RESEARCH ARTICLE

Editorial Process: Submission:05/05/2024 Acceptance:11/14/2024

The Association between *EPHX1* Gene Polymorphisms and Lung Cancer among Jordanian People

Tamara E. Al-Qudah[1], Said Ismail[1], Dana Abdeen[1], Nidaa A. Ababneh[2], Faisal Khatib[1], Mohamed K. Al-Essa[1], Feras Hawari[3], Nathir Obeidat[4], Nabil J. Alkayed[1,5]*, Yanal Shafagoj[1]

Abstract

Objective: Genetic susceptibility to lung cancer is the subject of extensive research. We investigated whether polymorphisms in the microsomal epoxide hydrolase (*EPHX1*) gene is linked to lung cancer susceptibility in Jordanian patients. **Methods:** In this case-control study a total of 218 subjects from Jordan University Hospital and King Hussein Cancer Center were screened (108 lung cancer patients and 110 matched controls). Polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) was used to detect the two common polymorphisms in *EPXH1* located in exons 3 and 4; namely, amino acid substitution from tyrosine to histidine at residue 113 of exon 3 (Tyr113His; low activity allele) and amino acid substitution from histidine to arginine at residue 139 of exon 4 (His139Arg; high activity allele). **Results:** There was no significant difference in genotype distribution between control subjects and lung cancer patients. In addition, no differences were found when evaluated according to age, gender or tobacco consumption. For exon 3 the adjusted OR was 0.970 (95% CI 0.473 - 1.991) for the Tyr113/His113 genotype and 0.692 (95% CI 0.301 – 1.590) for the Tyr113/Tyr113 genotype, respectively. For exon 4 the adjusted OR was 0.596 (95% CI 0.297-1.197) for the His139/Arg139 genotype and 0.882 (95% CI 0.117- 6.660) for the Arg139/Arg139 genotype, respectively. Logistic regression analysis did not show differences in *EPXH1* distributions between patients and controls when categorized according to predicted microsomal epoxide hydrolase activity. Therefore, common polymorphisms within *EPHX1* do not appear to be significant risk factors for lung cancer development in the Jordanian population. **Conclusion:** No associations were observed between lung cancer risk and *EPXH1* polymorphisms in the Jordanian population.

Keywords: *EPXH1* polymorphism- Jordan- lung cancer- microsomal epoxide hydrolase

Asian Pac J Cancer Prev, **25 (11)**, 3913-3919

Introduction

Lung cancer represents a significant healthcare burden due to its high incidence and mortality rate. As reported by the GLOBOCAN 2020 database, it is the second most common type of cancer in the world, accounting for 11.4% of all new cancer cases, and has the highest mortality rate, accounting for 18.0% of total cancer deaths. In men, it is the leading cause of cancer morbidity and mortality, while in women, it ranks third in terms of incidence, preceded only by breast and colorectal cancer, and second in mortality, following breast cancer. In most countries, the survival of lung cancer patients at 5 years after diagnosis is only 10% to 20%; this high mortality rate is largely attributed to delayed diagnosis [1, 2]. According to the 23rd annual report of the incidence of cancer in Jordan published in 2018, lung cancer is the fourth most common type of cancer among the Jordanian population and the most common cause of cancer deaths in Jordanian males [3].

Variuos risk factors have been identified, including active and passive tobacco smoking [4], genetic predisposition [5], and occupational exposure to certain chemicals and pollutants [6]. The most significant risk factor for the development of lung cancer is tobacco smoking, primarily due to the diverse carcinogenic agents present in the tobacco smoke, including polycyclic aromatic hydrocarbons (PAHs) and aromatic amines. It is estimated that around 90% of lung cancer cases can be linked to the long-term inhalation of tobacco smoke [4, 5], nevertheless only 10%–20% of long-term smokers will ultimately develop lung cancer, which strongly suggests

[1]*Department of Physiology and Biochemistry, School of Medicine, University of Jordan, Amman, Jordan.* [2]*Department of Pathology, School of Medicine, University of Jordan, Amman, Jordan.* [3]*Cancer Control Office, King Hussein Cancer Center, Amman, Jordan.* [4]*Department of Internal Medicine, School of Medicine, University of Jordan, Amman, Jordan.* [5]*Department of Anesthesiology and Perioperative Medicine, Knight Cardiovascular Institute, Oregon Health and Science University, Portland, OR, USA. *For Correspondence: yanals@ju.edu.jo. Tamara E. Al-Qudah and Yanal Shafagoj have equal contribution in this study.*

the involvement of host-related factors [4]. Studies suggest that nearly 8% of lung cancer cases can be attributed to genetic predispositions. Individuals with a family history of the disease face a 2.4-fold increased risk of developing lung cancer themselves [7]. The identification of genetic markers that are associated with increased susceptibility to lung cancer is an area of active research [8].

