Relationship between melanoma-associated antigen 1 (*MAGE-A1*) gene polymorphisms and colorectal cancer development

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ABSTRACT. In the Saudi population, colorectal cancer (CRC) is the first and third most common malignancy among males and females, respectively. The CRC risk is associated with the expression of certain genes, which can be used for prognoses and for finding screening-related biomarkers. One such gene is melanoma-associated antigen 1 (*MAGE-A1*); however, little is known about this gene or its expression as it relates to CRC susceptibility in Saudi patients. The aim of this study was to use the TaqMan genotyping assay to explore the relationship between *MAGE-A1* single nucleotide polymorphisms (SNPs), specifically rs3788745, rs3788749, and rs3788753, and CRC susceptibility in 192 Saudi patients with CRC and 192 healthy individuals (a control group). The rs3788749 SNP exhibited an increased prevalence in individuals with the CT and CT + TT genotypes, indicating a correlation between the presence of *MAGE-A1* polymorphism and susceptibility to CRC (p=0.038 and 0.026, respectively). The CRC group also showed a significant difference in allele frequency (T alleles) when compared to the control group (p=0.019). Categorizing the patients with CRC by tumor location revealed a high prevalence of *MAGE-A1* rs3788749 polymorphism in

patients with rectal localizations and the CT and CT + TT genotypes (p=0.014) and the T alleles (p=0.023). However, no significant differences were found in the clinical and demographic parameters related to *MAGE-A1* SNPs rs3788745 and rs3788753 in terms of the genotype and allele frequencies in patients with CRC when compared to healthy individuals. In conclusion, *MAGE-A1* rs3788749 polymorphism is associated with an increased susceptibility to CRC, and especially rectal cancer, suggesting that this polymorphism may serve as a rectal cancer biomarker for the early detection of CRC in Saudi patients.

Keywords: Colorectal cancer; Polymorphism; *MAGE-A1*; Saudi population

INTRODUCTION

Colorectal Cancer (CRC) is a heterogeneous disease; it occurs through combinations of different factors, such as environments, genetics and epigenetics (Kruk J and Czerniak U, 2013; Peters U et al., 2013). In the United States, it is the second-most major neoplasm and leads to death among both males and females. Approximately 140,000 Americans are diagnosed with this disease and more than 50,000 related deaths are recorded annually (Al-Thafar AK et al., 2017). In the Kingdom of Saudi Arabia (KSA), this malignancy affects both men and women and is the second-leading cause of cancer-related deaths in KSA citizens (Saudi Cancer Registry, 2011). Moreover, it is the first- and third-most common cancer among Saudi men and women, respectively (Al-Thafar AK et al., 2017). Additionally, CRC mortalities and incidences in the KSA have recently increased, leading to higher risks of increased CRC mortality rates in the KSA (Ibrahim EM et al., 2008). To improve these tests and decrease CRC deaths, several studies investigated genes associated with CRC regarding prognoses and screening-related biomarkers (Krupa R et al., 2011; Lurje G, Zhang W, Lenz HJ 2007; Peltekova VD et al., 2014) However, early CRC biomarkers that could help predict the presence of the disease were not identified. Despite this, the analysis of genetic variations may provide such an insight into the genetics of human CRC. For example, SNPs, the most common genetic variations in the human genome, may be excellent genotypic biomarkers for predicting CRC developments as they have the potential to be unique genetic markers for association-based approaches to discovering genetic components (Ulrich CM, Robien K, McLeod HL, 2003).

In addition, SNP patterns and profiles may assist in identifying comprehensive gene collections that may contribute to cancer developments and susceptibility (Semlali A et al., 2016). The presence of SNPs in several genes, such as CYP24-A1 (Chen XQ et al., 2017), TLR-9 (Semlali A et al., 2016). RETN (Alharithy RN, 2014), and TNFα (Kapitanovic S et al., 2014), was found to be associated with colon cancer risks. Moreover, SNPs were found to be located in different positions within genes, such as in promoters, exons, introns, and 5' or, 3' untranslated regions (UTRs) and these SNPs can alter gene expressions using various mechanisms (Deng N, Zhou H, Fan H, Yuan Y, 2017). Therefore, this study aimed to investigate the potential contribution of melanoma-associated antigen 1 (*MAGE-A1*) SNPs (rs3788753, rs3788749 and rs3788745) and expression levels in CRC among Saudi patients compared with healthy colorectal samples to identify a diagnostic biomarker for patients with CRC.

It should be noted that cancerous human antigens can generally be separated into five categories: cancer/testis (CT) antigens, overexpressed proteins, tumor viruses, mutated antigens and differentiation antigens (Stevanovic S, 2002). CT genes are a large category of testis-specific genes that are expressed in human cancers of diverse histological origins (Babatunde KA et al., 2017; Scanlan MJ, Simpson AJ, Old LJ, 2004). These include melanomas, ovarian cancers, colon cancers, pancreatic cancers, lung malignancies, renal malignancies and hematopoietic malignancies (Caballero OL and Chen YT, 2009). Among the CTAs identified to date, the MAGE family is one of the most widely studied antigen families that recognize expressions in a wide variety of

human tumors (Simpson AJG et al., 2005). “MAGE” was originally a “melanoma-associated antigen”; later it was discovered that its homologs formed a multi-gene family in mammalian genomes (Vanderbruggen P et al., 1991; Castelli C et al., 2000).

