might represent an immunological basis for vitiligo associated with HT.

**Keywords:** Vitiligo. Thyroiditis, Autoimmune. Sequence Homology, Amino Acid. Computational Biology.

#### Resumo

**Introdução:** o vitiligo é uma doença de despigmentação adquirida multifatorial, caracterizada por uma perda espontânea de melanócitos funcionais da epiderme. Vitiligo e tireoidite de Hashimoto (TH) ocorrem frequentemente em associação e parecem ser caracterizados por um processo autoimune. O vitiligo associado à TH sugere homologias genéticas entre eles. **Objetivo:** identificar homologia das sequências de proteína entre a proteína do melanócito (Pmel) e peroxidase da tireóide (TPO), usando ferramentas de bioinformática, para propor um mecanismo inicial que poderia explicar a produção de autoanticorpos de reação cruzada entre o melanócito e a TPO. **Metodologia:** foi realizada uma comparação entre a sequência de aminoácidos (AA) da Pmel e da TPO, disponível no banco de dados Basic Local Alignment Search Tool (BLAST) do National Center for Biotechnology Information (NCBI), a fim de encontrar regiões de homologia locais entre as sequências de AA. **Resultados:** a sequência de homologia entre a Pmel e a TPO variou de 21,0% (19 resíduos idênticos na sequência de cada 90 AA na sequência) a 55,0% (6 resíduos idênticos na sequência de 11 AA). Os alinhamentos idênticos apresentaram valores relativamente altos (E) devido à presença de alinhamentos curtos. **Conclusão:** os dados de Bioinformática sugerem uma possível ligação patológica entre Pmel e a TPO. A sequência de homologia entre Pmel e a TPO pode apresentar um mimetismo molecular sugerindo a possibilidade de cruzamento entre antígeno da Pmel e da TPO que pode representar uma base imunológica para a associação entre o vitiligo e a TH.

**Palavras-chave:** Vitiligo. Tireoidite Autoimune. Homologia de Sequência de Aminoácidos. Biologia Computacional.

#### INTRODUCTION

Vitiligo is a disease in which segmental depigmentation or generalized depigmentation of skin, mucosal surfaces and hair due to destruction of melanocytes. Vitiligo presents elevated frequencies of other autoimmune diseases and the presence of organ specific antibodies in affected patients, suggesting that these diseases involve shared genetic components<sup>1</sup>.

Thyroid dysfunction and autoimmune thyroid diseases have been reported in association with vitiligo, given that Hashimoto's thyroiditis (HT) and Graves' disease are the

most important and prevalent autoimmune thyroid diseases associated with vitiligo<sup>2</sup>.

The thyroid peroxidase (TPO) gene provides instructions for making an enzyme called TPO that plays a central role in the function of the thyroid gland. The TPO assists the chemical reaction that adds iodine to a thyroglobulin, fundamental in thyroid hormones production. The TPO was first identified as being the thyroid microsomal antigen involved in autoimmune thyroid disease<sup>3</sup>. The TPO is an important self-antigen in autoimmune thyroid disease.

The vitiligo occurs with a frequency of 0.38–2.9% in various populations, and the prevalence of autoimmune thyroiditis in individuals with vitiligo varies between 4.4 and 21.0% in different studies<sup>4</sup>.

Bioinformatics program currently available to

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construct the structure analyses of TPO component provides an essential framework for TPO assembly, and understanding of your molecular mechanisms. Similarly, molecular modeling has facilitated the understanding of molecular mimicry as a result of cross-immune response to similar melanocyte protein (Pmel) antigen with TPO human, since most of molecular mimicry is likely to involve T-cell mediation, and T cells generally recognize linear peptides that range from 8 to 20 AA in length<sup>5</sup>.

Until recently, little structure information about Pmel and TPO human were available and molecular model has been built on partial structures, with assembly guided by biochemical data. In this study, we assessed the protein sequence homology between Pmel and TPO, using bioinformatics tools, to propose an initial mechanism which could explain the production of cross-reacting autoantibodies to Pmel and TPO.

## METHODOLOGY

Were performed the comparison between the AA sequence of the MP and TPO human, available in the database of National Center for Biotechnology Information (NCBI) on Basic Local Alignment Search Tool (BLAST2p)<sup>6</sup>. The BLAST was introduced as a sequence alignment heuristic that was an order of magnitude faster than earlier approaches for analyzing biological information. Very quickly, this software became a landmark enabling technique for bioinformatics. Thus, the BLAST2p refers to a program used to generate alignments between a nucleotide or protein sequence, referred to as a query and nucleotide sequences against other database of nucleotide, referred to as subject sequences.

The expect value is a parameter that describes the number of hits one can expect to identify by chance when searching a database of a particular size (expect value considered as statistically significant with  $p < 0.05$ ). It decreases exponentially as the score of the match increases. The lower an expect value is, 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 expect 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.

### Sequence Homology searches – Protein database search and analysis

The sequence homology searching is a method of searching sequence databases by using alignment to a query sequence. By statistically assessing how well database and query sequences match one can infer homology and transfer information to the query sequence.

One protein-protein sequence alignment method, the BLAST2p program, was used to generate alignments

between Pmel 17 sequence, referred to as a “query” and TPO sequences within a database, referred to as “sbjct” sequences. This method is limited to searching for linear epitope homology, which will miss three-dimensional conformational homologies and possible cross-reactivity between protein and non-protein epitopes.

