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A Systematic Study on Mirror Repeats in CTLA4 and KCNJ11 Genes of Diabetes

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Authors' contributions

This work was carried out in collaboration among all authors. All kinds of In silico data preparation as well as drafting of the manuscript done by authors AY and RS. Both authors contribute equally. Author SY helped in the rectification & finalization of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

All cellular functions are controlled by the regulatory material which is constituted majorly by variety type of repeat elements. Among that types a unique pattern of bases form a Mirror repeat (MR) which thought to plays variety of roles at the genomic level that include regulation of gene expression, genomic integrity, triplex structure formation etc. The present research focused on identification of Mirror repeats in different genes types involved in both diabetes types. Two different genes CTLA4 (T1D) & KCNJ11 (T2D) were targeted to identify these sequence type. A manual method refers as FPCB is utilized to extract out MR sequences. The targeted gene sequences were divided into short regions so that it can be handled out easily while bioinformatics based analysis.

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Among the both types it was reported that both genes have different pattern of distribution of MR sequences. CTLA4 have 346 MR sequences in total in which MR235 have highest length of 50bps. In case of KCNJ11 a total no of 235 MR sequences were reported in which MR105 having highest length of 58bps. The present study will be helpful to elucidate the roles of MR sequences in diseases development & progression. It will be helpful for sequence based protein finding studies using bioinformatics tools as well as wet lab experiments.

Keywords: Mirror repeats (MR); CTLA4; KCNJ11; T2D; T1D; DNA.

1. INTRODUCTION

The fundamental question in biology is how genetic information transmits and replicates from cell to cell and organism to organism. It has been attributed to the fact that identifying DNA as genetic material and elucidating its transmission mechanism has laid the foundation for our current molecular understanding of biology [1]. The significant essentiality of DNA lies in the fact that it provides genetic specificity and structurally it is a long fibre of nucleotides [2]. Throughout the evolutionary period, DNA had been chosen over RNA as the carrier of genetic material which is attributed to the fact that it is much more stable than RNA in an aqueous environment [3]. The immense portion of information about the human genome collected from the past few decades has provided us with the opportunity to learn about unknown facts of DNA including the claim that non-coding repetitive sequences around different genes have a crucial role to play in genetic processes [4]. In approximately 30-90% of the genome, repetitive sequences refer to short and long DNA fragments that repeat numerous times throughout the genome across different locations [5,6]. This particular research focused on the identification of mirror repeats in the Homo sapiens Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and Potassium inwardly-rectifying channel subfamily J member 11(KCNJ11) genes which have significant role in the development and progression of Type 1 Diabetes & Type 2 Diabetes .Diabetes Mellitus a widespread metabolic condition, is a chronic disease confined to people of all ages and is characterized by high glucose levels resulting from abnormality in insulin secretion from the pancreas, insulin action, or sometimes both [7]. Our country is dealing with severe implications related to diabetes as some research shows by 2030 diabetes mellitus may impact up to 79.4 million people in India as concluded by various researches [8]. It is most commonly of two types, both are characterized by high glucose levels or hyperglycaemia [9]. T1D is represented by the absolute lack of insulin hormone and has an autoimmune basis that has its origin when pancreatic cells (beta cells) that produce insulin are destroyed by a T cell-mediated immune response [7]. Conversely, Type 2 Diabetes earlier known as non-insulin dependent DM resulted from impaired insulin secretion by pancreatic beta cells and insulin resistance in insulin-sensitive tissues [9]. Both types have genetic and environmental significance. Since approximately 70% of identical twins develop T1D by the age of 60 [10,11] strongly recommended that genetics entertain more dominant consequences than its environmental counterpart. Some of the genes concerned with T1D are HLA class II alleles (contribute to nearly 30-50%) of genetic risk [12], but other genes involved with smaller effects are insulin, PTPN22, CTLA4, IL2RA, IFHI, and others. Contrary to T1D, there is a strong interlinked association of both genetic and environmental factors, contributing to T2Ddevelopment [13]. Genes associated with Type 2 Diabetes (T2D) are CAPN10, TCF7L2, HNF1A, ABCC8, GCK, KCNJ11, and many more genes [14]. The rapid increase in the prevalence of diabetes (T1D & T2D) has been based on the changes in lifestyle and environmental factors, as well as their complex interaction contributing to the emergence of the disease [15] and these factors include infections, diet, intestinal microbiota, age, obesity, low physical activity, sedentary lifestyle and others [15,16]. In the present study CTLA4 (T1D) and KCNJ11 (T2D) genes were examined for Mirror repeat sequences as these genes shows great importance in context of diabetes. The major focus of present study is also to check the distribution of MR sequences in the targeted genes. Cytotoxic T-lymphocyte-associated protein 4, (CTLA-4) is located on chromosome 2 which belongs to the immunoglobulin super family and encodes a protein molecule that acts as a negative regulator of T cell activation. CTLA4 is to a great degree associated with thyroid autoimmunity. Decrement in the amount of soluble CTLA4 is majorly responsible for increased receptivity for T1D as suggested by various studies [17]. The KCNJ11 (Potassium