In the human genome, this family can be categorized into three subfamilies based on expression patterns and functions (Vanderbruggen P et al., 1991; Katsura Y and Satta Y, 2011): there are 12 MAGEA, 18 MAGEB and 7 MAGEC (Doyle JM, Gao J, Wang J, Yang M, 2010; Chomez P et al., 2001); members. Each subfamily comprises 1–15 genes [21, 23], and is expressed in highly proliferating cells, such as tumor, placenta, and germ line cells (Vanderbruggen P et al., 1991; Katsura Y and Satta Y, 2011). MAGEA, MAGEB and MAGEC genes are located on the X chromosome, and they encode tumor antigens that are important in cancer immunity. Peptides in the human MAGE homology domain (MHD) are 160–170 amino acids long (Vanderbruggen P et al., 1991; Katsura Y and Satta Y, 2011). Melanoma Associated Antigen-A1 (*MAGE-A1*) was identified as the first CTA in a melanoma patient (Vanderbruggen P et al., 1991).

Incidences of CRC in the Saudi population have increased in recent years. Consequently, screening processes are important for the early detection of CRC and decreasing the CRC death rate. Therefore, this study investigated the possibility of using *MAGE-A1* common primer sets to screen for CRC among Saudi patients. However, while *MAGE-A1* had many SNPs, there was no evidence these SNPs could be used as tumor prognosis factors among patients with CRC. To confirm the role of *MAGE-A1* polymorphisms in tumor prognoses of patients with CRC, three *MAGE-A1* SNPs (rs3788753, rs3788749 and rs3788745) were analyzed. Moreover, the allele and genotype frequencies of *MAGE-A1* polymorphisms and clinical factors, such as age, sex, and pathological classifications were analyzed among Saudi patients with CRC compared to matched normal colorectal tissues in order to determine potential influences on CRC susceptibility.

MATERIALS AND METHODS

Ethical approval certificate and data collection

This case-controlled study was reviewed by, and ethical approval was obtained from, the Ethics Review Committee of the Medicine College at the King Khalid University Hospital (KKUH) of King Saud University, Riyadh, Saudi Arabia. Written informed consent was obtained from participants after the objectives of this study were explained. Each participating subject completed and signed an informed consent questionnaire regarding his or her participation before blood sample was taken for genotyping purposes and a tissue biopsy was conducted for gene expression purposes. No patients received either immunotherapy or radiotherapy prior to sample collection, and the questionnaire collected demographic and clinical data, such as the age at which each patient was diagnosed with CRC, as well as each patient’s gender, family health history, smoking habits, tumor location, tumor grade and date of diagnosis. The questionnaire was written in both Arabic and English to ensure participants found it easy to understand, and the patients agreed that all data could be used for publishing.

Population and sample collection

To study *MAGE-A1* polymorphisms, the participants were divided into two groups. The first was a CRC group that comprised 192 Saudi patients (114 men and 78 women) with histological diagnoses of CRC and positive clinical, endoscopic, radiological and histopathological results for malignancies between 2011 and 2016 through the endoscopy service of the KKUH before they received any medical treatments for the disease. The second was a control group that comprised 192 age-matched healthy individuals (111 men and 81 women) who lacked any signs of malignancy. Whole blood specimens were collected from the individuals and genomic DNA was extracted from the blood to study the *MAGE-A1* polymorphisms of the CRC causes in comparison with normal groups.

DNA extraction from peripheral blood

A total of 3 ml of blood was taken from each study participant using an EDTA Vacutainer vial (BD Vacutainer Systems, Plymouth, UK), then stored at −80°C until required. Genomic DNA was immediately extracted from 200 µl of EDTA-anticoagulated whole blood in accordance with the manufacturer's instructions using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The isolated genomic DNA was preserved at −20°C until analysis, and the concentration of the DNA samples was validated via a NanoDrop 8000 spectrophotometer (Thermo Scientific, USA). Finally, DNA purity was measured in terms of A260/A280 and A260/A230 absorbance ratios.

MAGE SNP selections and DNA genotyping

TaqMan Allelic Discrimination assay kits (Thermo Fisher Scientific, Waltham, MA, USA) were used to genotype the three *MAGE-A1* polymorphisms: rs3788753 (C/G), rs3788749 (G/A), and rs3788745 (A/G). All primer sequences and probe mixtures that were used were designed by Thermo Fisher Scientific as per the manufacturer's protocol. *MAGE-A1* polymorphisms were selected based on three different criteria: *MAGE-A1* SNP positions, *MAGE-A1* SNP frequencies with cancer issues, and *MAGE-A1* SNP correlations with different malignances among patients of different ethnic backgrounds. For DNA genotyping, each sample was examined using a 96-Well Format QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Briefly, reaction mixtures were briefly investigated using a 10µL/reaction volume. Each reaction contained 2 µL of DNA (20 ng), 0.2 µL of 40× TaqMan® Genotyping SNP Assay (Applied Biosystems), 5.5 µL of 2X TaqMan PCR Master Mix (Applied Biosystems) and 2.3 µL of distilled water. For PCR amplifications, a Light Cycler 480 Instrument II Real Time PCR System was used.

The efficiency of genotyping samples was tested to confirm the genotyping quality with positive and negative controls. The laboratory staff that performed the genotyping assays was blinded to patient's information.

The statistical methods used for data analyses

Data analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 24, made by SPSS, Chicago, USA). p-values were considered statistically significant is they were below 0.05, and analyzed polymorphism frequencies were tested for patients with CRC and healthy controls using Hardy-Weinberg equilibrium (HWE). Odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for both genders and ages. X2 values were measured using chi-square tests to determine significant differences between the patients with CRC and the healthy individuals.

