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Original Research

Spexin gene polymorphism in type 2 diabetes mellitus patients of South Indian population

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Abstract

Background and aims: The purpose of the study is to assess the concentrations of spexin (SPX) and other specific biochemical parameters, subsequently spexin (SPX) gene polymorphisms among south Indian healthy controls and in T2DM and T2DM +HTN patient's blood samples, which were selected according to certain inclusion and exclusion criteria. **Material and method:** Named and kit methods are used for the evaluation of spexin, biochemical parameters, and for spexin (SPX) gene polymorphism. **Results:** In this prospective study, spexin concentration is a negatively associated biomarker, and elevated levels of glucose, insulin resistance, liver enzymes, and inflammation are independently associated with assessing the diabetic and arterial hypertension disease states. The SPX gene sequencing illustrates exon mutation in the 6th position and no polymorphism is detected in the 2nd, 3rd, 4th, and 5th exons. Whereas intronic mutations are observed in the 3rd and 5th positions. Furthermore, heterogeneous mutations are observed in the 8th and 13th samples. Samples 10 and 14 showed homozygous of -42G>A mutation in exon 6. Samples 11, 12, 18, 19, 21, and 22 showed a heterozygous mutation (-42G>R) in the intron that is present 5 ´ to the exon 6. There is a deletion mutation in the acceptor site of the intron present 5 ´ to the exon 4. **Conclusion:** This is the first study reporting an association between spexin gene polymorphisms and the levels of spexin peptides.

Keywords: diabetes mellitus, exon, hypertension, intron, HOMA-IR, mutation, and spexin gene.

Background and aims

Type 2 diabetes mellitus (T2DM) and hypertension (HTN) are common concomitants. Hypertension is twice as recurrent in patients with diabetes compared with those who do not have diabetes. In addition, patients with hypertension frequently show signs of insulin resistance and are at greater risk of diabetes mounting than in healthy persons [1]. The chief cause of morbidity and mortality in diabetes is cardiovascular disease, which is exacerbated by hypertension. Thus, diabetes and hypertension are intimately interwoven because of alike risk factors, such as endothelial dysfunction, vascular inflammation, arterial remodeling, atherosclerosis, dyslipidemia, and obesity. There is also considerable overlie in the cardiovascular complications of diabetes and hypertension-related primarily to micro and macrovascular disease [2].

Spexin (SPX), namely neuropeptide Q (NPQ), is a newly identified peptide hormone. Spexin was first acknowledged in the human genome via bioinformatic practice. In humans, the gene that encodes SPX is located on chromosome 12, specifically C12orf39. SPX gene encloses 6 exons and 5 introns, encoding a prepropeptide of 116 amino acids. In humans, the precursor of

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spexin contains a signal peptide, two prohormone cleavage sites, and a predicted processed peptide [3].

Gene structure analysis indicates that spexin is more closely related to galanin than kisspeptin. A ligand-receptor contact study illustrates that spexins activate human, xenopus, and zebrafish GALR2/3 family receptors, suggesting that spexins are natural ligands for GALR2/3. Spexin arbitrates multiple biologic progressions, improves glucose management in obese mice with type 2 diabetes, and amazingly diminishes hepatic triglyceride both biochemically and histologically in mice with hepatic steatosis. Preliminary studies in mice with diet-induced obesity, type 2 diabetes, and hepatic steatosis imply that the admin of spexin may be effective in handling these three circumstances [4].

The objective of the study is to assess the concentrations of spexin (SPX) and other specific biochemical parameters, subsequently spexin gene polymorphisms among south Indian healthy controls, T2DM, and T2DM +HTN patients' blood samples. This is the foremost study that attempted to identify the single nucleotide polymorphism in spexin gene of diabetic patients.

Materials and methods

Subjects

A cross-sectional study design was conducted in the department of Biochemistry in collaboration with the Department of General Medicine attached to R L Jalappa hospital and Research Centre of Sri Devaraj Urs Academy of Higher Education and Research. The ethical approval granted by the Institutional Ethics Committee in vides No SDUMC/KLR/IEC/28/2019-20. Written informed consent was obtained from all the study participants during their visit to Hospital.

