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Research article

Investigating the association of microRNA polymorphisms and lifestyle factors with the susceptibility to common gastrointestinal cancers in an Indian population- A case control study

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ABSTRACT

The cancers of the gastrointestinal (GI) tract have become a common diagnosis worldwide contributing to a large number of mortalities. Though potentially curable they are mostly fatal due to late diagnosis and lack of accurate diagnostic markers. microRNA, micromanagers of gene expression have been associated to have distinct roles as oncogenes or tumour suppressors in several cancers including GI cancers. These miRNAs are known to harbour single nucleotide polymorphisms (SNPs) that lead to loss or gain of its functions and have been found to be associated with altering susceptibility of several cancers. The current study aimed to investigate the role of miRSNPs in common gastrointestinal cancers. A case control study was designed which included 210 GI cancer cases and 230 cancer free controls. The miRSNPs were successfully genotyped using MassARRAY technique. Association analysis revealed that *miR-196*a; rs11614913, pre-mir-423; rs6505162, pre-mir-605; rs2043556, pre-mir-149; rs2292832 and pri-mir-30c; rs928508 polymorphisms significantly altered the risk of common GI cancers. Multifactor dimensionality reduction analysis demonstrated that miRSNPs alter GI cancer risk by interacting with exposures like diabetes mellitus, alcohol consumption, diet and socioeconomic status in the study subjects. In conclusion it was found that presence of miRNA polymorphism and certain lifestyle factors alters susceptibility to GI cancers significantly.

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1. Introduction

The GI tract has an actively renewing epithelium with the most rapid turnover rate in the entire human body, making it more prone to neoplastic changes [1]. Malignancies of the GI tract such as tumors of the primary GI tract like colon, rectum, stomach, esophagus, anus and tumors of the secondary GI tract like gallbladder, pancreas, liver and bile duct amount for 23 % of the world's cancer deaths. After years of research on understanding these tumors, they still exhibit high mortality rates despite being highly curable. Factors such as late diagnosis, improper staging and resistance to treatments contribute to this irony. The 2022 GLOBOCAN official release on cancer statistics lists GI cancers, viz. colorectal cancer (CRC), gastric cancer (GC) and esophageal cancer (EC) as the top contributors to cancer deaths worldwide with CRC and GC occupying the top 5 and EC finding its place among top 10. When considering the incidence and mortality rates of common GI cancers such as CRC, GC and EC it was noted that India was the fourth most affected country.

microRNAs (miRNAs) are small but indispensable molecules of the cellular machinery that orchestrate the expression of almost one third of the human genome. They are noncoding RNA molecules transcribed by the nuclear genome and measure up to 22 nucleotides. They carry out their regulatory function by often binding to the 3′UTR of mRNA and destabilizing them leading to mRNA degradation or repression of translation. Ever since their discovery, miRNAs have been frequently associated with cancers, this was supported by the evidence that more than half of the known miRNAs are located in the fragile regions of genome associated with cancers [2]. miRNAs are said to play dual roles as oncogenes and tumor suppressors in cancer. As an oncogene, they regulate genes involved in tumorigenesis, proliferation, apoptosis, angiogenesis, invasion and migration and facilitate the progression of cancers. As tumor suppressors, miRNAs downregulate oncogenes or upregulate tumor suppressor genes. Several miRNA signatures have been observed in many cancers including breast, leukaemia, lung, thyroid and stomach.

Single nucleotide polymorphisms (SNPs) are single base variations abundantly present in the genome whose effect varies depending on their genomic location. Generally, polymorphisms do not cause a disease but may increase or decrease the susceptibility to a disease [3]. miRNAs are well appreciated for their stringent conservation across several organisms making it difficult for SNPs to occur. However, the past decade has witnessed several research studies reporting the presence of polymorphisms in miRNAs. These microRNA polymorphisms (miRSNPs) constitute a novel class of polymorphisms with functional effects due to their sequence dependent regulatory system. Based on the region where the polymorphisms exist, they can be classified into, miRNA gene polymorphisms, SNPs in miRNA binding regions in mRNAs (binding site polymorphisms) and polymorphisms in miRNA biogenesis pathways. Presence of SNPs in miRNAs lead to loss of function or gain in function of miRNAs leading to dysregulation of gene expression causing disease phenotypes [4].

Carlo Corce et al., were the forerunners who found a germline mutation in *pri-mir-16*-1 that was associated with familial chronic lymphocytic leukaemia and found to decrease expression of the *miR-16-1* [5]. This was followed by a study on *miR-146a* polymorphism rs2910164 in papillary thyroid carcinoma (PTC) where the polymorphism increased cancer susceptibility by affecting miRNA expression [6] and till date a few miRNAs and their polymorphisms have been investigated in many cancers. Polymorphisms in *miR-196a, miR-499a, miR-149, miR-27a, miR-423* and *miR-let-7a2* have been found to be associated with the risk of common GI cancers but limited mostly to European and Chinese populations [7,8]. Thus, it is clear that microRNAs and their variants play an important role in altering the susceptibility to various cancers in different populations. They have immense potential to serve as population based genetic markers to predict risk, for early diagnosis and better management of disease outcome. In our previous studies we found that *miR-146a* rs2910164 polymorphism significantly increased the risk of CRC, whereas, *miR-27a* gene polymorphisms rs895819 and rs11671784 were not associated with GI cancers [9,10]. Therefore, we aimed to explore the role of various other microRNA polymorphisms in gastrointestinal cancers and analyse how they present themselves along with other environmental factors in subjects from the southern states of India.

2. Materials and methods

2.1. Study population and sample collection

The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (Deemed to be University) IEC-NI/13/APR/33/39 and was conducted as per ICMR ethical guidelines for biomedical research and the declaration of Helsinki.

The recruitment of subjects has been elaborately described in our earlier publication [10]. A total of 210 GI cancer patients and 230 cancer free controls were recruited for the study.

Around 3 mL of venous blood collected for routine clinical investigations was received from the Central laboratory of Sri Ramachandra Medical Centre and clinical profile of subjects was retrieved from the hospital medical records department. Basic characteristics of the subjects were obtained by interviewing them by means of a structured questionnaire. The identity and privacy of the participants were preserved throughout the study.

2.2. Selection of microRNA polymorphisms for the study

A total of 18 SNPs in 17 microRNA genes were identified from miRNA databases and literature. All the miRNAs were regulators of genes involved in cancer pathways. The polymorphisms selected were limited to the miRNA genes and flanking regions. The primary miRNA energy, SNP miRNA energy and $\triangle$G was obtained from miRNA SNP database V2.0.

2.3. SNP genotyping

Genotyping was done by using MassARRAY kits purchased from Agena Bioscience Inc, San Diego, CA, USA. The MassARRAY system implies the Matrix Assisted Laser Desorption/Ionisation-Time of Flight methodology (MALDI-TOF) [11]. The major steps involved in it are multiplex PCR, shrimp alkaline phosphatase (SAP) reaction, Extension reaction and MALDITOF MS.

The SNP IDs selected for the study were submitted to the assay design software for primer and assay design. The extension and PCR primers sequences were retrieved from the software and procured commercially from Integrated DNA Technologies, Inc. USA.

The multiplex PCR was set up with 4 μL of multiplex PCR cocktail and 2 μL of the appropriate genomic DNA sample (5–10 ng/μL) in each well of a 384-well microtiter plate. The plate was then centrifuged at 10,000 rpm for 1 min and was run in the thermocycler with the following reaction conditions; initial denaturation at 95 °C for 15 min, annealing at 56 °C for 30 s, extension at 72 °C for 1 min. Steps 1–3 were repeated for 45 cycles following which final extension was carried out at 72 °C for 3 min. The SAP reaction was done by adding 2 μL of the SAP mixture to each sample in the 384-well microtiter plate. The plate was incubated in a thermocycler at 37 °C for 40 min, 55 °C for 5 min and stored at 4 °C.

The iPLEX Gold reaction mixture was prepared as per manufacturer's instructions and 2 μL of it was added to each sample in the 384-well microtiter plate. The plate was sealed with the sealant and cycled in a ABI thermo cycler with the following reaction conditions; 94 °C for 30 s, 94 °C for 5 s, 5 cycles at 52 °C for 5 s and 80 °C for 5 s, 72 °C for 3 min and stored at 4 °C after completion of 40 cycles.

The samples were conditioned with 6 mg of clean resin per well to remove unwanted ions that could interfere with the MALDI-TOF MS analysis. The samples were then dispensed on to the SpectroCHIP by the Nano dispenser. The SpectroCHIP spotted with the samples was placed in the chip slot of the mass spectrometer. The results were viewed as cluster plots using Typer Tool 4.0.

2.4. Statistical analysis

The concordance of genotype frequencies with Hardy – Weinberg Equilibrium (HWE) was calculated using the chi-square test. SNPs with P value more that 0.05 was considered to be concurrent with HWE. The association of the genotypes and alleles with GI cancer was done by calculating the odds ratio with 95 % CI through Binary logistic regression while P value less than 0.05 was considered significant. The crude odds ratio was adjusted for covariates using multinomial regression. The statistical tests were done using SPSS v16.0. The interaction between the SNPs and environmental parameters were tested using Multifactor dimensionality reduction (MDR) analysis by MDR software v3.2 [12].