RESULTS

The basic clinical parameters of the study populations

As shown in Table 1, 192 cases with pathologically confirmed CRC and 192 normal volunteers were enrolled, and any patient who was not Saudi and/or who was a smoker was excluded from the study. There were no significant demographic variations between the CRC cases and the healthy controls; however, there were some clinical differences. For example, patients' ages, genders and tumor locations varied between groups. The median ages were 55.74 ± 12.34 years and 56.56 ± 11.21 years for healthy and cancer groups, respectively. Moreover, no significant differences were found in the male-to-female ratios of the CRC cases and the normal volunteers (the ratios were 114:78 and 111:81 for CRC and control individuals, respectively). Other clinical parameters for the populations, including the localizations of the patients' tumors and CRC therapy data, are shown in Table 1.

Table 1. Clinical characteristics of the subjects for genotyping.

Variables	Cancer	Control
Number	192	192
Gender		
Male	114	111
Female	78	81
Age		
Below 57	106	94
Above 57	86	98
Median Age	56.56± 11.21	55.74 ± 12.34
Localization		
Colon	116	---
Rectum	76	---

The relationship between select *MAGE-A1* polymorphisms and CRC risks among Saudi patients

As previously mentioned, to examine the correlation between *MAGE-A1* polymorphisms and colorectal cancer susceptibility among the Saudi population, three *MAGE-A1* SNPs (rs3788753, rs3788749 and rs3788745) were examined and their allelic and genotypic frequencies were compared between 192 CRC patients and 192 healthy control individuals. The allelic and genotype distributions of the examined SNPs, along with corresponding odds ratios, are shown in Table 3. Observations of genotypic frequencies did not result in any significant Hardy–Weinberg expectations for rs3788745 and rs3788753. The genotype frequencies of patients with CRC were 91% for TT, 8% for TC and 1% for CC for rs3788745 and 90% CC, 5% CG and 5% GG for rs3788753. The genotype frequencies of control individuals were 92%, 6% and 2% for TT, TC and CC for rs3788745, respectively, and 88%, 8% and 4% for CC, CG and GG for rs3788753, respectively. However, the heterozygous CT and a combination of CT + TT genotypes for the SNP rs3788749 showed a more than two-fold higher risk for CRC developments in patients with CRC, not the healthy control individuals. For CT, OR was 2.528, the CI was 1.023–6.248 and p= 0.038. For CT + TT, OR was 2.677, the CI was 1.091–6.570 and p= 0.026. Moreover, the T allele frequency was high in CRC cases, unlike the control cases; OR was 2.745, the CI was 1.140–6.609 and p= 0.019 (Table 2).

Table 2. Genotype frequencies of *MAGEA1* gene polymorphism in colorectal cases and controls.

SNP	Variants	Patients Cases	Controls	OR	CI	χ^2 Value	P- Value
rs3788745	TT	172 (0.91)	175 (0.92)	Ref			
	TC	16 (0.08)	11 (0.06)	1.480	0.668-3.280	0.94	0.33197
	CC	2 (0.01)	4 (0.02)	0.509	0.092-2.814	0.62	0.43034
	TC+CC	18 (0.9)	15 (0.08)	1.221	0.596-2.500	0.30	0.58472
rs3788749	T	360 (0.95)	361 (0.95)	Ref			
	C	20 (0.05)	19 (0.05)	1.056	0.554-2.011	0.03	0.86941
	CC	171 (0.90)	178 (0.96)	Ref			
	CT	17 (0.09)	7 (0.04)	2.528	1.023-6.248	4.28	**0.03849**
	TT	1 (0.01)	0 (0.00)	3.122	0.126-77.175	1.04	0.30832

	CT+TT	18 (0.10)	7 (0.04)	2.677	1.091-6.570	4.94	**0.02627**
	C	359 (0.95)	363 (0.98)	Ref			
	T	19 (0.05)	7 (0.02)	2.745	1.140-6.609	5.48	**0.01928**
	CC	173 (0.90)	166 (0.88)	Ref			
	CG	10 (0.05)	14 (0.08)	0.685	0.296-1.586	0.79	0.37517
rs3788753	GG	9 (0.05)	8 (0.04)	1.079	0.407-2.864	0.02	0.87790
	CG+GG	19 (0.10)	22 (0.12)	0.829	0.433-1.587	0.32	0.57042
	C	356 (0.93)	346 (0.92)	Ref			
	G	28 (0.07)	30 (0.08)	0.907	0.531-1.550	0.13	0.72133

Notes: P-Values in bold represent significant results (P < 0.05); Ref=Reference allele.

Comparison of *MAGE-A1* SNP genotype and allele allocations based on the clinical parameters of the CRC and control subjects

MAGE-A1 genotypic and allelic distributions were analyzed after correlations between genders, ages, and tumor locations and CRC diagnoses were determined. First, the relationship between CRC risks and individual SNPs was determined based on genders. The genotype frequencies of males (n=114) and females (n=78) with CRC, compared to those of healthy males and females, are presented in Table 4. No significant relationship was found between female and male patients with CRC and healthy male and female subjects (see Table 3 for males and females). Second, SNP correlations to the ages at which patients were diagnosed with CRC were analyzed and patients were categorized as ⩽ 57 (n=106) or > 57 (n=86) years of age. The allele and genotype allocations of individual SNPs and statistical analyses are shown in Table 6 and 7, respectively.

The results revealed no significant relationship regarding patients' ages, whether patients were above or below 57 years old. That is, ages did not appear to affect the genotype and allele allocations of the three *MAGE-A1* polymorphisms (Table 4). Third, correlations based on tumor locations (the colon or rectum) between colon cancer risks and individual *MAGE-A1* SNPs were assessed (Table 5). The SNP rs3788749 had a significant correlation with increased CRC risks among those diagnosed with CRC in the rectum. The CT and CT + TT genotype frequencies were similar in that the frequency was 12% for colon cancer patients and 4% for the control subjects. These genotypes increased the risk of CRC development by more than three times in Saudi patents (OR= 3.416, CI= 1.223–9.538, and p= 0.014). Furthermore, in the Saudi CRC population, T alleles were present more than three-fold higher than in the control population: OR= 3.264, CI= 1.193–8.930 and, p= 0.024 (Table 5).