inwardly-rectifying channel, subfamily J, member 11) present on chromosome 11 has no introns and it encodes the Kir6.2 ATP-sensitive potassium channel, which plays an important role in pancreatic beta cell insulin production and secretion [18-19]. The Kir6.2protein with the high-affinity sulfonylurea receptor 1 (SUR1) forms the KATP channel, which acts as a metabolic sensor in various tissues. Structurally, the large macromolecular complex KATP channel has a central pore formed by the tetramer of Kir6.2 surrounded by the four high-affinity SUR1 subunits, encoded by the ABCC8 gene located next to the KCNJ11, which is widely expressed in beta cells and neurons [20-21]. Mutations in KCNJ11 cause permanent neonatal diabetes mellitus, and hyperinsulinemia infancy, due to the permanent opening of KATP channels in the plasma membrane of the beta cells [22].

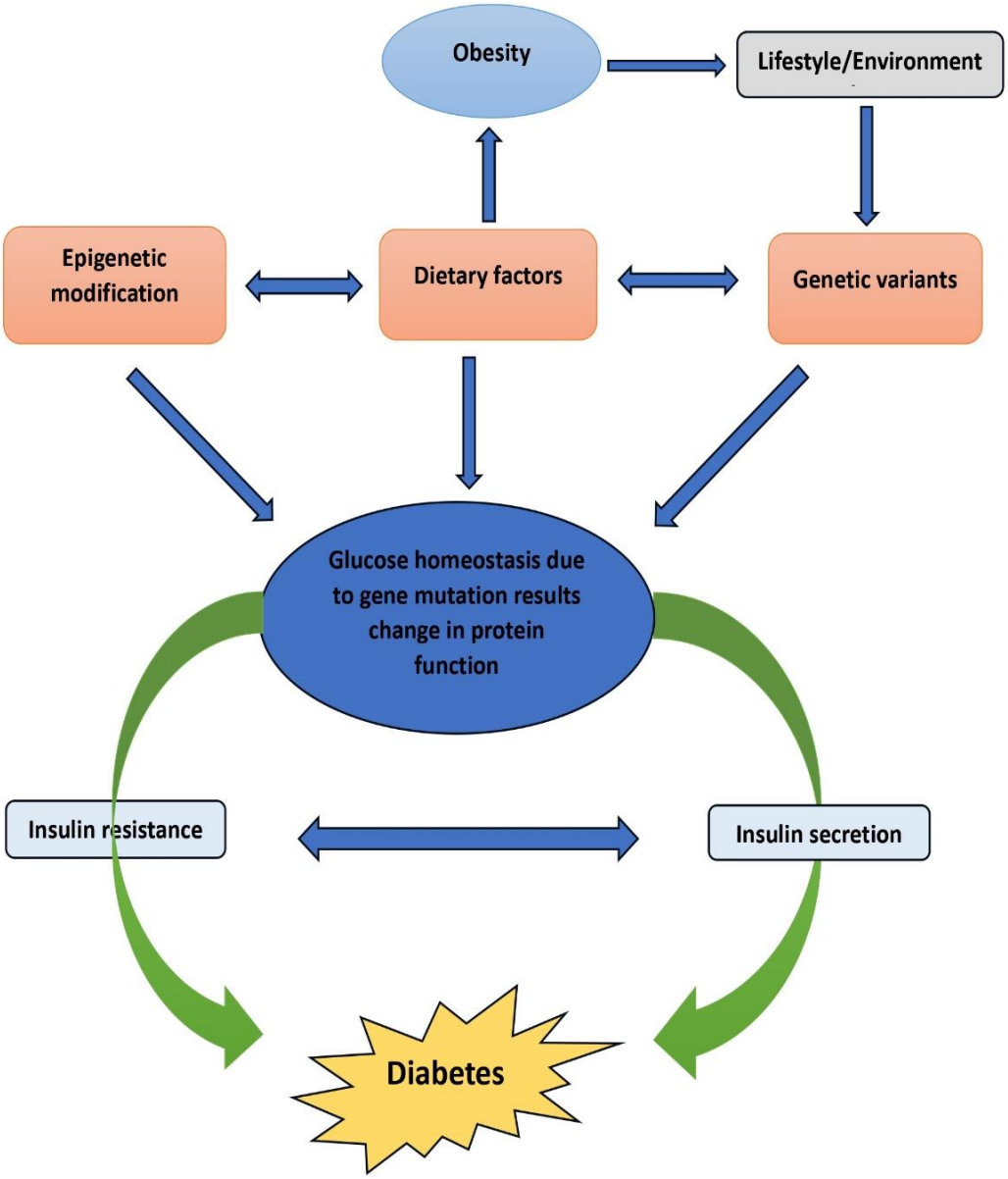

Fig. 1. Showing schematic representation of various factors associated with diabetes

2. MATERIALS AND METHODS

The human CTLA 4 and KCNJ11 genes which play an important role in T1D and T2D have been analysed to identify mirror repeat patterns using the FPCB bioinformatics approach [23-24]. These genes are selected on the basis of their roles in the development of diabetes. The given table enlists the number of regions of the particular complete gene sequence.

The complete process is divided into four basic steps: -

Step 1-The genetic sequences of the desired genes that are CTLA 4 and KCNJ11 are to be downloaded from the official website of NCBI (National Centre for Biotechnology Information) in FASTA format [25]. Then the sequence is divided into different regions that comprising of selected number of base pairs.

Table 1. Illustrates the distribution of regions in respective targeted genes of T1D and T2D

S. No.	No. of Regions in CTLA4 (NG_011502.1)	No. of Regions in KCNJ11 (NG_012446.1)
1	1-1000	1-1000
2	1001-2000	1001-2000
3	2001-3000	2001-3000
4	3001-4000	3001-4000
5	4001-5000	4001-5000
6	5001-6000	5001-6000
7	6001-7000	6001-7000
8	7001-8000	7001-8000
9	8001-9000	8001-9000
10	9001-10000	9001-10000
11	10001-11000	-
12	11001-12000	-
13	12001-13175	-