It has been established that lung cancer development is influenced by the complex interactions of various susceptible genes and environmental factors, however, identifying a specific gene polymorphism responsible for the disease's pathogenesis remains challenging. One of the studied genes associated with lung cancer is the microsomal epoxide hydrolase gene *EPHX1*

Epoxides, whether from exogenous or endogenous sources, must be continuously hydrolyzed and excreted from the body since they interact with DNA, lipids, and proteins, leading to organ damage and various diseases and cancers. Microsomal epoxide hydrolase (mEH) is an enzyme that catalyzes the hydrolysis of highly reactive epoxides to their corresponding less reactive and more water-soluble dihydrodiols, which can be eliminated easily from the human body. This hydrolysis is generally a detoxification reaction; however, in the case of some hydrocarbons, more highly reactive and mutagenic compounds are generated in the metabolic process [9, 10]. For instance, mEH converts PAHs into highly toxic, mutagenic, and carcinogenic epoxides like benzo[a]pyrene-7,8-diol-9,10 epoxide (BPDE), the ultimate carcinogen of benzo[a]pyrene (B[a]P) [11].

The enzyme mEH is found in all tissues, but its highest level of expression can be found in the lung, bronchial epithelial cells, liver, skin, and gonads [12]. The effect of the mEH gene (*EPHX1*) variability on susceptibility to different diseases and cancer types has been extensively studied, and several polymorphisms have been reported. Two relatively common single nucleotide polymorphisms (SNPs) in *EPHX1* have been shown to affect enzyme activity: one in exon 3, a T to C transition mutation leading to a tyrosine to histidine substitution at position 113 (Tyr113His), and the other in exon 4, an A to G transition mutation leading to a histidine to arginine substitution at position 139 (His139Arg). Tyr113His substitution, sometimes referred to as the "slow allele," is associated with a 40%–50% decrease in the in vitro activity of mEH, and His139Arg substitution, sometimes referred to as the "fast allele," is associated with a 25% increase in the in vitro enzyme activity [13-15]. Benhamou et al. classified enzyme activity into slow, intermediate, and fast based on the combination of these two polymorphisms [16]. An increase in mEH activity could hypothetically confer cancer protection through enhanced detoxification or pose a higher risk by facilitating carcinogen activation; the same applies to lowered mEH activity.

Because of the known effect of these two polymorphisms on mEH activity, several studies have explored their potential association with cancers and diseases. Alteration in mEH activity was associated with Crohn's disease [17], chronic obstructive pulmonary disease [18], emphysema [19], breast cancer [10], and colorectal cancer [20]. Several studies have been conducted to assess the association between *EPHX1* polymorphisms and lung cancer, but the results have been inconsistent. A study performed on a Danish population confirmed the association between lowered mEH activity and the risk of developing tobacco-related cancer among smokers in the general population [21]. A meta-analysis that assessed the association between lung cancer and *EPHX1* polymorphisms in an Asian population showed significant association between Tyr113His and His139Arg polymorphisms and lung cancer [22]. By contrast, others found that Tyr113His polymorphism among Caucasians was associated with decreased risk of lung cancer, while the His139Arg polymorphism was associated with a modest increase in risk of lung cancer among the same group [14].

The main aim of this study is to investigate a possible correlation between the *EPHX1* polymorphisms and lung cancer development in the Jordanian population. To our knowledge, there are no previous studies that have investigated the association between the *EPHX1* polymorphisms and lung cancer among this cohort.