Table 3. Genotype frequencies of *MAGEA1* gene polymorphism in colorectal cases and controls (gender: Male or Female)

Gender	SNP	Variant	patients Cases	Controls	OR	CI	χ^2 Value	P- Value
		TT	104 (0.92)	101 (0.93)	Ref			
		TC	8 (0.07)	5 (0.05)	2.200	0.738-6.560	2.09	0.14861
	rs3788745	CC	1 (0.01)	3 (0.02)	0.333	0.034-3.259	0.98	0.32203
		TC+CC	9 (0.08)	8 (0.07)	1.500	0.588-3.825	0.73	0.39348
Male		T	216 (0.96)	207 (0.95)	Ref			
		C	10 (0.04)	11 (0.05)	1.149	0.503-2.622	0.11	0.74212
		CC	101 (0.89)	104 (0.95)	Ref			
	rs3788749	CT	11 (0.10)	5 (0.05) 0.)	2.265	0.760-6.751	2.25	0.13330

		TT	1 (0.01)	0 (0.00)	3.089	0.124-76.706	1.02	0.31143
		CT+TT	12 (0.11)	5 (0.05)	2.471	0.840-7.267	2.86	0.09108
		C	213 (0.94)	213 (0.98)	Ref			
		T	13 (0.06)	5 (0.02)	2.600	0.911-7.421	3.41	0.06470
		CC	104 (0.91)	96 (0.88)	Ref			
		CG	6 (0.05)	6 (0.06)	0.923	0.288-2.960	0.02	0.89286
		GG	4 (0.04)	6 (0.06)	0.615	0.169-2.247	0.55	0.45872
	rs3788753	CG+GG	10 (0.09)	12 (0.12)	0.769	0.318-1.862	0.34	0.55988
		C	214 (0.94)	198 (0.92)	Ref			
		G	14 (0.06)	18 (0.08)	0.720	0.349-1.485	0.80	0.37181
		TT	68 (0.88)	73 (0.91)	Ref			
		TC	8 (0.10)	6 (0.08)	1.043	0.321-3.388	0.0002	0.94434
		CC	1 (0.01)	1 (0.01)	0.348	0.014-8.674	0.95	0.32907
	rs3788745	TC+CC	9 (0.12)	0.01) 7 (0.09)	0.894	0.286-2.791	0.04	0.84681
		T	144 (0.94)	152 (0.95)	Ref			
		C	10 (0.06)	8 (0.05)	0.781	0.264-2.305	0.20	0.65349
		CC	70 (0.92)	73 (0.97)	Ref			
		CT	6 (0.08)	2 (0.03)	3.129	0.611-16.025	2.06	0.15157
		TT	0 (0.00)	0 (0.00)				
Female	**rs3788749**	CT+TT	6 (0.08)	2 (0.03)	3.129	0.611-16.025	2.06	0.15157
		C	146 (0.96)	148 (0.97)	Ref			
		T	6 (0.04)	2 (0.03)	3.041	0.604-15.314	2.00	0.28294
		CC	69 (0.88)	70 (0.87)	Ref			
		CG	4 (0.06)	8 (0.10)	0.507	0.146-1.762	1.18	0.27812
		GG	5 (0.06)	2 (0.03)	2.536	0.476-13.516	1.27	0.26057
	rs3788753	CG+GG	9 (0.12)	10 (0.13)	0.913	0.350-2.385	0.03	0.85262
		C	142 (0.91)	148 (0.92)	Ref			
		G	14 (0.09)	12 (0.08)	1.216	0.544-2.719	0.23	0.63347

Ref=Reference allele

Table 4. Genotype frequencies of *MAGEA1* gene polymorphism in colorectal cases and controls (age: below 57 or above 57)

Age	SNP	Variant	patients Cases	Controls	OR	CI	χ^2 Value	P- Value
		TT	95 (0.91)	84 (0.92)	Ref			
		TC	9 (0.09)	5 (0.05)	1.987	0.663-5.955	1.55	0.21296
		CC	0	2 (0.03)	0.181	0.009-3.819	2.19	0.13916
	rs3788745	TC+CC	9 (0.09)	7 (0.08)	1.419	0.526-3.829	0.48	0.48752
		T	199 (0.96)	173 (0.95)	Ref			
Below 57		C	9 (0.04)	9 (0.05)	1.073	0.435-2.651	0.02	0.87809
		CC	93 (0.89)	85 (0.94)	Ref			
		CT	11 (0.11)	5 (0.06)	2.011	0.671-6.024	1.61	0.20483
	rs3788749	TT	0 (0.00)	0 (0.00)				
		CT+TT	11 (0.11)	5 (0.06)	2.011	0.671-6.024	1.61	0.20483

