INSULIN AND ISLET AMYLOID POLYPEPTIDE GENES IN DIFFERENT SPECIES

PAUL GAGNIUC[1], CONSTANTIN IONESCU-TIRGOVISTE[2]

[1]Institute of Genetics, University of Bucharest, Romania
[2]National Institute of Diabetes, Nutrition and Metabolic Diseases "N.C. Paulescu", Romania
Corresponding author: Paul Gagniuc, E-mail: paulgagniuc@yahoo.com

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Islet amyloid polypeptides (IAPP) are coexpressed with insulin by pancreatic beta-cells. In abnormal conditions, amyloid deposits disrupt the structural integrity of beta-cell membranes, thus leading to a reduction/loss of beta-cells. In order to understand the genetic basis of various diseases, especially polygenic diseases (diabetes, obesity and vascular disease), we made a rough analysis of INS gene from 11 species and IAPP gene from 9 species. For this study we used some methods previously presented by authors, such as Kappa Index of Coincidence (Kappa IC) patterns and in-depth analysis. Our results show some sequence features of these two genes that can not be detected by usual methods, such as sequence alignment. Although there is a high homology between insulin sequences, the proinsulin C-peptide differs between species. From an evolutionary standpoint, a comparison of these genes may lead to a better understanding of some diabetes-related mechanisms.

Key words: islet amyloid polypeptide gene; insulin gene; gene patterns; beta-cell.

INTRODUCTION

Pancreatic islets[1] (nearly one million in a normal human pancreas) represent 1-2% (about 1-1.5 grams) of the pancreas total mass and contain five known types of cells: α-cells (synthesizing glucagon hormone), β-cells (insulin, amylin and C-peptide), δ-cells (somatostatin), PP cells (pancreatic polypeptide) and ε-cells (ghrelin)[2]. Human Islet amyloid poly-peptide (IAPP, also known as amylin) and insulin are coexpressed in pancreatic beta-cells in response to meals[3]. Amylin (Figure 1E) contributes to glycemic control and exibits a role of guardian/partner to insulin[4] (one molecule of islet amyloid polypeptide for every 100 molecules of insulin). Insulin mediates cell signaling through activation with the Insulin Receptor[5] (Figure 1C,D), and causes cells in the liver, muscle, and fat tissue to store glucose from the blood as glycogen (glucose polymer). However, the immediate role of insulin is to eliminate excess glucose from the blood. Furthermore, by inhibiting the release of glucagon, insulin prohibits the use of fat as an energy source. In a feedback mechanism, insulin regulates long-term food intake whereas amylin decreases short-term food intake. Beta cells can respond quickly (in approximately 10 minutes) to glucose spikes in blood by releasing insulin reserves (simultaneously replacing the "stock" with new insulin reserves). One known pathway in which beta-cells respond to glucose spikes is through GIP/GIP-R and GLP-1/GLP1-R interaction. The gastric inhibitory polypeptide receptors (GIP-R) and glucagon-like peptide 1 receptors (GLP1R) are found on the surface of beta-cell membrane[6,7,8]. Thus, insulin release is stimulated by K-cells of the duodenum and small intestine which synthesize the gastric inhibitory polypeptide (GIP) and L-cells (also with intestinal localization) which synthesize glucagon-like peptide-1 (GLP-1). Otherwise, after a meal, K-cells and L-cells release GIP and GLP-1 into the

