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Evaluation of *DRD1* Gene Expression Pattern and rs774034163 Genetic Polymorphism in Patients with Gastric Cancer: A Case-Control Study in Iran

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Objective: Gastric cancer (GC) ranks among the most prevalent cancers of the gastrointestinal (GI) tract and is responsible for many cancer deaths annually. The etiology of GC is believed to result from the cumulative damaging effects of genetic, epigenetic, and environmental factors during the individual's lifetime. Dopamine receptor D1 (*DRD1*) represents the most abundant dopamine receptor in the central nervous system (CNS). Several dopaminergic pathways depend on the receptor to convey stimulatory dopamine signals. The current project has been designed to assess the gene expression and polymorphism of *DRD1* (rs774034163 A>T) among Iranian patients suffering from GC in Isfahan. Materials and Methods: ARMS PCR technique was used to assess rs774034163 A>T polymorphism in 91 paraffin blocks of stomach tissues (42 GC patients and 49 healthy subjects). Additionally, 41 samples (20 GC patients and 21 healthy subjects) were selected randomly to assess *DRD1* gene expression using the qRT-PCR technique. Results: The 'AT' genotype of rs774034163 was not significantly associated with GC (OR = 0.65, 95% CI = 0.214-1.970, and *p*-value = 0.446); the 'TT' genotype was also not observed in our population. Regarding allele frequency, the 'T' allele is not correlated with GC (OR = 0.677, 95% CI = 0.235-1.948, and *p*-value = 0.469). Furthermore, the expression of the DRD1 gene in patients and healthy individuals demonstrated no noticeable difference (*p*-value = 0.835). Conclusions: According to the results, rs774034163 A>T polymorphism is not associated with GC genotypically or allelically. Also, there is no correlation between increased gene expression levels of *DRD1* and the pathogenesis of GC.

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Introduction

Gastric cancer (GC) incidence varies globally; most cases manifest in low-income countries, half in Eastern Asia. Males are more susceptible to acquiring GC than females, supporting the hypothesis that environmental factors, heredity, and hormones may all have an impact. Various genetic and epigenetic changes have occurred over a prolonged period, leading to GC formation. It is well-known that *Helicobacter pylori* (*H. pylori*) infection is one of the leading risk factors for GC (Figueiredo et al., 2017). GC is classified into the following categories: hereditary diffuse (3%), gastric stump (7%), early-onset (10%), and sporadic (80%) (Sitarz et al., 2018; Skierucha et al., 2016). Geographic variations in incidence can be observed. Over 50% of all new cases are estimated to arise in developing countries. The threat ranges from an average risk difference of 15–20 times when comparing the predominantly and minimally at-risk populations (Sitarz et al., 2018). It is estimated that 95% of all cancerous lesions from the stomach occur as gastric adenocarcinomas (GAC) (Ajani et al., 2017).

Many genes (such as *CDH1*, *P53*, *P15*, *P16*, *APC*, *MLH1*, *c-MET*, *RB*, *RAS*, *RAF*, *AKT*, *ERBB2*, *TGF-β*, *VEGF*, *FGFR*, *PD-1L*, and *MMP9*) reported having an impact on GC etiology (Gao et al., 2016; Panarese et al., 2017; Zhu et al., 2020).

Dopamine belongs to the class of catecholamines. This hormone in the human body regulates both circulating hormones and neurotransmitters. It functions through a network of G-protein-coupled receptors that are classified into two subgroups: the first is the D1-like receptors (*DRD1* and *DRD2*), and the second is the D2-like receptors (*DRD2*, *DRD3*, and *DRD4*) (Sobczuk, Łomiak, & Cudnoch-Jędrzejewska, 2020). Dopamine D1 receptors participate in G-protein coupled receptor (GPCR)-independent and GPCR-mediated signaling. The latter consists of three distinct components: The ERK pathway, phospholipase C activation, and the cAMP/PKA signaling. Through the receptor tyrosine kinase, the dopamine D1 receptor has the potential to initiate the signaling process of AKT-GSK3. CREB activity and histone H3 are significant nuclear modulators of the dopamine D1 receptor pathway at the cellular level (Sobczuk, Łomiak, & Cudnoch-Jędrzejewska, 2020). In the stomach, dopamine is synthesized in G cells through DOPA decarboxylase and tyrosine hydroxylase enzymes. The kidney can efficiently excrete sodium load after oral sodium intake if the G cells in the stomach stimulate gastrin secretion by stimulating its receptor D1R, which is engaged during the procedure due to its interaction with the PPAR1 receptor (Xu et al., 2020).