The following TPO AA sequences were analyzed, with the respective NCBI sequence identification numbers (GI): thyroid peroxidase GI: 4680721, thyroid peroxidase [Homo sapiens] GI: 18539488, thyroid peroxidase [Homo sapiens] GI: 339871, thyroid peroxidase [Homo sapiens] GI: 339867, thyroid peroxidase [Homo sapiens] 339865, Thyroid peroxidase [Homo sapiens] GI: 63100775, and thyroid peroxidase [Homo sapiens] GI: 62865489. The following Pmel 17 AA sequences were analyzed, with the respective NCBI sequence identification numbers (GI): melanocyte protein Pmel 17 [Homo sapiens] GI: 125063, and Pmel 17 protein [Homo sapiens] GI: 190106.

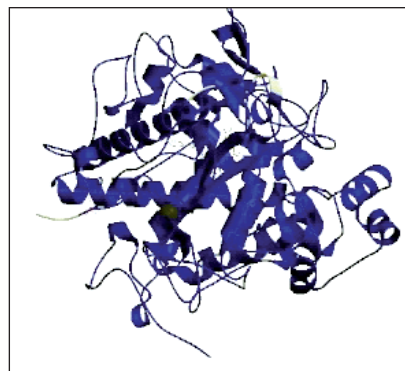
## RESULTS

### • TPO

Regarding the TPO accession number: AAA61217.2 the ID contained 922 AA in its protein sequence<sup>7</sup>. The TPO accession number: AAA61216.1 the ID had 876 AA protein sequences<sup>7</sup>. The TPO accession number: AAA61215.1 study allowed us to obtain an ID that comprised 933 AA protein sequences<sup>7</sup>. The TPO accession number: AAA97517.1 we were able to assemble an ID containing 933 AA protein sequences<sup>8</sup>. The TPO accession number: AAH95448.1 study allowed us to obtain an ID that comprised 933 AA protein sequences<sup>9</sup>. The TPO accession number: AAY16985.1 had ID of 933 AA protein sequences<sup>10</sup>.

The TPO gene contains 17 exons and covers at least 150 kb of chromosome 2<sup>8</sup>. Structure of TPO was built from the SWISS-MODEL that is a fully automated protein structure homology-modelling server, accessible via the ExPASy web server (Figure 1).

Figure 1 – The Structure of the Thyroid Peroxidase (TPO)



Fonte: Source <http://swissmodel.expasy.org/interactive/JhZ9D8/templates/>



mimicry because only five to six AA are necessary to induce an immune response<sup>18</sup>. Evaluating this way, due to of amino acids variation between proteins different, molecular mimicry should not happen from a probabilistic viewpoint. If we assume that five or six amino acid residues are used to induce a monoclonal antibody, the likelihood of 20 amino acids that occur in six identical residues between two proteins is 1 in 64 million. However, there is evidence of many molecular mimicry events that make this probability false. More than 5% of over 800 monoclonal antibodies derived from multiple RNA and DNA viruses, as well as from a large number of T cell clones, engage in such interactions. These cross-reactions, termed molecular mimicry, are against unique host proteins involved in autoimmune responses and diseases. Thus, molecular mimicry initiated as a cross-reacting with an appropriate host-antigen, can be a mechanism for instigating an autoimmune disease. Molecular mimicry provides an explanation for the genetic observation that identical twins rarely manifest the same autoimmune disease and the documented epidemiologic evidence that microbial and/or viral infections often precede autoimmune disorders<sup>19</sup>.

Epitopes are part of antigens that are recognized by T and B cells. Thus, the molecular mimicry could explain the development of autoimmune diseases through cross reactions of between epitopes and antibodies present in the body that promote an adverse autoimmune response<sup>20</sup>.

In this study, we analyzed the sequence homology between the AA sequences of six human TPO and two Pmel 17. We found that TPO and Pmel 17 share AA sequences homologies, where in some similar regions contain epitopes of both TPO and Pmel 17 highly alike. No studies have been found in medical literature assessing the protein homology between TPO and Pmel 17.

On the basis in sequence alignment between the Pmel 17 and the template structure, a three-dimensional model was generated through of SWISS-MODEL workspace that is an integrated web-based modeling expert system. The BLAST and FASTA packages of sequence comparison programs provide programs for comparing protein and DNA sequences to protein databases<sup>21</sup>. Use the BLAST for to identify homologous proteins and protein sequences based on excess sequence similarity between Pmel 17 and TPO. The expect value is a parameter that describes the number of hits one can expect to identify by chance when searching a database of a particular size. It decreases exponentially as the score of the match increases. This is because the calculation of the expect value takes into account the length of the query sequence. In our study the identical alignments presented relatively high expect values due to presence of short alignment.

Despite the limitations posed by the unavailability of complete proteome data for Pmel 17 proteins, because the BLAST is a method that is limited to searching for linear epitope homologies, losing three-dimensional conformational homologies and possible cross-reactivity between protein and non-protein epitopes, the homolo-

gies between the AA sequences of TPO, which are potential B – and T-cell epitopes of these antigens, and proteins of Pmel 17, were successfully identified. Thus, these observed homologies could be functionally important in molecular mimicry, receptor binding and cell signaling events involved in autoimmunity, and may have important implications for the understanding of the relationship between vitiligo and HT by formation of similar antibodies.

## CONCLUSION

In conclusion, bioinformatics data suggest a possible immunological link between vitiligo and HT. Therefore, through molecular mimicry the homologies between Pmel 17 polyproteins and thyroid self-proteins could be a mechanism of induction of a cross reactive immune response to self-antigens resulting in HT.

**Conflicts of interest:** The authors declare no conflicts of interest.

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Submetido em: 28/08/2016

Aceito em: 03/01/2017