University of Sistan and Baluchestan

Journal of Epigenetics

Online ISSN: 2676 - 4350

Homepage: https://jep.usb.ac.ir

Iranian Genetics Society

Assessing the Relationship of C-FOS Gene Expression and rs997415225 Polymorphism with Gastric Cancer in the Iranian Population of Isfahan Province

Sayed Ahmad Kaihan [1], Milad Mollaali [1], Sepideh Eisazaei [1], Mozhgan Sheikholeslami [1], Simin Hemati [2*]

[1] Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran. E-mail: sayedahmadkaihan2010@gmail.com; milad_mollaali@pgs.usb.ac.ir; sepideh.eisazaeis@gmail.com; mojgan.eslami2010@yahoo.com.

[2] *Corresponding author*, Department of Radiation Oncology, Isfahan University of Medical Science, Isfahan, Iran. E-mail: hematti@med.mui.ac.ir, Tel.: +98-313-235-8070.

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 11 September 2022
Revised: 22 January 2023
Accepted: 20 February 2023

Keywords:
Stomach Neoplasms;
Genes, fos;
Genetic Variation;
Real-Time Polymerase Chain Reaction;
Iran.

A B S T R A C T

Objective: Gastric cancer (GC) is an exceedingly prevalent cancer worldwide and one of the main reasons for death from cancer. The individuals' susceptibility to GC depends upon several genetic and epigenetic alterations that occur over their lifetime. The *C-FOS* is an oncoprotein that engages in tumorigenesis through its oncogenic roles. It modulates many cellular functions, and its aberrant expression can lead to several types of cancers. Materials and Methods: The Amplification Refractory Mutation System (ARMS) PCR technique was conducted to detect genotypic types of rs997415225 in 50 healthy controls and 45 patients with GC. Furthermore, the quantitative Real-Time PCR (qRT-PCR) technique was applied to determine *C-FOS* relative gene expression in 20 healthy controls and 20 cancer patients. Results: Genotypic types of rs997415225 did not seem to be correlated with GC ($P > 0.05$), but allele "A" frequency was correlated ($P = 0.048$). Also, there was a significant difference between the two groups regarding the *C-FOS* gene expression level ($P = 0.044$). Conclusions: It has been found that genotypic types of rs997415225 have no impact on GC; however, the presence of allele "A" is associated with a potential risk of GC development. In addition, the increased *C-FOS* gene expression was linked to the progression of this cancer; hence, preventing GC through *C-FOS* down-regulation might be a promising approach.

Introduction

Gastric cancer (GC) is an acute malignancy that arises from solid neoplasms. As the disease is often diagnosed at an advanced stage, patients suffering from the disease have a poor prognosis. Several treatments are available to treat GC, including radiation therapy, immunotherapy, targeted therapy, chemotherapy, and surgery (Chandra et al., 2021).

There is significant regional disparity in the incidence of GC throughout the world, with eastern European, South American, African, and Asian countries having a much higher prevalence. Western countries have experienced a consistent reduction in the absolute occurrence of GC over

DOI: 10.22111/jep.2023.43313.1043

Publisher: University of Sistan and Baluchestan

How to Cite: Kaihan, S. A., Mollaali, M., Eisazaei, S., Sheikholeslami, M., & Hemati, S. (2022). Assessing the Relationship of C-FOS Gene Expression and rs997415225 Polymorphism with Gastric Cancer in the Iranian Population of Isfahan Province. Journal of Epigenetics, 3(2), 21-28. DOI: 10.22111/jep.2023.43313.1043

the past decades, but proximal stomach cancer is on the rise. GC is one of the leading causes of death from cancer worldwide, affecting approximately one in thirteen individuals. Males are more susceptible to being affected by this form of cancer; accordingly, GC is commonly encountered within the Iranian male population and is considered the primary contributor of death through cancer in them. In a recent estimate presented by Iranian researchers, they predicted that the epidemiology of stomach cancer from 2008 to 2016 indicated that GC in women will decrease to 5.5% in 2025 (6.5% and 6.3% in 2008 and 2016, respectively); the GC in men will also reduce to 11.4% in 2025 (12.3% and 12% in 2008 and 2016, respectively); both sexes reported 9.6%, 9.2%, and 8.4% in 2008, 2016, and 2025, respectively. They report that in Isfahan Province, there was a 12.49 per 100,000 men and 6.04 per 100,000 women GC rate between 2014 and 2016. Various elements contribute to these regional disparities, including lifestyle factors (particularly smoking, alcohol consumption, and poverty), *Helicobacter pylori* (*H. pylori)* infection, and genetics-related variables. Several factors are involved in developing GC, with patients routinely categorized according to their clinical stage and histopathological characteristics. Before the advent of the genomic era, Lauren's classification of GCs into diffuse and intestinal subtypes was extensively adopted (Roshandel et al., 2021; Sung et al., 2021; Yeoh & Tan, 2022).