		C	197 (0.95)	175 (0.97)	Ref			
		T	11 (0.05)	5 (0.03)	1.954	0.666-5.735	1.54	0.21485
		CC	96 (0.91)	77 (0.85)	Ref			
		CG	4 (0.04)	8 (0.09)	0.401	0.116-1.382	2.22	0.13637
		GG	5 (0.05)	5 (0.06)	0.802	0.224-2.871	0.12	0.73422
	rs3788753	CG+GG	9 (0.09)	0) 13 (0.15)	0.555	0.225-1.367	1.67	0.19626
		C	196 (0.93)	162 (0.90)	Ref			
		G	14 (0.07)	18 (0.10)	0.643	0.310-1.332	1.43	0.23179
		TT	76 (0.89)	90 (0.92)	Ref			
		TC	7 (0.08)	6 (0.06)	1.169	0.362-3.773	0.07	0.79397
		CC	2 (0.02)	2 (0.02)	0.584	0.052-6.570	0.19	0.65987
	rs3788745	TC+CC	9 (0.11)	8 (0.08)	1.023	0.355-2.949	0.004	0.96682
		T	159 (0.94)	186 (0.95)	Ref			
		C	11 (0.06)	10 (0.05)	0.930	0.358-2.413	0.02	0.88138
		CC	77 (0.92)	92 (0.98)	Ref			
		CT	6 (0.07)	2 (0.02)	3.584	0.703-18.270	2.66	0.10303
		TT	1 (0.01)	0 (0.00)				
Above 57	**rs3788749**	CT+TT	7 (0.08)	2 (0.02)	4.182	0.844-20.720	3.56	0.05924
		C	160 (0.95)	186 (0.99)	Ref			
		T	8 (0.05)	2 (0.01)	4.650	0.973-22.214	4.44	0.05128
		CC	76 (0.88)	89 (0.91)	Ref			
		CG	6 (0.07)	6 (0.06)	1.171	0.363-3.782	0.07	0.79160
		GG	4 (0.05)	3(0.03)	1.561	0.339-7.196	0.33	0.56477
	rs3788753	CG+GG	10 (0.12)	9 (0.09)	1.301	0.503-3.368	0.30	0.58670
		C	158 (0.92)	184 (0.94)	Ref			
		G	14 (0.08)	12 (0.06)	1.359	0.611-3.023	0.57	0.45119

Ref=Reference allele

Table 5. Genotype frequencies of *MAGEA1* gene polymorphism in colorectal cases and controls (tumour location: colon or rectum)

Tumor location	SNP	Variant	patients Cases	Controls	OR	CI	χ^2 Value	P- Value
		TT	101 (0.89)	175 (0.92)	Ref			
		TC	10 (0.09)	11 (0.06)	1.575	0.646-3.838	1.01	0.31408
		CC	2 (0.02)	4 (0.02)	0.866	0.156-4.814	0.03	0.86964
	rs3788745	TC+CC	12 (0.11)	15 (0.08)	1.386	0.624-3.078	0.65	0.42079
		T	212 (0.94)	361 (0.95)	Ref			
		C	14 (0.06)	19 (0.05)	1.255	0.616-2.554	0.39	0.53081
Colon		CC	103 (0.92)	178 (0.96)	Ref			
		CT	8 (0.07)	7 (0.04)	1.975	0.696-5.605	1.69	0.19359
		TT	1 (0.01)	0 (0.00)	5.174	0.209-128.173	1.72	0.19000
	rs3788749	CT+TT	9 (0.08)	7 (0.04)	2.222	0.804-6.144	2.47	0.11570
		C	214 (0.96)	363 (0.98)	Ref			
		T	10 (0.04)	7 (0.02)	2.423	0.909-6.460	3.32	0.06839
	rs3788753	CC	102 (0.89)	166 (0.88)	Ref			

		CG	6 (0.05)	14 (0.08)	0.697	0.260-1.873	0.52	0.47263
		GG	7 (0.06)	8 (0.04)	1.424	0.501-4.045	0.44	0.50502
		CG+GG	13 (0.11)	22 (0.12)	0.962	0.464-1.993	0.01	0.91628
		C	210 (0.91)	346 (0.92)	Ref			
		G	20 (0.09)	30 (0.08)	1.098	0.608-1.984	0.10	0.75559
		TT	70 (0.92)	175 (0.92)	Ref			
		TC	6 (0.08)	11 (0.06)	1.364	0.486-3.830	0.35	0.55475
		CC	0	4 (0.02)	0.277	0.015-5.205	1.59	0.20736
	rs3788745	TC+CC	6 (0.08)	15 (0.08)	1.000	0.373-2.682	0.001	1
		T	146 (0.96)	361 (0.95)	Ref			
		C	6 (0.04)	19 (0.05)	0.781	0.306-1.994	0.27	0.60426
		CC	67 (0.88)	178 (0.96)	Ref			
		CT	9 (0.12)	7 (0.04)	3.416	1.223-9.538	6.08	**0.01368**
		TT	0 (0.00)	0 (0.00)	2.644	0.052-134.610	0.001	1
Rectum	**rs3788749**	CT+TT	9 (0.12)	7 (0.04)	3.416	1.223-9.538	6.08	**0.01368**
		C	143 (0.94)	363 (0.98)	Ref			
		T	9 (0.06)	7 (0.02)	3.264	1.193-8.930	5.89	**0.02373**
		CC	70 (0.92)	166 (0.88)	Ref			
		CG	4 (0.05)	14 (0.08)	0.678	0.215-2.131	0.45	0.50317
		GG	2 (0.03)	8 (0.04)	0.593	0.123-2.862	0.43	0.51075
	rs3788753	CG+GG	6 (0.08)	22 (0.12)	0.647	0.251-1.664	0.83	0.36300
		C	144 (0.95)	346 (0.92)	Ref			
		G	8 (0.05)	30 (0.08)	0.641	0.287-1.431	1.20	0.27430