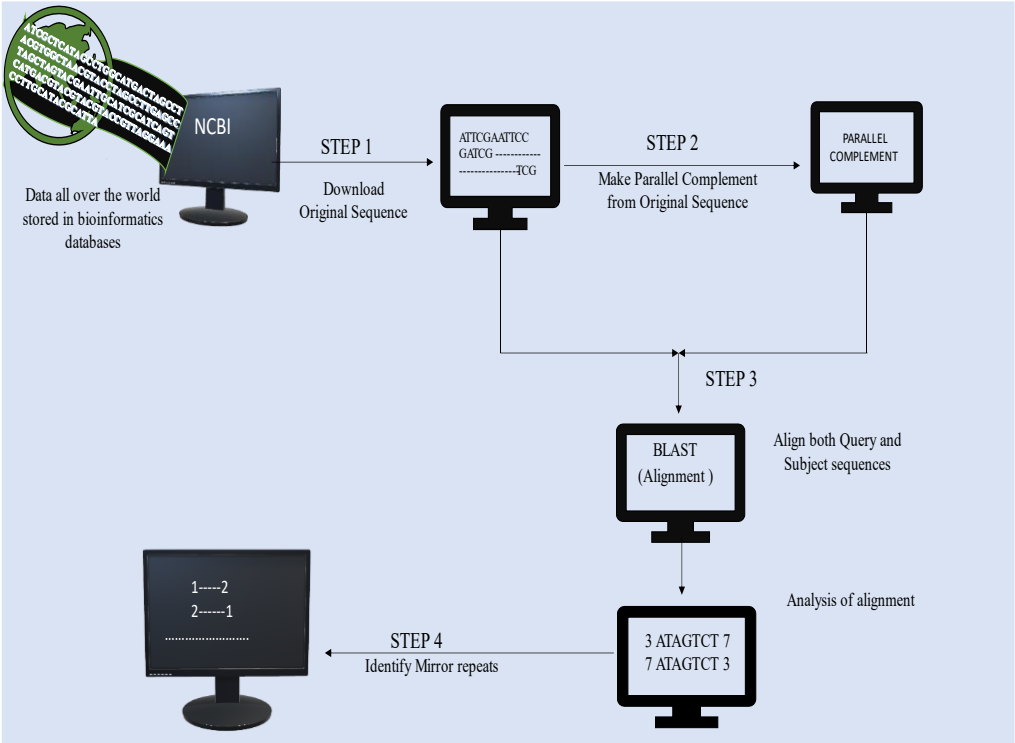

Fig. 2. Showing methodology for mirror repeats identification

Step 2- Here, each segment containing selected base pairs replicated to a bioinformatics tool called Reverse Complement which converts the original sequence to complement one.

Step3-Lastly, the original FASTA format query and complement sequences are compared through another bioinformatics tool called BLAST for comparing homologous sequences. Here, different algorithm parameters are set for example Max target sequences, expected threshold & word size, etc. Through the use of BLAST, we get to know if there are mirror repeats in query and complement sequences if the position of the pattern is exactly reversed. Multiple similar studies have been done in the past on mirror repeat identification in various phyla which support the present investigation [26-27].

Step 4- Result &Analysis.

3. RESULTS AND DISCUSSION

The obtained alignment results by FBCB analysis for the selected CTLA 4 and KCNJ11 genes contain several homologous alignment sequences (Mirror repeats) in different regions which may be providing a hint about their importance in context of T1D and T2D. The graphs given below depicted the distribution of the number of mirror repeat sequences in the respective regions of the genes. This particular study is supported by some of the previous research conducted for similar outcomes [28-32]. Mirror repeats with a minimum length of 7 bps were found in all the regions of both genes. In context of maximum length, MR235 of CTLA4 gene (50 bps) & MR105 of KCNJ11 gene (58 bps) were reported in the region 9 & 5 respectively.