Genetic susceptibility (e.g., inherited *CHD1* mutation) is the primary cause of diffuse forms of GC, whereas intestinal forms of GC are acquired and have 80-90% gene errors (Vauhkonen et al., 2006). Chromosomal instability (49.8%) is the most prevalent molecular subtype of GC. It is caused primarily by mutations in the *P53* gene and high levels of RTK-RAS amplification; genome stability (19.7%), microsatellite instability (21.7%), and Epstein-Barr virus (EBV) positivity (8.8%) make up the other molecular subtypes (Network, 2014; Onoyama et al., 2022).

A human stomach is the only natural repository for *H. pylori*. Infection with *H. pylori* usually arises during adolescence and persists throughout the host's life without antibiotics. The spread of the bacteria can occur through either fecal-oral or oral-oral ways. *H. pylori* is estimated to occur in half of the world's population frequently, and 15% of people with the infection acquire gastric ulcers; GC and peptic ulcers can occur even if the infection is asymptomatic (Alipour, 2021; Kayali et al., 2018). It is estimated that approximately 10 million Japanese have undergone eradication therapy against *H. pylori* for early gastric cancer (EGC) from 2000 to 2016; as a result of eradicating *H. pylori*, EGC has decreased dramatically (Tsuda et al., 2017).

FOS gene, with the official name: "Fos proto-oncogene, AP-1 transcription factor subunit," commonly known as *p55*, *AP-1*, and *C-FOS*, is mapped on 14q24.3 and its genomic sequence has about 10.5 kbp; furthermore, it is most expressed in the bone marrow (Ding et al., 2022; O'Leary et al., 2016). Four members of the FOS gene family are *FOSL1*, *FOSL2*, *FOSB*, and *FOS* (*C-FOS*). The AP-1 transcription factor complex is constructed by dimerization of the leucine zipper proteins encoded by these genes. AP-1 protein has led to the FOS proteins serving as transformation, differentiation, and cell proliferation regulators. Occasionally, *C-FOS* expression has also been associated with the apoptosis process (O'Leary et al., 2016).

The rs997415225 polymorphism occurs at the 5′UTR of the *C-FOS* gene, which maps to chr14:7527896; the reference allele is "G", while the variants are "A" and "T" (Sherry et al., 2001).

According to estimates, there were 112,000 new cases of stomach cancer in Iran in 2016, and 160,000 cases are expected to develop by 2025 (Roshandel et al., 2021).

The current research was intended to assess the *C-FOS* relative gene expression in GC patients recruited in Al-Zahra Hospital in Isfahan city, as well as whether the rs997415225 genotypic variants from the *C-FOS* gene have any association with GC.

Material and methods

Sample collection

From 2016 to 2018, 95 samples of stomach formalin-fixed paraffin-embedded (FFPE) tissues, including 45 samples of GC and 50 normal stomach FFPE tissues, were acquired from Al-Zahra Hospital in Isfahan after applying pathobiological confirmation tests. **Table 1**

displays the demographic characteristics, and **Table 2** shows the pathobiology criteria of GC patients.

Table 1 - A description of the demographic characteristics.

	Cases (N=45)		**Controls** (N=50)	
Mean age ± SD	59.41 ± 11.7		60.26 ± 10.32	
Age groups	Men (%)	Women (%)	Men (%)	Women (%)
27-46	5 (11.11)	1 (2.22)	4 (8)	1 (2)
47-60	7 (15.56)	11 (24.44)	11 (22)	3 (6)
61-74	11 (24.44)	4 (8.89)	14 (28)	8 (16)
75-89	5 (11.11)	1 (2.22)	7 (14)	2 (4)
Total	28 (62.22)	17 (37.78)	36 (72)	14 (28)

N = number, SD = Standard Deviation.