In a study, a total of 330 subjects in the age group of 40–60 years comprising both genders were subdivided into 3 clusters, namely healthy controls and T2DM, T2DM +HTN subjects with n=110 each. Whereas a genetic variance study was carried out in 30 subjects with n=10 each.

Inclusion criteria

Subjects with clinically proven T2DM with or without hypertension were included in the study. The criteria for diagnosis of T2DM as per the American Diabetic Association 2019 were considered with FBS ≥126 mg/dl, PPBS ≥200 mg/dl, and $HbA_{1c\geq}$6.5% [5].

Exclusion criteria

Familial hyperlipidemia, secondary diabetes, secondary hypertension, Patient with known cardiovascular diseases, Liver diseases, Patient with weight loss treatment, Pregnancy or breast feeding, surgical history of gall bladder removal, GIT disorders and CNS disorders, use of medications known to influence gastrointestinal tract, thyroid disorders, cancer patients and immune disorders [5].

Sample collection

Under aseptic conditions, 3 ml of venous blood samples were drawn from the antecubital vein from all study participants and transferred to plain and EDTA vacutainers. The plain vacutainers were allowed to refract for 20 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes to obtain a clear serum and EDTA vacutainers were processed to obtain plasma and stored at −80° until analysis.

Biochemical parameter

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a sphygmomanometer. The serum fasting blood sugar (FBS), postprandial blood sugar (PPBS), homeostatic model assessment- insulin resistance (HOMA-IR), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and C- reactive protein (CRP) were measured using Vitros 250 dry chemistry analyzer (Johnson and Johnson USA).

As per the provided commercial kit procedure, FBS by glucose oxidase peroxidase

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(GOD-POD) method. Serum ALT and AST by IFCC method, CRP by enzymatic heterogenous sandwich immunoassay method. The serum spexin concentration was measured by ELISA as per the protocol of commercial kit manufacturer instructions (Catalogue No KBH3507-Krishgen biosystems India).

DNA isolation

EDTA blood was taken into the Falcon tubes. The blood sample was mixed with erythrocyte lysis buffer and vortexed until the foam was formed. Then place it in the refrigerator for 30 minutes and then keep it at room temperature until it melts. Centrifuge the sample for 10 minutes and the supernatant was discarded. Add 12 ml of erythrocyte lysis buffer to it, mix well and centrifuge for 6 minutes till a white color pellet of heme was obtained, and further add 270 Ul of 20% SDS, and 30 Ul Proteinase K (10 mg/ml) mixed for 5 times. Samples were incubated in a boiling water bath at 37°C overnight. The next day, samples were taken from a water bath, and add 5 M 500 Ul NaCl and 11 ml isopropyl alcohol and gently mix until clear DNA is visible. The total precipitated thread-like DNA was transferred into a sterile microcentrifuge tube followed by the addition of 80% ethanol and centrifuged for 5 minutes at 12,000 rpm. The supernatant was discarded and the same step was repeated 3 times to obtain purified DNA and was allowed to dry for 30 minutes. Further, add 500 Ul of Tris-EDTA buffer to dissolve DNA, and to get a clear solution. The solution was incubated in a water bath at 65°C for 20 minutes. The overnight DNA solution was placed on the rocker the following day, isolated DNA was stored at –80°C.

PCR set up reactions

Reference sequence for spx gene (Accession No: NC_000012.12) was retrieved from the NCBI website. The primer pairs (forward and reverse) for each coding exon were designed and primers were purchased from (Bioserve Biotechnologies Pvt Ltd, India). Lyophilized primers were dissolved with tri buffer made up to final concentration which was followed by the instructions given in the kit. PCR master mix was purchased from (VNIR biotechnologies Pvt Ltd, Banglore). According to the instructions PCR master mix, the working stock was prepared by adding 2 µl of each gDNA (10–100 ng), 500 nM of each primer added to lyophilized VNIR 2X master mix for PCR, and the volumes were adjusted to 20 µl with deionized distilled water. PCR programs were: 95°C for 3 minutes (Activation), followed by 30 seconds of 95°C for denaturation, annealing 72°C for 45 seconds; 72°C extension for 1 minute, and the final extension was 72°C for 10 minutes.

