Genetic Analysis of colorectal carcinoma using high throughput SNP genotyping technique within the population of Jammu and Kashmir.

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Abstract

Background SNP genotyping has become increasingly more common place to understand the genetic basis of complex diseases like cancer. SNP-genotyping through massARRAY is a cost-effective method to quantitatively analyse the variation of gene expression in multiple samples, making it a potential tool to identify the underlying causes of colorectal carcinogenesis.

Methods In the present study, SNP genotyping was carried out using Agena mass ARRAY, which is a cost-effective, robust, and sensitive method to analyse multiple SNPs simultaneously. We analysed 7 genes in 492 samples (100 cases and 392 controls) associated with CRC within the population of Jammu and Kashmir. These SNPs were selected based on their association with multiple cancers in literature.

Results This is the first study to explore these SNPs with colorectal cancer within the J&K population. 7 SNPs with a call rate of 90% were selected for the study. Out of these, one SNP i.e. rs2229080 of *DCC* was found to be significantly associated with the current study and 6 were non-significantly associated with CRC within the studied population. The allelic OR observed for the variant rs2229080 of *DCC* was 1.5 (1.1–2.3 at 95% CI), p value = 0.02.

Conclusion This is the first study to find the relation of Genetic variants with the colorectal cancer within the studied population using high throughput mass ARRAY technology. It is further anticipated that the variants should be evaluated in other population groups that may aid in understanding the genetic complexity and bridge the missing heritability.

Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide, resulting in 1–2 million new cases each year (1). In 2018, nearly 10% of all cancer incidences were reported to be of CRC, resulting in about 900,000 deaths worldwide(2). The incidence of CRC has been associated with obesity, red meat consumption, and physical inactivity (3,4). In addition, the genetic factors and epigenetic changes also play a key role in the initiation and progression of CRC (5,6). Delay in the diagnosis of CRC is a major hurdle in the management of CRC, which is evident by the rise in new cases each year. Therefore, it is critical to identify markers that may help in the early prognosis and development of therapeutic interventions accordingly.

In India, CRC accounted for about 43,000 deaths in the year 2018 (7). In the Jammu and Kashmir (J&K) region, there is a spike in the incidence of gastric, oesophagus and CRC in recent years (8,10). The rise in the incidence of these disorders may be as a result of lifestyle and food consumption. However, the genetic aspect of gastro-intestinal cancer cannot be overruled (11). According to the J&K based hospital report, CRC accounted for the second most common type of cancer, with an incident rate of 16.8%, in the region (8). In this regard, identification of the CRC related genetic variants in the region of J&K is necessary for the proper prognosis and CRC management, which can be achieved through single-nucleotide polymorphisms (SNP) genotyping.

SNP genotyping is a powerful tool that has identified the genetic basis of complex disease, including CRC (5). The studied SNPs hence provides key insights on the molecular pathogenesis of cancer that can be further translated to the identification of cancer and therapeutic biomarkers (12,13). Identifying the role of these genetic variants may provide valuable insights on prognosis and optimize therapies for the treatment of CRC. The Agena Bioscience MassARRAY® System provides genotype data for several user-defined SNPs in a large number of DNA samples in a high-throughput and cost-effective manner (14). In this study, we have carried out SNP genotyping using Agena MassARRAY® to identify multiple SNPs and samples simultaneously.

The previous study in Chinese population has showed that rs2229080 of *DCC* (Deleted in Colorectal Carcinoma Netrin1 Receptor) was associated with low breast cancer risk (15). In contrast, *DCC*rs2229080 displays no significant association with esophageal cancer risk in the region of J&K (16). In the present study, we analysed 7 genes and found out that rs2229080 of *DCC* was significantly associated with colorectal cancer in the current studied population of J&K region. Taken together, our study investigated the role of cancer-related genetic variants in CRC in the population of J&K. The study of these genetic variants may provide valuable insights on the proper prognosis and CRC management. However, more large-scale sample size studies are required that will further support the present study.

Material And Methods

Sample Collection

This study was approved by the Institutional Ethics Review Board (IERB) of Shri Mata Vaishno Devi University (SMVDU). All details were recorded in a pre-designed proforma and the written informed consent was obtained from each participant before conducting the study.

A total of 492 participants including 100 colorectal cancer patients and 392 healthy controls (age and sex-matched) were recruited from the Jammu and Kashmir region of India. All the participants recruited for the study were obtained from hospitals and various clinics of J&K. 2ml of venous blood was collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes from all the participants. The clinical parameters of both cases and controls are provided in **Supplementary Table 1**.