Materials and Methods

Selection of subjects

A total of 218 Jordanian subjects were included in this study, 108 lung cancer patients and 110 healthy controls. The mean ages of the lung cancer patients and controls were 60.1±11.6 years and 58.5±12.3 years, respectively. Patients were recruited from Jordan University Hospital (JUH) and King Hussein Cancer Center (KHCC); all were inpatients admitted for diagnosis and treatment of lung cancer. These patients were diagnosed by a pulmonologist and the diagnosis was confirmed by histopathology. Controls were recruited from patients visiting the family medicine clinic at JUH. The control group was matched to lung cancer cases by age and gender and had no history of- cancer or chronic respiratory disease.

All participants completed a questionnaire providing their name, address, age, occupation, detailed smoking history, medical history with an emphasis on the respiratory system, and family history of cancer. The detailed smoking history included number of years of smoking, average number of cigarettes per day, age at which smoking started, exposure to passive smoking, and time since cessation of smoking for ex-smokers. Patients were recruited regardless of age, gender, smoking status, type of primary lung cancer, or cancer stage. . From each participant, 5 ml venous blood was collected in EDTA tubes.The study was approved by the institutional review boards of the University of Jordan and the JUH. All participants were informed about the purpose and protocol of the study and asked to sign an informed consent form. The study was conducted in full accordance with the tenets of the Declaration of Helsinki statement for case-control studies.

DNA Extraction

Blood samples were obtained from participants by trained personnel via venipuncture; 5 ml venous blood was collected in EDTA tubes. Within 24 hours, DNA was

extracted from the blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Isolated DNA was stored at -20° C until use.

EPHX1 polymorphism analysis

Genotyping was performed by investigators who were blinded to the subjects' case or control status to detect both polymorphisms in *EPHX1*, as previously described by Smith and Harrison [16]. We performed two separate PCR reactions using the thermal cycler PTC-100 (MJ Research, Inc., USA) followed by RFLP. The two primer pairs used were m*EPHX1*F (5'GATCGATAAGTTCCGTTTCACC) and m*EPHX1*R (5'-ATCCTTAGTCTTGAAGTGAGGAT) for exon 3 and m*EPHX1*F (5'-ACATCCACTTCATCCACGT) and m*EPHX1*R (5'-ATGCCTCTGAGAAGCCAT) for exon 4.

PCR was performed in a total volume of 50µl reaction mix, consisting of 5µl 10X PCR buffer, 2.5 mM MgCl2, 2 µl dNTPs, 100 ng of each primer, 1 IU of Taq DNA polymerase (Promega, USA), and 250 ng genomic DNA. Reaction conditions were as follows: 95 ˚C for 5 min, 35 cycles consisting of 94 ˚C for 1 min, 56 ˚C for 1 min, and 72˚C for 1 min, and finally 72˚C for 5 min.

PCR products were then digested with EcoRV and RsaI (New England Biolabs, USA) for exon 3 and exon 4 respectively. Sixteen microliters of the PCR product were digested with 10 units of the corresponding enzyme and incubated at 37°C overnight. Then the fragments were separated by electrophoresis using a 3% agarose gel in 1×TBE buffer. The gels were then stained with ethidium bromide and transilluminated with ultraviolet light.

Fragments obtained were as follows: for exon3; wild-type genotype TT (140bp+22bp), heterozygous genotype TC (162bp+140bp+22bp), and homozygous mutant genotype CC (162bp), and for exon4; wild-type GG (164bp+46bp), heterozygous genotype AG (210bp+164bp+46bp), and homozygous mutant genotype AA (210bp).

Statistical analysis

Statistical analysis was conducted on SPSS version 23.0 and re-validated on R version 4.3.3. Descriptive statistics were utilized to characterize the sample. Categorical variables were showcased by frequencies and percentages, while continuous variables were described by means +/- standard deviations. Odds ratios (OR) between variables of interest and cases were derived from a univariate binary logistic regression. Adjusted ORs were derived from a multivariate binary logistic model. A p-value less than 0.05 was considered statistically significant. . Allele and genotype frequencies for both polymorphisms were calculated and tested for their distribution according to the Hardy-Weinberg equilibrium.

Results

The characteristics of the study population, consisting of 108 lung cancer patients and 110 controls, are presented in Table 1. Notably, there were no significant differences between the cases and controls regarding age and gender. The average age for cases was 60.1±11.6 years, while for controls it was 58.5±12.3 years. The percentage of males was 83.3% for cases and 83.6% for controls. In relation to smoking habits, 57.4% of cases were current smokers compared to 70% of controls. However, it is worth noting that 13% of cases did not provide a response regarding current smoking status. Among controls, the average pack-years for current smokers was 47.6 ± 42.4, whereas among cases it was 81.7 ± 53.8.

