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DACT1 variants and colorectal cancer

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According to GLOBOCAN, colorectal cancer is the third most frequent cancer and the second most common cause of cancer death worldwide [1]. Several risk factors, such as advanced age, family history of cancer, sex, alcohol, red meat, genetic, and epigenetic, mainly contribute to CRC prevalence [2]. Wnt/β-catenin is one of the most common pathways activated in many cancers and plays a key role in cell proliferation, apoptosis, Ca^{2+} homoeostasis, and differentiation [3]. The aberrant activation of the Wnt/β-catenin pathway is responsible for more than 90% of colorectal cancer cases [4]. The DACT family proteins (Dapper Antagonist of Catenin) consist of three members: DACT1, DACT2, and DACT3 [5]. DACT1 (also known as Dapper1/Dpr1) negatively regulates the Wnt/β-catenin pathway by interacting with Dishevelled (Dvl), a key mediator of Wnt signalling, and promoting its lysosomal degradation; therefore, *DACT1* can act as a potential tumour-suppressor gene [6]. Given the role of *DACT1* in Wnt pathway regulation and colorectal cancer pathogenesis, it is possible the *DACT1* single nucleotide polymorphisms (SNPs) may contribute to the risk of developing colorectal cancer. Huang *et al*. have shown that different genotypes of rs863091 affect the expression of *DACT1* in gastric cancer [7]. However, the association of genetic variations in the *DACT1* with colorectal cancer is unknown. We therefore hypothesized links between *DACT1* rs863091 and rs11541 SNPs with colorectal cancer.

We tested our hypothesis with 221 cases of colorectal cancer (118 males and 103 females, mean/SD age 49.5 ± 12.2) and 186 cancer-free controls (82 males and 104 females, 48.4 ± 12.9: sex and age differences $p = 0.061$ and $p = 0.406$, respectively). All subjects were selected from referrals to Taleghani Hospital, Tehran, Iran, between 2006 and 2015. All patients were diagnosed and confirmed based on histopathological tests, clinical examination, and colonoscopy on isolated biopsies. Noncancerous individuals were randomly taken from the people who visited the hospital for a routine check-up with no malignancy. Patients with a history of malignancies (self-reported history), radiotherapy, and previous chemotherapy treatment were excluded. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved the current research) Code: IR. SBMU. MSP.REC.1397.632. All cases and controls in this project provided written informed consent.

The genotype data for *DACT1* rs863091 and rs11541 was obtained according to UCSC Genome Browser (https://genome.ucsc.edu/) and dbSNP (https://www.ncbi.nlm.nih.gov/projects/) databases. The *DACT1* rs863091 and rs11541 variants are located in the coding exon 4 and 3-UTR of the *DACT1* gene, respectively. We used several online databases, such as HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and mirSNP (http://bioinfo.bjmu.edu.cn/mirsnp/search/), to predict the potential functional characteristics of these SNPs. Five 5 ml peripheral blood was collected into EDTA. We used the salting-out method for genomic DNA extraction, then extracted DNA was stored at −20℃. The quality of DNA was evaluated by 1% agarose gel electrophoresis prepared in 0.5X TBE buffer (tris/borate/EDTA). The purity and concentration of the extracted DNA were measured by NanoDrop Spectrophotometer (Aosheng, China). *DACT1* rs863091 and rs11541 genotyping were performed by tetra-primer amplification refractory mutation system polymerase chain reaction (TP-ARMS-PCR) method. Primer1 online software (http://primer1.soton.ac.uk/primer1.html) was employed for designing primers. The primer sequences for genotyping rs863091 were: Forward inner (Fi) GGCTTCTGAGGA ACGGGAGCGTTTGTTTC, Reverse inner (Ri) CCTGTGAGA CACCGCCCGGGGCTATA, Forward Outer (Fo) GCGGTGGA TCTGAGCTAGATGCCGTCAA, Reverse Outer (Ro) CCTCTT GCTTTCGGCTTGTTCGGCCTTT. The primer sequences for genotyping rs11541 were: Fi: TCTTAAAAAACAGCCC TTCCACAAAC, Ri: AATCCAGTCCAGATTGGACCTTTGAA CC, Fo: CTACTCATGCACAAAACATGCATATATTGG, Ro: AGTTTTGGGATAAAATTTTGGTCCTTGG. PCR reactions were prepared in a 25 µl reaction volume, including 2 µl of genomic DNA with DNA concentration of 100–200 ng,

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12.5 µL of 2X Taq PCR Pre-Mix (BioFact, Korea), 1 µL of respective primers with a final concentration of 10 pmol, and 6.5 µl ddH2O. XP thermal cycler apparatus (BIOER, China) was used to amplify genomic DNA. Briefly, amplification was performed with an initial denaturing step at 95℃ for 5 min followed by 32 cycles of denaturation at 95℃ for 1 min, annealing temperature at 60℃ for 30 sec for rs863091and 56℃ for 30 sec for rs11541, and extension at 72℃ for 1 min with a final extension at 72℃ for 5 min. The amplification products were evaluated by electrophoresis on a 2% agarose gel containing RedSafe stain dye (CinnaGen, Iran). Finally, we confirmed the genotype of 10% of the samples by Sanger sequencing using an ABI 3730xl DNA analyser (Macrogen, Korea) (Figure 1).