DNA quantification by gel electrophoresis

Allow making the solution to lukewarm and 0.1 mg/ml ethidium bromide was added. The agarose gel was then poured into the gel-casting tray and allowed to harden. The agarose gel was positioned in an electrophoresis tank with 1X TAE buffer. DNA samples were mixed with a 6X DNA loading dye and loaded onto an agarose gel. The agarose gel was electrophoresed at 2 volts/cm and the images were captured in the gel documentation system (Bio-Rad Gel Dock) [6].

DNA purification

Samples were preceded for purification using QIAGEN QIAquick PCR Purification Kit (cat. No.28104). For the removal of primers, nucleotides, enzymes, mineral oil, salts, agarose, ethidium bromide, and other impurities from DNA samples, weight DNA fragments generated by non-specific amplification. 100 µl PCR product was added to 500 µl of binding buffer and mixed thoroughly. QIAquick spin column was placed into a 2 ml collection tube and the sample was loaded into the column and subjected to centrifugation for 30–60 seconds at 13,000 rpm. The flow-through was discarded and the QIAquick spin column was placed back in the collection tube. Add 0.75 ml wash buffer PE to the QIAquick spin column and centrifuge for 30–60 seconds at 13,000 rpm and the flow through was discarded

and the column was placed back in the collection tube and again subjected for 1-minute centrifugation. QIAquick spin column was placed in a sterile 1.5 ml vial, to elute DNA, add 50 ul of elution buffer to the center of the column, and centrifuged for 1 minute at 13,000 rpm. The eluted DNA was subjected again for centrifugation for 1 minute at 13,000 rpm and stored at −20°C.

Sanger di-deoxy chain termination method

Purified samples were taken for sequencing. DNA sequencing was performed for all the 6 exons of SPX gene with BigDye® Terminator v3.1 cycle sequencing kit using applied Biosystems™ MiniAmp™ Plus thermal cycler using Big Dye™ Terminator V3.1 kit according to the manufacturer's instructions. The results obtained from sequencing of all the exons were recorded and subjected for data analysis to identify any polymorphism in applied Biosystems 3730xL Analyzer.

Statistical analysis

The results of the study were presented as mean±SD and to know the significance between and among verified parameters in study groups. One-way analysis of variance (ANOVA) with post hoc Bonferroni analysis was used to compare the values between the three groups. Pearson correlation (r) is used to correlate between the parameters. The level of significance by 'p'-value ≤0.05 was considered significant in the analysis. Statistical data of the study was analyzed using a licensed version of SPSS software 22.0.

Results

In a prospective study of south Indian people, all the results were expressed in mean with standard deviation. First of all, the age and systolic and diastolic blood pressures of control individual were 45.5±10.0 years and 114.6±7.7 mmHg, 80.5±7.2 mmHg, whereas, for T2DM, 52.2±7.3 years and 136±516.9 mmHg, 82.6±2.9 mmHg and for T2DM +HTN patients were, 53.5±7.0 years and 151.1±12.3mmHg and 88.9±7.1 mmHg, respectively, with regard to systolic BP, there was a significant difference among all study subjects and the same was with diastolic BP as well.

In healthy controls, FBS, PPBS, and HOMA-IR values were 85.1±0.8, 99.5±1.4 mg/dl, and 7.03±0.1 μIU/ml correspondingly. While in T2DM outcomes were 191.7±7.0, 233.4±8.1 mg/dl, and 10.06±0.1 μIU/ml. Whilst in T2DM + HTN patients 191.9±5.8, 237.9±5.7 mg/dl, and 8.57±0.2 μIU/ml correspondingly. Results clearly reveal a positive correlation between sugar concentration and diabetic conditions. HOMA-IR correlates significantly with high insulin resistance in diabetic patients compared with hypertensive subjects.

In this study, we also examined the associations of serum liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in healthy controls, T2DM and for T2DM + HTN subjects, the outcomes for ALT were 19.9±0.7, 27.5±1.3 and 29.5±1.4 U/l whereas for AST 20.4±0.6, 27.5±1.3 and 32.8±2.3 U/l, respectively. Our results suggest that these serum liver enzymes were positively associated with an increased risk of T2DM and for T2DM + HTN.

Spexin concentration was assessed as 1.54±0.42, 0.40±0.13, and 0.23±0.07 ng/ml in control, T2DM, and T2DM + HTN individuals correspondingly. A significant difference is observed in spexin levels. Spexin concentration is less in patients compared with healthy individuals, amongst the patients, T2DM individuals showed higher values.

