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IRS2 Gly1057Asp Polimorfizmi ile Özofagus Kanseri Arasında İlişki Yoktur

No Association Between *IRS2 Gly1057Asp* Polymorphism and Esophageal Cancer

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ÖZET

Amaç: Bu çalışmanın amacı *IRS2 Gly1057Asp* varyantı ile özofagus kanseri yatkınlığı arasındaki ilişkiyi ortaya çıkarmaktır.
Materyal ve Metot: Çalışma 70 özofagus kanseri ve 100 sağlıklı kontrol örneğini içeren vaka-kontrol çalışmasıdır. Genomik DNA, periferal kandan izole edilmiş olup, gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) temelli SNP genotipleme için ticari olarak dizayn edilmiş Taqman assay kullanılmıştır**.**
Bulgular: Genotip dağılımında hasta ve kontrol örnekler arasında anlamlı bir farklılık saptanmamıştır.
Sonuç: *IRS2 Gly1057Asp* varyantı ile özofagus kanser gelişim riski arasında anlamlı bir ilişki belirlenememiştir.

Anahtar Kelimeler: IRS2 Gly1057Asp, rs1805097, Özofagus kanseri .

ABSTRACT

Objective: The aim of this study was to figure out the relationship between *IRS2 Gly1057Asp* variant and susceptibility to esophageal cancer.
Material and Method: A case-control study was conducted to select 70 esophageal cancer patients and 100 healthy control samples. Genomic DNA was extracted from peripheral whole blood samples and Real-Time Polymerase Chain Reaction (RT-PCR) based SNP genotyping was performed using predesigned Taqman assay.
Results: There was no significant difference in the frequency distribution of genotypes between patients and control group.
Conclusion: There is no significant correlation between the IRS2 Gly1057Asp variant and the risk of esophageal cancer.

Keywords: IRS2 Gly1057Asp, rs1805097, Esophageal cancer.

INTRODUCTION

Esophageal cancer (EC) is one of the most common malignancy of upper digestive tract and it is the seventh leading cause of cancer-related mortality worldwide (Alaouna et al., 2019). EC is usually diagnosed at advanced stages because of the lack of early detection methods and genetic markers and overall survival rate for 5 years ranges from 15% to 25% (Pennathur at al., 2013). Although environmental factors including nutrition and tobacco consumption may have an important role in EC development, determination of genetic markers that would predispose an individual to EC may require. Numerous genomic aberrations that are mainly associated with carcinogen, alcohol folate metabolism and DNA repair genes have been identified in EC development but genetic variants that may affect EC susceptibility are still unclear (Alaouna et al., 2019; Lin et al., 2018).

Insulin receptor substrate (IRS) proteins are the main docking proteins in insulin signaling. IRS2 is widely expressed in human tissues and expression profile of IRS2 is associated with cancer progression and metastasis (Gorgisen et al., 2017). Overexpression of IRS2 is correlated with increased cell adhesion in colorectal carcinoma, invasiveness of breast cancer cells and liver tumor progression (Boissan at al., 2005; Chan and Lee, 2008; Day et al., 2013; Porter et al., 2013)

IRS2 gene is located on chromosome 13q34 and *Gly1057Asp* (rs1805097) is the most common variation that has been associated with polycystic ovary syndrome (PCOS), insulin resistance and obesity (Mammarella et al, 2000; Lautier et al, 2003; Lin et al., 2014). Although, there are numerous studies that have shown the functional effects of *IRS2 Gly1057Asp* variation on metabolic diseases, its effect on cancer susceptibility is still controversial. Yin et al. showed that *IRS2 Gly1057Asp* variation is associated with decreased risk of colorectal cancer while Hu et al. did not observe significant connection between *IRS2 Gly1057Asp* variation and colorectal cancer development (Hu et al., 2014; Yin et al., 2017). These differences may due to the sample size and populations. Therefore, population-based studies are important to clarify the exact role of *IRS2 Gly1057Asp* variation in cancer susceptibility.

Since IRS2 regulates the metabolic functions of the cells, it is highly plausible that *IRS2* gene polymorphism may play a role in EC development. We could not find any study that assessed association with this variant and EC in the literature. In this study, it was aimed to investigate the association of *IRS2 Gly1057Asp* variant with EC in Turkish population.