Table 2. Illustrates the region-wide distribution of mirror repeats in selected genes

S.No.	Region/s	No. of mirror repeats in the CTLA4 (NG_011502.1)	No. of mirror repeats in the KCNJ11 (NG_012446.1)
1	Region 1	33	24
2	Region 2	21	17
3	Region 3	22	21
4	Region 4	28	23
5	Region 5	30	28
6	Region 6	22	21
7	Region 7	28	20
8	Region 8	26	33
9	Region 9	27	30
10	Region 10	27	18
11	Region 11	25	-
12	Region 12	31	-
13	Region 13	26	-

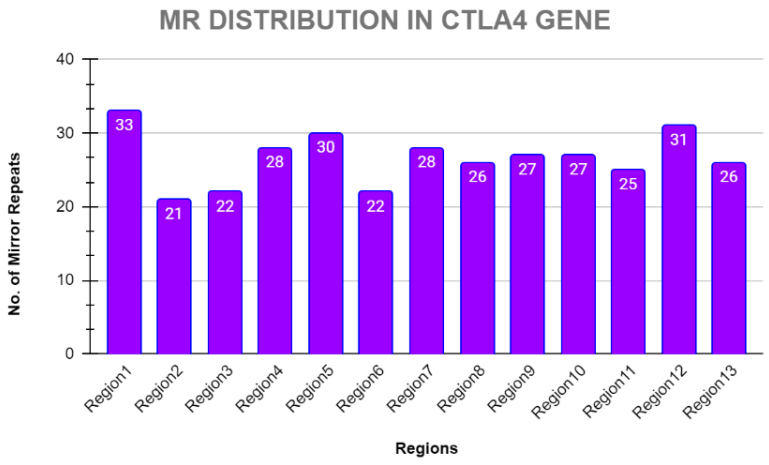

Fig. 3. Graph showing the presence of mirror repeats in different regions of the CTLA4

Table 3. Depicted pattern of some selected MR sequences in the CTLA4 gene

MR No.	Sequence	Length	Position
MR20	AGGGGTCT—GTCCCAGGTGGAGCTTGGATCTGAGGA	35	632
MR42	TGTATC---TGGAGGTACCCTATGT	22	387
MR97	AAAATAAGATAATAGGAGAAAA	22	804
MR115	TTTAA-TCTTCACTTTTGAATTT	22	295
MR125	TTCTTGTGATTTTAGttttttt	22	518
MR132	TTGGCTTGTTTTGTTCAGTT	20	892
MR141	TCTCCTGTTTTTTCTTCTCTTCATCCCTGTCTTCT	35	226
MR163	TTTAAATATGTAAAGATT----- AAATAAACACTCTTAGAAGTATTTAAATTT	48	186
MR175	TAGAAATTCAGACCAGAGTGATAGAT	26	744
MR190	TAACAACATCTTCCACTCTACAACAAT	27	178
MR196	ATATCATCTCATCTAATTATCT-CT-TACTATA	31	428
MR201	AGGGGAG-GCTGGGGGGTGTGGAGAGGGGA	28	639
MR202	TGGGGGTGTGGAGAGGGGAAGGGGT	25	648
MR235	AGATGAGGCAATAAATGAAGA— GGAAGGACAGTGGTAAAGAACGCACTAGA	50	800
MR248	CCTGTCAGGATTTCTTTTTGACAGTCC	27	315
MR300	TTTGAATATTGTGTTCAAATTTT	23	202
MR346	ACCTCCAAATATTATAATCCTTCA	24	1075