Due to failure in isolation of DNA in sufficient quantity or failure of genotyping, not all subjects could be analyzed: only 102 patients and 95 controls were analyzed for exon 3, 92 patients and 93 controls were analyzed for exon 4, and 88 patients and 85 controls were eligible for a combined analysis of both exon 3 and exon 4. The X2 analysis revealed that the gene frequencies for the exon 4 polymorphism were consistent with Hardy-Weinberg equilibrium in both cases and controls, in contrast to the exon 3 polymorphism, which did not meet this criterion.

As shown in Table 2, there was no statistically significant differences in the distribution of genotypes between cases and controls for the exon 3 polymorphism, and no association was observed when data was adjusted for age, gender, and smoking status. The prevalence of the His113/His113 genotype was lower in the lung cancer cases (14.7%) than in the controls (17.9%), but this difference did not reach statistical significance. The adjusted OR calculated relative to subjects with the Tyr113/Tyr113 was 0.692 (95% CI 0.301 – 1.590). The heterozygous Tyr113/His113 genotype was higher in the cancer group (24.5%) compared to the control group (22.1%), but the difference was again not statistically significant. The adjusted OR, calculated relative to subjects with the Tyr113/Tyr113, was 0.970 (95% CI 0.473 - 1.991).

Table 2 also shows the mEH exon 4 genotype distribution for cases and controls; there was no statistically significant difference between cases and controls before and after adjustment of data for age, gender, and smoking status. The adjusted OR was 0.596 (95% CI 0.297-1.197) for the His139/Arg139 genotype and 0.882 (95% CI 0.117- 6.660) for the Arg139/Arg139 genotype, respectively.

Table 3 displays the classification of combined

Table 1. Characteristics of the Study Population

	Cases = 108	Controls = 110
Age (years ± SD)	60.1 ± 11.6	58.5 ± 12.3
Gender		
Male	90 (83.3%)	92 (83.6%)
Female	18 (16.7%)	18 (16.4%)
Smoking status		
Smoker	62 (57.4%)	77 (70.0%)
Non-smoker	15 (13.9%)	20 (18.2%)
Ex-smoker	17 (15.7%)	13 (11.8%)
Unknown	14 (13.0%)	0 (0.0%)
Packs/year		
Current smokers	81.7± 53.8	47.6 ± 42.4
Ex-smoker	58.2 ±40.9	52.7 ± 33.9

Table 2. Analysis of *EPHX1* Exon 3 and Exon 4 Genotypes in Lung Cancer Patients and Controls and adjusted OR* (95% CI)

EPHX1	Cases		Controls		rOR	95% CI	P value	aOR*	95% CI	P value
	N	%	N	%						
EXON 3										
Tyr113/Tyr113	61	59.8	57	60.0	ref	ref	Ref	ref	ref	ref
Tyr113/His113	26	24.5	21	22.1	0.952	0.466 - 1.945	0.893	0.97	0.473 - 1.991	0.934
His113/His113	15	14.7	17	17.9	0.706	0.309 - 1.611	0.408	0.692	0.301 - 1.590	0.386
Total	102	100	95	100.0						
EXON 4										
His139/His139	69	75	62	66.7	ref	ref	ref	ref	ref	ref
His139/Arg139	21	22.8	29	31.2	0.586	0.293 - 1.174	0.132	0.596	0.297 - 1.197	0.146
Arg139/Arg139	2	2.2	2	2.2	1	0.137 - 7.325	1	0.882	0.117 - 6.660	0.903
Total	92	100	93	100						

*Adjusted for age, gender, and smoking status.

