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Molecular study of the KCNJ11 gene and its correlation with Prakriti to preventing and managing type 2 diabetes

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ABSTRACT

In Ayurveda, every individual is believed to possess a unique entity known as *Prakriti,* which distinguishes them from others physically, physiologically, and psychologically. This entity also determines an individual's response to a particular stimulus, and it is believed that such responses are not solely determined by genetics. The present research aims to validate the Ayurvedic concept of *Prakriti* from a modern molecular perspective to strengthen the personalized and precise treatment approach. A study was conducted to investigate the role of the KCNJ11gene in the susceptibility of individuals to type 2 diabetes mellitus (T2DM) with their metabolic status. The research involved allele mining on three major *Prakriti* groups - *Vata, Pitta*, and *Kapha* - in 112 patients with T2DM and 112 healthy individuals. The KCNJ11 gene, responsible for insulin secretion membrane pore formation, was analyzed to determine the susceptibility of different *Prakriti* types to T2DM. The MutPred tool predicted the molecular cause of disease-related amino acid substitution. According to the study, only *Pitta* and *Kapha Prakriti* were diagnosed with diabetes, while all three *Prakriti* types were present in the control group of healthy individuals. A protein model was prepared, and the changes resulting from mutations were observed for each group in their protein sequence, both as synonymous and non-synonymous mutations. Ultimately, these changes contributed to the manifestation of T2DM. Based on the findings, it appears that *Prakriti* groups may experience changes in protein function due to nonsynonymous mutations and differences in amino acids at the protein level.

1. Introduction

Ayurveda is an ancient Indian system of healthcare that covers a wide range of topics related to health and lifestyle. It provides detailed information on various diseases, including diabetes, although its terminology is not widely recognized due to the lack of empirical evidence.[1] The literature of Ayurveda has been in practice in India since 1500 B.C. While Modern medicine has gained popularity due to its ability to provide immediate relief through fast-acting drugs, the fundamental principles of Ayurvedic treatment are still highly relevant today. However, the challenge lies in validating these principles from a contemporary perspective and using reliable and easily understandable language.[2] Ayurveda suggests that people with different *Prakriti* types may have unique health and disease situations, influencing their susceptibility to illnesses, diagnosis, recommended diet and lifestyle, and reaction to medications and surroundings.[3] In Ayurveda, the term "*Prakriti*" refers to the inherent constitution of the human body, which is determined by the balance of three doshas: principles of movement

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(*Vata*), metabolism (*Pitta*), and strength (*Kapha*) from birth to death.[4] Any imbalance of these doshas can lead to pathological consequences, known as "*Vikruti*." Recent studies suggest that *Prakriti* has a clear genomic and epigenomic basis, making it a promising approach to personalized medicine. Phenotyping an individual involves assessing various characteristics such as body frame, food and bowel habits, disease resistance, memory retention, and metabolism.[5] The latest development in medical science now allows for precision medicine. This means that medical treatment can be tailored to suit the unique characteristics of each patient.[6] However, this does not necessarily mean producing one-of-a-kind drugs or medical equipment for each patient.[7] Instead, patients are categorized into smaller groups based on their specific susceptibility to a particular illness and how they respond to potential treatment options. Recent studies conducted through Ayurgenomics have demonstrated that individuals with different *Prakriti* types (*Vata, Pitta*, and *Kapha*)[8] exhibit significant differences at the biochemical and gene expression level.[9]

The global number of prevalent cases of type 2 diabetes estimated by Global Burden of Disease (GBD) reported 437.9 million cases in 2019, representing a 49 % increase since 1990.[10] There are various factors that can influence diabetes, including genetics and the environment.[11,12] Environmental factors such as obesity, inactivity, high blood pressure, abnormal lipid levels, and aging are also significant risk factors.[13] Mutations in the genomic DNA, whether harmful or not, can contribute to developing diabetes.[14] Certain non-harmful types of mutations called single nucleotide polymorphisms (SNPs) have been linked to an increased risk of various forms of diabetes.[15] Although more than 40 gene mutations are associated with an increased risk of type 2 diabetes, they only account for 10 % of the disease's overall genetic makeup.[16] Some examples of these genes are PPARG, GLUT2, TCF7L2, and CAPN10.[16] Among the many genes that play a role in diabetes, ABCC8, KCNJ11, and PPARG are the most studied[17], and they primarily affect insulin activity, glucose metabolism, pancreatic beta cell function, as well as other metabolic factors such as energy intake and expenditure, and lipid metabolism.[18]

Potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11), which forms a compartment of the ATP-sensitive potassium (K-ATP) channel in the beta cells of the islets, is associated with the susceptibility to various types of diabetes.[19] The KCNJ11 gene is situated on the short arm of chromosome 11 (11p15.1) and does not have an intronic region.[19] During the study, the molecular information was located on Annotation Release 109, GRCh38.p12 (NCBI). K-ATP channels in the cell membranes respond to glucose levels in the bloodstream by opening or closing. Glucose is the primary source of energy for most cells in the body. When the K-ATP channels close due to an increase in glucose, insulin is released by the beta cells into the bloodstream. This helps regulate blood sugar levels.[20] The KCNJ11 gene is essential in type 2 diabetes susceptibility by affecting insulin secretion concerning metabolic status.[21] The structure of the KCNJ11 gene, which is 390 amino acids long and lacks intronic regions, makes it ideal for molecular research. Most studies on the KCNJ11 gene have been conducted on Caucasian and East Asian populations. However, this study has been carried out on the Indian population and their constitution (*Prakriti*) to examine the impact of non-synonymous single nucleotide polymorphisms (nsSNPs) on the structure and function of the Kir6.2 membrane protein coded by the KCNJ11 gene.

2. Material and methods

2.1. Sample size and criteria of subject selection

A study was conducted on 112 individuals with diabetes who received treatment at the Endocrine and Metabolism department of Sir Sunderlal Hospital, Institute of Medical Sciences, B.H.U, Varanasi, India. Control subjects were also included in the study, with 112 participants selected from the B.H.U employee community, each from a different department. This research analyzed the dominant *Prakriti* of *Vata*, *Pitta*, and *Kapha*, based on Ayurveda's text descriptions.[22] Male and female participants between the ages of 40 and 70 were included in the study after providing written informed consent. *Prakriti* was assessed using a proforma developed by Tripathi and Gehlot.[23] The details of the control subject and patient inclusion and exclusion criteria were previously published.[4] Our earlier study showed the clinical and biological characteristics of diabetic patients and control subjects.[4] A blood sample of 3 mL was collected for further investigation. The Institutional Ethics Committee approved the research through letter No. Dean/2015–2016/1572 dated 30-12-2015.

The primary *Prakriti* was determined through a reliable and validated questionnaire by considering the relative percentage difference between the top two *Prakritis* with a minimum of 10 %. During the selection process for *Prakriti* types, random selection was utilized to prevent any potential bias towards specific *Prakritis*. The dominant Dosha in an individual was determined by calculating the total score for each Dosha using a formula.

$$\%DOSHA = \frac{Marks\ scored\ by\ an\ individual\ for\ a\ Dosha}{Total\ marks\ allotted\ to\ that\ Dosha} x100$$

2.2. Genomic DNA extraction

Blood samples were collected from individuals with different *Prakriti* types to conduct a molecular study. This included both case and control groups, and DNA was extracted from these samples using the Qiagen DNA blood Medikit (QIAGEN, Valencia, CA) (Cat. No. 51183) according to the manufacturer's instructions. The purity of the DNA was measured using the Nanodrops instrument. The isolated DNA was stored at −20 °C for polymerase chain reaction (PCR).

2.3. PCR amplification

For the primer synthesis of KCNJ11, gene sequences were downloaded from the NCBI database.

Then, allele mining nested PCR primers were developed to cover the total length of the gene using the Primer 3 software. A total of 11 sets of primers were developed with at least 100bp overlap. All primer sequences used for the study are shown in Table 1. Details of the base pair sequences are given in supplementary file S1. These 11 sets of primers were used to amplify PCR products from samples of two control and six diabetic cases, including three *Pitta* and three *Kapha*. To standardize the PCR amplification, we used gradient PCR for each primer pair with a control sample and determined the annealing temperature for all 11 primers. The PCR reaction mixture and amplification condition for gradient PCR are given in supplementary file S2. The amplified PCR products were analyzed for potential polymorphism in the *Pitta* and *Kapha* sample categories by separating them on a 4 % metaphor agarose gel.

2.4. Sanger sequencing

After separation, we eluted the PCR products from all eight samples using QIAGEN's gel elution kit (Cat No./ID: 28,704 QIAquick Gel Extraction Kit) following the manufacturer's instructions. Next, we cleaned the eluted PCR products using the protocol of QIAGEN's PCR cleanup kit (Cat No./ID: 28,106 QIA quick PCR Purification Kit). Finally, we sent the products to Macrogen, Inc, Beotkkot-ro, Geumcheon-gu Seoul, Korea, for Sanger sequencing. Each PCR product was sequenced in both forward and reverse directions to minimize sequencing errors.