Comparison between Saudi Arabian individuals and those in other ethnics groups

The Saudi Arabian individuals examined in this study were genotyped to compare the samples of the residents of Riyadh region with results in other ethnics groups obtained from the literature (Table 6). We studied 190, 185 and 188 samples from Riyadh region, for the rs3788745, rs3788749, and rs3788753 SNP genotypes, respectively. The allelic and genotypic frequencies for rs3788745 and rs3788749 SNPs were significantly different among Chinese (Han Chinese in Beijing), Japanese (Japanese in Tokyo), Nigerian (Yoruba in Ibadan), Kenyan (Maasai in Kinyawa), and Utah residents of Northern and Western European ancestry for rs3788749 compared to those of the Saudi Arabian population (P<0.005). For *MAGE-A1* rs3788753, the Saudi population (CRS) was significantly different from Chinese (HCB), Japanese (JPT), Nigerian (YRI), Kenyan (MKK), Utah residents of Northern and Western European ancestry (CEU) populations. Moreover, as presented in Figures 1 and 2, regional linkage disequilibrium (LD) plots were constructed using SNP annotation and Proxy Search (SNAP, http://www.broadinstitute.org/mpg/snap/ldplot.php). The maximum r2 values for the determined SNPs were 0.911 for rs3788749 and 0.494 for rs3788753. Nevertheless, there was no LD data found in the literature for rs3788745.

Table 6. *MAGEA1* Allele and genotype frequencies in Riyadh region and other populations

SNP	Population	Genotype frequency (N)			Allele frequency		X^2	P value
		TT	TC	CC	T	C		
rs3788745	CRS (n=190)	0.921 (175)	0.058 (11)	0.021 (4)	0.950	0.050	-	-
	HCB (n=90)	0.400 (36)	0.244 (22)	0.356 (32)	0.522	0.478	73.41313	**<0.005**
	JPT (n=88)	0.409 (36)	0.182 (16)	0.409 (36)	0.500	0.500	78.3675	**<0.005**

rs3788749	YRI (n=120)	0.750 (90)	0.133 (16)	0.117 (14)	0.817	0.183	14.26769	**<0.005**
	CRS (n=185)	0.962 (178)	0.038 (7)	-	0.981	0.019	-	-
	CEU (n=120)	0.100 (12)	0.100 (12)	0.800 (96)	0.150	0.850	222.2001	**<0.005**
	HCB (n=v)	0.067 (6)	0.178 (16)	0.756 (68)	0.156	0.844	200.6116	**<0.005**
	JPT (n=88)	0.045 (4)	0.114 (10)	0.841 (74)	0.102	0.898	218.5015	**<0.005**
rs3788753	YRI (n=120)	-	-	1.000 (120)	-	1.000	290.7495	**<0.005**
	CRS (n=188)	0.883 (166)	0.074 (14)	0.883 (8)	0.920	0.080	-	-
	CEU (n=120)	0.967 (116)	0.033 (4)	-	0.983	0.017	5.524966	**0.019**
	HCB (n=88)	0.409 (36)	0.250 (22)	0.341 (30)	0.534	0.466	55.27718	**<0.005**
	JPT (n=88)	0.409 (36)	0.182 (16)	0.409 (36)	0.500	0.500	62.97618	**<0.005**
	YRI (n=120)	0.750 (90)	0.133 (16)	0.117 (14)	0.817	0.183	7.389096	**0.007**

Abbreviations: CRS: Saudi population residing in the Riyadh region of central Saudi Arabia. **CEU:** Utah residents with Northern and Western European ancestry from the CEPH collection. **HCB:** Han Chinese in Beijing, China. **JPT:** Japanese in Tokyo, Japan. **YRI:** Yoruba in Ibadan, Nigeria.