Based on Genome Reference Consortium Human Build 38 patch release 14 (GRCh38.p14) indexed in the NCBI database, the *DRD1* gene is mapped to 5q35.2 and contains two exons. Its DNA length is 4147 base pairs (bp), mRNA length is 4031 bp, and protein length is 446 amino acids (aa). The brain, placenta, and prostate tissues have the most *DRD1* gene expression in the human body (Sayers et al., 2022). Currently, there are 2507 SNP in dbSNP and 62 CNV in dbVar for the *DRD1* gene. The rs774034163 A>T variation is present in 5′UTR of *DRD1* (chr5-175443131 A>T; *DRD1* RefSeqGene: NG_011802.1:g.6030T>A; *DRD1* transcript: NM_000794.5:c.-32T>A). The allele A is ancestral. According to the ExAC project in NCBI dbSNP, the allele T frequency in Global, Asian, American, European, African, and other populations was reported to be 0.000009, 0.00005, 0.00000, 0.00000, 0.0000, and 0.000, respectively. Until the writing of this article, the ClinVar database has not identified any clinical significance for it (Huo et al., 2015). The rs774034163 identified as Variant of Uncertain Significance (VUS) in Franklin database [available at: https://franklin.genoox.com/clinical-db/variant/snp/chr5-175443131-A-T-hg38?app=acmg-classification], so further investigation will be needed to determine its putative role as (likely) benign or (likely) pathogenic in populations.

Iran is one of the regions where GC is prevalent. The prevalence of non-cardiac GC among Iranian children is 42% due to a positive diagnosis of *H. pylori*. Additionally, the frequency of occurrence differs from region to region in Iran due to geographical circumstances, so it is generally viewed to be lower in the south and higher in the north (Moosazadeh, Lankarani, & Afshari, 2016; Moradzadeh & Anoushirvani, 2020). Reducing the risk of GC can be achieved by controlling *H. pylori* infection, minimizing salt consumption, providing

nutritious fruits and vegetables, avoiding tobacco and alcohol, and ensuring food safety (Sun et al., 2018).

As mentioned above, the *DRD1* gene is essential in regulating nerve activity, which can have a role in sending signals from organs (such as the stomach) to the CNS. The pathogenic role of rs774034163 A>T has not been reported in population studies. Therefore, in this study, we intend to examine the *DRD1* gene expression and evaluate rs774034163 A>T polymorphism status for the first time in Iranian patients with GC in Isfahan Province.

Materials and Methods

Patients

From 2016-2018, Isfahan's Al-Zahra Hospital provided 91 pathologically confirmed paraffin blocks of stomach specimens from Iranian GC patients for this study; 42 were stomach tissues with GC, and 49 were healthy margin of stomach tissues. Patients were enrolled in this study based solely on the histological types of GC; all other variables, such as gender, age, cancer stages, and histological grades, were randomly selected. **Table 1** presents the characteristics of the statistical population, and **Table 2** provides detailed clinical characteristics.

Table 1 - A description of the demographic characteristics of samples for polymorphism and gene expression studies.

Characteristics	Polymorphism study		Gene expression study	
	Case (N=42)	Control (N=49)	Case (N=20)	Control (N=21)
Gender (N)				
Male	27	34	13	14
Female	15	15	7	7
Age (year)				
Mean ± SD	60 ± 11.55	60.18 ± 13.96	59.75 ± 14.65	63.52 ± 13.80
Minimum	30	27	30	32
Maximum	81	84	81	84

N = Number, SD = Standard Deviation.