3. Results

3.1. SNPs selected for the study

A total of 18 miRSNPs; *miR-196a*; rs11614913, *miR-499a*; rs3746444, *miR-149*; rs2292832, *miR-25*; rs41274221, *miR-26a-1*; rs7372209, *miR-218-2*; rs11134527, *miR-124-1*; rs531564, *miR-92a-1*; rs9589207, *miR-608*; rs4919510, *miR-605*; rs2043556, *miR-34b/c*; rs4938723, *miR-603*; rs11014002, *miR-30c*; rs928508, *miR-423*; rs6505162, *miR-Let7a2*; rs629367, *miR-27a*; rs895819, rs11671784 and *miR-146a*; rs2910164 were identified from miRNA databases and literature. . SNPs rs2910164, rs11671784 and rs895819 were analysed in our earlier study [9,10]. In the current study the results will be included in the MDR analysis to check for SNP-SNP interactions and Genotype-Phenotype interactions. The results of the 15 SNPs have been presented below.

3.2. Distribution of miRNA polymorphisms and compliance with hardy weinberg equilibrium

Features of the study population such as age, sex and basic characteristics have been represented already. The genotype and allele frequencies of all the 16 miRSNPs were calculated and tested for HWE using chi square test. All the polymorphisms which were concordant with HWE in the control group were included for the study. It was found that polymorphisms rs3746444, rs531564, rs4919510 and rs9589207 present in *miR-499*, *miR-124-1*, *miR-608* and *miR-92a-1* genes respectively were not in HWE in the control group and were excluded from further analysis. HWE concordance in the control group is the standard criteria for association analysis. The HWE deviation could be due to genotyping error or population stratification effect. All the other polymorphisms were in HWE. The *miR-25* rs41274221 polymorphism was not observed in our study population (Table 1).

3.3. Association of miRNA polymorphisms with common GI cancers

3.3.1. Association of miR-196a polymorphism rs11614913 with GI cancers

The dominant model showed an increase in GI cancer risk in model a ($OR^a = 1.52$, $p^a = 0.051$). Further the analyses revealed that 'TT', 'T' genotype and alleles significantly increased the risk of GI cancers (CC vs TT; OR = 2.35, p value = 0.024, $OR^a = 2.58$, $P^a = 0.020$, $OR^b = 2.31$, $P^b = 0.035$ & C Vs T; OR = 1.44, p value = 0.021, $OR^a = 1.52$, $P^a = 0.013$, $OR^b = 1.4$, $p^b = 0.041$) while the recessive model showed that the 'CC' genotype had a protective effect (Recessive; OR = 0.47, p value = 0.041, $OR^a = 0.44$, $p^a = 0.038$, $OR^b = 0.48$, $p^b = 0.050$) against GI cancer. In the subgroup'others' we found that the 'TT' genotypes significantly increased the risk of these cancers. However, due to the small numbers further warranting of these results is needed for the others group (Table 2). The allele frequencies obtained from the study were compared with frequencies from worldwide populations obtained from 1000 genomes

Table 1
Frequency distribution of the genotypes and HWE analysis of the selected miRNA polymorphisms.

microRNA	SNP	Base Change	Control Genotypes N (%)			MAF	HWE	Case Genotypes N (%)			MAF	HWE
			Wt	Hz	Mu		X^2 (p value)	Wt	Hz	Mu		X^2 (p value)
miR-92a-1	rs9589207	G/A	199(99.5)	0(0)	1(0.5)	0.01	200(**0.00**)	198(99)	0(0)	2(1)	0.01	200(**0.000**)
miR-608	rs4919510	C/G	93(46.5)	77(38.5)	29(15.0)	0.34	4.22(**0.040**)	93(46.7)	87(43.7)	19(9.5)	0.31	0.04(0.835)
miR-605	rs2043556	T/C	118(59)	65(32.5)	17(8.5)	0.25	3.25(0.071)	126(63)	67(33.5)	7(3.5)	0.20	0.28(0.6)
miR-34b/c	rs4938723	C/T	123(61.5)	45(22.5)	5(2.5)	0.22	0.53(0.467)	152(76)	45(22.5)	3(1.5)	0.26	0.026(0.873)
miR-603	rs11014002	C/T	157 (159.3)	43(38.4)	0 (2.3)	0.11	2.902(0.089)	155(77.5)	37(18.5)	8(4.0)	0.46	7.625(**0.006**)
miR-30c	rs928508	A/G	69(34.5)	92(46.0)	39(19.5)	0.43	0.692(0.401)	54(27.0)	106(53.0)	40(20.0)	0.47	0.851(0.356)
miR-423	rs6505162	A/C	63(31.7)	89(44.7)	47(23.6)	0.46	1.98(0.1596)	72(36.2)	82(41.2)	45(22.6)	0.43	5.121(**0.024**)
miR-Let7a2	rs629367	A/C	129(64.5)	62(31)	9(4.5)	0.20	0.195(0.659)	121(60.5)	65(32.5)	14(7)	0.23	1.597(0.206)
miR-27a	rs11671784	G/A	183(96.3)	7(3.7)	0(0)	0.02	0.07 (0.796)	172(94)	10(5.5)	1(0.5)	0.19	3.51 (0.061)
miR-196a	rs11614913	C/T	97(48.5)	79(39.5)	24(12)	0.32	1.569(0.06)	115(57.5)	73(36.5)	12(6.0)	0.24	0.0008(0.927)
miR-499	rs3746444	A/G	91(45.5)	10(5)	99(49.5)	0.52	161.94(**0.00**)	104(52)	5(2.5)	91(45.5)	0.47	180.42(**0.000**)
miR-149	rs2292832	T/C	62(31)	90(45)	48(24)	0.47	1.83(0.177)	64(32)	90(45)	46(23)	0.46	1.72(0.190)
miR-25	rs41274221	C/T	200(100)	0(0)	0(00)	0.10	220(**0.000**)	202(100)	0(0)	0(0)	0.00	–
miR-26a-1	rs7372209	C/T	150(75)	45(22.5)	5(2.5)	0.14	0.53(0.467)	152(76)	45(22.5)	3(1.5)	0.13	0.03(0.873)
miR-218-2	rs11134527	A/G	60(30)	95(47.5)	45(22.5)	0.46	0.398(0.528)	60(30.0)	92(46)	48(24)	0.47	1.176(0.278)
miR-124-1	rs531564	C/G	188(94)	10(5)	2(1)	0.04	13.5(**0.000**)	179(89.5)	19(9.5)	2(1.0)	0.06	3.05(0.081)

Wt, wild type, Hz, Heterozygous, Mu, Mutant, MAF, Minor allele frequency, HWE, Hardy Weingberg Equilibrium. bold p values in controls represent SNPs not in HWE.

Table 2
Association analysis of *miR-196a* rs11614913 SNP with GI cancers.

Cancer Site	Model	OR	95 % CI	p Value	OR[a]	95 % CI	p[a] Value	OR[b]	95 % CI	p[b] Value
GIC	Dominant	1.42	096–2.11	0.079	1.52	1–2.31	**0.051**	1.36	0.90–2.05	0.142
	Recessive	0.47	0.23–0.97	**0.041**	0.44	0.21–0.96	**0.038**	0.48	0.23–1.00	**0.050**
	CC Vs CT	1.27	0.84–1.93	0.259	1.35	0.87–2.10	0.180	1.21	0.78–1.87	0.391
	CC Vs TT	2.35	1.12–4.95	**0.024**	2.58	1.16–5.75	**0.020**	2.31	1.06–5.04	**0.035**
	C Vs T	1.44	1.06–1.97	**0.021**	1.52	1.09–2.11	**0.013**	1.4	1.01–1.93	**0.041**
GC	Dominant	1.21	0.71–2.05	0.479	1.3	0.72–2.34	0.381	1.18	0.69–2.02	0.557
	Recessive	0.3	0.09–1.02	**0.053**	0.34	0.08–1.31	0.117	0.3	0.1–1.04	0.058
	CC Vs CT	1.01	0.59–1.75	0.966	1.15	0.62–2.13	0.653	0.99	0.57–1.74	0.977
	CC Vs TT	3.38	0.96–11.8	0.057	3.1	0.78–12.2	0.107	3.26	0.91–11.7	0.069
	C Vs T	1.37	0.90–2.09	0.140	1.38	0.87–2.21	0.172	1.35	0.88–2.06	0.175
CRC	Dominant	1.51	1–2.56	0.122	1.64	0.95–2.83	0.075	1.56	0.91–2.68	0.110
	Recessive	0.7	0.29–1.7	0.436	0.59	0.24–1.50	0.274	0.7	0.28–1.74	0.445
	CC Vs CT	1.47	0.84–2.59	0.179	1.54	0.86–2.75	0.144	1.53	0.85–2.75	0.157
	CC Vs TT	1.67	0.67–4.13	0.275	2.12	0.79–5.64	0.134	1.78	0.69–4.59	0.233
	C Vs T	1.4	0.92–2.11	0.116	1.52	0.99–2.34	0.056	1.43	0.93–2.19	0.107
EC	Dominant	1.16	0.49–2.75	0.739	1.26	0.52–3.04	0.604	1.22	0.49–3.03	0.664
	Recessive	0.69	0.15–3.17	0.642	0.66	0.14–3.08	0.597	0.7	0.15–3.30	0.655
	CC Vs CT	1.09	0.44–2.71	0.860	1.21	0.48–3.06	0.694	1.15	0.44–3.03	0.772
	CC Vs TT	1.49	0.31–7.08	0.62	1.46	0.3–7.16	0.639	1.58	0.30–8.21	0.587
	C Vs T	1.18	0.60–2.32	0.629	1.26	0.63–2.49	0.516	1.22	0.60–2.49	0.580
Others	Dominant	2.97	1.03–8.57	**0.044**	2.87	0.98–8.36	**0.054**	2.73	0.92–8.13	0.071
	CC Vs CT	2.28	0.79–6.61	0.129	2.29	0.78–6.7	0.131	2.07	0.69–6.22	0.194
	C Vs T	3.07	1.17–8.05	**0.023**	2.88	1.09–7.61	**0.033**	2.88	1.08–7.7	**0.035**

CI; Class Interval, OR; Crude Odds Ratio, OR[a], P[a]; adjusted for age and sex, OR[b], P[b]; adjusted for Diabetes, diet and region, p value - significant values are bold. GIC; Gastrointestinal cancers, GC; Gastric cancer, CRC; Colorectal cancer, EC; Esophageal cancer.