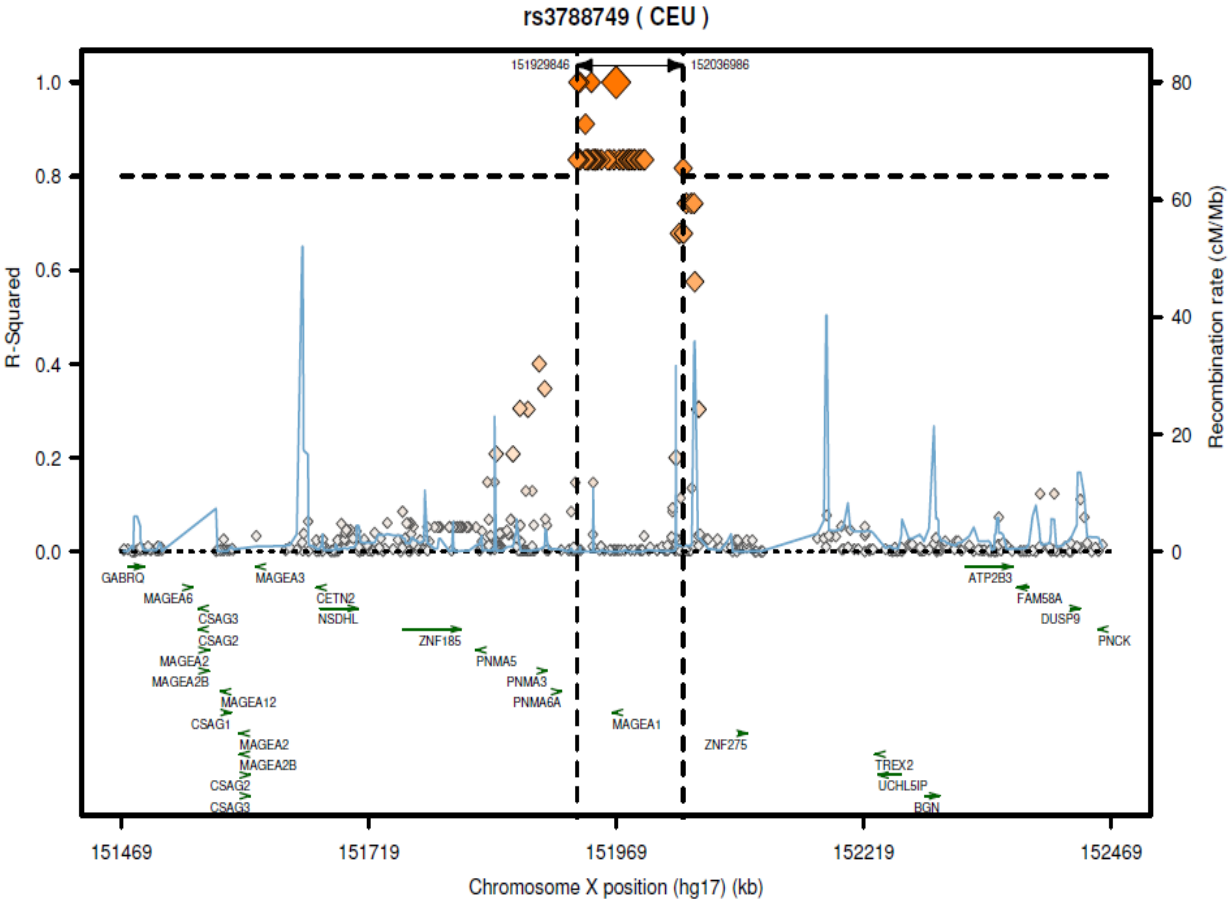

Figure 1: Regional LD plot for the MAGE-A1 rs3788749 SNP.

Abbreviations: LD: Linkage Disequilibrium; MAGE: Melanoma Associated Antigen; SNP: Single-Nucleotide Polymorphism; CEU: Utah Residents with Northern and Western European Ancestry from the CEPH Collection; CEPH: Centre D"Etude Du Polymorphism Human.

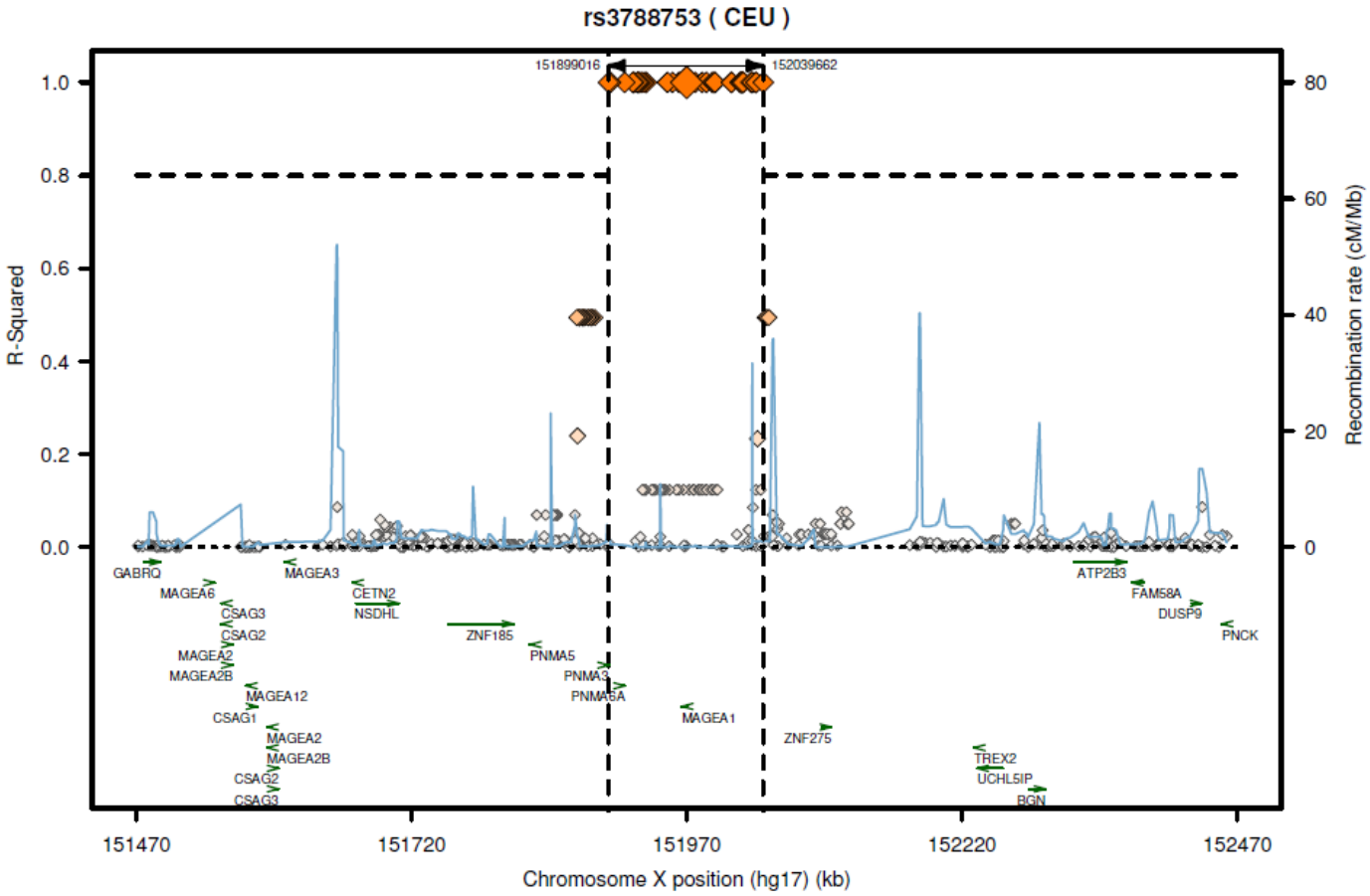

Figure 2: Regional LD plot for rs3788753 SNP in MAGE-A1.