Table 3. Analysis of mEH Activity in Lung Cancer Patients and Controls and adjusted OR* (95% CI)

mEH Activity**	Cases		Controls		rOR	95% CI	P value	aOR*	95% CI	P value
	N	%	N	%						
Low	32	36.4	29	34.1	Ref	ref	Ref	ref	ref	ref
Intermediate	39	44.3	34	40	1.247	0.617 - 2.518	0.539	1.227	0.603 - 2.498	0.572
High	17	19.3	22	25.9	0.71	0.302 - 1.667	0.431	0.707	0.299 - 1.675	0.431
Total	88	100	85	100						

*Adjusted for age, gender, and smoking status; ** Low, individuals with His113/His113 and His139/His139 genotypes, His113/His113 and His139/Arg139 genotypes or Tyr113/His113 and His139/His139 genotypes; Intermediate: individuals with Tyr113/Tyr113 and His139/His139 genotypes, Tyr113/His113 and His139/Arg139 genotypes or His113/His113 and Arg139/Arg139 genotypes; High: individuals with Tyr113/Tyr113 and His139/Arg139 genotypes, Tyr113/Tyr113 and Arg139/Arg139 genotypes or Tyr113/His113 and Arg139/Arg139 genotypes. No individuals were genotyped as homozygous for both variant alleles

genotypes of exon 3 and exon 4 into three levels of predicted mEH activity—low, intermediate, and high—based on the classification by Benhamou et al. [20]. In cases, the percentages of predicted low, intermediate, and high mEH activities were 37.8%, 43.3%, and 18.9%, respectively, while in controls, they were 35.6%, 39.1%, and 25.3%. Data were adjusted for age, gender, and smoking status. No significant association was found between any of the predicted mEH activity levels and lung cancer.

Discussion

Globally, the mean age of onset for lung cancer is 66 years, with about 5%–10% of patients younger than 50 years [4]. In our study, the mean age for patients with lung cancer was 61.1 years, with a range of 26–87 years; 18% of our patients were younger than 50 years. These deviations from global trends can be attributed to the high prevalence of smoking in Jordan or to genetic variations. There is a notable difference in the male-to-female ratio across regions, with ratios ranging from 1.2 in North America to 5.6 in North Africa [1]; in our study, lung cancer was 5.2 times more frequent in males than in females. Surprisingly, the control group had a higher percentage of current smokers (70%) than the cases (57.4%). However, it is worth noting that 13% of cases did not provide an answer regarding smoking status, which could potentially explain this discrepancy. These patients probably refused to answer due to social or familial concerns. Current smokers in both groups are considered heavy smokers (more than 20 packs/year). However, our results show a higher value among patients when compared to controls (81.7 ± 53.8 vs. 47.6 ± 42.4 packs/year). Around 57% of lung cancer patients in our study were smokers and 15.7% were ex-smokers; notably, the prevalence of smoking was considerably higher among male patients, 60.6% of whom were smokers, compared to only 11.1% of female patients. These results highlight the depth of the smoking epidemic in Jordan and the need for more extensive education programs and stricter regulations to control this epidemic. Smoking prevalence in Jordan is extremely high; it was estimated by the Tobacco Industry Interference Index to be around 70% among young adults, which is the second highest prevalence in the world [23] and several studies have reported smoking prevalence in Jordanian males to be between 40% and 70% [24-26].

Given lung cancer's poor prognosis, research interest has been focused on the identification of asymptomatic individuals with increased risk of lung cancer; that is, "Who is going to develop lung cancer?" Efforts are underway to discover reliable blood biomarkers. One of the genes studied extensively to assess its association with lung cancer is the microsomal epoxide hydrolase gene (*EPHX1*), with inconsistent results among different ethnicities. In this study, the *EPHX1* polymorphism

was investigated in Jordanian lung cancer patients and controls.

Our findings on exon 3 polymorphism revealed that the frequency of the His113/His113 genotype was slightly lower in lung cancer cases than in controls. However, this difference did not reach statistical significance, most probably due to the small sample size. The Tyr113/His113 genotype was marginally more prevalent in the cancer group than in the control group, yet this difference was not statistically significant. Similar to our results, a study conducted on Mexican Americans and African Americans revealed that the Tyr113/His113 and His113/His113 genotypes did not appear to influence lung cancer risk among these ethnicities [27]. A meta-analysis of 28 studies did not find a significant correlation between exon 3 polymorphism and lung cancer risk; however, the subgroup analysis by ethnicity revealed a potentially increased risk of lung cancer associated with the Tyr113/His113 and His113/His113 in Asians, but not in White or African ethnicities [28]. Similar results among Asians were reported by Yu et al. in their meta-analysis their eight included studies were performed on Chinese, Japanese, and Indian populations. They showed that His113/His113 genotype carriers have 29% higher risk of lung cancer than Tyr113/Tyr113 carriers in Chinese populations [26]. Gsur et al. reported that among White Austrians the exon 3 polymorphism was associated with a significantly decreased risk of lung cancer [29]. A Finnish study that included 230 lung cancer cases and a large control group (n = 2105) concluded that the Tyr113/His113 and His113/His113 genotypes posed a decreased lung cancer risk compared with the wild-type genotype [30].