DNA isolation and ARMS PCR assay

The total DNA was extracted from 95 FFPE stomach tissues (45 cases and 50 controls) via the DNA Extraction Kit (Ziaviz, Tabriz, Iran) following the supplier's instructions.

The rs99741522 was detected using the ARMS PCR assay. This process involves i) one cycle of denaturing at 95 °C for 10 min, ii) 35 cycles of 95 °C for 30 s, 58.2 °C for 40 s, and 72 °C for 5 min, and iii) one cycle of polymerization at 72 °C for 10 minutes. The PCR products were confirmed through electrophoresis on 2% agarose gels. The polymorphism primers have been designed using Primer3 Online Software (Untergasser et al., 2012) and are provided in **Table 3**.

Table 2 - The pathobiological features of GC patients.

Pathobiological criteria		Count (N=45)
Histological type	Adenocarcinoma	37
	Adenocarcinoma papillary type	1
	Signet-ring cell carcinoma	1
	Adenocarcinoma collide type	1
	Poorly adenocarcinoma	1
	N/A	4
Histological grade	Well-differentiated	6
	Low moderately differentiated	1
	G2 moderate	1
	Poorly differentiated G1	3
	Poorly differentiated G2	8
	Poorly differentiated G3	19
	N/A	7
Cancer stage	PT1	1
	PT2	5
	PT3	21
	PT4a	9
	PT4b	1
	N/A	8
Perineural invasion	Present	10
	Absent	5
	Not identified	23
	N/A	8
Lymph vascular invasion	Present	21
	Absent	3
	Not identified	17
	N/A	4

N/A = not available, N = Number.

Table 3 - The primer sequences were employed in ARMS PCR.

SNP	Sequences (5′-3′)	Product size (bp)	Tm (°C)
rs997415225	F-inner(common): GTTGAGCCCGTGACGTTTAC	375	58.2
	R-outer: CTTACCTGCGCGTTGACA		
	R-inner(wild): ATCATCGTGGCGGTTAGGC	272	
	R-inner(mutant): ATCATCGTGGCGGTTAGGT	272	

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

Total RNA extraction, cDNA synthesis, and qRT-PCR

An RNA Extraction Kit (Pars Tous, Mashhad, Iran) was employed to isolate RNA from 40 formalin-embedded stomach tissues (20 cases and 20 controls). Following this, the concentration and the purity of RNA were quantified through ScanDrop® 250 (Analytik Jena, Jena, Germany), and its consistency was qualified via 1% agarose gel electrophoresis. The final step of the process is the generation of cDNA by converting 10 µg of

total RNA using the cDNA synthesize kit (Pars Tous, Mashhad, Iran) according to the supplier's directions.

The SYBR Green-based qRT-PCR assay was carried out via the StepOne™ Real-time PCR System (Applied Biosystems, USA). These are the processes involved in qRT-PCR reactions: the initial incubation at 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 58.2 °C (*C-FOS*) or 60 °C (*RNA 18S*) for 1 min; ultimately, a melting curve was generated covering the range 58.2-95 °C. The relative gene expression was assessed according to the $2^{-\Delta\Delta ct}$ formula (Livak & Schmittgen, 2001) that utilized the *RNA 18S* gene as an internal control for the normalization of the *C-FOS* gene. The primer sequences for *C-FOS* gene expression were also designed using Primer3 Online Software; they are summarized in **Table 4**.

Table 4 - The primer sequences were employed in qRT-PCR.

Genes	Sequences	Product size (bp)	Tm (^{0}C)
C-FOS	F: CACTCCAAGCGGAGACAGAC	139	58.2
	R: AGGTCATCAGGGATCTTGCAG		
RNA 18S	F: GTAACCCGTTGAACCCCATT	112	60
	R: CCATCCAATCGGTAGTAGCG		

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

Statistics

Statistical analysis was conducted through SPSS 26.0 (SPSS, Chicago, IL). The polymorphism status was determined using Multinominal Logistic Regression test. Linear Regression test was used to forecast the impact of rs997415225 SNP on gender and age groups. Mann–Whitney U test was employed to assess the comparison between healthy and patient groups' gene expression data. The significance thresholds for all statistical tests are based on $P < 0.05$.