Phase 3 project and it showed that allele frequency recorded in the study population was found similar to the South Asian populations (Supplementary Fig. 1).

3.3.2. Association of pre-mir-149 polymorphism rs2292832 with GI cancers

Association analysis showed that the 'CC', 'C' genotype and alleles of this polymorphism significantly increased the risk of GI cancers on the whole in both unadjusted and adjusted models **(TT Vs CC; crude OR = 1.80, p = 0.032 & T Vs C; crude OR = 1.38, p**

Table 3
Association analysis of *Pre-mir-149* rs2292832 SNP with GI cancers.

Cancer Site	Model	OR	95 % CI	p Value	OR[a]	95 % CI	p[a] Value	OR[b]	95 % CI	p[b] Value
GIC	Dominant	1.5	0.97–2.33	0.070	1.4	0.89–2.25	0.145	1.68	1.06–2.63	**0.029**
	Recessive	0.67	0.43–1.05	0.077	0.64	0.40–1.02	0.063	0.63	0.39–1.0	**0.05**
	TT Vs TC	1.35	0.84–2.17	0.218	1.25	0.76–2.05	0.383	1.44	0.87–2.38	0.153
	TT Vs CC	1.8	1.05–3.07	**0.032**	1.77	1.01–3.11	**0.046**	2.1	1.18–3.75	**0.012**
	T Vs C	1.38	1.04–1.82	**0.024**	1.37	1.02–1.83	**0.036**	1.47	1.1–1.97	**0.009**
GC	Dominant	1.43	0.80–2.56	0.224	1.37	0.71–2.63	0.353	1.62	0.89–2.95	0.117
	Recessive	0.73	0.40–1.33	0.301	0.74	0.38–1.43	0.369	0.73	0.4–1.34	0.307
	TT Vs TC	1.32	0.71–2.48	0.383	1.27	0.63–2.56	0.499	1.5	0.78–2.89	0.226
	TT Vs CC	1.63	0.80–3.32	0.177	1.61	0.74–3.49	0.23	1.82	0.87–3.82	0.111
	T Vs C	1.3	0.90–1.90	0.155	1.28	0.84–1.93	0.249	1.37	0.94–2.01	0.104
CRC	Dominant	1.53	0.86–2.69	0.147	1.36	0.75–2.45	0.307	1.68	0.92–3.05	0.091
	Recessive	0.6	0.33–1.11	0.104	0.59	0.32–1.12	0.109	0.55	0.29–1.05	0.068
	TT Vs TC	1.32	0.72–2.43	0.377	1.18	0.63–2.22	0.601	1.37	0.72–2.6	0.341
	TT Vs CC	1.98	0.96–4.05	0.063	1.79	0.85–3.78	0.124	2.45	1.11–5.37	**0.026**
	T Vs C	1.44	1–2.09	**0.052**	1.38	0.95–2.01	0.096	1.54	1.05–2.26	**0.027**
EC	Dominant	2.04	0.83–5	0.121	1.84	0.73–4.6	0.195	2.32	0.87–6.16	0.092
	Recessive	0.97	0.38–2.49	0.956	0.98	0.38–2.52	0.965	1.01	0.38–2.66	0.985
	TT Vs TC	2.41	0.85–6.88	0.1	2.33	0.80–6.73	0.119	2.92	0.92–9.22	0.068
	TT Vs CC	1.66	0.58–4.78	0.347	1.46	0.49–4.36	0.498	1.81	0.59–5.57	0.303
	T Vs C	1.37	0.74–2.53	0.314	1.31	0.70–2.43	0.4	1.39	0.73–2.63	0.318
Others	Dominant	1.13	0.39–3.30	0.822	1.13	0.38–3.35	0.828	1.68	0.54–5.26	0.370
	Recessive	0.42	0.12–1.49	0.177	0.42	0.12–1.51	0.185	0.27	0.06–1.21	0.087
	TT Vs TC	0.85	0.28–2.6	0.778	0.87	0.28–2.73	0.811	1.11	0.35–3.54	0.865
	TT Vs CC	2.15	0.49–9.46	0.31	2.26	0.51–10.1	0.284	4.47	0.77–25.9	0.095
	T Vs C	1.42	0.73–2.78	0.303	1.42	0.72–2.79	0.315	1.83	0.90–3.73	0.095

CI; Class Interval, OR; Crude Odds Ratio, OR[a], P[a]; adjusted for age and sex, OR[b], P[b]; adjusted for Diabetes, diet and region, p values-significant values are bold. GIC; Gastrointestinal cancers, GC; Gastric cancer, CRC; Colorectal cancer, EC; Esophageal cancer.

= 0.024). The recessive model indicated that the wild type genotype had a positive effect on the carriers in the adjusted models (**OR^b = 0.63, p^b = 0.05**). Further, the subgroup analysis done to check the effect of the polymorphism on the individual GI cancer groups showed that the polymorphism influenced the risk of CRC. The 'CC' genotype and 'C' allele increased the risk of CRC significantly in the adjusted (model b) and unadjusted models (**TT Vs CC; OR^b = 2.45, p^b = 0.026 & T Vs C; crude OR = 1.44, p = 0.052, OR^b = 1.54, p^b = 0.027**). Moreover, the heterozygous genotype 'TC' was found to increase the risk of esophageal cancer when adjusted for DM, region and diet, but the results were only marginally significant (**TT Vs TC; OR^b = 2.92, p^b = 0.068**) (Table 3**).** The allele frequencies of the control groups were compared to the 1000 genomes Phase 3 worldwide populations and it was found to be same as the Gujarati Indian population (Supplementary Fig. 2).

3.3.3. Association of pre-mir-423 polymorphism rs6505162 with GI cancers

The rs6505162 polymorphism had no association with GI cancers as a whole in any of the genetic models. However, the analysis with subgroup cancers showed a strong association between CRC and the polymorphism. The ORs indicated that the 'AA' genotype of the SNP increased the risk of CRC in the adjusted model (**OR^b = 2.39; p^b = 0.037).** Similarly, the allelic model also suggested that the 'A' allele significantly influenced the risk of CRC **(Crude OR = 1.44, p = 0.045, OR^b = 1.6; p^b = 0.013) (**Table 4**).** The allele frequencies of the control groups were compared to the 1000 genomes Phase 3 worldwide populations and found similar to the Indian Telugu in UK, Punjab from Lahore and Srilankan Tamil in UK populations (Supplementary Fig. 3).

3.3.4. Association of pri-mir-30c polymorphism rs928508 with GI cancers

The association analysis showed that AA & AG genotypes reduced GI cancer risk (Dominant**; OR^b = 0.64, p^b = 0.048 & GG Vs AG; OR^b = 0.62, p^b = 0.045**). In the gastric cancer subgroup the dominant model showed a similar risk reducing effect (**OR = 0.49, p = 0.024**; **OR^a = 0.51, p^a = 0.052**; **OR^b = 0.47, p^b = 0.019**) The heterozygote 'AG' genotype also reduced the risk of gastric cancer in our study population in the co dominant model (**GG Vs AG; OR = 0.47, p = 0.022**). The results were consistent even after adjusting for covariates (**GG Vs AG; OR^a = 0.51, p^a = 0.052, OR^b = 0.47, p^b = 0.019**). There was no association of the polymorphism with other GI cancers (Table 5). In the esophageal cancer subgroup the recessive model showed an increased risk (**OR^b = 2.7, p^b = 0.045**) and therefore due to small size of the EC subgroup this results need to be validated further for consideration. The allele frequencies derived from the current study were similar to those of the South Asians (Supplementary Fig. 4).

3.3.5. Association of pre-mir-605 polymorphism rs2043556 with GI cancers

The 'CC' genotype of rs2043556 SNP was found to increase the risk of GI cancers drastically **(OR = 2.57, p = 0.043)** whereas the 'TT' genotype was found to play a protective role **(OR = 0.39, p = 0.042).** Similar outcomes were observed in the adjusted ORs **(Recessive; OR^a = 0.29, p^a = 0.011, OR^b = 0.35, p^b = 0.027 & TT Vs CC; OR^a = 3.25, p^a = 0.016, OR^b = 2.93, p^b = 0.028).** There was no association of the polymorphisms with the sub group cancers (Table 6). The allele frequencies of the control groups were compared to the 1000 genomes Phase 3 worldwide populations and found similar to South Asian populations (Supplementary Fig. 5).