In this potential study of a middle-aged population, we observed elevated baseline CRP levels in people who developed T2DM and T2DM + HTN, among them HTN patients showed a lofty quantity of inflammatory protein. The evaluated values were 6.35±0.2, 14.7±1.0, and 26.5±2.1 mg/dl in healthy controls, T2DM, and T2DM +HTN, respectively. All the results were analyzed with Pearson and Bonferroni correlation to know the significance within and among the tested parameters. More purposely, Pearson correlation is best used to describe linear relationships. The Bonferroni method can be used to compare different groups at the baseline. The outcomes were displayed in Tables 1, 2, 3, and 4.

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Table 1: Biomarker spexin and biochemical variant levels in healthy, Type 2 diabetes mellitus (T2DM), Type 2 diabetes mellitus with hypertension (T2DM +HTN) individuals.

Variables	Controls (mean±SD) N=110	Type 2 diabetes mellitus (T2DM) (mean±SD) N=110	Type 2 diabetes mellitus with hypertension (T2DM +HTN) (mean±SD) N=110)	P=value
Age (years)	45.5±10.0	52.2±7.3	53.5±7.0	0.001
Systolic (mmHg)	114.6±7.7	136.5±16.9	151.1±2.3	0.001
Diastolic (mmHg)	80.5±7.2	82.6±2.9	88.9±7.1	0.001
Fasting blood sugar (FBS) (mg/dL)	85.1±0.8	191.7±7.0	191.9±5.8	0.001
Post prandial blood sugar (PPBS) (mg/dL)	99.5±1.4	233.4±8.1	237.9±5.7	0.001
Homeostatic model assessment- Insulin resistance (HOMA-IR) (uIU/ml)	7.03±0.1	10.06±0.1	8.57±0.2	0.001
Alanine aminotransferase (ALT) (U/L)	19.9±0.7	27.5±1.3	29.5±1.4	0.001
Aspartate aminotransferase (AST) (U/L)	20.4±0.6	27.5±1.3	32.8±2.3	0.001
SPEXIN (ng/ml)	0.79±0.03	0.65±0.03	0.48±0.02	0.001
C-reactive protein (CRP)	6.35±0.2	14.7±1.0	26.5±2.1	0.001

Table 2: Significance in between evaluated variables and studied groups of South India

Variables	Controls Vs Type 2 diabetes mellitus (T2DM) (N=110))	Control Vs Type 2 diabetes mellitus with hypertension (T2DM +HTN) (N=110)	Type 2 diabetes mellitus (T2DM) & Type 2 diabetes mellitus with hypertension (T2DM +HTN) (N=110)
Age (years)	0.001^{***}	0.001^{***}	>0.05
Systolic (mmHg)	0.001^{***}	0.001^{***}	0.001^{***}
Diastolic (mmHg)	0.002^{***}	0.001^{***}	0.001^{***}
Fasting blood sugar (FBS) (mg/dL)	0.001^{***}	0.001^{***}	>0.05
Post prandial blood sugar (PPBS) (mg/dL)	0.001^{***}	0.001^{***}	>0.05
Homeostatic model assessment- Insulin resistance (HOMA-IR) (uIU/ml)	0.001^{***}	0.001^{***}	0.001^{***}
Alanine aminotransferase (ALT) (U/L)	0.001^{***}	0.001^{***}	>0.05
Aspartate aminotransferase (AST) (U/L)	0.007^{**}	0.001^{***}	0.006^{**}
SPEXIN (ng/ml)	0.003^{**}	0.001^{***}	0.001^{***}
C-reactive protein (CRP) (mg/dL)	0.001^{***}	0.001^{***}	0.001^{***}

The mean difference is significant at the <0.05 level. Highly significant indicates $p \leq 0.01^{***}$.