Results

Polymorphism findings

Table 5 compares genotypic and allelic frequencies within patients and healthy individuals. Analysis of the influence of rs997415225 on the gender and age groups of the population is provided in **Table 6**. Furthermore, **Fig. 1** illustrates the status of the PCR product loaded on 2% agarose gel for this polymorphism.

Table 5 - The rs997415225 status based on genotypic and allelic frequencies within case and control groups.

		Cases (N=45)	Controls (N=50)	OR	95% CI	*P*-value [a]
Genotypic frequency	G G	37 (82.2%)	45 (90%)	Reference	Reference	Reference
	A G	0 (0%)	2 (4%)	0.001	0.001-0.001	-
	A A	8 (17.8%)	3 (6%)	3.24	0.803-13.104	0.099
Allelic frequency	G	74 (82.2%)	92 (92%)	Reference	Reference	Reference
	A	16 (17.8%)	8 (8%)	2.486	1.009-6.129	0.048*

N = Number, CI = Confidence interval, OR = Odd ratio, * = significant at $P < 0.05$, [a] = Multinominal Logistic Regression test.

Table 6 – The prediction of the impact of rs997415225 SNP on gender and age groups.

Variable	Standardized Coefficients Beta	95% CI for B (Lower Bound, Upper Bound)	*P*-value [a]
Gender	0.135	(-.093, 0.437)	0.188
Age groups	-0.159	(-264, 0.032)	0.122

CI = Confidence interval, a = Linear Regression test.

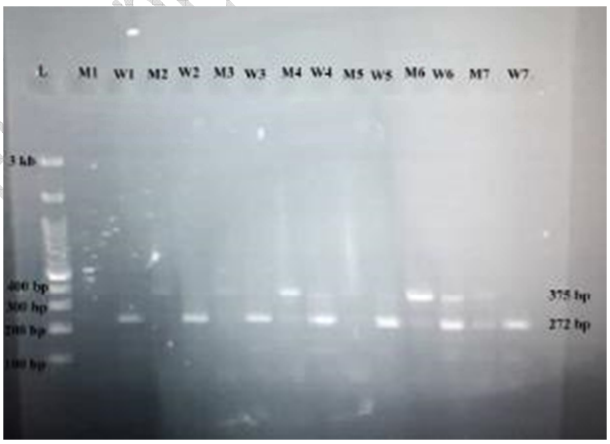

Fig. 1 - Electrophoresis on 2% agarose gel for rs997415225. The control band is 375 bp (observed in all samples); the homozygote wild (GG), heterozygote (AG), and homozygote mutant (AA) bands are 272 bp. GG indicates that only the primer "W" is detected, AA indicates that only the primer "M" is detected, and AG indicates that both primers ("W" and "M") are detected. Consequently, in the present figure, samples 1-6 are homozygote wild (GG), and sample 7 is heterozygote (AG). L: Ladder (size marker) 100-3000 bp, M: mutant allele, and W: wild allele.

Gene expression findings

Gene expression data are compared in **Table 7**.

Table 7 - An analysis of relative gene expression differences in the *C-FOS* gene across patients and non-patients.

Gene	Groups	N	Expression mean ± SD	*P*-value[a]
C-FOS	Cases	20	0.204 ± 0.069	0.044*
	Controls	20	0.074 ± 0.024	

N = Number, * = significant at $P < 0.05$, [a] = Mann–Whitney U test, SD = Standard Deviation.