Table 4
Association analysis of *Pre-mir-423* rs6505162 SNP with GI cancers.

Cancer Site	Model	OR	95 % CI	p Value	OR^a	95 % CI	P^a Value	OR^b	95 % CI	p^b Value
GIC	Dominant	1.21	0.80–1.84	0.359	1.12	0.72–1.73	0.618	1.29	0.84–1.99	0.243
	Recessive	0.93	0.58–1.49	0.762	0.98	0.60–1.61	0.944	0.82	0.50–1.34	0.434
	CC Vs AC	1.22	0.78–1.92	0.385	1.14	0.71–1.82	0.599	1.26	0.79–2.0	0.342
	CC Vs AA	1.20	0.71–2.05	0.498	1.09	0.62–1.92	0.765	1.45	0.82–2.56	0.207
	C Vs A	1.12	0.86–1.12	0.401	1.05	0.8–1.39	1.054	1.22	0.92–1.59	0.166
GC	Dominant	1.24	0.71–2.15	0.446	1.17	0.64–2.17	0.609	1.33	0.75–2.35	0.323
	Recessive	0.87	0.46–1.65	0.665	1.05	0.51–2.15	0.897	0.79	0.41–1.53	0.482
	CC Vs AC	1.22	0.67–2.22	0.522	1.23	0.63–2.41	0.545	1.28	0.69–2.36	0.434
	CC Vs AA	1.29	0.63–2.64	0.495	1.13	0.51–2.51	0.758	1.64	0.76–3.58	0.211
	C Vs A	1.12	0.86–1.46	0.401	1.05	0.79–1.39	0.712	1.22	0.92–1.59	0.166
CRC	Dominant	1.34	0.78–2.31	0.282	1.28	0.74–2.24	0.379	1.53	0.87–2.68	0.139
	Recessive	0.57	0.29–1.15	0.118	0.57	0.28–1.17	0.126	0.51	0.24–1.05	0.69
	CC Vs AC	1.17	0.66–2.07	0.603	1.12	0.62–2.03	0.698	1.26	0.71–2.35	0.410
	CC Vs AA	1.90	0.88–4.08	0.101	1.81	0.82–4.01	0.145	2.39	1.05–5.4	**0.037**
	C Vs A	1.44	1.01–2.05	**0.045**	1.41	0.98–2.03	0.067	1.596	1.1–2.3	**0.013**
EC	Dominant	1.37	0.56–3.32	0.491	1.3	0.53–3.20	0.566	1.39	0.53–3.6	0.502
	Recessive	2.09	0.85–5.14	0.107	2.22	0.89–5.53	0.087	2.06	0.8–5.3	0.134
	CC Vs AC	2.50	0.80–7.82	0.115	2.5	0.79–7.93	0.120	2.44	0.74–8.1	0.145
	CC Vs AA	0.73	0.27–1.99	0.544	0.64	0.23–1.79	0.398	0.74	2.5–2.195	0.592
	C Vs A	0.76	0.43–1.34	0.347	0.72	0.41–1.28	0.267	0.76	0.41–1.38	0.362
Others	Dominant	0.57	0.18–1.78	0.330	0.52	0.16–1.65	0.267	0.68	0.21–2.23	0.521
	Recessive	1.90	0.71–5.1	0.203	2.1	0.76–5.74	0.148	1.62	0.55–4.75	0.383
	CC Vs AC	0.70	0.20–2.41	0.566	0.65	0.19–2.29	0.502	0.88	0.24–3.19	0.841
	CC Vs AA	0.42	0.12–1.52	0.185	0.38	0.10–1.39	0.145	0.48	0.12–2.03	0.320
	C Vs A	0.59	0.31–1.11	0.099	0.54	0.28–1.03	0.062	0.67	0.34–1.30	0.234

CI; Class Interval, OR; Crude Odds Ratio, OR^a, P^a; adjusted for age and sex, OR^b, P^b; adjusted for Diabetes, diet and region, p values-significant values are bold. GIC; Gastrointestinal cancers, GC; Gastric cancer, CRC; Colorectal cancer, EC; Esophageal cancer.

Table 5
Association analysis of *Pri-mir-30c* rs928508 SNP with GI cancers.

Cancer Site	Model	OR	95 % CI	p Value	OR^a	95 % CI	p^a Value	OR^b	95 % CI	p^b Value
GIC	Dominant	0.69	0.45–1.06	0.90	0.68	0.44–1.06	0.095	0.64	0.41–0.99	**0.048**
	Recessive	1.04	0.63–1.71	0.880	1	0.59–1.68	0.999	1.04	0.62–1.74	0.881
	GG Vs AG	0.67	0.43–1.05	0.082	0.65	0.41–1.06	0.082	0.62	0.38–0.98	**0.045**
	GG Vs AA	0.75	0.43–1.33	0.327	0.76	0.41–1.39	0.375	0.71	0.39–1.28	0.255
	G Vs A	0.84	0.64–1.11	0.229	0.85	0.63–1.14	0.272	0.82	0.61–1.08	0.166
GC	Dominant	0.49	0.26–0.91	**0.024**	0.51	0.25–1.01	**0.052**	0.47	0.25–0.88	**0.019**
	Recessive	1.12	0.58–2.15	0.738	1.02	0.49–2.10	0.955	1.15	0.59–2.24	0.683
	GG Vs AG	0.47	0.24–0.85	**0.022**	0.48	0.23–0.99	**0.046**	0.45	0.23–0.88	**0.019**
	GG Vs AA	0.54	0.25–1.21	0.133	0.58	0.24–1.43	0.237	0.5	0.22–1.14	0.100
	G Vs A	0.84	0.64–1.11	0.229	0.85	0.63–1.14	0.272	0.82	0.61–1.09	0.166
CRC	Dominant	0.80	0.46–1.39	0.424	0.77	0.43–1.37	0.372	0.76	0.43–1.36	0.358
	Recessive	0.83	0.41–1.65	0.591	0.82	0.40–1.67	0.586	0.79	0.39–1.61	0.515
	GG Vs AG	0.73	0.41–1.32	0.302	0.71	0.38–1.29	0.259	0.7	0.38–1.28	0.247
	GG Vs AA	1.00	0.46–2.19	0.996	0.99	0.43–2.25	0.974	1	0.45–2.23	0.998
	G Vs A	0.96	0.66–1.38	0.808	0.94	0.64–1.38	0.761	0.95	0.65–1.39	0.795
EC	Dominant	1.19	0.49–2.90	0.695	1.21	0.49–2.95	0.680	0.92	0.36–2.35	0.856
	Recessive	2.27	0.90–5.75	0.083	2.24	0.87–5.75	0.093	2.73	1.02–7.26	**0.045**
	GG Vs AG	1.97	0.67–5.8	0.217	1.99	0.67–5.91	0.216	1.53	0.49–4.84	0.467
	GG Vs AA	0.61	0.22–1.71	0.349	0.65	0.23–1.88	0.429	0.48	0.16–1.42	0.183
	G Vs A	0.79	0.43–1.46	0.45	0.799	0.43–1.48	0.476	0.65	0.34–1.24	0.191
Others	Dominant	0.66	0.23–1.92	0.448	0.74	0.25–2.16	0.582	0.59	0.20–1.80	0.356
	Recessive	0.50	0.11–2.26	0.370	0.45	0.09–2.05	0.302	0.54	0.12–2.54	0.439
	GG Vs AG	0.55	0.18–1.63	0.278	0.59	0.19–1.78	0.353	0.49	0.15–1.52	0.215
	GG Vs AA	1.36	0.25–7.33	0.723	1.48	0.27–8.21	0.655	0.77	0.12–5.35	0.786
	G Vs A	1.00	0.51–1.95	0.990	1.1	0.55–2.14	0.818	0.93	0.45–1.89	0.830

CI; Class Interval, OR; Crude Odds Ratio, OR^a, P^a; adjusted for age and sex, OR^b, P^b; adjusted for Diabetes, diet and region, p values-significant values are bold. GIC; Gastrointestinal cancers, GC; Gastric cancer, CRC; Colorectal cancer, EC; Esophageal cancer.

Table 6
Association analysis of *Pre-mir-605* rs2043556 SNP with GI cancers.