Abbreviations: LD: Linkage Disequilibrium; MAGE: Melanoma Associated Antigen; SNP: Single-Nucleotide Polymorphism; CEU: Utah Residents with Northern And Western European Ancestry from the CEPH Collection; CEPH: Centre D"Etude Du Polymorphism Human.

DISCUSSION

Susceptibility to CRC, a multifactorial disease, arises due to both environmental and genetic factors (Park Y et al., 2016). This cancer affects both males and females, and the CRC incidence has increased in Saudi Arabia in recent years. The early detection of CRC can allow patients to receive proper treatment, thereby reducing CRC morbidity, mortality, and incidence (Tan SC, 2018); however, appropriate screening methods are crucial for this detection. The occurrence of CRC is commonly associated with genetic polymorphisms in different genes (Ulrich CM, Robien K, McLeod HL, 2003), such as PITX1 (Gunathilake MN et al., 2018) and TLR-9 (Semlali A et al., 2016). One gene, *MAGE-A1*, is not expressed in normal tissues except the testis, but it is expressed in tumor tissues or tumor cells (Vanderbruggen P et al., 1991; Feichtinger J et al., 2012).

This unique expression pattern and the antigenic properties of the protein expressed by *MAGE-A1* allow this gene to be used as an immunotherapeutic target and cancer biomarker (Lee AK and Potts PR, 2017). The main hypotheses of the present study were that *MAGE-A1* is important in cancer initiation and that its genetic changes are associated with increased CRC risks. Consequently, the principal objective of this study was to investigate the effects of gene polymorphisms, specifically rs3788753, rs3788749, and rs3788745, and their associations with increased CRC risks in Saudi patients.

The results for *MAGE-A1* genetic polymorphism revealed no relationship between the allelic and genotypic distributions of the *MAGE-A1* SNPs rs3788745 and rs3788753 regarding CRC risks in the Saudi population. However, a significant relationship was evident between minor genetic and allelic allocations in the *MAGE-A1* SNP rs3788749 in terms of CRC risks in Saudi patients. According to the NCBI's SNP database, rs3788749 is an intron SNP, and it may increase *MAGE-A1* gene expression through its independent functioning as a binding site for transcription factors (Deng N, Zhou H, Fan H, Yuan Y, 2017; Zhu X, Asa SL, Ezzat S, 2009). Nevertheless, many recent studies have reported that this "intron-mediated enhancement" can lead to increases in gene transcription, gene stability, and the efficiency of mRNA translation. For example, Zhu et al (2009) determined that polymorphic sequences in intron 2 of the FGFR 2 gene was related to a constitutive histone acetylation at multiple sequences in this intron that harbored putative transcription binding sites (Zhu X, Asa SL, Ezzat S, 2009).

Significant correlations were found between the rs3788749 SNP and cancer of the rectum, but no significant correlations were found between the SNP and cancer of the colon at the genetic and allelic levels. This finding suggested that this polymorphism is a specific marker for rectal cancer and therefore could be useful as a rectal cancer biomarker for the early detection of CRC in Saudi patients. By contrast, the three *MAGE-A1* SNP locus genotypes showed no correlation with clinical parameters, such as gender and age, in Saudi males and females.

No previous studies have reported an association between *MAGE-A1* genetic polymorphisms and CRC susceptibilities. Wang et al. (2004) demonstrated an association between the distribution of *MAGE-A1* SNPs and hepatocellular carcinoma in a Chinese population, but the tested SNPs were not the same as those examined in the present study (Wang LP et al., 2004). A literature search for the *MAGE-A1* SNPs rs3788745 and rs3788749 revealed a similar profile for the Riyadh populations and other ethnic groups and a similar profile to that described for SNPs in other genes, such as TLR4 (Kohailan M et al., 2017) and TLR6 (Kohailan M et al., 2016). At the expression level, different studies have shown a higher expression of the *MAGE-A1* gene in colon cancer tissues than in normal colon tissues (Feichtinger J et al., 2012; Hofmann O et al., 2008) ; Hilal NR, Novikov DV, Novikov VV, Karaulov AV, 2017).

The MAGE-A family members, such as the *MAGE-A1* gene, could be involved in the progression of gastric cancer, as determination of their expression profiles appears to have prognostic value for patients with gastric cancer (Lian Y et al., 2017). To date, no previous studies have examined the relationship between the genetic variations of the *MAGE-A1* rs3788753, rs3788749, and rs3788745 SNPs among Saudi patients with CRC. Consequently, the strength this work is its novelty; it is the first study that validates the correlation between genetic polymorphism of the *MAGE-A1* gene and CRC development risk, particularly in the Saudi ethnic population, who suffer from several genetic diseases due to consanguinity. However, this work also has some limitations. First, the number of samples is small; therefore, larger sample sizes and studies of other ethnic populations are required to confirm the associations between CRC progression and *MAGE-A1* SNPs, especially SNPs located in regulatory regions of the gene. Second, studies are also needed on the function of these genetic variants on CRC development; therefore, we propose to conduct future investigations of the effect of these *MAGE-A1* SNPs on CRC cell proliferation, apoptosis, and migration in order to understand the mechanisms of action of these polymorphisms in the regulation of CRC development.

CONCLUSION

The genotypes and alleles of the rs3788749 *MAGE-A1* SNP were associated with CRC susceptibility in the Saudi population. This suggests that this polymorphism may serve as a rectal cancer biomarker for the early detection of CRC. However, further investigations are necessary to determine the mechanisms and the functional aspects this relationship between rs3788749 polymorphisms and CRC.

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DISCLOSURE

The authors report no conflicts of interest in this work.

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