RUNX3 gene polymorphisms and haplotypes in Mexican patients with colorectal cancer

S. Suárez-Villanueva[1], M.L. Ayala-Madrigal[1], J. Peregrina-Sandoval[2], N. Macías-Gómez[3], R. Ramírez-Ramírez[1], R. Muñiz-Mendoza[1], J.M. Moreno-Ortiz[1], M. Centeno-Flores[4], V. Maciel-Gutiérrez[4], E. Cabrales[5] and M. Gutiérrez-Angulo[1,6]

[1]Doctorado en Genética Humana and Instituto de Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México
[2]Laboratorio de Inmunobiología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México
[3]Laboratorio de Genética Humana, Centro Universitario del Sur, Universidad de Guadalajara, Ciudad Guzmán, Jalisco, México
[4]Hospital Civil de Guadalajara "Dr. Juan I. Menchaca", Guadalajara, Jalisco, México
[5]Hospital Civil de Guadalajara "Fray Antonio Alcalde", Guadalajara, Jalisco, México
[6]Departamento de Clínicas, Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlán de Morelos, Jalisco, México

Corresponding author: M. Gutiérrez-Angulo
E-mail: melva73@hotmail.com

Genet. Mol. Res. 14 (4): 15505-15510 (2015)
Received June 29, 2015
Accepted July 23, 2015
Published November 30, 2015
DOI http://dx.doi.org/10.4238/2015.November.30.28

ABSTRACT. We analyzed a possible association between *RUNX3* gene polymorphisms and haplotypes in Mexican patients with colorectal cancer (CRC). Genomic DNA samples were obtained from the peripheral blood of 176 Mexican patients with CRC at diagnosis and from 195 individuals that formed the control group. The polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism. Association was estimated by odds ratio (OR). The haplotypes and linkage disequilibrium

were established using the Arlequin v3.5 software. We found that the *RUNX3* polymorphisms analyzed were in Hardy-Weinberg equilibrium. The *RUNX3* rs2236852 AA genotype and A allele showed association with CRC (OR = 0.39, 95%CI = 0.21-0.73, P < 0.01; OR = 0.65, 95%CI = 0.49-0.87, P < 0.01, respectively), while the rs6672420, rs11249206, and rs760805 polymorphisms did not show significant association with CRC. The TA haplotype (SNPs rs760805 and rs2236852) showed an increased risk for CRC (OR = 2.52, 95%CI = 1.47-4.30, P < 0.001). In conclusion, we found that the AA genotype and A allele of rs2236852 polymorphism confer a decreased CRC risk, while the TA haplotype appears to increase the risk of CRC development in Mexican patients.

Key words: *RUNX3* gene; Colorectal cancer; Polymorphisms; Mexican population

INTRODUCTION

The human *RUNX3* gene (GenBank accession No.: NT_004610.18), which maps to chromosome 1p36.1, contains six exons and its overall size is approximately 67 kb. The RUNX3 protein is required for neurogenesis of dorsal root ganglia and is part of the WNT and TGF-β pathways in intestinal epithelia (Tsunematsu et al., 2009). The inactivation of *RUNX3* by epigenetic mechanisms, loss of heterozygosity, homozygous deletion, and genetic variants has been associated with gastric, bladder, lung, and colorectal cancer (CRC) (Bae and Choi 2004; Kim et al., 2005; Zhang et al., 2008; Soong et al., 2009; Fan et al., 2011; Lee et al., 2013). Approximately 250 variants of *RUNX3* have been reported (Sherry et al., 2001). In this study, Mexican patients with CRC were screened for four polymorphisms, namely rs6672420 (exon 1, c.53 A>T), rs11249206 (intron 1, T>C), rs760805 (intron 3, T>A), and rs2236852 (intron 4, G>A), previously shown to be associated with several types of cancer.

MATERIAL AND METHODS

Populations

The present study included 176 patients (98 men and 78 women; mean age: 57.8 years; range: 20-96 years) selected by the oncology service at the Hospitales Civiles de Guadalajara, Mexico, according to their clinical and histopathological CRC criteria. The ratio of colon cancer and rectal cancer was 1.55:1. A control group was composed of 195 healthy blood donors (126 men and 69 women; mean age: 34 years; range: 19-56 years) without familial history of cancer from the Hospital Civil “Juan I. Menchaca” of Guadalajara, Mexico. All subjects provided written informed consent before donating 5-10 mL peripheral blood each. This research was approved by the Ethics Committee of Centro Universitario de los Altos, Universidad de Guadalajara (CUA/CINV/035/2013).