Cancer Site	Model	OR	95 % CI	p Value	OR^a	95 % CI	P^a Value	OR^b	95 % CI	P^b Value
GIC	Dominant	1.17	0.79–1.76	0.435	1.17	0.76–1.78	0.481	1.17	0.77–1.78	0.451
	Recessive	0.39	0.16–0.97	**0.042**	0.29	0.11–0.76	**0.011**	0.35	0.14–0.89	**0.027**
	TT Vs TC	1.03	0.67–1.57	0.899	0.97	0.62–1.52	0.885	1.01	0.65–1.57	0.956
	TT Vs CC	2.57	1.03–6.43	**0.043**	3.25	1.25–8.48	**0.016**	2.93	1.12–7.66	**0.028**
	T Vs C	1.29	0.92–1.8	0.138	1.34	0.94–1.91	0.102	1.31	0.93–1.88	0.124
GC	Dominant	1.09	0.64–1.86	0.757	0.94	0.52–1.71	0.840	1.2	0.69–2.10	0.517
	Recessive	0.44	0.12–1.53	0.196	0.27	0.07–1.05	0.058	0.37	0.10–1.32	0.124
	TT Vs TC	0.96	0.55–1.68	0.884	0.72	0.38–1.37	0.318	1.04	0.58–1.86	0.892
	TT Vs CC	2.26	0.63–8.06	0.210	3.4	0.85–13.67	0.084	2.69	0.73–9.93	0.136
	T Vs C	1.29	0.92–1.8	0.138	1.34	0.94–1.91	0.102	1.31	0.93–1.85	0.124
CRC	Dominant	1.22	0.72–2.09	0.463	1.2	0.69–2.09	0.510	1.27	0.73–2.20	0.403
	Recessive	0.57	0.19–1.74	0.321	0.47	0.15–1.48	0.195	0.49	0.15–1.56	0.224
	TT Vs TC	1.12	0.64–1.98	0.686	1.06	0.59–1.897	0.855	1.15	0.64–2.06	0.650
	TT Vs CC	1.84	0.59–5.73	0.295	2.14	0.67–6.88	0.200	2.05	0.62–6.74	0.237
	T Vs C	1.27	0.81–1.98	0.299	1.3	0.82–2.06	0.260	1.33	0.84–2.10	0.222
EC	Dominant	1.08	0.45–2.62	0.863	1.05	0.43–2.57	0.911	1.15	0.46–2.87	0.774
	TT Vs TC	0.86	0.35–2.09	0.734	0.79	0.32–1.96	0.614	0.87	0.34–2.20	0.770
	T Vs C	1.35	0.63–2.90	0.438	1.38	0.64–2.97	0.417	1.48	0.67–3.24	0.330
Others	Dominant	1.51	0.55–4.12	0.426	1.37	0.495–3.80	0.544	1.03	0.37–2.91	0.951
	TT Vs TC	1.19	0.43–3.29	0.732	1.03	0.37–2.91	0.951	1.12	0.39–3.22	0.839
	T Vs C	1.75	0.71–4.32	0.222	1.68	0.65–4.17	0.261	1.92	0.76–4.84	0.170

CI; Class Interval, OR; Crude Odds Ratio, OR^a, P^a; adjusted for age and sex, OR^b, P^b; adjusted for Diabetes, diet and region, p values-significant values are bold. GIC; Gastrointestinal cancers, GC; Gastric cancer, CRC; Colorectal cancer, EC; Esophageal cancer.

3.4. Lack of association of miR polymorphisms with GI cancers

The association analysis of polymorphisms *miR-26a-1*; rs7372209, *miR-218-2* rs11134527, *miR-34b/c* rs4938723, *miR-603* rs11014002 and *mir-Let7a2* rs629367 revealed that they were not associated with altering the susceptibility of GI cancers or its cancer sub groups (Supplementary Table 1 - Supplementary Table 5)

3.5. Multifactor dimensionality reduction analysis to identify interaction between miRSNPs

MDR analysis was conducted to identify models that elevate the risk of gastrointestinal cancers. This analysis produced the best-fit models, with total risk quantified as odds ratios (ORs) and p-values. Entropy-based interaction graphs were generated from the MDR analysis for each dataset. The entropy values in the cells corresponding to individual SNPs represent their primary independent effects. The entropy values marked on the lines connecting two SNPs indicate the nature of their interaction: blue lines denote a high degree of redundancy, green lines indicate a reduced level of redundancy, and gold lines signify independence or additivity. Additionally, interaction dendrograms were created for each MDR analysis dataset. These dendrogram interaction graphs were constructed using hierarchical cluster analysis to reveal the presence, strength, and nature of epistatic effects. If the line connecting two factors is further to the right, it indicates a stronger interaction effect, represented by a black line in figures. A red line between factors signifies a high degree of synergy, while an orange line indicates a lesser degree of synergy.

MDR analysis was done to detect SNP-SNP interactions that might possibly increase the risk of GI cancers and subgroup cancers. In the GI cancer analysis, it was observed that polymorphisms rs11134527, rs2292832, rs6505162, rs928508, rs895819 increased the odds of GI cancer for a person having these polymorphisms together. Table 7 clearly illustrates the significant models and the consistent rise in odds ratio (OR) values for each model as SNPs are added sequentially to the analysis, highlighting the effect of SNP-SNP interactions. The entropy and dendrogram figures also showed the possible interactions between the SNPs to increase the risk of GI cancers (Fig. 1A & B).

MDR modelling was done for GC, CRC and EC subgroups. Tables 8, 9 and 10 represent the SNP-SNP interactions observed in GC, CRC and EC respectively. The results were further elaborated in the entropy and dendrograms. The rs11134527, rs2292832, rs6505162, rs928508, rs895819 polymorphisms showed significant interaction towards increasing the risk for GC (Fig. 2A & B). SNPs rs11134527, rs11614913, rs2292832, rs6505162, rs895819 showed significant

interaction towards increasing the risk for CRC (Fig. 3A & B). MiRSNPs rs11134527, rs2292832, rs6505162, rs928508, rs895819 showed significant interaction towards increasing the risk for EC (Fig. 4A & B). The analysis revealed risk instilling roles of several SNPs that were not observed in the earlier association analysis. This shows that polymorphisms act differently based on the presence of other SNPs and influence each other's role towards the development of the disease.

3.6. Multifactor dimensionality reduction analysis to identify interaction between SNP and environmental factors

MDR analysis was performed to examine the environmental factors in the study that had the propensity to alter susceptibility to GI cancers. The analysis was done for GI cancers as a whole and for the individual subgroup GI cancers.

In GI cancers, socioeconomic status (SES) along with polymorphisms rs2292832, rs629367, rs6505162, rs928508 increased the risk of GI cancers. There was a steady increase in the OR values indicating the increase in risk of GI cancer with the increase in number of risk factors (Table 11). The entropy diagram and dendrogram showed diabetes mellitus (DM) and SES to be involved in GI cancer risk (Fig. 5A and B).

Similarly, rs11134527, rs928508, rs2910164 and socioeconomic status of an individual determined the amount of risk towards GC (Table 12). The entropy diagram also revealed that sex interacted with rs629367 to increase GC risk. The entropy diagram and dendrogram provided a better view on the networking among the different SNPs and environmental factors (Fig. 6A and B). Table 13 represents the models and respective ORs for CRC cancers. In CRC, polymorphisms rs11134527, rs11614913, rs2292832, rs6505162, rs895819 and factors like alcohol drinking, diet and SES were modelled in the entropy diagram and dendrogram (Fig. 7A and B). The esophageal cancers showed no remarkable interactions between SNPs and environmental factors.

4. Discussion

The initial screening of the polymorphisms showed that *miR-499*; rs3746444, *miR-124-1*; rs531564, *miR-608*; rs4919510 and *miR-92a-1*; rs9589207 polymorphisms deviated from HWE and therefore were excluded from further investigations. Deviation from HWE mostly indicates errors in genotyping though theoretically it might be an indicator of inbreeding and population stratification [13,14]. Association analysis yielded quite a few interesting results that are discussed below.

miR-196a is widely recognized for its oncogenic role, as it affects carcinogenesis and tumor progression by regulating processes such as proliferation, invasion, metastasis, and apoptosis. It is mostly correlated with the prognosis of several cancers making it a

Table 7
Interaction analysis of miRSNPs in GI cancers.

Factors	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
rs11614913	0.5537	0.4681	6/10	1.48	0.99–2.36	0.0586
rs11614913, rs2910164	0.5918	0.4842	5/10	2.3	1.44–63.67	0.0004
rs11614913, rs2292832, rs2910164	0.6415	0.4719	3/10	3	1.9–4.40	<0.0001
rs2292832, rs629367, rs6505162, rs928508	0.7036	0.5811	7/10	5.39	3.45–8.40	<0.0001
rs11134527, rs2292832, rs6505162, rs928508, rs895819	0.7865	0.4744	4/10	12.66	7.63–21.02	<0.0001

CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

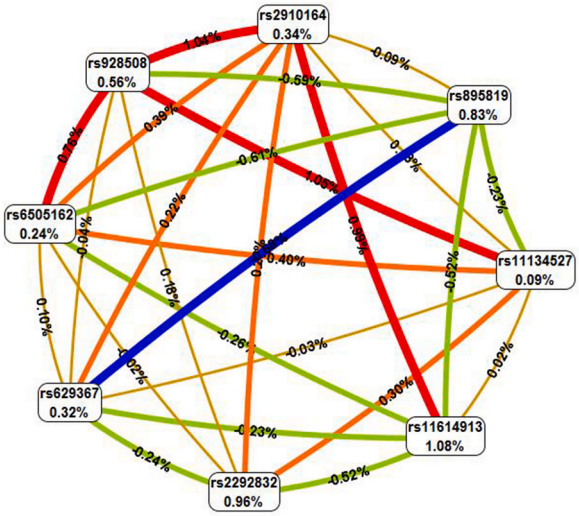

Fig. 1A. Entropy diagram depicting interactions between miRSNPs in GI cancers. Values inside SNP cells indicate the main independent effects. Values marked connecting lines between two SNPs represent the entropy of interaction. Blue lines indicate a high degree of redundancy, green lines a reduced degree of redundancy and gold lines represent independence or additivity.