Table 3: Pearson correlation between biochemical variables and biomarker spexin in Type 2 diabetes mellitus (T2DM)

Variables	Spexin	
	r	P
Age (years)	0.09	0.30
Systolic (mmHg)	- 0.09	0.34
Diastolic (mmHg)	0.02	0.83
Fasting blood sugar (FBS) (mg/dL)	0.002	0.98
Post prandial blood sugar (PPBS) (mg/dL)	0.24**	0.09
Homeostatic model assessment- Insulin resistance (HOMA-IR) (uIU/ml)	0.11	0.22
Alanine aminotransferase (ALT) (U/L)	0.15	0.98
Aspartate aminotransferase (AST) (U/L)	0.006	0.34

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 4: Pearson correlation between biochemical variables and biomarker spexin in Type 2 diabetes mellitus with hypertension (T2DM+HTN)

Variables	Spexin	
	r	p
Age (years)	- 0.061	0.52
Systolic (mmHg)	0.05	0.54
Diastolic (mmHg)	0.16	0.08
Fasting blood sugar (FBS) (mg/dL)	-0.06	0.58
Post prandial blood sugar (PPBS) (mg/dL)	-0.08	0.39
Homeostatic model assessment- Insulin resistance (HOMA-IR) (uIU/ml)	0.01	0.87
Alanine aminotransferase (ALT) (U/L)	0.02	0.83
Aspartate aminotransferase (AST) (U/L)	0.04	0.61

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 5: Spexin gene primers and the sequence information gathered from the NCBI Primer BLAST.

Sl No.	FP (5' – 3')	RP (5' – 3')	PCR amplicon
SPX_exn2	GAAGGTAGAAGGGAAACACCTC	CATTGCAGCTCAGTCCTCTATT	365bp
SPX_exn3	TGTCCACTCTGCTCTTTCTT	TCCTAGGGGTCTTCCATTAT	231bp
SPX_exn4	GGAGGAGCTGAGGTTTAAG	GTGAGGAGACGCTTTCTTT	238bp
SPX_exn5	TGCTGCATGTTAGAATAGGA	CACAGAATCCCGAAAGTAAG	228bp
SPX_exn6	TGTGTCCCAAGCTGAGAAAA	AACAGGACCTGAAGCAATGAAA	453bp

Furthermore, our study also deals with genetic variance among 30 subjects (10 healthy controls and 10 T2DM, 10 T2DM + HTN). The extracted DNA was amplified by PCR with primers and the sequence information of these primers was gathered from the NCBI Primer-BLAST and displayed in Table 5.

The amplification was assessed by running the amplicons along with the DNA markers on 1.5% Agarose gel electrophoresis and the

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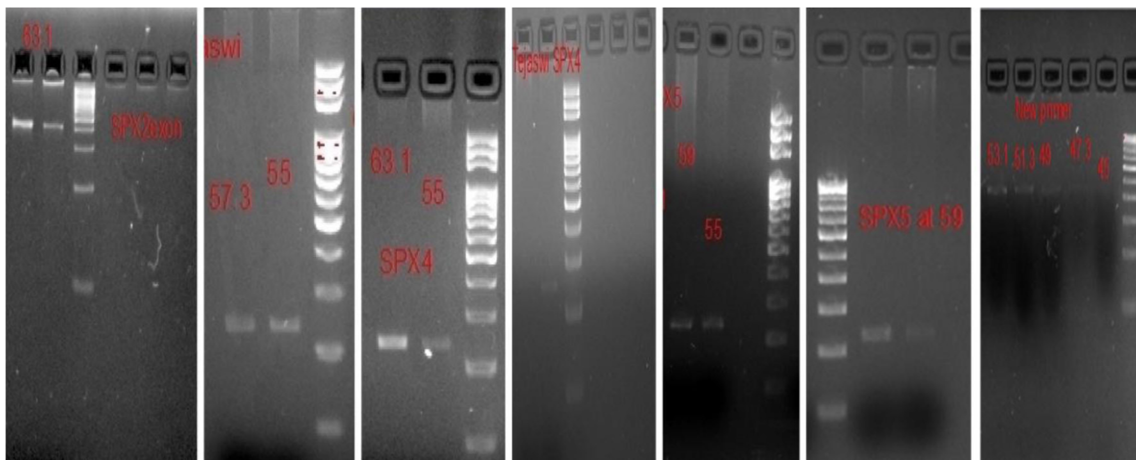

Figure 1: The ethidium bromide stained 1.5% agarose gels of amplified mutated spexin DNA samples.