Discussion

Our study revealed that the genotypic types of rs997415225 do not appear statistically significant concerning GC, but allele "A" frequency correlates significantly to this cancer (**Table 5**). As shown in **Table 6**, there is no direct correlation between the rs997415225 genotypes and gender ($P < 0.188$) or age groups ($P < 0.122$). The *C-FOS* gene expression differs statistically between patients and the healthy group (**Table 7**). Researchers have explored the role of *C-FOS* expression in several diseases and cancers through various molecular approaches; most studies show that *C-FOS* expression is increasing (Abarrategi et al., 2018; Manios et al., 2020). A research conducted by a Korean group on the expression status of *C-FOS* protein in GC revealed that the expression of this protein was decreased in some pathological criteria such as shorter survival, lymphatic invasion, lymph node metastasis, and advanced cancer stage. Also, they concluded that the reduced expression of *C-FOS* displays tumor suppressor function in GC, and they proposed that its suppressor activity may be due to the pro-apoptotic activity (Jin et al., 2007). Furthermore, numerous studies have assessed the effect of various drugs on the activity of the *C-FOS* gene in humans and other model organisms (de Medeiros et al., 2005; Köylü et al., 2021; Orihuel et al., 2021). Lee et al. reported that a compound in chili peppers called dihydrocapsaicin (DHC) inhibited and attenuated the *C-FOS* gene expression and the phosphorylation of the p70S6K1-S6 pathway in response to the epidermal growth factor (EGF). Consequently, they concluded that DHC is a viable natural cancer-preventive agent because it blocks the *C-FOS* signaling pathways (Lee et al., 2019). An investigation conducted by Gutiérrez et al. revealed that nuclear *C-FOS* and proliferative activity reduced with aging in the mammary gland, and the nuclear ERα46/ERα66 ratio did not fall below 1. As a result of the carcinogenic transformation of N-nitroso-N-methyl urea (NMU), nuclear *C-FOS* and proliferative activity were elevated (Gutiérrez et al., 2020). A study conducted by Babu et al. using qRT-PCR has shown that estradiol-17β (E_2), treated in human breast cancer MCF-7 cells, elevates the expression of *c-Jun* mRNA two-fold, and Fra-1 and *C-FOS* expression by 1.5- and one-fold, respectively. In contrast, the antiestrogen tamoxifen (TMX) significantly reduced *C-FOS*, *Fra-1*, and *c-Jun* mRNA levels by 0.4-, 0.6-, and 0.6-fold, respectively (Babu et al., 2013).

The rs997415225 polymorphism has not documented bibliography data in variation databases (such as dbSNP, ClinVar, and gnomAD), and neither has an article with this polymorphism been indexed in the Google Scholar citation database and other similar databases. Accordingly, to the best of our knowledge, this study is the first report at the global level that deals with the impact of this polymorphism on a disease, and its research outputs might be regarded as a pioneering contribution to defining the direction for forthcoming studies of GC and other related diseases. A study reported two SNPs at the *C-FOS* promoter, rs7101 at -60 (T/C, reference allele: "T") and rs2239615 at -135 (A/T, reference allele: "A"), which could be linked to knee-osteoarthritis (Huber et al., 2019). A study demonstrated that the *C-FOS* rs7101T mutant allele positively correlates with schizophrenia; nevertheless, the rs1063169 SNP was significantly less prevalent than the control group (Boyajyan et al., 2015). Another study conducted by Chinese researchers on colorectal cancer revealed that having a "T" allele at rs1063169 and a "T" allele at rs7101 in FOS was associated with an increased risk of colorectal cancer. Additionally, they found that homozygous variants were more likely to be related to this cancer than heterozygous variants (Chen et al., 2019).

Our study is the first report investigating the etiological role of rs997415225 polymorphism in cancer. Further research with large groups of sample sizes is crucial to clarify all aspects of this

SNP in gastric cancer. Therefore, we recommend more advanced analyzes, such as Sanger sequencing, to confirm the mutation. In addition to genetic and transcriptomic studies, future studies are expected to be carried out at the proteomic level to create a complete plan for this genetic change in GC.

5. Conclusion

In summary, our result showed that although the allele "A" frequency is statistically significant, it makes it possible that the AA genotype might contribute to the development of gastric cancer if our population size expands, based on the AA genotype odd ratio (OR = 3.486). In addition, *C-FOS* overexpression was observed. Since our study was restricted to small sample size, more extensive samples will be required to provide evidence supporting a robust correlation. Much information is available regarding the genetic and epigenetic alterations that underlie GC, which should make it feasible to enhance the clinical treatment of GC; hence, *C-FOS* targeting by validated agents may represent an intriguing alternative for CG therapy. It is urgent to identify and investigate additional SNP loci affiliated with the pathogenesis of GC in order to improve the prevention and treatment of GC and diminish its prevalence. Our study reported the first evidence for the role of rs997415225 regarding cancer; therefore, its results can provide a starting point for future research involving this polymorphism.