Statistical analysis for calculating allele and genotype frequencies for both variants was conducted by MEDCALC online calculator (www.medcalc.org/calc/odds_ratio) and SNPStats online software (https://www.snpstats.net). Chi-square test was applied for estimating the deviation from the Hardy–Weinberg equilibrium (HWE) in both patients and healthy controls. The statistical significance was set at the *P*-value <0.05.

The results of genotype and allele frequencies of *DACT1* polymorphisms are shown in Table 1. The SNPs' genotype frequencies in both patients and control subjects were consistent with the Hardy–Weinberg equilibrium. In a standard model, the *DACT1* rs863091 CT genotype was more frequent in patients, whilst in a dominant model, TT+CT was more frequent, and in an over-dominant model, CT was less frequent. However, there was no difference in allele frequencies. There were no associations between any *DACT1* rs11541 variants in allelic or genotypic frequencies with colorectal cancer in any inheritance models.

The aberrant activation of the Wnt pathway in CRC and involvement of *DACT* family members in this pathway have been established. DACT1 is a negative regulator for this pathway that inhibits the activation of

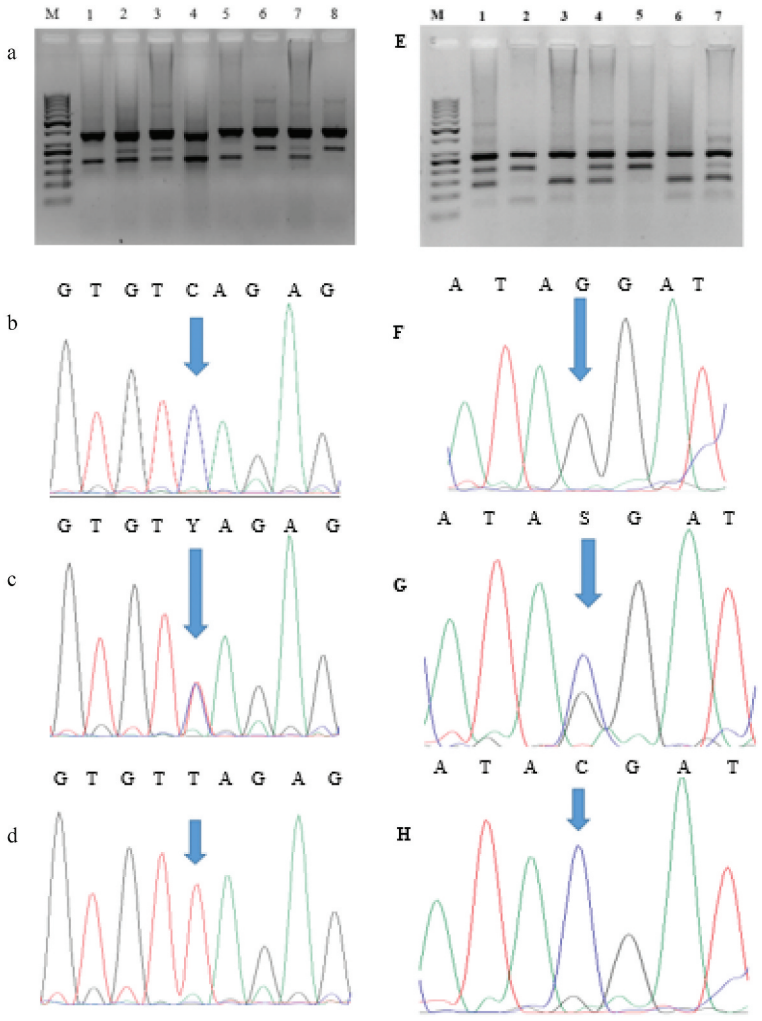

Figure 1. Gel electrophoresis pattern to identify rs863091 (a) and rs11541 polymorphisms (e). Figure (a); Lane M: 50 bp DNA marker; Lanes 1, 4, and 5 showing CC genotype; Lanes 2, 3, and 7 showing CT genotype; Lanes 6 and 8 showing TT genotype. Electropherograms show rs863091 different genotypes (b, c and d show CC, CT and TT genotypes, respectively). Figure (e); Lane M: 50 bp DNA marker; Lanes 1, 4, and 7 showing CG genotype; Lanes 3 and 6 showing GG genotype; Lanes 2 and 5 showing CC genotype). Electropherograms show different rs11541 genotypes (f, g and h showing the GG, CG and CC genotype, respectively).

Table 1. Genotype and allele frequencies for rs863091 and rs11541 SNPs in patients and controls.