Genotyping

Genomic DNA was isolated from peripheral blood by CTAB/DTAB (Gustincich et al., 1991). The analysis of *RUNX3* variants was performed by PCR-RFLP. PCR conditions included an

initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 65.5°C for rs6672420 and rs2236852 and 62°C for rs11249206 and rs760805, and 30 s extension at 72°C. The final extension was at 72°C for 5 min. The primer sequences used were designed according to Zhang et al. (2008). The restriction enzymes were *Bts*cI for rs6672420, *Nde*I for rs11249206 and rs2236852, and *Bts*1107 for rs760805. The digested products were visualized in 6% silver-staining polyacrylamide gel.

Statistical analysis

Genotypes and allele frequencies were determined by counting. Hardy-Weinberg equilibrium (HWE) analysis wasperformed by the chi-square test. In both groups, haplotypes were inferred by means of Bayesian algorithm for unknown gametic phase, and the linkage disequilibrium (LD) between SNP pairs was measured by correlation (r^2) using the Arlequin v3.5 software (Excoffier and Lischer, 2010). Risk effects in genetic associations were evaluated by odds ratio (OR). Significance was considered if $P < 0.05$ and $r^2 > 0.33$ for linkage disequilibrium (Ardlie et al., 2002). For haplotypes only, $P < 0.005$ after Bonferroni's correction was considered to be significant.

RESULTS

The demographic factors of CRC patients and controls are showed in Table 1. All four polymorphisms were in HWE in the control group. The association analysis related the AA genotype and A allele of rs2236852 (intron 4, G>A) polymorphism to a significantly decreased CRC risk (OR = 0.39, 95%CI = 0.21-0.73, $P < 0.01$; OR = 0.65, 95%CI = 0.49-0.87, $P < 0.01$, respectively). No statistical intergroup differences for genotype and allele frequencies of rs6672420, rs11249206, and rs760805 polymorphisms were found (Table 2). The LD analysis for all SNPs showed disequilibrium for rs760805 and rs2236852, with $r^2 = 0.70$ for controls and $r^2 = 0.44$ for patients. Among the haplotypes inferred from these SNPs, the most frequent was found to be TG, constructed by combining the wild alleles. Additionally, the TA haplotype appeared to confer a 2.52-fold increased risk for CRC (Table 3). These results were presented at the 64th Annual Meeting of The American Society of Human Genetics (Suarez-Villanueva et al., 2014).

Table 1. Demographic data of CRC patients and controls.

	Cases [N = 176 (%)]	Controls [N = 195 (%)]
Gender		
Female	78 (44)	69 (35)
Male	98 (56)	126 (65)
Smoking		
No	77 (44)	83 (43)
Yes	86 (49)	112 (57)
NA	13 (7)	
Alcohol consumption		
No	84 (47.7)	46 (24)
Yes	75 (42.6)	149 (76)
NA	17 (9.7)	
Physical activity		
No	87 (49)	76 (39)
Yes	63 (36)	119 (61)
NA	26 (15)	
Diabetes		
No	122 (69)	193 (99)
Yes	37 (21)	1 (0.5)
NA	17 (10)	1 (0.5)

NA = not available.

Table 2. Analysis of the association between SNPs of *RUNX3* gene and colorectal cancer.

SNP ID	Genotype /Allele	CRC patients		Control group		P value*	OR (95%CI)
		N	(%)	N	(%)		
rs6672420	TT	87	49.4	115	59.0		1.00 (reference)
	TA	77	43.8	70	35.9	0.08	1.45 (0.95-2.23)
	AA	12	6.8	10	5.1	0.30	1.59 (0.66-3.84)
	T	251	71.3	300	76.9		1.00 (reference)
	A	101	28.7	90	23.1	0.08	1.34 (0.96-1.86)
rs11249206	TT	64	36.4	82	42.1		1.00 (reference)
	TC	87	49.4	97	49.7	0.53	1.15 (0.74-1.78)
	CC	25	14.2	16	8.2	0.05	2.00 (0.99-4.06)
	T	215	61.1	261	66.9		1.00 (reference)
	C	137	38.9	129	33.1	0.10	1.29 (0.95-1.74)
rs760805	TT	51	29.0	69	35.4		1.00 (reference)
	AT	100	56.8	92	47.2	0.99	1.47 (0.93-2.33)
	AA	25	14.2	34	17.4	0.99	0.99 (0.53-1.87)
	T	202	57.4	230	59.0		1.00 (reference)
	A	150	42.6	160	41.0	0.66	1.07 (0.80-1.43)
rs2236852	GG	44	25.0	28	14.4		1.00 (reference)
	GA	92	52.3	102	52.3	0.47	0.57 (0.33-1.0)
	AA	40	22.7	65	33.3	<0.01	0.39 (0.21-0.73)
	G	180	51.1	158	40.5		1.00 (reference)
	A	172	48.9	232	59.5	<0.01	0.65 (0.49-0.87)

*P < 0.05.