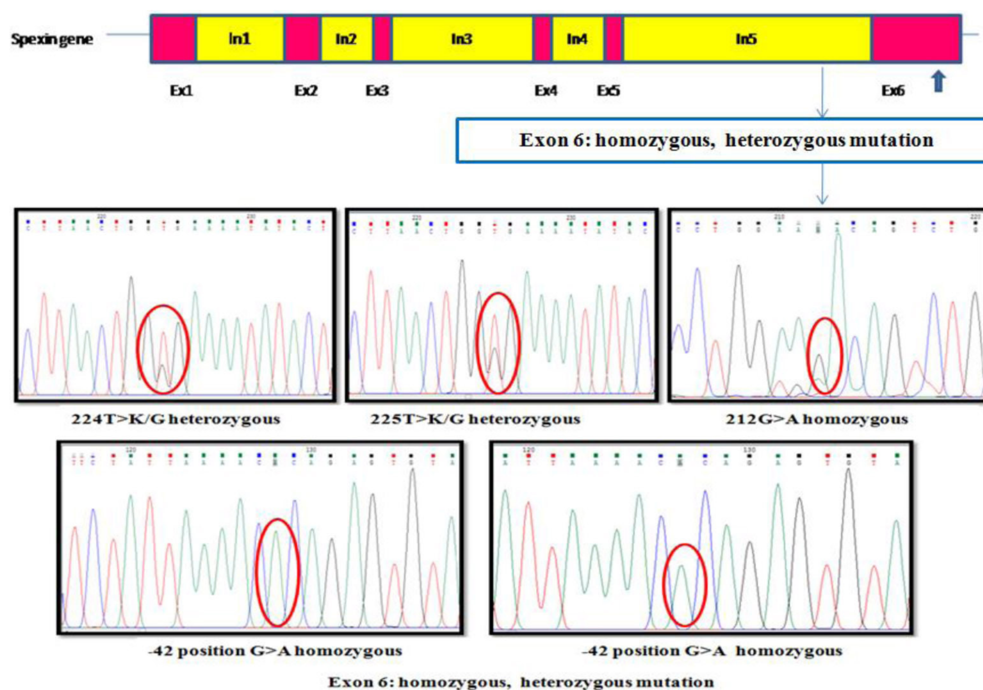

Figure 2: Sixth exonic homozygous and heterozygous mutation.

bands were visualized in Bio-Rad Gel Doc System. The amplified DNA products that exhibited prominent and clear bands on the gel were subjected to further purification. The ethidium bromide-stained agarose gel photographs were displayed in Figure 1.

Subsequently, DNA sequencing was done by fluorescence-labeled dNTPs were added to amplicons as it was synthesized. The information was translated to a sequence in an electropherogram. Fluorescent fragments were generated by the incorporation of dye-labeled ddNTPs. All dissimilar ddNTP (ddATP, ddCTP, ddGTP, or ddTTP) will bear a different-colored dye. All terminated fragments (those ending with a ddNTP), therefore, contain a dye at their 3′ end. The products from this reaction were injected into one capillary and were renowned as individual nucleotides with unique fluorophores that were recognized by the laser. The information from the laser was captured by photomultiplier tubes to generate an electropharagram that represents the unique sequence.

After sequencing, the NCBI database exploited for the genetic polymorphism verification, as an integrated part of NCBI, the contents of a database of single nucleotide polymorphisms are cross-linked to records in other information