Fig. 1B. Dendrogram depicting interactions between miRSNPs in GI cancers. Strongly interacting SNPs are placed closer and weekly interacting SNPs are placed far apart.

Table 8
Interaction analysis of miRSNPs in Gastric cancers.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
rs11134527	0.5741	0.4348	3/10	1.65	0.95–2.84	0.0724
rs11614913, rs2910164	0.6136	0.4518	3/10	2.74	1.52–4.93	0.0006
rs11134527, rs928508, rs2910164	0.6759	0.494	7/10	4.00	2.25–7.11	<0.0001
rs11134527, rs6505162, rs928508, rs2910164	0.7607	0.4711	3/10	10.61	5.24–21.48	<0.0001
rs11134527, rs2292832, rs6505162, rs928508, rs895819	0.8558	0.4276	4/10	41.93	15.98–110.0	<0.0001

CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

Table 9
Interaction analysis of miRSNPs in Colorectal cancers.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
rs2292832, rs6505162	0.6228	0.471	4/10	2.77	1.46–5.28	0.0015
rs11134527, rs2292832, rs928508	0.7046	0.6209	10/10	5.65	2.99–10.68	<0.0001
rs11134527, rs2292832, rs928508, rs2910164	0.7758	0.497	3/10	12.32	5.56–27.32	<0.0001
rs11134527, rs11614913, rs2292832, rs6505162, rs895819	0.8699	0.4908	8/10	64.46	19.33–215	<0.0001

CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

Table 10
Interaction analysis of miRSNPs in Esophageal cancers.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	P Value
rs2043556	0.6028	0.4089	4/10	2.39	0.85–6.75	0.0906
rs4919510, rs928508	0.686	0.4571	4/10	5.41	1.77–16.57	0.0012
rs2043556, rs2292832, rs4919510	0.7824	0.4147	2/10	15.45	3.51–68.00	<0.0001
rs11134527, rs6505162, rs928508, rs895819	0.8866	0.43	6/10	∞	–	<0.0001
rs11134527, rs2292832, rs6505162, rs928508, rs895819	0.9572	0.4729	8/10	∞	–	<0.0001

CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

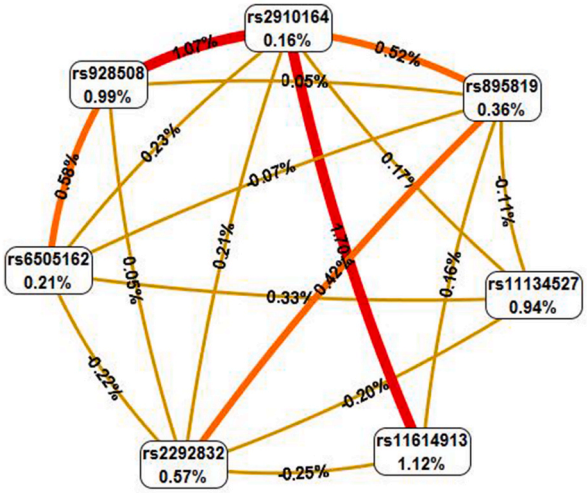

Fig. 2A. Entropy diagram depicting interactions between miRSNPs in Gastric cancers. Values inside SNP cells indicate the main independent effects. Values marked connecting lines between two SNPs represent the entropy of interaction. Blue lines indicate a high degree of redundancy, green lines a reduced degree of redundancy and gold lines represent independence or additivity.

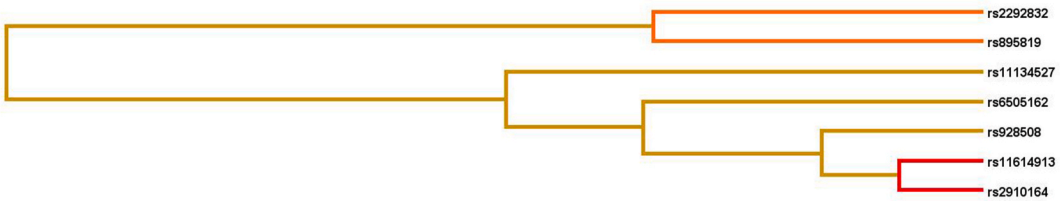

Fig. 2B. Dendrogram depicting interactions between miRSNPs in Gastric cancers. Strongly interacting SNPs are placed closer and weekly interacting SNPs are placed far apart.

potential diagnostic marker. *miR-196a* has been identified to have strong oncogenic roles in most of the cancers of the GI tract such as EC [15], GC [16] and CRC [17]. rs11614913 present in miR-196a is one of the earliest miRSNPs to be studied for its functional effects and role in altering susceptibility of malignant phenotypes. In the current study, we observed that the homozygous mutant genotype (TT) and allele (T) influenced the risk of GI cancers as a group which is an indicator of its probable role in the GI subgroups also. In addition, the recessive model showed that the 'CC' and 'CT' genotypes had a protective effect on its carriers. Likewise, the odds of acquiring gastric cancer were higher in the 'TT' carriers and lower in the 'CC'/'CT' carriers and these results were almost statistically significant. Similarly, in the subgroup others it was seen that the 'T allele increased cancer risk. The current study is a forerunner in bringing out the association of the SNP with GC in an Indian subpopulation.

Our findings align with several other studies that highlight the role of rs11614913 in influencing gastric cancer (GC) susceptibility, although a few studies dispute this role, noting that the effect alleles differ by population. The 'CC' genotype was associated with a higher risk of GC among Chinese individuals with a history of alcohol abuse, H. pylori infection, and familial cancer history [18]. This contrasts with a large study from the same population, which found that the genotype was linked to a lower risk of GC and better survival, supporting our results [19]. In addition, it is important to mention a study conducted in 2014 stated that the polymorphism

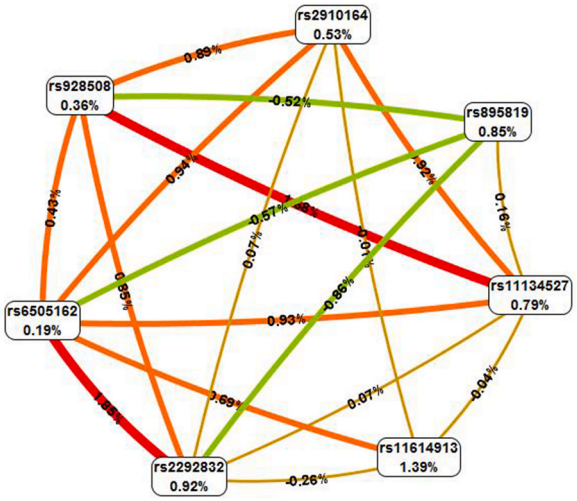

Fig. 3A. Entropy diagram depicting interactions between miRSNPs in Colorectal cancers. Values inside SNP cells indicate the main independent effects. Values marked connecting lines between two SNPs represent the entropy of interaction. Blue lines indicate a high degree of redundancy, green lines a reduced degree of redundancy and gold lines represent independence or additivity.

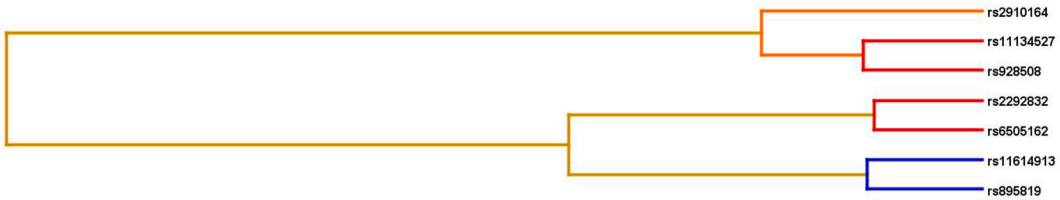

Fig. 3B. Dendrogram depicting interactions between miRSNPs in Colorectal cancers. Strongly interacting SNPs are placed closer and weekly interacting SNPs are placed far apart.

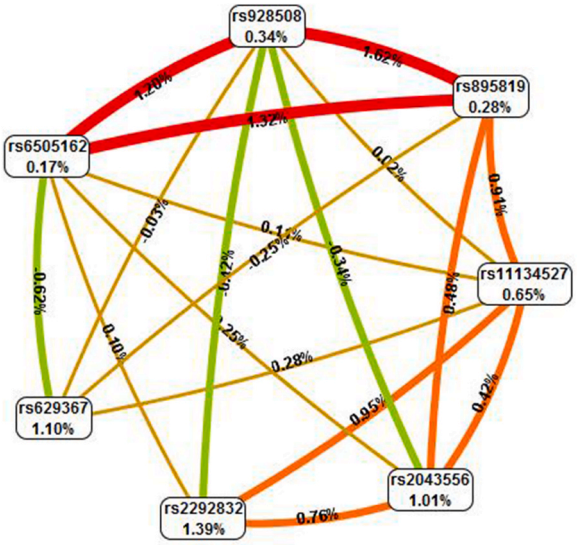

Fig. 4A. Entropy diagram depicting interactions between miRSNPs in Esophageal cancers.