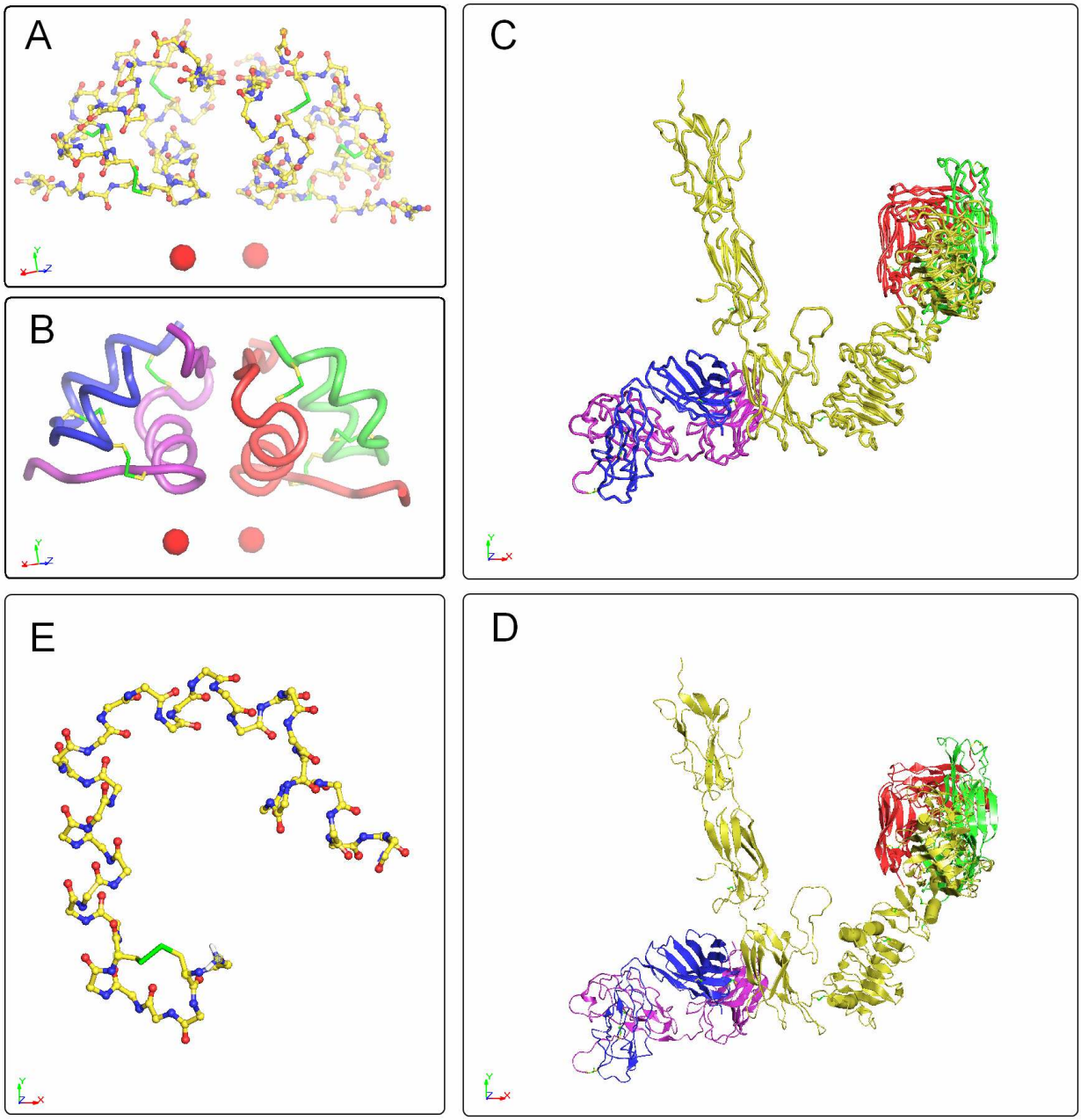

Fig. 1. Representation (3D) of some proteins involved in diabetes. (**A**) Ball and stick model of two insulin molecules (crystals) (*Homo sapiens*). Zinc atoms are the red bubbles, (**B**) a second method of viewing two insulin molecules (*Homo sapiens*) by using the solid string model. Insulin molecule 1 (chain A (green – 21 amino acids), chain B (red 30 aa)) and insulin molecule 2 (chain C (blue – 21 aa), chain D (purple – 30 aa)), (**C**) Insulin receptor from *Homo sapiens* (Solid string and Ribbon (solid) model). Chain A (green – 220 amino acids), chain B (red – 219 aa), chain C (blue – 215 aa), chain D (purple – 214 aa), chain E (yellow – 807 aa), (**D**) Insulin receptor (*Homo sapiens*), a second view by using Solid string, Ribbon (cartoon) and cartoon model, (**E**) human form of amylin molecule. Chain A (37 aa). Atoms: red (O), green (S-S bond), yellow (C), blue (N). Atom color applies to **A**.

bloodstream, thus interacting with GIP-R and GLP1-R on the beta-cell membrane. Among other roles, GIP and GLP-1 increase the release of insulin from beta-cells (so called incretin effect).