DNA extraction and ARMS PCR technique

Ninety-one paraffin blocks of stomach tissues (42 cases and 49 controls) were DNA extracted using the DNA Extraction Kit (Ziaviz, Tabriz, Iran), according to the instructions provided by its supplier. ARMS PCR was used to examine rs774034163 polymorphism in GC. The procedure comprises the following phases: one cycle for initial denaturation at 95 °C for 5 minutes; 35 cycles of 95 °C for 30 s, 61 °C for 40 s, and 72 °C for 35 s; and a final extension cycle at 72 °C for 5 min. The amplified amplicons were electrophoresed using 2% agarose gel. Primer3 Online Software (Kõressaar et al., 2018) was employed for designing polymorphism primers, which can be found in **Table 3**.

Table 2 - Clinicopathological status of GC patients for both polymorphism and gene expression studies.

Characteristics	Polymorphism (N=42)		Gene expression (N=20)	
	Male	Female	Male	Female
Histological types				
Intestinal	27	15	13	7
Histological grades				
Well	5	6	2	1
Moderately	5	3	4	1
Poorly	4	5	6	5
N/A	13	1	1	-
Cancer stages				
I	1	1	-	-
II	3	1	4	-
III	18	9	7	5
IV	4	4	1	2
N/A	1	-	1	-

N = Number, N/A = Not available data

RNA extraction and qRT-PCR

RNA was isolated from 41 paraffin blocks of stomach tissues (20 cases and 21 controls) using the RNA Extraction Kit (Pars Tous, Mashhad, Iran), according to the manufacturer's instructions, after traditional deparaffinization with ethanol and xylene solutions (Körbler et al., 2003). In addition, ScanDrop® 250 (Analytik Jena, Jena, Germany) was employed to quantify the purity and concentration of RNA; subsequently, its consistency was validated through 1% agarose gel electrophoresis. Using a cDNA synthesize kit (Pars Tous, Mashhad, Iran), cDNA was synthesized by reverse transcribing 10 µg of total RNA into cDNA, according to the manufacturer's instructions.

Table 3 - The primer sequences were utilized for the detection of rs774034163.

SNP	Sequences	Amplicon size (bp)	Annealing Tm (°C)
rs774034163	F outer: 5′-CACGGGATTGACTTGGATTGC-3′	413	61
	R outer (common): 5′-CTTACCTGCGCGTTGACA-3′		
	F inner (w): 5′-CTTGGGAACTTGAGGGGTGT-3′	273	
	F inner (m): 5′-CTTGGGAACTTGAGGGGTGA-3′	273	

SNP = Single nucleotide polymorphism, R = Reverse, F = Forward, Tm = melting temperature, bp = base pair, m = mutant, w = wild.

Table 4 - The primer sequences were employed for evaluating *DRD1* relative gene expression.

Genes	Sequences	Amplicon size (bp)	Annealing Tm (^{0}C)
DRD1	F: 5′-GACCTTGTCTGTACTCATCTCCT-3′	118	61
	R: 5′-GTCACAGTTGTCTATGGTCTCAG-3′		
RNA 18S	F: 5′-GTAACCCGTTGAACCCCATT-3′	112	60
	R: 5′-CCATCCAATCGGTAGTAGCG-3′		

R = Reverse, F = Forward, Tm = melting temperature, bp = base pair

Table 5 - The genotypic and allelic distribution frequencies of the rs774034163 polymorphism for GC patients and healthy subjects.

		Cases (N=42)	**Controls** (N=49)	**OR**	**95% CI**	***p*-value** [a]
Genotype distribution	AA	36 (85.7%)	39 (79.6%)	Reference	Reference	Reference
	AT	6 (14.3%)	10 (20.4%)	0.65	0.214-1.970	0.446
	TT	0 (0%)	0 (0%)	-	-	-
Allele frequency	A	78 (92.9%)	88 (89.8%)	Reference	Reference	Reference
	T	6 (7.1%)	10 (10.2%)	0.677	0.235-1.948	0.469

OR = Odd ratio, CI = Confidence interval, N = Number, [a] = Multinomial Logistic Regression test.