Conflict of interests

The authors declared no conflict of interests.

Acknowledgments

The authors appreciatively acknowledge the contributions of participants and Al-Zahra Hospital of Isfahan. Additionally, they are grateful to the University of Sistan and Baluchestan for providing financial sponsorship of this work.

Ethical consideration

All participants in Al-Zahra Hospital in Isfahan have signed a Consent Form.

References

Abarrategi, A., Gambera, S., Alfranca, A., Rodriguez-Milla, M. A., Perez-Tavarez, R., Rouault-Pierre, K., Waclawiczek, A., Chakravarty, P., Mulero, F., Trigueros, C., Navarro, S., Bonnet, D., & García-Castro, J. (2018). c-Fos induces chondrogenic tumor formation in immortalized human mesenchymal progenitor cells. *Sci Rep*, *8*(1), 15615. https://doi.org/10.1038/s41598-018-33689-0

Alipour, M. (2021). Molecular Mechanism of Helicobacter pylori-Induced Gastric Cancer. *J Gastrointest Cancer*, *52*(1), 23-30. https://doi.org/10.1007/s12029-020-00518-5

Babu, R. L., Naveen Kumar, M., Patil, R. H., Devaraju, K. S., Ramesh, G. T., & Sharma, S. C. (2013). Effect of estrogen and tamoxifen on the expression pattern of AP-1 factors in MCF-7 cells: role of c-Jun, c-Fos, and Fra-1 in cell cycle regulation. *Molecular and Cellular Biochemistry*, *380*(1), 143-151. https://doi.org/10.1007/s11010-013-1667-x

Boyajyan, A., Zakharyan, R., Atshemyan, S., Chavushyan, A., & Mkrtchyan, G. (2015). Schizophrenia-associated Risk and Protective Variants of c-Fos Encoding Gene. *Recent Adv DNA Gene Seq*, *9*(1), 51-57. https://doi.org/10.2174/2352092209666150223113334

Chandra, R., Balachandar, N., Wang, S., Reznik, S., Zeh, H., & Porembka, M. (2021). The changing face of gastric cancer: epidemiologic trends and advances in novel therapies. *Cancer Gene Therapy*, *28*(5), 390-399. https://doi.org/10.1038/s41417-020-00234-z

Chen, H., Ji, L., Liu, X., & Zhong, J. (2019). Correlation between the rs7101 and rs1063169 polymorphisms in the FOS noncoding region and susceptibility to and prognosis of colorectal cancer. *Medicine (Baltimore)*, *98*(26), e16131. https://doi.org/10.1097/md.0000000000016131

de Medeiros, M. A., Carlos Reis, L., & Eugênio Mello, L. (2005). Stress-Induced c-Fos Expression is Differentially Modulated by Dexamethasone, Diazepam and Imipramine. *Neuropsychopharmacology*, *30*(7), 1246-1256. https://doi.org/10.1038/sj.npp.1300694

Ding, S., Gan, T., Xiang, Y., Zhu, X., Jin, Y., Ning, H., Guo, T., Zhao, S., Xie, J., & Yuan, Z. (2022). FOS gene associated immune infiltration signature in perivascular adipose tissues of abdominal aortic aneurysm. *Gene*, *831*, 146576. https://doi.org/10.1016/j.gene.2022.146576

Gutiérrez, A., Sambuco, L., Álvarez, L., Núñez, M., Bergoc, R., Zotta, E., Martín, G., & Randi, A. (2020). Expression of estrogen receptor α variants and c-Fos in rat mammary gland and tumors. *J Steroid Biochem Mol Biol*, *199*, 105594. https://doi.org/10.1016/j.jsbmb.2020.105594

Huber, R., Kirsten, H., Näkki, A., Pohlers, D., Thude, H., Eidner, T., Heinig, M., Brand, K., Ahnert, P., & Kinne, R. W. (2019). Association of Human FOS Promoter Variants with the Occurrence of Knee-Osteoarthritis in a Case Control Association Study. *Int J Mol Sci*, *20*(6). https://doi.org/10.3390/ijms20061382

Jin, S. P., Kim, J. H., Kim, M. A., Yang, H. K., Lee, H. E., Lee, H. S., & Kim, W. H. (2007). Prognostic significance of loss of c-fos protein in gastric carcinoma. *Pathol Oncol Res*, *13*(4), 284-289. https://doi.org/10.1007/bf02940306