2.5. Allele mining

We conducted allele mining on the KCNJ11 gene, which is responsible for insulin secretion membrane pore formation, to determine the

Table 1
Primer designed for KCNJ 11 gene sequence using tool NCBI primer 3.

S. No.	Primer ID	Forward Primer (5'————3')	Primer ID	Reverse Primer (5'————3')
1.	KCNJ F1	AGGTCCGGTTAGTGGGAGAG	KCNJ R1	GAGTTCGAGACCAGCCTGAC
2.	KCNJ F2	TCAAGGGTGAGGCTGTTTTT	KCNJ R2	GCTGGCCTCACTTCTGAGAT
3.	KCNJ F3	GGAAGAGTCTGGTGGGGAGT	KCNJ R3	CACAGGAAGGACATGGTGAA
4.	KCNJ F4	GCCACACACATTGCTCATCT	KCNJ R4	CACCCACACGTAGCATGAAG
5.	KCNJ F5	GACCCTCATCTTCAGCAAGC	KCNJ R5	GGTGTTGCCAAACTTGGAGT
6.	KCNJ F6	ATCATCGTCATCCTGGAAGG	KCNJ R6	CCTGCTGAGGCCAGAAATAG
7.	KCNJ F7	CCAGGGTGTTACAAGGCACT	KCNJ R7	GTAGGCTCCACAGCACCAAC
8.	KCNJ F8	CTTGGTCCCTGAAAAAGCAC	KCNJ R8	AAAGATATCCACCCCCAAGC
9.	KCNJ F9	TGAGGAGAGGGGGTACTGTG	KCNJ R9	GACAGGAGAGGGGAAAGTCC
10.	KCNJ F10	CTTCAAGAGGCGCCATAGAC	KCNJ R10	AGACCAGGCACTTCAGCATT
11.	KCNJ F11	CGAATCCTGGCTCTAGCTGT	KCNJ R11	CCAGAGGTGATGGGGAACTA

susceptibility of different *Prakriti* types to T2DM. After sequencing, 8 separate fasta files were created for each sample and primer, representing the 8 samples by combining the forward/reverse reads of each sample. To prepare the KCNJ11 reference in the Indian population, we first mapped all Indian control samples (2 samples) to the KCNJ11 reference sequences using BWA and created a bam file. Similarly, we created mapping (.bam) files for each case of sample *Pitta* (3) and *Kapha* (3). The created bam files were manually checked for the correct mapping on the reference using the software Tablet, which is a (.bam) bam alignment file viewer. The bam files were loaded in the CLC genomic workbench along with the Human KCNJ11 reference sequence. The consensus sequence for the Indian KCNJ11 and two other samples (*Pitta, Kapha*) was called from the created bam file using the CLC genomic workbench, taking the option of filling N in place of unmapped reads (gap) rather than filling the bases with the KCNJ11 reference. The sample control consensus sequence was further used as a control Indian population KCNJ11 against the reference KCNJ11, and SNPs were further identified.

We created two sample sets representing *Pitta* and *Kapha* samples. In two sets, we have taken the known KCNJ11 as a reference, Indian population KCNJ11 fasta sequences, along with the consensus fasta sequences of the respective sample. These sets were used as input in Clustal W, and Multiple Sequence Alignment (MSA) by fixing the order of input as sequence (known Human KCNJ 11 reference), second Indian population KCNJ11, and remaining are the sample-specific (*Pitta, Kapha*) consensus sequences. We removed the unknown bases ("N") from the entire consensus called sequences to make the alignment more specific. The alignment file was manually investigated to identify the SNPs specific to the Indian population KCNJ 11, not the reference human KCNJ11 gene. After the gene level alignment study, only the gene's open reading frame (ORF) was considered to identify the changes at the mRNA level and SNP present in this region. The ORF was translated into protein using the Sequence Manipulation Tool (Freely available), the amino acid sequences were compared, and variation at the protein level was predicted. After the SNP search by proteomic tools, a protein model was prepared for both *Pitta* and *Kapha* samples, highlighting the location of the synonymous mutation on the protein structure. Additionally, a mutation prediction tool was utilized to analyze the impact of the non-synonymous mutation on the protein's structure and function. The changes occurring due to mutation were viewed for each group in their protein sequence as synonymous and non-synonymous mutation, which finally contributes to T2DM manifestation.

2.6. Molecular cause of disease-related amino acid substitution

To determine the molecular cause of disease-related amino acid substitution, we used MutPred. This web-based tool considers various factors such as protein structure, function, and evolution to make predictions. It also uses SIFT, PSI-BLAST tools, and some structural disorder prediction algorithms. Additionally, through functional analysis, MutPred can predict DNA-binding sites, catalytic domains, calmodulin-binding targets, and posttranslational modification sites (http://mutpred.mutdb.org/).