Table 3. Analysis of the *RUNX3* haplotype in colorectal cancer.

Haplotype (rs760805/rs2236852)	Chromosomes		P value*	OR (95%CI)
	CRC patients	Control group		
TG	156	205		1.00 (reference)
AA	128	133	0.15	1.27 (0.92-1.74)
TA	46	24	<0.001	2.52 (1.47-4.30)
AG	24	28	0.69	1.13 (0.63-2.02)

*Bonferroni's correction; P < 0.005.

DISCUSSION

The *RUNX3* gene, like the other members of the *RUNX* family, can function either as a tumor suppressor or as an oncogene. In CRC, *RUNX3* appears to function as a tumor suppressor due to its inactivation by epigenetic mechanisms in 41.5% of individuals with CRC (Soong et al., 2009). In this report, we found that carriers of the AA genotype or A allele of rs2236852 (intron 4, G>A) polymorphism have a decreased CRC risk. In Chinese patients, the corresponding AG and GG genotypes were associated with an increased risk for gastric cancer but not bladder neoplasm (Zhang et al., 2008; Wu et al., 2009). Although the rs2236852 polymorphism is located in an intron 4 region without apparent regulatory sequences (Flicek et al., 2013), a query of the TF search profile databases (Heinemeyer et al., 1998) using a program that searches for sequence fragments highly correlated to transcription factor binding sites showed a Sp1 transcription factor site located four nucleotides upstream of this polymorphism. Therefore, the protective effect of rs2236852 (intron 4, G>A) polymorphism in CRC may be related to changes in gene expression, or to combination with other haplotype variants.

For rs6672420, rs11249206, and rs760805 SNPs, no association with CRC risk was observed. However, Slattery et al. (2011) reported that the AA and TT genotypes of the rs6672420

SNP were associated with an increased risk for colon (OR = 1.24, 95%CI = 1.07-1.45) and rectal (OR = 1.24, 95%CI = 1.07-1.45) cancer, respectively. Wu et al. (2009) analyzed the rs11249206 and rs760805 polymorphisms in patients with gastric cancer and reported that the CC genotype for the former (OR = 1.75, 95%CI = 1.03-2.99) and the AA genotype for the latter (OR = 1.82, 95%CI = 1.14-2.92) increased the risk for such a neoplasm. A study of 368 Chinese patients with bladder cancer found that the rs760805 SNP AA genotype was associated with an increased risk (OR = 1.97, 95%CI = 1.44-2.69), which was even greater in patients aged >65 years (OR = 2.83, 95%CI = 1.77-4.53), males (OR = 2.16, 95%CI = 1.52-3.07), smokers (OR = 3.46, 95%CI = 2.16-5.54), and drinkers (OR = 2.60, 95%CI = 1.58-4.27) (Zhang et al., 2008).

The increased risk for CRC in carriers of the TA haplotype may be due to additional *RUNX3* variants in LD with our tested SNPs, rather than the A (rs760805) and G (rs2236852) alleles which were also found to increase CRC risk when considered individually. Association studies of *RUNX3* haplotypes in bladder and gastric cancer have been described. An analysis of ten *RUNX3* SNPs (including those reported here) revealed an increased risk for bladder cancer in individuals with TATCCCAAAA or AGCTTGAGAG haplotype (Zhang et al., 2008). Furthermore, *RUNX3* haplotypes of promoter-located SNPs have been associated with gastric cancer and inflammatory bowel disease (Lim et al., 2011; Guo et al., 2011). In conclusion, our results suggest that the AA genotype and A allele of rs2236852 polymorphism confer a decreased CRC risk, while the TA haplotype (rs760805 and rs2236852) increases the risk for CRC development in Mexican patients.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr. H. Rivera for the critical review of the manuscript. S. Suárez-Villanueva, R. Ramírez-Ramírez and R. Muñiz-Mendoza are Ph.D. students in Human Genetics and were supported by CONACyT fellowships.

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