MATERIAL and METHODS

Study subjects

This study was approved by Ethical Committee of Van Education and Training Hospital (2020/05-12/03/2020). Informed written consent was obtained from each individual and our study confirmed to the standards of the Declaration of Helsinki. We recruited 100 healthy controls and 70 esophageal cancer patients. In control group, subjects were randomly selected among volunteers visiting the medical center for physical examinations without esophageal cancer and they were matched to the cases by age, gender, and region. Subjects who had history of cancer also excluded from study in control group. All patients were recently diagnosed with esophageal cancer based on the clinical manifestations, with endoscopic and pathologic examinations. Exclusion criteria for the patients were receiving chemo- and radiotherapy treatments and suffering from other malignancies.

DNA extraction and SNP genotyping

Genomic DNA was isolated from peripheral blood using GF-1 Blood DNA Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer' s instructions. IRS2 rs1805097 single nucleotide polymorphism (SNP) was genotyped by Real-Time Polymerase Chain Reaction (RT-PCR) technology using predesigned Taqman assay (Thermo Scientific, USA) (Assay ID: C_144336620). RT-PCR amplification was performed in a 25 µL containing 2,25 µL at the concentration of 3-20 ng genomic DNA diluted in distilled water, 12,50 µL of 2xTaqpath ProAmp Master Mix (2X) and 1,25 µL of 20X Taqman SNP Assay mix. SNP Genotyping reaction was carried out in a 96 well-optical plate on a StepOnePlus System (Thermo Scientific, USA) as follows: initial denaturation of 95◦C for 10 min followed by 40 cycles of denaturation 95◦C for 15 s, annealing/extension of 60◦C for 1 min. The alleles were labeled with fluorescent probes VIC®-dye and FAMTM dye (Thermo Scientific, USA).

Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test of normality. According to results, Non-parametric tests were preferred. The differences in proportions between groups were compared by using Chi-Square or Fisher's Exact test, where appropriate. Continuous variables were compared using Mann-Whitney U test among categories of grouping variables. To assess the agreement between genotypes observed and those predicted by the Hardy-Weinberg equilibrium, the likelihood ratio test (G statistic) was used. In order to define independent risk factors of esophageal cancer, multivariant logistic regression analysis was used and adjusted for ages and gender, odds ratios were calculated. p-values less than 0.05 were considered significant. Frequencies (percentages), mean ± standard deviation

and median (minimum-maximum) were given as descriptive statistics. For statistical analyses, we used SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA). Significance was assumed for a p value of <0.05.

RESULTS

Table 1 summarizes the baseline characteristics of our study group. There were no significant differences in gender and age distributions. The mean age of patients was 58.47±10.03 and age of control was 60.14±10.09. We examined the possible association between IRS2 Gly1057Asp and EC using logistic regression analysis with different models. In EC group, Gly/Gly, Gly/Asp and Asp/Asp genotype frequencies were detected as 38.5%, 42.2% and 53.8%, respectively (Table 2). In logistic regression analysis Gly/Gly was considered as a non-exposed reference, odd ratios were 1.168 (95% CI: 0.609-2.240) for Gly/Asp carriers and 1.829 (95% CI: 0.609-5.487) for Asp/Asp carriers. Although, Asp/Asp genotype has 1.829 fold higher risk for EC development, it was not statistically significant (p=0,282). We also did not observe statistically significant association between IRS2 Gly1057Asp and EC cancer in dominant and recessive models. In dominant model, genotype frequency of Gly/Gly was 38.5% and Gly/Asp+Asp/Asp genotype frequency was 44.3% in patient group and odd ratio was determined as 1.273 (95% CI: 0.690 – 2.349; p=0,440). In recessive model, 62 (40%) patients had Gly/Gly+Gly/Asp genotype and 8 (53.3%) patients had Asp/Asp genotype which 1.714 fold increased the risk of EC compare to Gly/Gly+Gly/Asp genotype (95% CI: 0.592 – 4.968) but it was not statistically significant (p=0.321) (Table 2).