3. Results

The study conducted on patients diagnosed with type 2 diabetes and secondary complications, which also includes control subjects without a family history or medication use. The aim was to analyze genetic differences based on *Prakriti*. The study found that only individuals with *Pitta* and *Kapha Prakriti* were diagnosed with diabetes, while all three *Prakriti* types (*Vata, Pitta,* and *Kapha*) were present in the control group of healthy individuals.

To ensure maximum accuracy during sequence reading, 11 primers were designed, each with a 20bp forward and reverse sequence. The amplification temperature of the KCNJ 11 gene was standardized, and the final amplification results on control samples were observed. Primers 1, 4, 5, 6, 10, and 11 had a final amplification temperature of 57 °C (Fig. 1a), while primers 2, 3, 7, 8, and 9 had a final amplification temperature of 65.2 °C (Fig. 1b).

The 11 sets of primers designed for the KCNJ11 gene were divided into two groups based on their amplification temperature, and PCR products were then separated on 4 % metaphor Agarose gel to study any possible polymorphism in two *Prakriti Pitta*, *Kapha* samples. The experiment observed no insertion deletion because all bands were monomorphic (Supplementary file S3). So, samples from two study groups were sequenced for SNP search.

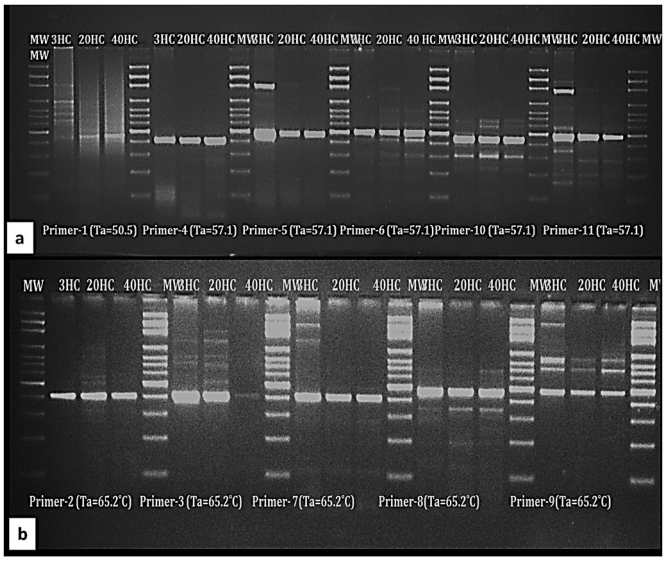

Fig. 1. Primers 1, 4, 5, 6, 10, and 11 show specific amplification temperature (57 °C), whereas Primers 2, 3, 7, 8, and 9 show amplification temperature (65.2 °C) specific bands.

3.1. Genomic study for Prakriti-wise allele mining

The PCR-cleaned product was sent for Sanger sequencing, and sequencing for each PCR product was done using both forward and reverse directions to minimize the chance of error during sequencing. After the assembly of primers, a complete gene map was prepared for type 2 diabetic patients. These sequences were aligned through Clustal W (free software), a multiple sequence alignment tool to see variations at the level of sequence. The reference sequence, control sequence, and finally, the diabetic case samples for the *Pitta* and *Kapha Prakriti* groups were aligned to search for SNP (Fig. 2).

3.2. Protein sequence study

Protein sequence alignment was done for *Kapha and Pitta* diabetic patients with reference sequence and Indian population control (Fig. 3). Only an open reading frame was considered for the study to observe amino acid variations. The gene map was prepared by primer walking, translated to protein sequence, and then aligned with the reference sequence and diabetic control sequence.

Variations were found in the case sample sequence of three *Prakriti* types, which may be responsible for protein level changes. Amino acid variations affecting protein function may explain how type 2 diabetes manifests in different *Prakriti* types. Table 2 summarizes the result obtained from a synonymous and non-synonymous mutation in *Pitta, Kapha*, with reference to control samples.

Further, we conducted computational structural modeling of *Pitta* and *Kapha Prakriti* to understand the protein structural modification. The mutation was highlighted in blue in the protein model, while amino acid symbols are red (Fig. 4).

These 3D models were designed using SWISS-MODEL software. Furthermore, an analysis of amino acid substitution prediction by the MutPred server (Table 3) was done. The Mutpred software predicted mutations and identified a harmful mutation in *Pitta Prakriti*. Specifically, a non-synonymous mutation at position 265 results in a loss of catalytic residue at N265. Additionally, this mutation causes an increase in disorder and glycosylation, a decrease in helix formation, and an increase in loop formation.