In their cycle, IAPP peptides are also "broadcasted" into the blood circulation from pancreatic islets and later are broken by peptidases in the kidney. Nevertheless, pancreatic islets of patients width type 2 diabetes, show amyloid deposits deriving from IAPP[9,10].

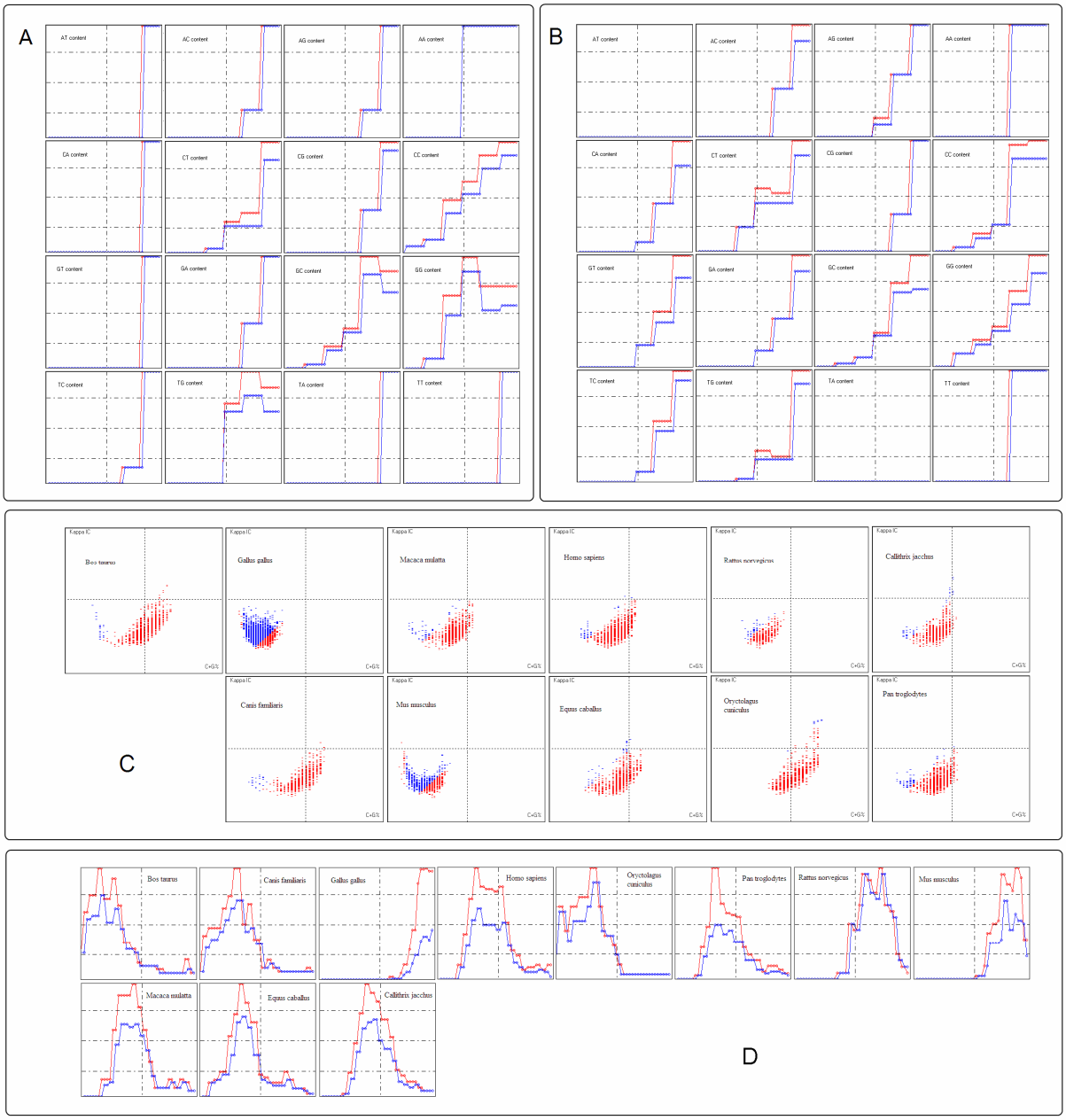

Fig. 2. Analysis of insulin gene in different species. (A) dinucleotide analysis for *Bos taurus* insulin gene, (B) dinucleotide analysis for *Homo sapiens* insulin gene. Red lines represent dinucleotide islands while blue lines represent clusters of dinucleotide islands, (C) Insulin gene patterns from 11 species. Red areas represent C+G values higher than Kappa IC values whereas blue areas represent Kappa IC values higher than C+G content, (D) (C+G)% islands and clusters of insulin gene from 11 species. Red lines represent (C+G)% islands while blue lines represent clusters of (C+G)% islands.

Amyloid deposits disrupt the structural integrity of beta-cell membranes, perhaps preventing diffusion of insulin crystals (Figure 1A,B) outside the cell membrane[11,12].

These mechanical defects, gradually lead to a reduction/loss of beta-cells and insulin production. Beta cells also release a byproduct of insulin production, namely C-peptide[13,14]. The presence of C-peptides in the bloodstream, prevents neuropathy (kidney disease) and vascular deterioration. In addition to their anti-inflammatory properties, C-peptides are even more important as their levels are used to assess the viability of beta-cells.