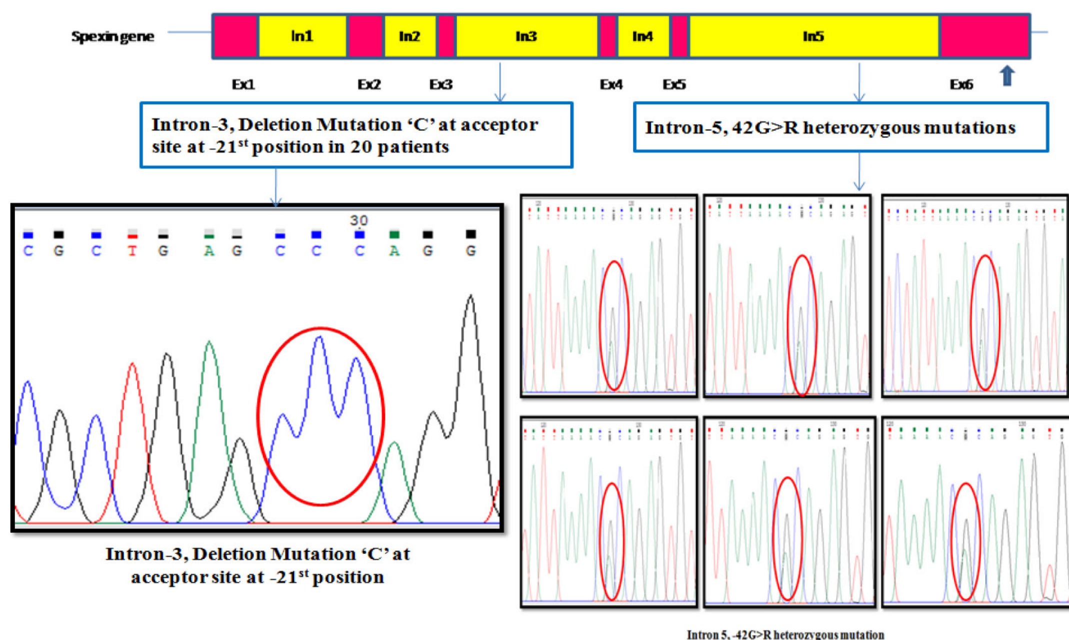

Figure 3: Intron 3 deletion mutation and intron 5 heterozygous mutation

resources such as GenBank, LocusLink, the human genome sequence and PubMed. The result sets from queries in any of these resources will point the user back to the relevant records in dbSNP. Only a few samples show variation in the sequence. Genetic background may accelerate the growth of T2D and arterial hypertension in the context of rapid nutrition transition. Epidemiologic studies and randomized clinical trials show that these were mostly avoidable through diet and lifestyle amendments.

The varied sequencing results of the SPX gene showed exon mutation in the 6th position and no polymorphism was detected in the 2nd, 3rd, 4th, and 5th exons. Whereas intronic mutations were observed in the 3rd, and 5th positions. Furthermore, heterogeneous mutations were observed in the 8th and 13th samples. In these samples stop codon (T>K, where K=T or G) mutated, and that codes for glycine, because of that translation continues up to 17 amino acids further until a stop codon stops the translation.

In the alignment, there seems to be A but in the chromatogram, it looks like it's G because of G>R mutation it looks like it was Asp to Asn mutation but it is difficult to say anything about this position as per the chromatogram. The data looks noisy to conclude whether it is G or R in this position.

Samples 10 and 14 showed homozygous of -42G>A mutation in exon 6. Samples 11, 12, 18, 19, 21, and 22 showed a heterozygous mutation (-42G>R) in the intron that was present 5' to the exon 6. The significance of this intronic mutation was not known but might cause an early metabolic syndrome.

There was a deletion mutation in the acceptor site of the intron present 5' to the exon 4. In this, there was the deletion of one C in the polypyrimidine tract at -21st position from the intron-exon boundary. Whether this deletion makes any abruption in the splicing was affected or it leads to alternate splicing, not very clear as it needs further experimental investigation. Exonic and intronic homozygous and heterozygous mutations along with fluorescence electrogram were put on a show in Figures 2 and 3.

Discussion

Metabolic disorders including T2DM and arterial hypertension diseases are closely related to the aging process. Obesity and insulin resistance as the early preconditions and their effects associated with metabolic diseases are recurrently established among the aged [7]. The decline in lean body mass and increase in body fat, particularly visceral adiposity that often goes with

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aging, may add to the progress of insulin resistance [8].

Hypertension is a silent destroyer as rarely any signs can be seen in its early stages until a severe medical crisis takes place like a heart attack or chronic kidney disease. Since people are ignorant of extreme blood pressure, it is barely through measurements that detection can be done. Although the majority of patients with hypertension remain asymptomatic, some people with HTN report headaches, lightheadedness, vertigo, altered vision, or fainting episode [9].

Cardiovascular (CV) risk aspects such as obesity, hypertension, and dyslipidemia are widespread in patients with diabetes mellitus, placing them at raised danger for cardiac events. In addition, many studies have found biological mechanisms associated with diabetes mellitus that autonomously boost the risk of CVD in diabetic patients [10, 11].