Model	Allele/ genotype	Cases N (%)	Controls N (%)	OR (95% CI)	*P*
rs863091 C ≥ T	CC	103 (55.1)	108 (65.8)	1.00	-
	CT	77 (41.2)	47 (28.7)	1.71 (1.09–2.7)	0.019
	TT	7 (3.7)	9 (5.5)	0.81 (0.29–2.3)	0.69
Dominant	CC	103 (55.1)	108 (65.9)	1.00	-
	TT+CT	84 (44.9)	56 (34.1)	1.57 (1.02–2.4)	0.04
Recessive	CC+CT	180 (96.3)	155 (94.5)	1.00	-
	TT	7 (3.7)	9 (5.5)	0.67 (0.24–1.8)	0.43
Over-dominant	CC+TT	110 (58.8)	117 (71.3)	1:00	-
	CT	77 (41.2)	47 (28.7)	1.74 (1.11–2.7)	0.014
Allele	C	283 (75.7)	263 (80.2)	1.3 (0.90 – 1.9)	0.15
	T	91 (24.3)	65 (19.8)	0.76 (0.53–1.1)	0.15
rs11541 C ≥ G	GG	105 (53.9)	90 (59.6)	1.00	-
	CG	77 (39.5)	52 (34.4)	1.26 (0.80–2.0)	0.29
	CC	13 (6.7)	9 (6)	1.23 (0.50–3.0)	0.64
Dominant	GG	105 (53.9)	90 (59.6)	1.00	-
	CC+CG	90 (46.1)	61 (40.4)	1.26 (0.82–1.9)	0.28
Recessive	GG+CG	182 (93.3)	142 (94.0)	1.00	-
	CC	13 (6.7)	9 (6.0)	1.13 (0.47–2.7)	0.78
Over-dominant	GG+CC	118 (60.5)	99 (65.6)	1:00	-
	CG	77 (39.5)	52 (34.4)	1.24 (0.8–1.9)	0.33
Allele	C	287 (73.6)	232 (76.8)	1.19 (0.83–1.7)	0.33
	G	103 (26.4)	70 (23.2)	0.84 (0.59 – 1.2)	0.33

OR = odds ratio, CI = confidence interval.

Dvl and promotes its degradation [8]. Recently, the association between different gene SNPs of Wnt pathway with various cancers have been extensively studied [9]. The data presented here demonstrated that the *DACT1* rs863091 CT genotype increased the likelihood of colorectal cancer. We observed that the genotype frequencies showed a significant difference under the dominant and over-dominant inheritance models. Reports of previous studies on *DACT1* rs863091 SNPs in various cancers are conflicting. Huang *et al*. reported that the *DACT1* rs863091 T allele and TT+CT genotypes were linked with a decreased likelihood of gastric cancer in a Han Chinese population. They also showed that this association was observed, especially in younger individuals, demonstrating this variant's protective role against gastric cancer [7]. Fernandez-Rozadilla *et al*. detected no association between rs863091 and colorectal cancer in a Spanish cohort [10]. The rs863091 is a synonymous SNP, Val [GTC]>Val [GTT], and locates in exon 4 of the *DACT1*. The growing scientific evidence highlighted that synonymous SNPs (sSNPs) could affect the RNA processing, protein expression, and enzymatic activity through different mechanisms such as codon usage bias, aberrant mRNA splicing, translation fidelity, the stability of mRNA and protein folding [11,12]. According to HaploReg v4.1, rs863091 is the binding position for FOXP3, BDP1 and EBF transcription factors. The role of these transcription factors in colorectal cancer progression has been confirmed; for example, Sun *et al*. showed that *FOXP3* was overexpressed in colorectal cancer and its upregulation significantly correlated with poor prognosis [13]. Several studies have reported that genetic variations in miRNAs' binding sites are associated with different malignancies, including colorectal cancer [14]. The rs11541 is located in the 3′-UTR region of the *DACT1*. According to the mirSNP, rs11541 locates at the *hsa-miR-3692-3p* binding site and affects the binding properties. Results based on the HaploReg v4.1 indicated that some transcription factors, including TCF4 and HNF4, bind at the rs11541 location. TCF4 (T-cell factor4) is a DNA binding protein that interacts with β-catenin and promotes epithelial-mesenchymal transition (EMT) and proliferation in the Wnt pathway, which subsequently leads to an increase in malignant transformation [15]. We failed to observe any link between rs11541 allele and genotype frequencies and colorectal cancer. The present investigation suffers from limitations such as a relatively small sample size (so confirmation in a larger sample size is required) and its single point case-control design that cannot address the prospective risk of colorectal cancer, merely a link. Nevertheless, this work represents an advance in biomedical science because it points to the possible importance of *DACT1* rs863091 in colorectal cancer, and so its potential use in diagnosis.

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Disclosure statement

The authors state no conflict of interest and have no other relevant affiliations or financial involvement.

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