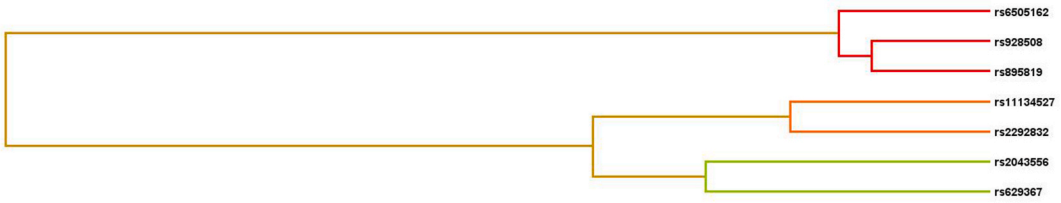

Fig. 4B. Dendrogram depicting interactions between miRSNPs in Esophageal cancers. Strongly interacting SNPs are placed closer and weekly interacting SNPs are placed far apart.

Table 11
Interaction analysis of miRSNPs and Environmental factors in GI Cancers.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
rs2292832, SES	0.6035	0.4881	4/10	2.25	1.48–3.40	0.0001
rs2043556, rs2292832, SES	0.6446	0.503	4/10	3.33	2.15–5.14	<0.0001
rs2292832, rs6505162, rs928508	0.7038	0.5953	7/10	5.39	3.45–8.40	<0.0001
rs2292832, rs629367, rs6505162, rs928508, SES	0.7813	0.5544	6/10	13.49	8.05–22.59	<0.0001

SES; Socioeconomic Status, CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

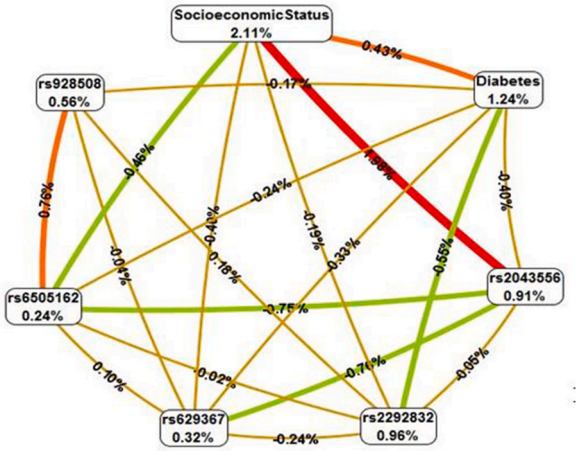

Fig. 5A. Entropy diagram depicting interactions between miRSNPs and Environmental factors in GI cancers.

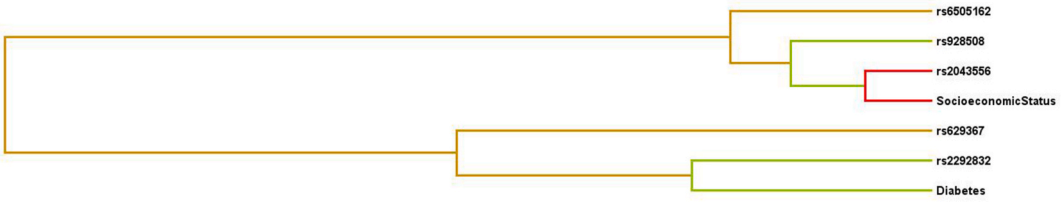

Fig. 5B. Dendrogram depicting interactions between miRSNPs and environmental factors in GI cancers.

Table 12
Interaction analysis of miRSNPs and Environmental factors in GC.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
rs6505162, SES	0.6283	0.4827	4/10	2.78	1.58–4.89	0.0003
rs629367, rs6505162, SES	0.6895	0.4975	4/10	4.69	2.58–8.51	<0.0001
rs11134527, rs928508, rs2910164, SES	0.7664	0.5233	4/10	12.52	5.88–26.67	<0.0001

Socioeconomic Status, CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

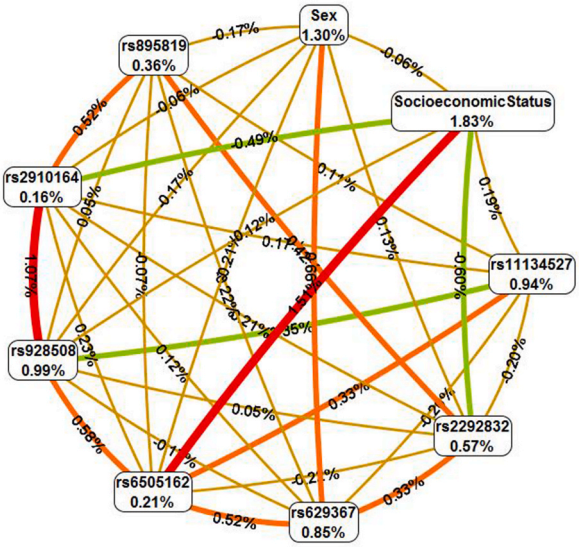

Fig. 6A. Entropy diagram depicting interactions between miRSNPs and Environmental factors in gastric cancers.

Fig. 6B. Dendrogram depicting interactions between miRSNPs and environmental factors in gastric cancers.

Table 13
Interaction analysis of miRSNPs and Environmental factors in CRC.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	OR	CI	p Value
Drinking, Diet	0.6292	0.4692	3/10	3.57	1.72–7.43	0.0004
rs11134527, rs2292832, rs928508	0.7053	0.5489	7/10	5.65	2.98–10.68	<0.0001
rs11134527, rs2292832, rs928508, SES	0.8034	0.5931	7/10	18.91	8.14–43.9	<0.0001

SES; Socioeconomic Status, CV; Cross validation, Bal; Balanced, Acc; Accuracy, OR; Odds Ratio, CI; Class interval. Significant p values are bold.

had no role in being susceptible to GC in the Chinese [20]. Such seemingly farcical results from the same population leave us with an inconclusive role of the SNP in the Chinese populations. Further, the 'CC' genotype elevated the risk of GC in Greeks [21], Chinese [22], Korean females [23] and in aged Japanese [24] while, the polymorphism lacked any significance in the Romanian population [25]. A meta-analysis performed on the existing studies showed that the 'CC' genotype reduced the risk of GC [26] supporting our findings while 2 other meta-analysis suggested that the polymorphism had no effect on GC risk [27,28].

miR-423 has been recognized as a contributor to various normal lipid metabolism and cancer pathways. Expression studies have shown abnormal expression patterns of miR-423 in cancers such as OSCC and HNSCC. Similar to other miRNAs, miR-423 exhibits dual roles in different cancer tissues. It was found to be upregulated in the early stages of CRC and ovarian cancer, highlighting its potential as a diagnostic marker.The rs6505162 polymorphism present in this miRNA has been widely studied in ECs and has exhibited race dependant roles [29,30]. In our study, the polymorphism had significant association with CRC where the 'AA' genotype actively increased the odds of acquiring CRC in its carriers in the adjusted models. Further, it was also noticed that the 'A' allele influenced the risk of CRC in all the models showing the prominent role of the SNP in CRC. A similar scenario was observed in the Chinese population where the 'AA' genotype was associated with the metastasis of CRC [31]. In addition, the study by Xing et al., showed the influence of the SNP on survival rate and recurrence free survival in CRC patients with higher BMI and TNM stages [32]. Though, there were studies where the SNP was associated with EC [33,34] it was not evident in our study. It is also important to note at this juncture that a single study on EC from a north Indian population also did not find any association of the SNP with EC risk [35]. Our study was the first to report the association of *miR-423* SNP with risk of CRC and also to report the MAF from a South Indian population.

miR-30c, a member of the miR-30 family, is essential for lipid metabolism, adipogenesis, cell proliferation, and differentiation. It

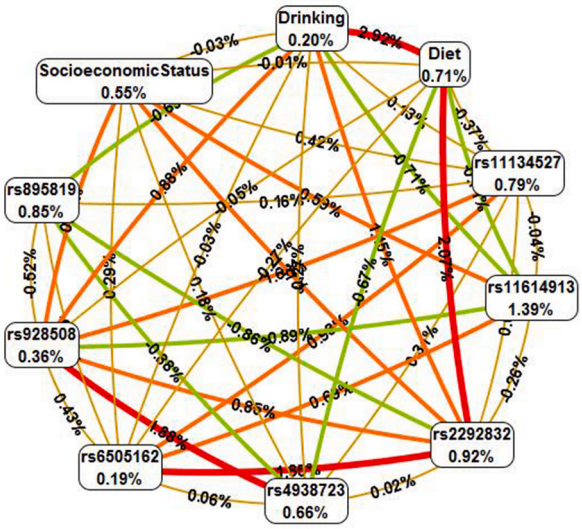

Fig. 7A. Entropy diagram depicting interactions between miRSNPs and Environmental factors in Colorectal cancers.