Kayali, S., Manfredi, M., Gaiani, F., Bianchi, L., Bizzarri, B., Leandro, G., Di Mario, F., & De' Angelis, G. L. (2018). Helicobacter pylori, transmission routes and recurrence of infection: state of the art. *Acta Biomed*, *89*(8-s), 72-76. https://doi.org/10.23750/abm.v89i8-S.7947

Köylü, A., Altunkaynak, B. Z., & Delibaş, B. (2021). Effects of tacrolimus on c-fos in hippocampus and memory performances in streptozotocin model of Alzheimer's disease of rats. *Turk J Med Sci*, *51*(4), 2159-2166. https://doi.org/10.3906/sag-2008-291

Lee, J. S., Kim, Y. A., Jang, Y. J., Oh, Y., & Byun, S. (2019). Dihydrocapsaicin Inhibits Epithelial Cell Transformation through Targeting Amino Acid Signaling and c-Fos Expression. *Nutrients*, *11*(6). https://doi.org/10.3390/nu11061269

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-

Delta Delta C(T)) Method. *Methods*, *25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Manios, K., Tsiambas, E., Stavrakis, I., Stamatelopoulos, A., Kavantzas, N., Agrogiannis, G., & A, C. L. (2020). c-Fos/ c-Jun transcription factors in non-small cell lung carcinoma. *J BUON*, *25*(5), 2141-2143. https://www.jbuon.com/archive/25-5-2141.pdf

Network, C. G. A. R. (2014). Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*, *513*(7517), 202-209. https://doi.org/10.1038/nature13480

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., . . . Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*, *44*(D1), D733-745. https://doi.org/10.1093/nar/gkv1189

Onoyama, T., Ishikawa, S., & Isomoto, H. (2022). Gastric cancer and genomics: review of literature. *J Gastroenterol*, *57*(8), 505-516. https://doi.org/10.1007/s00535-022-01879-3

Orihuel, J., Gómez-Rubio, L., Valverde, C., Capellán, R., Roura-Martínez, D., Ucha, M., Ambrosio, E., & Higuera-Matas, A. (2021). Cocaine-induced Fos expression in the rat brain: Modulation by prior Δ(9)-tetrahydrocannabinol exposure during adolescence and sex-specific effects. *Brain Res*, *1764*, 147480. https://doi.org/10.1016/j.brainres.2021.147480

Roshandel, G., Ferlay, J., Ghanbari-Motlagh, A., Partovipour, E., Salavati, F., Aryan, K., Mohammadi, G., Khoshaabi, M., Sadjadi, A., Davanlou, M., Asgari, F., Abadi, H., Aghaei, A., Ahmadi-Tabatabaei, S. V., Alizadeh-Barzian, K., Asgari, A., Asgari, N., Azami, S., Cheraghi, M., . . . Bray, F. (2021). Cancer in Iran 2008 to 2025: Recent incidence trends and short-term predictions of the future burden. *Int J Cancer*, *149*(3), 594-605. https://doi.org/10.1002/ijc.33574

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*, *29*(1), 308-311. https://doi.org/10.1093/nar/29.1.308

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, *71*(3), 209-249. https://doi.org/10.3322/caac.21660

Tsuda, M., Asaka, M., Kato, M., Matsushima, R., Fujimori, K., Akino, K., Kikuchi, S., Lin, Y., & Sakamoto, N. (2017). Effect on Helicobacter pylori eradication therapy against gastric cancer in Japan. *Helicobacter*, *22*(5). https://doi.org/10.1111/hel.12415

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3--new capabilities and interfaces. *Nucleic Acids Res*, *40*(15), e115. https://doi.org/10.1093/nar/gks596

Vauhkonen, M., Vauhkonen, H., & Sipponen, P. (2006). Pathology and molecular biology of gastric cancer. *Best Pract Res Clin Gastroenterol*, *20*(4), 651-674. https://doi.org/10.1016/j.bpg.2006.03.016

Yeoh, K. G., & Tan, P. (2022). Mapping the genomic diaspora of gastric cancer. *Nature reviews cancer*, *22*(2), 71-84. https://doi.org/10.1038/s41568-021-00412-7