In *Kapha Prakriti,* three mutation sites were identified for sample no.7. The first mutation at position 78 leads to a gain of catalytic residue, glycosylation, a loss of sheet, a gain of disorder, and a gain of the helix. At position 247, there is a gain of glycosylation, phosphorylation, sheet, a loss of loop, and growth of catalytic residue. At 291 positions, there is a gain of phosphorylation, ubiquitination, glycosylation, and helix. Similarly, for sample 6 of *Kapha Prakriti*, three positions of non-synonymous mutations were found: the first at position 89 caused a gain of methylation, MoRF binding, ubiquitination, and catalytic residue, and a loss of glycosylation, and stability. In the second one at position 97, there is a gain of MoRF binding, loss of glycosylation, a gain of the helix, loss of stability, and gain of methylation. The third position at amino acid residue 112 caused a gain of ubiquitination, sheet, and disorder and a loss of stability and glycosylation.

The probability of deleterious mutation scores for N265S, P78S, P247T, T291P, T89K, P97A, and A112G was 0.705, 0.554, 0.546, 0.585, 0.764, 0.432 and 0.413, respectively. Loss of catalytic residue was predicted (P = 0.1289) for N265S. Gain of catalytic residue (P = 0.007), a gain of glycosylation at P78 (P = 0.025), a loss of sheet (P = 0.0817), a gain of disorder (P = 0.2457), and a gain of the helix (P = 0.2684) were predicted at P78S. The gain of methylation (P = 0.0101), gain of ubiquitination (P = 0.023), and gain of catalytic residue (P = 0.0463) were predicted for amino acid substitution at T89K along with loss of glycosylation (P = 0.0493). The gain of ubiquitination (P = 0.0633), gain of the sheet (P = 0.0827), gain of disorder (P = 0.086), along with loss of stability (P = 0.0966), and loss of glycosylation (P = 0.1247) at A112G mutation were predicted. This implied that some nsSNPs may account for potential structural and functional alterations of KCNJ11. After the SNP search by proteomic tools, a protein model was prepared, and the changes occurring due to mutation were viewed for each group in their protein sequence as synonymous and non-synonymous mutation, which finally contributed to type 2 diabetes manifestation (Fig. 4).

4. Discussion

Genetic variations at a single base position in DNA, known as Single-nucleotide polymorphisms (SNPs), are responsible for about 90 % of human genetic diversity. SNPs are abundant in the exon region of the human genome, and act as biological markers with great potential to predict an individual's health.[24] Studies have found that most non-synonymous SNPs (nsSNPs) are associated with hereditary genetic diseases. *In silico* analysis can help identify which nsSNPs are harmful and which are neutral.[25] The location of the SNP in a functionally significant domain, such as the ATP-binding domain, can significantly affect the protein's function.[26] Our study focuses on the polymorphism associated with the ATP-sensitive potassium (K_{ATP}) channel protein subunit Kir6.2.

Recent studies on pathophysiology have shown that insulin trafficking of K_{ATP} channels can be affected by mutations in ATP binding cassette subfamily C member 8. Mutations in the KCNJ11 gene, which encodes the Kir 6.2 protein and plays a critical role in the ATP-dependent potassium channel of pancreatic β-cells, can also result in abnormal insulin secretion and diabetes.[11] Furthermore, the study showed that higher levels of methylation may increase the risk of developing diabetes by suppressing the KCNJ11 gene.

The study used an *in-silico* method to detect nsSNPs associated with type 2 diabetes in the KCNJ11 gene coding for Kir 6.2 protein that had