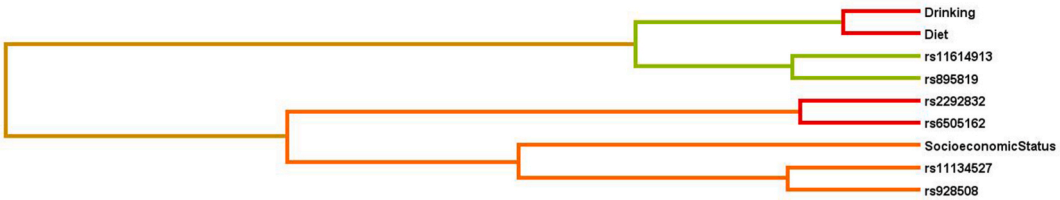

Fig. 7B. Dendrogram depicting interactions between miRSNPs and environmental factors in Colorectal cancers.

has a dual role in cancer, acting as both a tumor suppressor and an oncogene. miR-30c is actively involved in cancers affecting the breast, colon, bladder, lung, ovary, prostate, liver, endometrium, and Myc-induced B lymphoma. It is known to regulate the expression of the KRAS gene and inhibit the KRAS-MAPK pathway, which helps to impede cell proliferation, invasion, and metastasis [36,37]. Several studies have found this polymorphism to be associated with risk, survival and chemoresistance of lung cancers [38–41]. However, there is only a single report on the association of the polymorphism with GC. This Chinese study demonstrated that the 'AA' genotype instilled an increased risk of GC and also influenced upregulation of miR-30c expression [42]. In our study, the GIC group showed that the 'AG' genotype decreased the disease risk significantly in all the models. Moreover, the sub group cancer analysis disclosed new findings which were never reported before. The dominant and 'AA vs AG' models showed a significantly reduced risk of GC in all the models hinting a protective role of the 'AG' genotype in GC. Another interesting revelation was that the recessive model indicated a significant risk of EC in the adjusted model which was also the first report on the SNPs association with EC. At this point it is noteworthy to state that the current study was first to screen for this polymorphism in the Indian continent and observe its MAF and effects in GI cancers.

miR-605 is well acclaimed for its tumor suppressing role in multiple cancers by preventing expression of genes involved, in cell proliferation, invasion and metastasis. miR-605 primarily functions as a tumor suppressor, playing a key role in regulating various signaling pathways. It has been found to be downregulated in melanoma tissues, which helps to inhibit cancer progression. Additionally, miR-605 prevents epithelial-mesenchymal transition (EMT) and metastasis by interfering with TRAF6 in the NF-kB pathway [43]. The rs2043556 housed within this miRNA has been associated with risk of GI cancers and BC [44,45,46]. Similarly, the 'AG' and 'GG' genotypes increased the risk of GC in people of Brazil [47] indicative of a potential role of the SNP in GI cancers. In the current study, the 'CC' genotype remarkably increased the odds of the carrier to acquire GI cancers and on the other hand the recessive model (CC vs TT + TC) conferred a protective role against GI cancer on its carriers. Likewise, the ORs achieved in the GI cancer sub groups indicated an increase in disease risk of GC and CRC but the results were statistically non-significant. This is the 1st attempt to bring out the significance of miR-605 polymorphism in cancers from India. To the best of our knowledge, this study is the forerunner in screening for rs2043556 and reporting its MAF from an Indian sub population.

miRNA polymorphisms in *miR-27a*; rs895819 & rs11671784 [10] *miR-26a-1*; rs7372209, *miR-218-2*; rs11134527, *miR-34b/c*; rs4938723, *miR-603*; rs11014002 and *Let-7a2*; rs629367 were not associated with the risk of GI cancers or the subgroups GC, CRC, EC and 'others. These results may seem to do away with the importance of these polymorphisms in predicting GI cancer risk but it is important to note that all these polymorphisms were well addressed for their role in GI cancers in several studies which is evident from

the review of literature. The lack of association could perhaps reflect on underlying reasons like ethnic differences and dependence on other risk factors. The polymorphism rs41274221 in *miR-25a* had only the 'CC' genotype in the study population which was concordant with the allele frequencies depicted in the Ensemble database [48] but discordant with the study by Zhou et al., where the 'A' allele of the polymorphism reduced the risk of GC by breaking the oncogenic character of *miR-25* [49].

In order to address an important objective of the study which was 'to elucidate the interaction between miRSNPs and environmental factors' we opted for Multifactor dimensionality reduction (MDR) analysis which was the first tool to detect non additive gene-gene interactions and genotype-phenotype interactions in polygenic human diseases [50]. It is basically a non-parametric method which uses a highly creative constructive induction algorithm which reduces the dimensions of the SNP data into a single feature making it easier to identify interactions that point out to two possible outcomes; high risk and low risk [51]. Moreover, MDR analysis is also a powerful tool in detecting valuable interaction even in the presence of defective data with genotyping error, missing data, phenocopy and genetic heterogeneity [52,53]. There are several evidences which have shown the efficiency of MDR in detecting significant genotype phenotype interactions which were not observed in the conventional regression analysis [53–55].

The SNP-SNP interaction analysis conducted in this study examined the polymorphisms miR-218-2; rs11134527, miR-149; rs2292832, miR-423; rs6505162, miR-30c; rs928508 and miR-27A; rs895819 within a single model, which significantly elevated the risk of gastrointestinal cancers.This analysis brought to light, the polymorphisms which did not appear in the association analysis done previously. *miR-218-2*; rs11134527 and *miR-27a*; rs895819 were not associated with GI risk individually but in the presence of the above SNPs they worked towards increasing the individual's susceptibility to GI cancers. The ORs also showed a steady increase in risk with addition of each SNP to the model indicating how a person's risk towards GI cancer varies based on the presence of different miRSNPs. The MDR analysis done in the GC and EC group found that *miR-218-2*; rs11134527, *miR-149*; rs2292832, *miR-423*; rs6505162, *miR-30c*; rs928508 and *miR-27a*; rs895819 interacted together significantly to increase the risk of GC and EC. The MDR analysis done in the CRC group presented miR-SNPs *miR-218-2*; rs11134527, *miR-196a*; rs11614913, *miR-149*; rs2292832, *miR-423*; rs6505162, and *miR-27a*; rs895819, to have an increased risk of CRCs. It is important to highlight that the SNP miR-30c (rs928508) was excluded from the colorectal cancer (CRC) model, while miR-196a (rs11614913) was carefully included, illustrating the differences in the models based on the specific disease groups. Consequently, the MDR analysis produced novel SNP-SNP models that could potentially be used to quantitatively predict an individual's susceptibility in the future.

MDR analysis was also employed to detect interactions between miRSNPs and environmental factors recorded in the study. In GI cancer group socioeconomic status (SES) interacted with *miR-149*; rs2292832, *Let-7a2*; rs629367, *miR-423*; rs6505162, and *miR-30c*; rs928508 to increase the risk of GI cancer. It is important to note that *Let-7a2*; rs629367 was added only in this model and not in the SNP-SNP model showing that SES is an important influencer and determinant of cancer risk. Further the entropy diagram and dendrograms also exhibited mild interactions between diabetes mellitus and the polymorphisms. SES was also modelled along with *miR-218-2*; rs11134527, *miR-30c*; rs928508, *miR-146a*; rs2910164 and with *Let-7a2*; rs629367, *miR-423*; rs6505162 separately in the GC subgroup. Thus, there were two different models increasing the GC risk. Moreover, the entropy diagram and dendrogram showed that *Let-7a2*; rs629367 interacted with sex in GC. Likewise, *miR-218-2*; rs11134527, *miR-149*; rs2292832, *miR-30c*; rs928508 and SES were put together as a significant model affecting CRC risk. The entropy diagram and dendrogram revealed a strong interaction between *miR-149*; rs2292832 and diet while *miR-34b/c*; rs4938723 weakly interacted with alcohol drinking to result in elevated CRC risk. The impact of SES on cancer burden has been well established earlier. SES is an important factor that reflects on an individual's exposure to environmental pollutants, availability of medical facilities, cancer awareness, nutrition and various other lifestyle practices. Our study also showed that SES of a person magnified the effect of SNPs and increased an individual's cancer risk [56–60]. The MDR analysis yielded such pronounced results which were not demonstrated earlier making the study one of its kind. The importance of mathematical modelling in genomic datasets is well highlighted by the above results that can be employed for construction of cancer prediction kits for the future generations where genome sequencing will be a common method in medicine, made accessible to the common man.

5. Conclusion

The study demonstrated that miRSNPs alter GI cancer risk and it is enhanced by the presence of risk factors like socioeconomic status, diabetes mellitus, diet and alcohol consumption in the Indian subpopulation. However, the role of these polymorphisms requires a comprehensive study across various populations to arrive at the exact potential of these SNPs as SNP biomarkers for GI cancer risk prediction. Determining how SNPs influence the health of an individual and later transforming this knowledge into the development of new treatment approaches will revolutionize the management of polygenic diseases like cancers. Moreover, these results are sure to attract attention towards implying these polymorphisms for future research.

CRediT authorship contribution statement

Charles Emmanuel Jebaraj Walter: Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Zioni Sangeetha Shankaran:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Sai Sushmitha Kontham:** Formal analysis. **Kotteeswaran Ramachandran:** Formal analysis. **Nandini Prakash:** Formal analysis. **Thanka Johnson:** Validation, Conceptualization. **Sri Nisha JR:** Validation.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board. The study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, IEC-NI/13/APR/33/39. Written Informed consent was obtained after explaining the study to the participant before recruitment.

Data availability statement

Not Applicable.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Charles Emmanuel Jebaraj W reports administrative support, article publishing charges, and equipment, drugs, or supplies were provided by Sri Ramachandra Institute of Higher Education and Research (Deemed to be University). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41519.

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