Regarding exon 4 polymorphism, our data showed that the His139/Arg139 genotype was lower in lung cancer cases than in controls; however, this difference did not reach statistical significance. Several studies have reported similar results. Yin et al. reported no association between exon 4 polymorphism and lung cancer in a Taiwanese population [31]. Gsur et al. also reported no association between exon 4 polymorphism and the risk of lung cancer among White Austrians [29]. On the other hand, the His139/Arg139 and Arg139/Arg139 genotypes were associated with an increased risk of lung cancer among Mexican Americans but not among African Americans [27]. In addition, a meta-analysis showed significant association between the His139/Arg139 and Arg139/Arg139 genotypes and lung cancer among Asians [32]. Another study reported a significant association between early-onset lung cancer before age 45 years and the presence of the His139/Arg139 genotype [4].

The predicted mEH can be evaluated by coupling both *EPHX1* polymorphisms and evaluating its association with lung cancer. In this regard, our data show no association between the predicted enzyme activity and lung cancer susceptibility. Kiyohara et al. reported a decreased risk for lung cancer with low predicted activity [10]. Lee et al, on the other hand, reported an increased risk of developing tobacco-related cancer among smokers with low predicted activity [25]. Several other studies, however, reported no association between predicted enzyme activity and lung cancer [13, 30, 33].

Several factors can cause these conflicting results: differences in ethnic composition among the study populations, methodological differences in genotyping methods, statistical analyses, and the selection of patients and controls, differences in statistical power largely due to variations in sample size, gene–gene interaction, and exposure to environmental pollutants [3, 13,18].

This study has certain limitations. First, the number of patients and controls was relatively small, and we were unable to get the results for a fair number of our subjects due to a failure to isolate DNA in sufficient quantity or a failure of genotyping. In addition, we did not address the relationship between the *EPHX1* polymorphism and the clinical parameters of lung cancer (prognosis, stages, and metastasis). The deviation from Hardy–Weinberg equilibrium in exon 3 but not exon 4 is attributable to the genotyping technique used rather than selection bias [29].

In conclusion, this study provides a first glimpse into the association between mEH genetic polymorphism and the risk of lung cancer in the Jordanian population. Our results suggest that *EPHX1* exon 3 and exon 4 polymorphism have no influence on lung cancer risk.

Author Contribution Statement

N.J.K., S.I. and Y.S. conceptualized the study. S.I. funding acquisition. N.J.K., S.I., Y.S., F.H. and N.O. design of the work. F.H. and N.O. provided access to lung cancer patients. D.A. and N.A.A. Interviewed the patients and collected the blood samples. D.A. and N.A.A. performed the experiments. M.K.E supervised the data collection and experiments. F.K., Y.S. and T.E.Q. formal analysis and interpretation of data. T.E.Q. wrote the original draft. All authors critically reviewed and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Funding

This work was supported by a generous grant from the Deanship of Scientific Research of the University of Jordan.

Informed Consent Statement

Informed consent was obtained from all participants included in the study.

Ethical Declaration

The study was approved by the institutional review boards of the University of Jordan and the JUH