DNA Extraction

The genomic DNA was isolated from the blood samples, using the manufacturer's protocol of Qiagen™ DNA isolation Kit (Catalogue No. #51206, Hilden, Germany). Genomic DNA was quantified using Eppendorf's Bio Spectrometer™ (Hamburg, Germany) at wavelength 260 nm and 280 nm and the ratio of OD260nm / OD280nm was taken as a criterion to check the purity of DNA. The quality of the genomic DNA was checked by agarose gel electrophoresis (Bio-Rad Gel Doc™ EZ imager)

Genotyping

Agena massARRAY platform was used for SNP genotyping, in Central Analyzer Mass array facility at SMVDU. It is a robust, cost-effective and highly sensitive tool for genotyping of SNPs and involves multiplex PCR[1]. Customized forward, reverse and single base extension primers were designed using Agena Design Suite V.2.0. Multiplex PCR was used to detect a variation in initially targeted region. 1 µl of genomic DNA (concentration of 10 ng/ul) was loaded in 384 well PCR plates and dried at 85°C for 10 minutes. After drying, the reaction mixture was prepared containing dNTPs, primers pool (forward & reverse), reaction buffer and DNA polymerase. After completion of first PCR, the reaction was treated with shrimp alkaline phosphatase (SAP). The multiplex PCR reaction was then subjected to single base extension reaction using mass modified ddNTPs and primers (pooled single extension primers). PCR cycle was adopted from Gabriel et al 2009 [1]. Further the final PCR product is treated with cationic resin and then energy reaction to keep check the quality of genotyping and transferred to spectro-chip. The transferred product then fired to MT analyser. The data was then processed and analysed by preinstalled Typer Analyzer v.4.0. The genotyping results were recognized by replicating 10% of random samples and the concordance rate were 98.3%. In the reaction of 384 well plates one negative and one positive control were added to check the quality of reaction mixture.

Genotyping Quality Control

SNPs having call rate above 90% were included for statistical analysis [2]. Hardy-Weinberg Equilibrium (HWE) among cases and controls were used for assessing the quality of genotypes after analysis.

Statistical analysis

The statistical analysis was performed using Plink V.1.0962 with a maximum of 10,000 permutations [3]. Each SNP was subjected to Hardy Weinberg Equilibrium (H.W.E) and significant association of SNPs was evaluated by 3×2 chi square tests for genotypic frequencies between cases and controls. Further logistic regression analysis was performed using SPSS V.23 in order to obtain corrected odds ratio (OR), confidence interval (CI) and p-value as level of significance from confounding factors like age and BMI. The power of the study was calculated using PS: power and sample size calculation (PS version 3.1.6) software (41).

Results

The current case-control association study included a total of 492 participants with 100 colorectal cases and 392 healthy controls. In the current study, the patient cohort included 56 males and 44 females, with a median age of 62.87 (± 9.8) years and median BMI of 20.75 (± 0.869) kg/cm³ respectively. Healthy controls constituted 280 males and 112 females with median age and median BMI of 48.81 (± 15.3) years and 24.9 (± 0.869) kg/cm³ respectively. The clinicopathological characteristics of all the participants are shown in (Supplementary Table 1).

In the current study, genetic variants that were not studied in association with CRC in the population of Jammu and Kashmir but are associated with other types of cancer were evaluated. These genetic

variations were studied to know whether they show increased risk or reduced risk with colorectal cancer in the population of Jammu and Kashmir. This is the first study to find the association of colorectal cancer in the population of Jammu and Kashmir.

We analysed 7 SNPs using mass ARRAY™. After stringent quality check, these SNPs having genotyping quality call greater than 90% (Table 1).

Table 1
SNPs showing call rate 90% and above (Candidate Genes selected for study)

Sr.	SNP	Gene	Cases	Controls	pValue	OR (95%CI)	HWE
1	rs2234593	<i>WT1</i>	G = 0.2246 T = 0.7754	G = 0.08857 T = 0.9114	4.81E- 05	2.981(1.731–5.136)	1
2	rs1799966	<i>BRCA1</i>	T = 0.5909 C = 0.4091	T = 0.4615 C = 0.5385	0.02292	1.685(1.073–2.647)	0.5584
3	rs2229080	<i>DCC</i>	C = 0.4593 T = 0.5407	C = 0.3545 T = 0.6455	0.02241	1.546(1.063–2.251)	0.2323
4	rs1801133	<i>MTHFR</i>	G = 0.6265 A = 0.3735	G = 0.1905 A = 0.8095	4.11E- 21	7.129(4.644–10.94)	3.46E- 14
5	rs10046	<i>CYP19A1</i>	G = 0.07447 A = 0.9255	G = 0.6889 A = 0.3111	1.54E- 42	0.03634(0.02017– 0.06545)	1.77E- 10
6	rs8034191	<i>HYKK</i>	T = 0.2817 C = 0.7183	T = 0.1875 C = 0.8125	0.03521	1.699(1.035–2.791)	0.285
7	rs1042522	<i>TP53</i>	G = 0.4947 C = 0.5053	G = 0.04651 C = 0.95349	1.84E- 34	20.07(11.26–35.75)	0.06011

Out of the selected variants rs2229080 of *DCC* was found to be significantly associated with colorectal cancer in the current studied population. The allelic OR observed for the variant rs2229080 of *DCC* was 1.5 (1.1–2.3 at 95% CI), p value = 0.022.