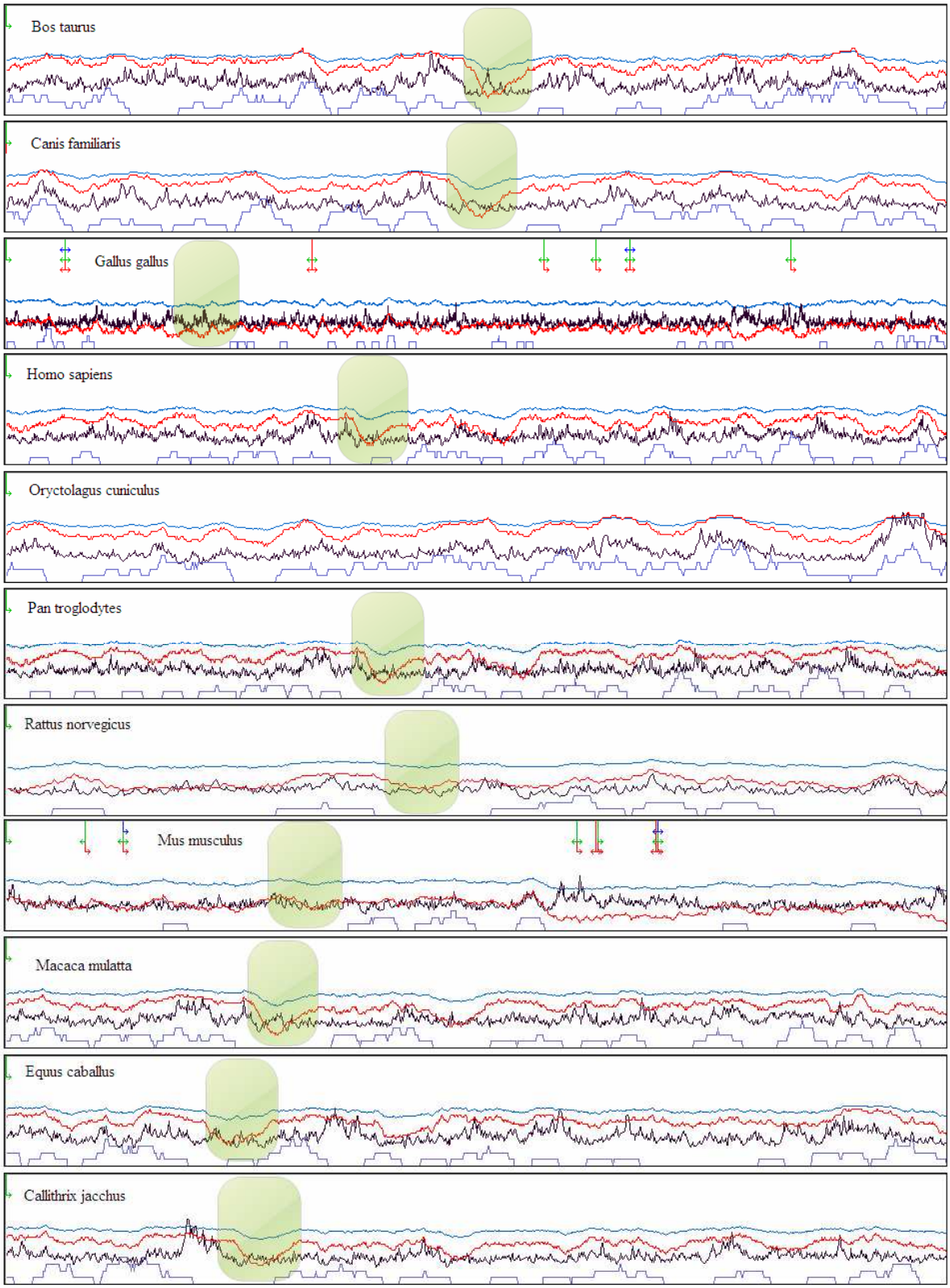

Fig. 3. DNA sequence analysis of insulin gene (INS gene) from 11 species. Transparent rectangles show the areas in which there is a depletion of (C+G)% and a very large value of Kappa IC. The arrows indicate the chain direction in which a particular motif sequence was found. Green arrows indicate the “ATG” motif, red arrows “TATA” motif and blue arrows “AATAAA” motif.