Data Availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication

of this article.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. https://doi.org/10.3322/caac.21660.
2. Nooreldeen R, Bach H. Current and future development in lung cancer diagnosis. Int J Mol Sci. 2021;22(16):8661. https://doi.org/10.3390/ijms22168661.
3. Jordan Cancer Registry. Cancer Incidence in Jordan [Internet]. Ministry of Health; Jordan; 2018.
4. Graziano C, Comin CE, Crisci C, Novelli L, Politi L, Messerini L, et al. Functional polymorphisms of the microsomal epoxide hydrolase gene: A reappraisal on a early-onset lung cancer patients series. Lung Cancer. 2009;63(2):187–93. https://doi.org/10.1016/j.lungcan.2008.05.004.
5. Rosenberger A, Illig T, Korb K, Klopp N, Zietemann V, Wölke G, et al. Do genetic factors protect for early onset lung cancer? A case control study before the age of 50 years. BMC Cancer. 2008;8(12):1–12. https://doi.org/10.1186/1471-2407-8-60.
6. Costa AAS, da Veiga GL, da Costa Aguiar Alves B, Gascón TM, Pereira EC, Azzalis LA, et al. Lung cancer biomarkers. A literature review. J Bras Patol e Med Lab. 2022;58(12):1–10. https://doi.org/10.1900/JBPML.2022.58.415.
7. Bashir NA, Ragab ES, Khabour OF, Khassawneh BY, Alfaqih MA, Momani JA. The association between epidermal growth factor receptor (EGFR) gene polymorphisms and lung cancer risk. Biomolecules. 2018;8(3): 53-61. https://doi.org/10.3390/biom8030053.
8. Yang IA, Holloway JW, Fong KM. Genetic susceptibility to lung cancer and co-morbidities. J Thorac Dis. 2013;5(SUPPL 5): 54-62. https://doi.org/10.3978/j.issn.2072-1439.2013.08.06.
9. Gautheron J, Jéru I. The multifaceted role of epoxide hydrolases in human health and disease. Int J Mol Sci. 2020;22(1):1–17. https://doi.org/10.3390/ijms22010013.
10. Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y. *EPHX1* polymorphisms and the risk of lung cancer: A HuGE review. Epidemiology. 2006;17(1):89–99. https://doi.org/10.1097/01.ede.0000187627.70026.23.
11. To-Figueras J, Gené M, Gómez-Catalán J, Piqué E, Borrego N, Corbella J. Lung cancer susceptibility in relation to combined polymorphisms of microsomal epoxide hydrolase and glutathione S-transferase P1. Cancer Lett. 2001;173(2):155–62. https://doi.org/10.1016/S0304-3835(01)00626-7.
12. Erkisi Z, Yaylim-Eraltan I, Turna A, Görmüs U, Camlica H, Isbir T. Polymorphisms in the microsomal epoxide hydrolase gene: Role in lung cancer susceptibility and prognosis. Tumori. 2010;96(5):756–63. https://doi.org/10.1177/030089161009600519.
13. Tilak AR, Kumar S, Jain M, Pant MC, Das BC, Guleria R, et al. Association of functionally important polymorphism of microsomal epoxide hydrolase gene (*EPHX1*) with lung cancer susceptibility. Cancer Invest. 2011;29(6):411–18. https://doi.org/10.3109/07357907.2011.587070
14. El-Sherbeni AA, El-Kadi AOS. The role of epoxide hydrolases in health and disease. Arch Toxicol. 2014;88(11):2013–32. https://doi.org/10.1007/s00204-014-1371-y.
15. Baxter SW, Choong DYH, Campbell IG. Microsomal epoxide hydrolase polymorphism and susceptibility to ovarian cancer. Cancer Lett. 2002;177(1):75–81. https://doi.org/10.1016/S0304-3835(01)00782-0.
16. Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A. Association between lung cancer and microsomal epoxide hydrolase genotypes. Cancer Res. 1998;58(23):5291–3.
17. De Jong DJ, Van Der Logt EMJ, Van Schaik A, Roelofs HMJ, Peters WHM, Naber THJ. Genetic polymorphisms in biotransformation enzymes in Crohn's disease: Association with microsomal epoxide hydrolase. Gut. 2003;52(4):547–51. https://doi.org/10.1136/gut.52.4.547.
18. Yang Q, Huang W, Yin D, Zhang L, Gao Y, Tong J, et al. *EPHX1* and GSTP1 polymorphisms are associated with COPD risk: a systematic review and meta-analysis. Front Genet. 2023;14(5). https://doi.org/10.3389/fgene.2023.1128985.