StepOne™ Real-time PCR System (Applied Biosystems, USA) was employed for conducting the qRT-PCR technique, which utilized SYBR Green as the indicator dye. In qRT-PCR reactions, the following processes are executed: the initial denaturation for 10 min at 95 °C; 40 cycles of 95 °C for 15 s, annealing & extension at 61 °C (*DRD1*) or 60 °C (*RNA 18S*) for 30 s; finally, a melting curve was established spanning 60-95 °C. The $2^{-\Delta\Delta ct}$ method (Livak & Schmittgen, 2001) quantified the relative gene expression by normalizing *DRD1* (NM_000794.5) with *RNA 18S*, which acted as the internal standard.

Table 4 presents the primer sequences designed using Primer3 Online Software (Kõressaar et al., 2018) for quantifying *DRD1* gene expression.

Statistics

The Multinomial Logistic Regression and Mann-Whitney U tests were applied to assess polymorphism and gene expression data, respectively. SPSS 26 (IBM Corp., Armonk, NY, USA) software was employed to execute all tests, and the *p*-values < 0.05 were significant.

Results

Polymorphism findings

Table 5 demonstrates the results of genotypes and alleles associated with the rs774034163 polymorphism in GC patients and healthy individuals.

Gene expression findings

A comparative analysis of the *DRD1* relative gene expression between GC patients and healthy individuals is provided in **Table 6**.

Table 6 - Comparative analysis between GC patients and healthy subjects in terms of relative gene expression of the *DRD1*.

Gene	Groups	N	Expression mean ± SD	***p*-value** [a]
DRD1	Case	20	3.35 ± 2.05	0.835
	Control	21	1.45 ± 1.09	

SD = Standard Deviation, N = Number, [a] = Mann–Whitney U test.

Discussion

According to the research findings presented in Table 5, the genotypes and alleles associated with the rs774034163 polymorphism and GC etiology could not be linked. In addition, concerning Table 6, no notable difference in the expression level of the *DRD1* gene was shown in the case and the control groups. So, the results, as mentioned earlier, reveal that *DRD1* (at the mRNA level) and its genetic polymorphism (rs774034163) may not be a chance of developing GC among Iranian patients of Isfahan province.

Numerous *in vivo* and *in vitro* examinations suggested that dopamine plays an integral role in drug resistance, tumor angiogenesis, apoptosis, and proliferation in diverse cancers, notably ovarian cancer, breast cancer, gastric cancer, and glioma (Ganguly et al., 2010; Lan et al., 2017; Moreno-Smith et al., 2011; Sarkar et al., 2008; Sobczuk, Łomiak, & Cudnoch-Jędrzejewska, 2020). Kang Yang et al. tested glioblastoma cells for antitumor activity utilizing a dopamine receptor agonist targeting the *DRD1* gene, alone or combined with temozolomide (TMZ). It is claimed that activating *DRD1* suppresses glioblastoma cell growth, which may represent a more promising approach to developing future treatments for glioblastoma (Yang et al., 2020).

It is evident from epidemiological analyses that cancer incidence may differ among individuals directly impaired by conditions caused to dysregulation of the dopamine system compared with normal individuals. It has been reported that individuals with schizophrenia are at a lower risk of prostate or colorectal cancer than the general population. Breast cancer results have been contradictory (Li et al., 2018; Sobczuk, Łomiak, & Cudnoch-Jędrzejewska, 2020).

A study conducted by Chen et al. in colorectal cancer demonstrated that dopaminergic immunoregulation significantly affects differentiating $CD8^+$ cells into $CD103^+$ TRM cells, which in turn regulates resistance to the tumor-induced by TRM cells (Chen et al., 2022).

A recent study on female patients with Rheumatoid arthritis (RA) demonstrates elevated levels of *DRD1* expression in their B cells. *DRD1* stimulates *IL-8* and *CCL3* production in these patients. After stimulation with D1-like receptors, these patients' memory B cells express more *CD95*. Also, *DRD1* expression levels are high in B cells at various stages of maturation (Wieber et al., 2022).

A study based on two independent cohorts in Chinese populations revealed neither the genotypic variation nor the allelic frequency of *DRD1* gene polymorphisms (rs5326, rs4867798, and rs4532) was significantly associated with schizophrenia phenotypes (Huo et al., 2015).