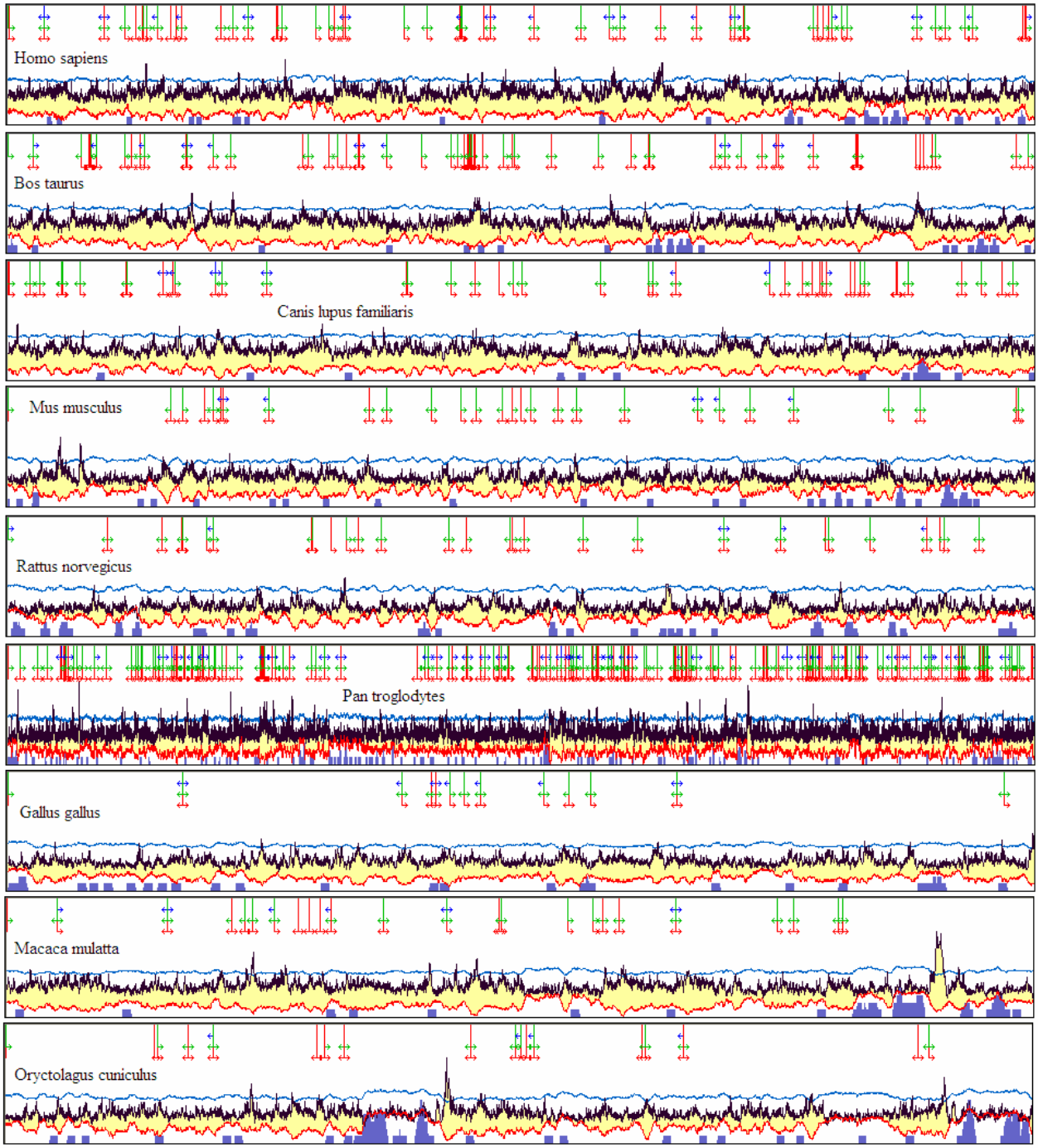

Fig. 4. Islet amyloid polypeptide gene in 9 species. The arrows indicate the chain direction in which a particular motif sequence was found. Green arrows indicate the "ATG" motif, red arrows "TATA" motif and blue arrows "AATAAA" motif.

Little is known about the incidence of diabetes in other species. For instance, it is known that diabetes affects 1 in 500 dogs or 1 in 400 cats[15,16,17]. Nevertheless, the pancreatic islets architecture differs between species[18]. Continuing our work[19,20] on "in-depth analysis" and Kappa Index of Coincidence (Kappa IC), for the first time presented by authors[21,22], we show an

analysis of Insulin and Islet Amyloid Poly-Peptide genes.

RESULTS AND DISCUSSION

In this study we focused on a parallel analysis of Insulin genes (INS gene – coding the proinsulin precursor from *Bos taurus*, *Canis familiaris*, *Gallus Gallus*, *Homo sapiens*, *Oryctolagus cuniculus*, *Pan troglodytes*, *Ratus norvegicus*, *Mus musculus*, *Macaca Mulatta*, *Equus caballus* and *Callithrix jacchus*) and Amylin genes (from *Bos taurus* (NC_007303.5), *Canis lupus familiaris* (NC_006609.3), *Homo sapiens* (NC_000012.11), *Ratus norvegicus* (NC_005103.2), *Mus musculus* (NC_000072.5), *Pan troglodytes* (NC_006479.3), *Gallus gallus* (NC_006088.2), *Macaca mulatta* (NC_007868.1), *Oryctolagus cuniculus* (NC_013676.1)) by using two new methods of DNA data mining. There is a strong homology between insulin sequences of different species. However, the proinsulin C-peptide differs between species. First, we made an analysis on dinucleotide islands/clusters by a method we have described previously[19-22] (Figure 2A,B,D). Our second method uses gene patterns (based on our Kappa IC method[21,22]), that capture all the features of a DNA sequence (Figure 2C and Figure 5), such as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs). In figure 5, the left side of a pattern shows DNA sequences (each point representing a sequence of 30b - the sliding window length) based on A+T nucleotides while the right side shows DNA sequences based on C+G nucleotides. In Figure 3 and 4, red lines represent the (C+G)% content, blue lines represent TM (melting temperature[23]), black lines represent Kappa IC values and blue bars represent CpG islands. In the insulin gene we noticed an area which shows a depletion of (C+G)% and a very large value of Kappa IC, embedded between two CpG islands. These types of areas are difficult to observe by sequence alignment methods. However, this area is missing from Oryctolagus cuniculus insulin gene (Figure 3). Also, in *Ratus norvegicus* and *Mus musculus* these areas are difficult to establish. Islet amyloid polypeptide genes are generally characterized by a low C+G content and a large Kappa IC value. Inside IAPP genes we noticed many Kappa IC peaks, which also corresponds to A/T short repeats (Figure 4).