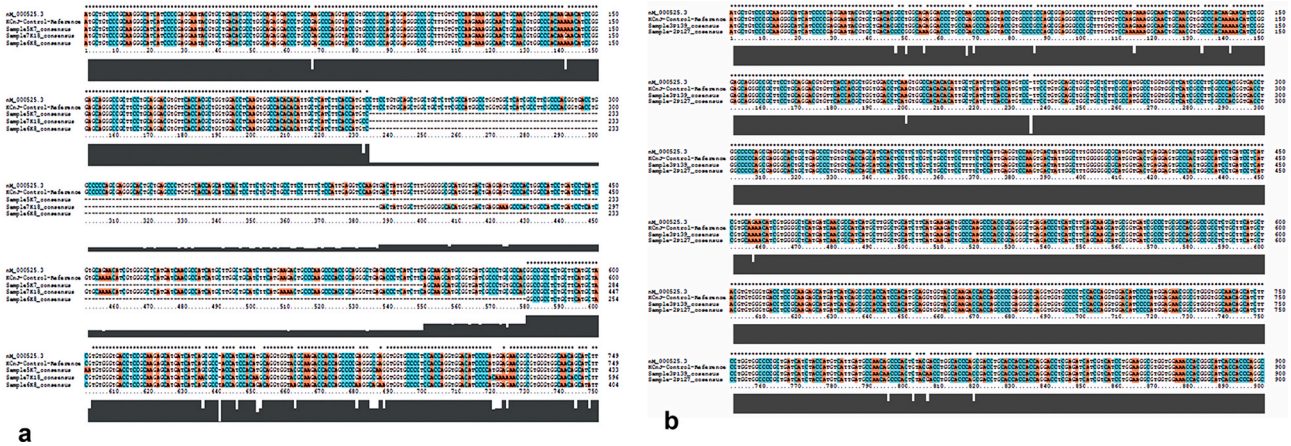

Fig. 2. Multiple sequence alignments for *Kapha* (A) and *Pitta* (B) *Prakriti* case samples with control and reference sequences.

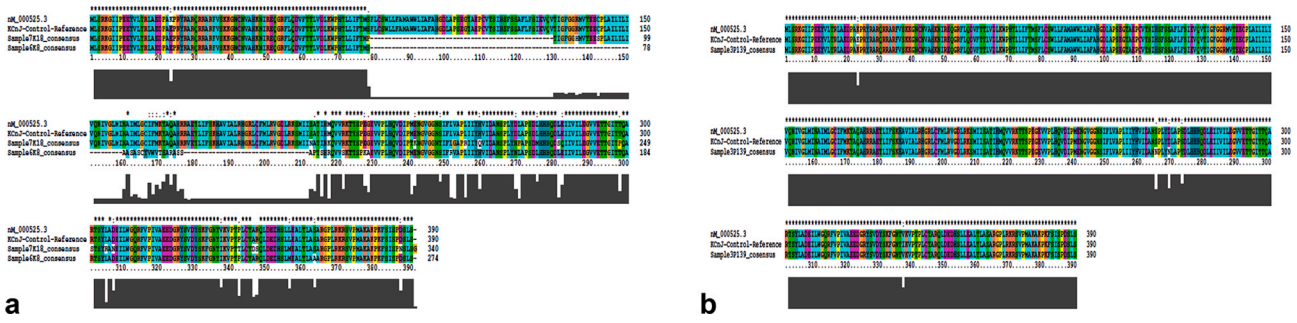

Fig. 3. Protein sequence alignment for *Kapha* (A) and *Pitta* (B) *Prakriti* samples with control and reference sequences.

Table 2
Synonymous and non-synonymous mutations in *Pitta and Kapha* with reference to control.

S. No.	Synonymous mutation	Non- Synonymous mutation
Kapha		
1.	Lys → Glu Glu → Lys Ser → Thr His → Gln Asp → Asn Leu → Met Glu → Gln Asp → Asn Leu → Met	Pro → Thr Ser → Pro Thr → Pro
2.	Ile → Val Phe → Trp Met → Val Thr → Ser Glu → Arg Glu → Lys Asp → Asn Leu → Met Ser → Ala	Lys → Thr Ala → Pro Gly → Ala
Pitta		
1.	Asp → Asn Ser → Thr	Ser → Asn