Discussion

Colorectal cancer (CRC) is the 3rd most persistent cancer and a prominent cause of cancer-related morbidity and mortality worldwide (4, 5). CRC evolves due to the progressive accumulation of genetic and epigenetic modification in the colonic epithelium, transforming them into colorectal adenomas and adenocarcinomas (6).

Genetics is an important risk factor associated with CRC. So, in the present study, genetic elucidation among cases and controls was explored. We analyzed seven genes in 492 samples consisting of 100 cases and 392 controls. All the seven SNPs had a call rate above 90%. These identified variants were rs2229080 of *DCC*, rs10046 of *CYP19A1*, rs1042522 of *TP53*, rs10228682 of *POT1*, rs10069690 of *TERT*, rs1051266 of *SLC19A1*, and rs1026071 of *ARTNL*. One SNP showing significant association with CRC within the population of our study was rs2229080 of *DCC*.

DCC (netrin-1), initially discovered in CRC, encodes the netrin-1 receptor, a member of the cell's immunoglobulin superfamily adhesion molecules, has been characterized as a potential tumor suppressor gene (7, 8). As soon as *DCC* bind to the netrin-1 receptor, it induces cell migration and proliferation. In the absence of netrin-1, *DCC*'s intracellular domain is cleaved by a caspase that induces apoptosis in a caspase-9-dependent pathway (9). *DCC* is frequently silenced or inactivated in various human cancers due to epigenetic silencing or loss of heterozygosity at chromosome 18q21 region (8, 10). Loss of *DCC* gene expression was shown to be an independent prognostic factor in colorectal (12), AML (11), and gastric cancer (13, 14) patients. Various studies have been carried out that demonstrate a significant association of *DCC* polymorphism with esophageal, colorectal, and gastric cancer risk (15–18). The deletion at 18q21 loci (containing *DCC* gene) is an essential step in GBC progression (19). A genome-wide association study (GWAS) also suggested *DCC* as a candidate gene deliberating GBC predisposition in a Japanese population (20). Some previous studies reported that there was no effect of GWAS reported SNPs on GBC risk. On the contrary, a significant association of rs2229080 and *DCC* rs714 was observed with GBC risk (21). The rs714 is associated with decreased *DCC* expression and loss of heterozygosity (LOH) in various cancers (22, 23). Further, rs2229080, a missense variation replacing Arg to Gly at *DCC* codon 201, was reported to increase the risk of colorectal cancer (24) and neuroblastoma (25). Moreover, this SNP was suggested to be associated with loss of *DCC* protein expression and LOH's target and indicating that the codon 201 polymorphism may interfere with the *DCC* transition or transcription (26).

To the best of our knowledge, to date, no study has been conducted on the role of the rs2229080 of *DCC* in colorectal cancer within the population of J&K. This is the first prelude study that investigated the possible correlation between the rs2229080 of *DCC* polymorphism and susceptibility to colorectal cancer.

Conclusion

In the present study, we explored the link between environmental factors, genetics, and colorectal cancer. This study is the first to investigate the relation of genetic variants associated with different cancers but not with colorectal cancer within Jammu and Kashmir. The present study could provide insights into genetic variation associated with the risk of developing colorectal cancer. Hence, if investigated further in the large cohort, this can unravel the biological significance of these SNPs in colorectal cancer among the Jammu and Kashmir populations.

Declarations

Acknowledgement.

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Contributions

R.S and R.K planned the work. B.S, AB and S.V performed experimental work in lab. G.R, A.B, R.J, S.V, A.A and D.B helped in sample collection. S.A gave the histopathologically conformation of the samples. B.S performed the statistical analysis, wrote the manuscript and R.J drafted the manuscript according to journal. R.S, R.K, and R.A finally refined the manuscript. All authors finally revised and approved the manuscript.

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Availability of data and materials

Data generated and analyzed during study is not available publicly but can be made available from the corresponding author upon reasonable request.

Ethics approval and consent for participation

The study was approved by the Institutional Ethics Review board (IERB) of Shri Mata Vaishno Devi University (SMVDU). The written informed consent was obtained from each participant before conducting the study. In this study all experimental research work was performed according to guidelines issued by Institutional Ethics Review board committee.

Consent for publication.

Not Applicable

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Competing interest

The authors declare no competing interest.

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