Table 4. Depicted pattern of some selected MR sequences in the KCNJ11 gene

MR No.	Sequences	Length	Position
MR5	GACCTCC-CCTCTCCTTCTCCAG	23	177
MR15	GCCACCCTCAGCTGTGGCC-CCCACCG	27	458
MR16	CCATCCTGACCCGCCCCCTCCTGCC	25	499
MR18	TTCGGACCCTGCCCATACCCCTGCCTGGGTT	31	698
MR84	GACCGCCTAATTTCCGCCAG	20	922
MR97	GGGAGGACGGCCAGCC- GCGAGCGCCCGGGCGGCGGGAGGG	41	407
MR99	GCCGGGTCGCGCGCAGGTCCG	21	589
MR105	GGGGGATCCGGAAAGCGGCGGGG- GCGCTCCGGGAGGGGTGGAGTAGGACATAGGGGG	58	690
MR111	GGGGTGGTCAGCTGGTGGGG	20	945
MR123	AAGAAGTGAAGTGGGACCCAGGTGGAGGTAAGGAA	35	374
MR133	TCCTTCTCGTCTGC-CTTCCT	21	914
MR136	GACCACCAGCCCCGAGGGCGAGGTGGTGCCCCT- CCACCAG	41	234
MR157	GGTCCCTGAAAAAGCACCTGG	21	164
MR161	GGGACAGCCTGGGGCTGACAGGG	23	274
MR164	TGGAAGGGGAGGAAGCAGGGGAAGGT	26	324
MR173	GGGGGTGGATA--- TCTTTGGGTTGCTGGAAAAGGTGTGGG	41	610
MR183	GCGGGGAGGGCAGGGGAGGGACG	23	907
MR184	GGAGGGCAGGGGAGGGACGGAAGG	24	911
MR187	GGGGGACTTTCCCCTCTCCTGTCTCAGGTGG	31	958
MR227	TCTTGCCCACTGATGGTGCTACCTCCATC-TGG- GGGCACCCTTTCT	47	737

The subsequent tables demonstrate the presence of some selected mirror repeats from different regions of the CTLA4 and KCNJ11 genes along with the information regarding their lengths and position in the genes. The rest of the information about remaining sequences, their patterns, length & size, etc. were provided in the supplementary data file.

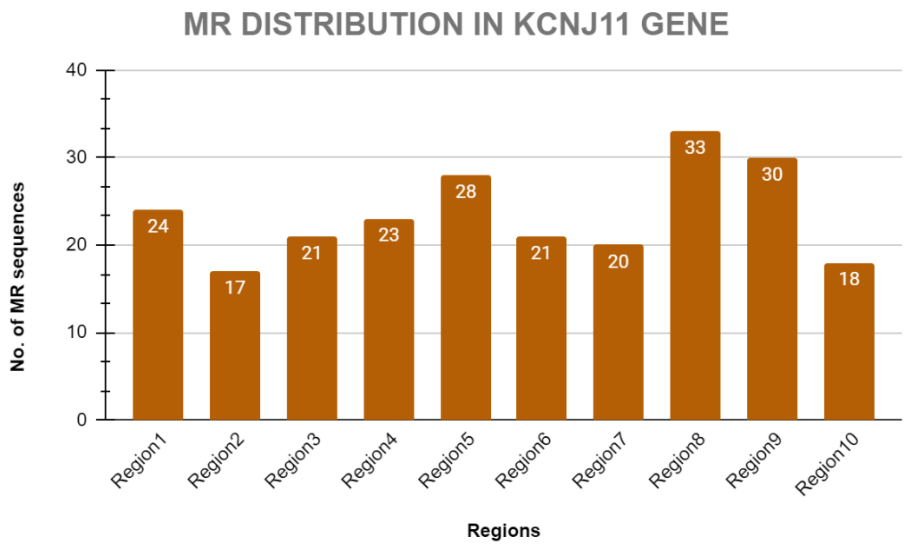

Fig. 4. Graph showing the mirror repeat in the different regions of the KCNJ11

4. CONCLUSION

This particular study focused on the identification and distribution of MR sequences in some selected genes (CTLA4 & KCNJ11) of diabetes. We have comprehended 346 and 235 mirror repeats within CTLA4 and KCNJ11 genes respectively through the utilization of FPCB approach. Through this investigation, it was also concluded that these identified MR sequences varies in their length and their distribution in the genes is also not uniform. Previous studies suggest the roles of MR sequences in gene expression, genomic instability, epigenetic modification and protein binding etc., but the involvement of mirror repeats in the development and progression of diabetes is need to be understood, future research sholds brighter pathways for the reasoning of the same.

SUPPLEMENTARY MATERIALS

Supplementary materials available in this link:
https://ikprress.org/index.php/AJOCR/libraryFiles/downloadPublic/20

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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