In 2009, Weihua Huang et al. demonstrated that the *DRD1* gene was linked to nicotine addiction by differentially expressing alleles A and G of the rs686 polymorphism in the 3′-UTR region of *DRD1*. No specific mechanism has been identified to explain its underlying causes; miRNA targeting is hypothesized to be responsible. From the two miRNAs predicted by computational analysis, miR-504 displays a stronger affinity for rs686A in the *DRD1* 3′-UTR, while miR-296 is more compatible with rs686G. Additionally, it was found that a significant expression difference between the two alleles of *DRD1* occurs due to miR-504 binding directly to the 3′-UTR region of the gene. By targeting the 3′-UTR of *DRD1*, miR-504 regulates the expression of *DRD1* and leads to the particular expression of *DRD1* (Huang & Li, 2009).

Xu et al. demonstrated that D1R mRNA is expressed in G cells, and its expression increases after NaCl concentrations are increased from 90 mM to 145 mM and 170 mM. This effect is similar to that of NaCl on gastrin mRNA. In the antrum of the stomach, human G cells also express the D1R protein (Xu et al., 2020).

Amjadi et al. (2023) in a study investigated the expression of *DRD1-DRD5* and *COMT* genes in peripheral blood and stomach tissue (via RT-qPCR) and plasma dopamine protein (via ELISA) in patients with gastric adenocarcinoma. They observed that *DRD1-DRD3* expression was higher in tumor samples than in healthy samples. In addition, the expression of *DRD1-DRD4* and *COMT* in the peripheral blood of patients was elevated. Finally, they reported that the plasma dopamine protein level in patients was significantly lower compared to the healthy group (Amjadi et al., 2023).

Reyhani et al. (2022) evaluated the relative expression pattern of the *DRD1* gene in glaucoma disease via RT-qPCR in Zahedan, Iran. Their study showed a significant relationship between the

increased expression of *DRD1* (46%) in patients and glaucoma disease (Reyhani et al., 2022).

A study was conducted by Kordi-Tamandani et al. (2013) regarding the methylation status of four genes, *DRD1*, *DRD2*, *DRD4*, and *DRD5*, in patients with schizophrenia in Zahedan, Iran. The results indicated that the *DRD1* gene has a similar methylation pattern in patients and healthy individuals. However, the methylation pattern between these two groups is statistically significant for other genes (*DRD2*, *DRD4*, and *DRD5*) (Kordi-Tamandani, Sahranavard, & Torkamanzehi, 2013).

Our present study had some limitations that should be considered in future studies: the sample size needs to be expanded to accurately report the status of this SNP in the community of Isfahanian patients suffering from GC. Also, the age of participants needs to be narrow and close to each other; this is important because the expression of some genes depends on age. It is better to use Sanger sequencing to confirm the presence of the mutation. This study also did not examine *DRD1* protein expression; thus, we recommend that this be achieved with Western blotting or other similar techniques.

Clinicians deal with many obstacles when managing GC patients. Hence, developing effective cancer diagnosis and treatment methods may be possible based on an individual's genetic profile. SNP studies in a wide range of populations allow investigators to hypothesize that a particular genotype may be associated with a specific type of disease or cancer to a certain extent. Further molecular analyses via high-throughput techniques can accurately evaluate the putative impact of *DRD1* genetic variations (specially rs774034163) on individuals engaged with GC.

Conclusion

To our knowledge, few studies in humans and other organisms have been performed explicitly focused on the expression of *the DRD1* gene at the mRNA level in GC. Interestingly, the polymorphism studied here, rs774034163, has not yet been studied in any cancers or diseases. Hence, our findings may serve as a starting point for future investigations of the *DRD1* gene and its desired polymorphism. The rare genotypes of a particular polymorphism might be notable if the sample size expands. In light of this, we recommend conducting further studies to verify our current findings by increasing the sample size and applying other inclusion criteria (e.g., the tumor size and familial history, smoking and alcoholic status, and *H. pylori* infection).

Conflict of interests

The authors declared no conflict of interest.

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Ethical Statement

Everyone who participated in this project had to fill out an informed consent form from Al-Zahra Hospital of Isfahan.

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