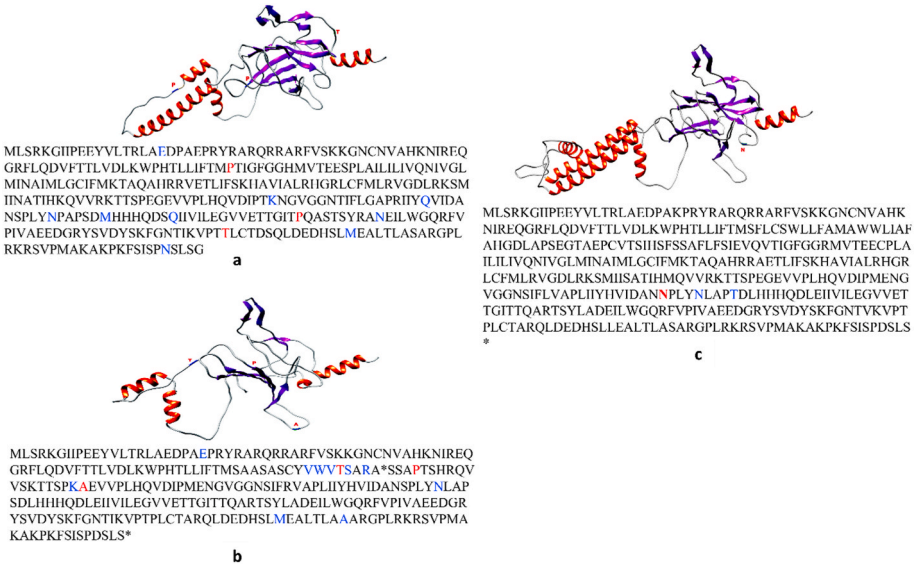

Fig. 4. Sequences and model of Kir 6.2 protein for *Kapha* (a and b) and *Pitta* (c) samples showing non-synonymous mutations.

not been previously documented. Kir 6.2 protein is a channel pore-forming protein which is 390 amino acids long with a specific domain architecture. To better understand polygenic diseases like type 2 diabetes, specific genomic studies are required. According to the Makhzoom et al.[27] studies on the genetic factors associated with metabolic syndrome risk, KCNJ11 rs5219 polymorphisms in the Syrian

Table 3
Analysis of the effect of nsSNPs in KCNJ11 structure, function, and evolution by MutPred server (http://mutpred.mutdb.org/).

Sample	Non-synonymous Mutation	Probability of deleterious Mutation	Top five features
***Pitta* (S3)**	**N265S**	0.705	Loss of catalytic residue at N265 (P = 0.1289) Gain of disorder (P = 0.1525) Gain of glycosylation at N265 (P = 0.2002) Loss of helix (P = 0.2662) Gain of loop (P = 0.2754)
***Kapha* (S7)**	**P78S**	0.554	Gain of catalytic residue at P78 (P = 0.007) Gain of glycosylation at P78 (P = 0.025) Loss of sheet (P = 0.0817) Gain of disorder (P = 0.2457) Gain of helix (P = 0.2684)
	P247T	0.546	Gain of glycosylation atP247 (P = 0.0273) Gain of phosphorylation at P247 (P = 0.0764) Gain of sheet (P = 0.1208) Loss of loop (P = 0.2237) Gain of catalytic residue at T246(P = 0.229)
	T291P	0.585	Gain of phosphorylation at T290 (P = 0.0644) Gain of ubiquitination at K287 (P = 0.123) Gain of glycosylation at T294 (P = 0.1275) Gain of helix (P = 0.132) Loss of stability (P = 0.1481)
***Kapha* (S6)**	**T89K**	0.764	Gain of methylation at T89 (P = 0.0101) Gain of MoRF binding (P = 0.0204) Gain of ubiquitination at T89 (P = 0.023) Gain of catalytic residue at T89 (P = 0.0463) Loss of glycosylation at T89 (P = 0.0493)
	P97A	0.432	Gain of MoRF binding (P = 0.0366) Loss of glycosylation at P97 (P = 0.0388) Gain of helix (P = 0.062) Loss of stability (P = 0.092) Gain of methylation at R101 (P = 0.1174)
	A112G	0.413	Gain of ubiquitination at K111 (P = 0.0633) Gain of sheet (P = 0.0827) Gain of disorder (P = 0.086) Loss of stability (P = 0.0966) Loss of glycosylation at P110 (P = 0.1247)

population are linked to an increased likelihood of developing T2DM. The study also highlights the significant impact of genetic factors on risk allele carriers, particularly when coupled with environmental factors. These observations suggest a potential correlation between phenotypic traits (*Prakriti*) and SNPs or epigenetic factors that could influence drug response and form the basis of personalized medicine approaches.

So far, no crystal structure is available for this protein in any database. In this study, 87 % coverage was achieved after sequencing the KCNJ11 gene. By comparing the Indian population control sequence with diabetic samples, several synonymous and nonsynonymous mutations were identified. The study was based on different *Prakriti* phenotypes, and damage was predicted in protein kir 6.2 for different *Prakriti* types.