19. Smith CAD, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet. 1997;350(9078):630–3. https://doi.org/10.1016/S0140-6736(96)08061-0.
20. Kiss I, Orsós Z, Gombos K, Bogner B, Csejtei A, Tibold A, et al. Association between allelic polymorphisms of metabolizing enzymes (CYP 1A1, CYP 1A2, CYP 2E1, mEH) and occurrence of colorectal cancer in Hungary. Anticancer Res. 2007;27(4 C):2931–7.
21. Lee J, Dahl M, Nordestgaard BG. Genetically Lowered Microsomal Epoxide Hydrolase Activity and Tobacco-Related Cancer in 47,000 Individuals. Cancer Epidemiol Biomarkers Prev. 2011;20(8):1673–82. https://doi.org/10.1158/1055-9965.EPI-10-1165.
22. Yu W, Lin Z, Qu B. Impact of Epoxide Hydrolase 1 Polymorphisms on Lung Cancer Susceptibility in Asian Populations. Cell Biochem Biophys. 2015;71(2):813–20. https://doi.org/10.1007/s12013-014-0268-y
23. Al Zawawi MM, Hammad Z, Mohammad Al-Ibrahim M, Khalifeh F, Al-Zawawi M. Tobacco Industry Interference Index in Jordan 2019 (Country Status implementation of the World Health Organization Framework Convention on Tobacco Control Article 5.3) [Internet]. Available from: https://www.who.int/tobacco/wntd/2012/article_5_3_fctc/en/
24. Alkouri O, Khader Y, Al-Bashaireh AM. Prevalence of Cigarettes and Waterpipe Smoking among Jordanians, Refugees, and Migrants in Jordan and Its Associated Factors: A Secondary Data Analysis. Int J Environ Res Public Health. 2022;20(1) 82-91. https://doi.org/10.3390/ijerph20010082.
25. Damsees R, Jaghbir M, Salam M, Al-Omari A, Al-Rawashdeh N. Unravelling the predictors of late cancer presentation and diagnosis in Jordan: a cross-sectional study of patients with lung and colorectal cancers. BMJ Open. 2023;13(5):1–8. https://doi.org/10.1136/bmjopen-2022-069529.
26. ALQudah MA, ALFaqih MA, Hamouri S, Al-Shaikh AF, Haddad HK, Al-Quran WY, et al. Epidemiology and histopathological classification of lung cancer: A study from Jordan, retrospective observational study. Ann Med Surg. 2021;65(5):102-06. https://doi.org/10.1016/j.amsu.2021.102330.
27. Wu X, Gwyn K, Amos CI, Makan N, Hong WK, Spitz MR. The association of microsomal epoxide hydrolase polymorphisms and lung cancer risk in African-Americans and Mexican-Americans. Carcinogenesis. 2001;22(6):923–8. https://doi.org/10.1093/carcin/22.6.923.
28. Zhang P, Zhang Y, Yang H, Li W, Chen X, Long F. Association between *EPHX1* rs1051740 and lung cancer susceptibility: a meta-analysis. Int J Clin Exp Med. 2015;8(10):17941–9.
29. Gsur A, Zidek T, Schnattinger K, Feik E, Haidinger G, Hollaus P, et al. Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. Br J Cancer. 2003;89(4):702–6. https://doi.org/10.1038/sj.bjc.6601142.
30. Voho A, Metsola K, Anttila S, Impivaara O, Järvisalo J, Vainio H, et al. *EPHX1* gene polymorphisms and individual

susceptibility to lung cancer. Cancer Lett. 2006;237(1):102–8. https://doi.org/10.1016/j.canlet.2005.05.029.

31. Yin L, Pu Y, Liu TY, Tung YH, Chen KW, Lin P. Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. Lung Cancer. 2001;33(2–3):133–41. https://doi.org/10.1016/S0169-5002(01)00182-9.
32. Xu X, Hua H, Fan B, Sun Q, Guo X, Zhang J. *EPHX1* rs2234922 polymorphism and lung cancer susceptibility in Asian populations: A meta-analysis. J Thorac Dis. 2015;7(7):1125–9. https://doi.org/10.3978/j.issn.2072-1439.2015.07.03
33. Lin P, Wang SL, Wang HJ, Chen KW, Lee HS, Tsai KJ, et al. Association of CYP1A1 and microsomal epoxide hydrolase polymorphisms with lung squamous cell carcinoma. Br J Cancer. 2000;82(4):852–7. https://doi.org/10.1054/bjoc.1999.1011

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.