The universal secular drift of increased diabetes incidence likely has multiple etiologies, which may proceed through multiple mechanisms. Previous reports reveal that sugar concentration is a significant statistical determinant of diabetes prevalence rates worldwide [12, 13]. In a parallel way, we acknowledged that sugar concentration appears to be uniquely correlated to diabetes prevalence free of other metabolic syndromes.

Insulin resistance is a trademark of obesity, diabetes, and cardiovascular events, and leads to many of the abnormalities associated with metabolic syndrome. The quantitative measurement of insulin sensitivity is not regularly used during biochemical examinations for diagnostic purposes, but the budding significance of insulin resistance has led to its wider application in research studies [14].

The HOMA-IR has been established to be a robust medical and epidemiological means for the assessment of insulin resistance. It is a useful method not only for diagnosing insulin resistance but also for follow-up during the treatment of patients with T2DM [15].

The liver plays an important role in the protection of standard glucose levels during fasting as well as in the postprandial period, and its task in the pathogenesis of T2DM has fascinated much interest. Indeed, hepatic dysfunction resulting from insulin-resistance syndrome may lead to the development of T2DM [16].

The liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are regularly employed in the evaluation of liver function and are considered markers of hepatocellular health. ALT is the precise marker of liver pathology and is found primarily in this organ. AST is found in other tissues and is a less specific marker of liver function [16]. A number of prospective studies have examined the associations between concentrations of AST and ALT and the incidence of type 2 diabetes. This study provides evidence that elevated AST and ALT levels may be associated with increased risk for T2DM. As the scientific confirmation is insufficient, more research is needed into the prognostic significance of liver function tests for incident cardiovascular aspects [17–19].

Earlier reports have established that small raises in inflammatory marker CRP predict the probability of mounting cardiovascular events both in diabetic and nondiabetic populations. In addition, in healthy subjects, amplified levels of CRP predict the risk of developing T2DM. Previous evidence reveals that CRP, besides its predictive role in formative cardiovascular risk, may represent an active participant in atherogenesis [20, 21].

Several single-nucleotide polymorphisms (SNPs) have been already associated with an increased risk of hypertension and T2DM, while their search is still ongoing. In addition, novel links between these disorders come from epigenetic studies. In this study, we analyzed the genetic variation in the SPX gene (Accession No: NC_000012.12) and analyzed whole exon and intron sequences among healthy controls and type 2 diabetes mellitus and arterial hypertension patients in the south Indian population. Previously very few researchers worked on genetic polymorphisms.

Xia et al. [22] investigated the short tandem repeat (CA)n polymorphism in G-protein–coupled receptor kinase 5 (GRK5) rs10886471 and further discussed its role in the T2DM risk of Chinese Hainan Island individuals.

Likewise, Abdul Azeez et al. [23] identified single nucleotide polymorphisms (SNPs) on

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chromosome 9p21.3 conferring the risk for CAD (coronary artery disease) in individuals of Caucasian ancestry.

Guerini et al. [24] focused on a complex of soluble-N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which play a key role in metabolic syndrome and insulin resistance, involved in endothelial dysfunction and heart disease. They assessed genetic variants of the SNARE genes are associated with ischemic heart disease (IHD) or not.

Recently Chen et al. [25] not only provide new information on the signal transduction for gastric expression of SPX but also unveil a novel feedback interaction between insulin and glucose for SPX regulation in the mouse model, which may have practical allegations on appetite control and energy balance in mammalian species. All the preceding statements are in agreement with our results.

Conclusion

In this prospective study, spexin concentration was a negatively associated critical biomarker and elevated levels of sugars, insulin resistance, liver enzymes, and inflammation are independently associated for assessing the diabetic and arterial hypertension states. This is the first study reporting an association between spexin gene polymorphisms and the levels of spexin peptides. A susceptible genetic background appears to be necessary for the development of ailment complications. Western lifestyles, obesity, dietary supplements, exposure to organic pollutants serve as non-genetic environmental factors which influence T2DM and diabetic vascular complications. Additional research is needed to better understand the disease process and its effects on health in order to improve the medical management of the patients.

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Conflict of interest

The authors declare no conflict of interest.

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