Table 1. Demographic features of patients and controls

		Patients		Control		
		Mean±S.Dev.	Median (Min.-Max.)	Mean±S.Dev.	Median (Min.- Max.)	p
Age		58.47±10.03	58.0 (41.0-85.0)	60.14±10.09	60.0 (28.0-89.0)	0.289
Sex	**Male**		32 (42.1)		44 (57.9)	0.825
	Female		38 (40.4)		56 (59.6)	
Age	**< 60**		38 (45.8)		45 (54.2)	0.233
	≥ 60		32 (36.8)		55 (63.2)	

Table 2. Genotype frequencies of IRS2 Gly1057Asp variant in esophageal cancer patients and controls

Models	*IRS2* Gly1057Asp	Patients	Control	p[a]	OR (95% CI)	p[b]
Co-dominant	Gly/Gly	35 (38.5%)	56 (61.5%)		1 (reference)	
Heterozygote	Gly/Asp	27 (42.2%)	37 (57.8%)	0.544	1.168 (0.609-2.240)	0.641
Homozygote	Asp/Asp	8 (53.8%)	7 (46.7%)		1.829 (0.609-5.487)	0.282
Dominant	Gly/Gly	35 (38.5%)	56 (61.5%)	0.440	1(reference)	
	Gly/Asp+Asp/Asp	35 (44.3%)	44 (55.7%)		1.273 (0.690 – 2.349)	0.440
Recessive	Gly/Gly+Gly/Asp	62 (40.0%)	93 (60.0%)	0.316	1(reference)	
	Asp/Asp	8 (53.3%)	7 (46.7%)		1.714 (0.592 – 4.968)	0.321

a: Compared by chisquare test ; b: Compared by logistic regression adjusted for age and sex.

DISCUSSION

Insulin signaling controls the glucose homeostasis and energy metabolism through complex signaling pathway. IRS proteins are the key players of this pathways and regulate the responses of the cells to the microenviromental stimuli (Gorgisen et al., 2017).

During insulin signaling, IRS2 is phosphorylated by insulin receptor (IR) or insulin like growth factor receptor (IGFR) at YXXM motifs which binding of PI3K-p85 to pYXXM motifs activates PI3K, which results in activation of AKT pathway is a hallmark pathway for carcinogenesis. In addition to IR and IGFR, IRS2 can also interact with growth hormone, leptin, vascular endothelial growth factor (VEGF), cytokine receptors. One common feature of these receptors is the induction of tumorigenesis (Gibson et al., 2007; Gorgisen et al, 2019). Therefore, it was suggested that IRS2 may have a pivotal role in cancer development. Transgenic mice with mammary specific overexpression of IRS2 causes rapid tumor formation and it also induces metastatic lung tumors and lung metastasis decreases in IRS2-/- mice (Nagle et al. 2004; Dearth et al, 2006). Overexpression of IRS2 and low IRS1 expression is found to be associated with poor outcomes in lung adenocarcinoma and squamous cell carcinoma patients (Piper et al., 2019).

To date there are numerous polymorphisms are identified in IRS2 gene. Among them, *IRS2 Gly1057Asp* was associated with various diseases. *IRS2 Gly1057Asp* variation is located close to two putative tyrosine phosphorylation sites at the positions 1042 and 1072. This non-conservative amino acid substitution may lead to change the tertiary structure and function of the protein and result in impaired signal transduction. Although, some studies showed that it did not affect the binding affinity of PI3K, it may affect splicing, transcriptional regulation, and post-translational modification of IRS2 (Mammarella et al., 2000; D'Alfonso et al, 2003; Wagner et al., 2004).

Up to date, effect of *IRS2 Gly1057Asp* in cancer development has been reported in different cancer types such as colorectal, breast, ovarian and endometrial tumors. Yukseloglu et al. did not observe significant association between colorectal cancer and *IRS2 Gly1057Asp* variant in Turkish population (Yukseloglu et al., 2014). In the same population, Cayan et al. showed that IRS2 1057Asp allele twofold increased risk for the development of endometrium cancer while they did not find any significant linked between this variant and ovarian cancer (Cayan et al., 2010; Cayan et al., 2011). Another study in Polish familial breast cancer cases also found no association between *IRS2 Gly0157Asp* and breast cancer development (Wagner et al., 2004). In concordance with these studies, our result did not support an association between 1057Asp allele of IRS2 and EC susceptibility.

The major strength of our study is the first preliminary study that examined the association between *IRS2 Gly1057Asp* and EC development with different models in the literature to our knowledge. However, there were several limitations in our study. First, sample size might be relatively small and second, we did not investigate the correlations between histological subtypes of EC and *IRS2 Gly1057Asp*. Therefore, our results need to be confirmed in larger sample size with different populations and effects of *IRS2 Gly1057Asp* in histological subtypes of EC need to be defined to clarify the exact role of *IRS2 Gly1057Asp* in EC.

CONCLUSION

There is no significant correlation between the IRS2 Gly1057Asp variant and the risk of esophageal cancer.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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