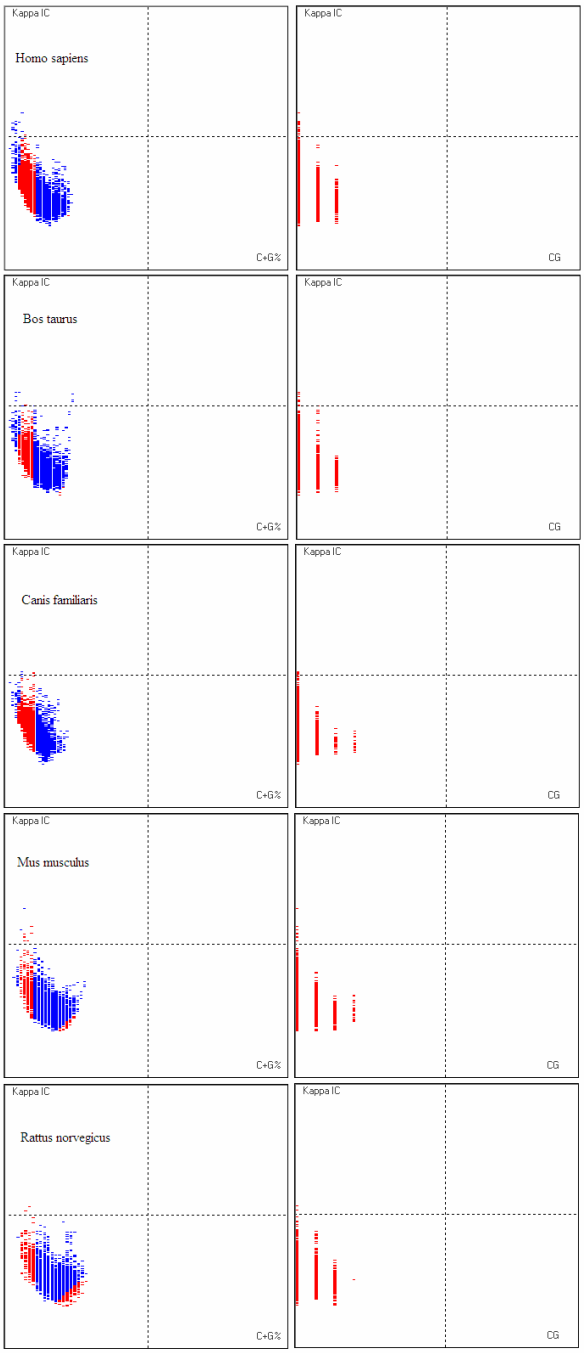

Fig. 5. IAPP gene patterns from 5 species. Left column shows Kappa IC/(C+G)% gene patterns. Right column shows Kappa IC/CpG gene patterns.

This suggests that IAPP genes have been under strong selection pressure during evolution.

MATERIALS AND METHODS

In-depth analysis of different dinucleotide structures is made through repeated tests with different dinucleotide thresholds[19]. For this stage of the experiment we used

GCLUSTER, our software design for "in-depth analysis" (sliding windows of 30b and thresholds between 20% and 60%). In the second stage of the experiment we used DNAKAPPA[21] (sliding windows of 30b). For 3D molecular structures we used 2DTG, 2L86 and 4INS PDB files (Protein Data Bank).

CONCLUSION

Researchers in biology increase their reliance on advanced bioinformatic methods and complex software. In order to understand the genetic basis of various diseases, especially polygenic diseases (diabetes, obesity and vascular disease), we made a rough analysis of INS gene from 11 species and for IAPP gene from 9 species. Our results show some sequence features of the two genes that can not be detected by usual methods, such as sequence alignment.

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