In this study, we found non-synonymous mutations in the protein sequence of individuals with *Kapha Prakriti* (samples 7 and 6) samples of type 2 diabetic patients. These mutations significantly change the protein's function and structure; thus, they are of great interest. In sample 7, the mutations were found on 78,247,291 positions, leading to changes in amino acid residue: Serine to Proline, Threonine to Proline, and Proline to Threonine. In sample 6, the mutations were found on 89,97,112 positions, leading to changes in amino acid residue Lysine to Threonine, Alanine to Proline, and Glycine to Alanine. Proline is not well-suited for the helical structure, but it can introduce kinks into α-helices and is mainly found on the surface of proteins. On the other hand, threonine can be in a protein's interior and surface and form hydrogen bonds with various polar substrates using its reactive hydroxyl group. With its hydrogen side chain, Glycine has more flexibility to reside in protein structures off-limits to other amino acids, such as tight turns. Alanine is a non-polar amino acid that aids in substrate recognition or specificity, especially in interactions with other non-reactive atoms, like carbon. Lysine is a polar amino acid that frequently plays a crucial role in protein structure.[28] All three non-synonymous mutations in *the Kapha Prakriti* sample reside in the cytoplasmic region. In *Pitta Prakriti*, the non-synonymous mutation was found at position 265, where the amino acid residue Serine changed to Asparagine. Serine is an amino acid found both inside a protein and on its surface. Asparagine typically prefers to be on the surface of proteins and exposed to water. Serine is an amino acid found both inside a protein and on its surface. Asparagine typically prefers to be on the surface of proteins and exposed to water. These mutations in protein structure and function are related to the *Prakriti* of type 2 diabetic individuals and can lead to disease conditions. Recent epigenome-wide association studies have identified mutations in several genes, including preproinsulin and the ATP-sensitive potassium (K_{ATP}) channel, as the primary genes responsible for diabetes. Mutations in KCNJ11 and GATA binding protein 6 (GATA6) have also been linked to neonatal diabetes mellitus (NDM) and gestational diabetes mellitus (GDM).[12] In addition, by altering DNA methylation, the gut microbiota can influence epigenetic pathways and potentially impact genes related to NDM's glucose metabolism. In a separate study, the offspring of GDM and non-GDM mothers were compared for epigenetic differences.[29] The findings showed significant alteration in 51 genomic regions; five genes' methylation was linked to GDM. Several genes, such as KCNJ11, Hepatocyte nuclear factor 1α (HNF1A), and glucokinase (GCK), polymorphic variants, increased the risk of GDM. Thus, it was concluded that an interplay between genetic, epigenetic, and environmental factors causes GDM.

This is the first-time approach that has been used to identify disease-causing nsSNPs in KCNJ11. By creating a model of the protein's structure, we were able to visualize the location of nsSNPs in three-dimensional space. Our findings could help develop more effective medications that would be more precise for constitution-type individuals. Research on precision medicine will improve clinical accuracy and ultimately benefit healthcare delivery systems. Our molecular findings support the functions of *Ayurveda*, which tells that each constitution type, *Vata, Pitta*, and *Kapha,* has varied metabolic power, which is directly related to the function of gene KCNJ11, based on the metabolic environment of the cell. According to the *Prakriti*-based molecular studies on type 2 diabetes, it is evident that in *Kapha Prakriti,* major non-synonymous mutations were found. *Ayurveda* considers diabetes a *Kapha* predominant disorder, and *Kapha Prakriti* individuals are more prone to the disease. Our study establishes the molecular confirmation of the Ayurvedic basis of disease manifestation with reference to T2DM through in-silica tools.

5. Conclusions

A molecular study has investigated the KCNJ11 gene's correlation

with an individual's *Prakriti* and the manifestation of type 2 diabetes. This study revealed significant differences in the number and location of mutations and how they affect the protein's structure and function. Interestingly, individuals with *Kapha Prakriti* tend to experience a greater negative impact on the protein's function due to genetic factors. According to Ayurvedic principles, type 2 diabetes often starts with an imbalance in the *Kapha* dosha. People with a *Kapha*-dominant *Prakriti* who follow a *Kapha*-promoting lifestyle are at a higher risk of developing this disease compared to individuals with other types of *Prakriti*. Integrating *Prakriti* into medicine can help manage diabetes better and may even help prevent or reverse the disease, allowing for comprehensive metabolism correction beyond mere symptom management.

6. Limitation

The study focused on Eastern Uttar Pradesh, India. Future studies could involve a larger sample size and gather samples from various regions within the country or globally to achieve more precise outcomes.

Authors' contribution

The research was conducted by S.S., who also drafted the original article. S.G. contributed to designing and drafting the article. N.K.A. provided the necessary subjects for the study based on the criteria from his OPD and assisted in drafting the manuscript. G.S., a biostatistician, did the data analysis. S.K.S., D.S., and P.K. critically reviewed and edited the article. R.S. conceptualized, critically examined, and edited the final manuscript.

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Institutional review board statement

The Institute of Medical Sciences Ethics Committee has also approved the research work via letter No. Dean/2015–2016/1572 dated 30-12-2015.

Informed consent statement

Written informed consent was obtained from each participant.

Data availability statement

This article includes the data generated and analyzed for this study.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2024.01.004.

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