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CYP1A1 Genetic Variations and Lung Cancer Risk in a Population of Libyan Males

Najah A. Fares, Othman A. El-Ansari, Mohamed A. Al-Griw*

Developmental Biology Division, Zoology Department, Faculty of Science, University of Tripoli, , P.O. Box 13160, Tripoli, Libya
*Corresponding Author, E-mail: m.algriw@uot.edu.ly

Abstract

Alterations in genes encoding the xenobiotic-metabolizing enzymes contribute to the variability in susceptibility to various cancers. In this study, we assessed the possible association between the *CYP1A1* variants and lung cancer (LC) risk in a population of Libyan males. For this study, we selected 20 unrelated healthy controls and 32 patients with LC. DNA samples from the controls and patients were screened by DNA-PCR and direct DNA sequence analysis to search for genetic sequence variations in *CYP1A1* gene (exon 7 and 3' non-coding region). *CYP1A1* mutations were identified in 11.5 % adult subjects and cases analyzed, and all were males. Overall, 11 *CYP1A1* mutations were documented in this study implicating exon 7 and 3' non-coding region. Nonsense, missense, and frame-shift mutations accounted for, respectively, 27.3 %, 63.6 % and 9.1 % of all *CYP1A1* mutations. Three missense mutations namely *CYP1A1*2B/m2 (*rs1048943*)*, *CYP1A1*4/m4 (*rs1799814), and *CYP1A1*2A/m1 (*rs4646903) have already been reported. The remaining mutations have not been described previously. We observed two apparently heterozygous carriers of mutation *CYP1A1*2B/m2* (*CYP1A1* 4889A/G [462Ile/Val] genotype) in control group. We also observed two heterozygotic genotypes one containing *mutation m4* (*CYP1A1* 4887C/A [461Thr/Asp]) *and another* containing *mutation m1* (6235T/C) in cancer group. The mutations *m2, m4,* and *m1* accounted for, respectively, 18.2 %, 9.1 % and 9.1 % of all *CYP1A1* mutations. Comparing the clinical features showed that PLT and WBC counts were lower in *CYP1A1* mutant than in *CYP1A1* wild type, but they have not reached statistical significant ($P > 0.05$). The average age of *CYP1A1* mutant was lower than in *CYP1A1* wild type. Overall, these findings suggest that genetic alterations in the metabolic gene *CYP1A1* are too rare to be of clinical

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relevance in this study, implying different pathways for the LC risk with respect to *CYP1A1* polymorphisms as a risk factor for LC at least in this study.

Keywords: Lung cancer; Cytochrome P450, Family 1; Subfamily A; Polypeptide 1 (*CYP1A1*) gene; Genetic Polymorphism; Risk.

المستخلص

تساهم التغيرات الوراثية في مورثات إنزيمات أيض المواد الكيميائية xenobiotic في زيادة القابلية بالإصابة بأنواع مختلفة من السرطانات. هدفت هذه الدراسة إلى تحديد العلاقة بين حدوث التغيرات في مورثة الـ CYP1A1 وسرطان الرئة في عينة من الليبيين. تم فحص الـ DNA للمجموعة الضابطة ومجموعة مرضى سرطان الرئة قيد الدراسة. استخدمت تقنية الـ DNA–PCR وتقنية تحليل تسلسل الـ DNA للكشف عن الاختلافات في تركيب مورثة الـ CYP1A1 (أكسون 7 والمنطقة 3 غير المشفرة). أظهرت النتائج أن 11.5٪ من عينات الدراسة توجد بها طفرات في مورثة الـ CYP1A1. تم تحديد 11 طفرة في الإكسون 7 والمنطقة 3 غير المشفرة من المورثة وكانت كالتالي: nonsense (27.3٪) و missense (63.6٪) و frame–shift (9.1٪). كما أوضحت النتائج انه يوجد ثلاثة طفرات من نوع الـ missense وهى كالتالي: CYP1A1*2B/m2 وCYP1A1*4/m4 و CYP1A1*2A/m1). كما لُوحظ أن في المجموعة الضابطة يوجد أثنين كان تركيبهما متباين اللاقحة heterozygous من طفرة الـ CYP1A1*2B/m2 (4889A/G [642Ile/Val]). أما في مجموعة المرضى، أظهرت النتائج أن اثنين في حالة heterozygous، وواحد يحتوي على طفرة CYP1A1/m4 (4887C/A [461Thr/Asp]). والأخرى تحتوي على طفرة m1 (6235T/C). شكلت الطفرات m2 وm4 وm1 النسب التالية 18.2 % و9.1% و9.1% على التوالي. أظهرت النتائج السريرية أن أعداد PLT وWBC كانت أقل في عينات الدراسة التي وجدت بها طفرات في مورثة الـ CYP1A1 مما كانت عليه في عينات المجموعة الضابطة، لكنها لم تصل إلى فروق معنوية ($P > 0.05$). كما أظهرت النتائج أن متوسط أعمار عينات الدراسة التي حُددت بها طفرات أقل مما هو عليه في عينات الدراسة والتي لم تحدد بها طفرات. تشير نتائج هذه الدراسة إلى أن تعدد أشكال مورثة الـ CYP1A1 نادر جداً وليس ذا أهمية سريرية، مما يعني أن هناك مسارات آخري محتملة لزيادة احتمال الإصابة بسرطان الرئة.

Introduction

Globally, lung cancer (LC) is a leading cause of death, with smoking being the largest single cause [1,2]. Cancers can be initiated by DNA damage caused by environmental factors, such as polycyclic aromatic hydrocarbons (PAHs), and some adverse habits including tobacco smoking and alcohol use [3,4]. Tobacco smoking has long been established as a risk factor for LC, even though fewer than

20% of smokers develop the disease. Smoking is responsible for 85 - 90% of LCs [2], yet < 20% of lifelong smokers develop LC, suggesting that other factors, including genetics, may play a role [2]. It has been shown that exposures to environmental and occupational PAHs are risk factors for LC [5]. However, not all of those who have been exposed to the risk factors will develop LC, suggesting that there is individual variation in cancer susceptibility in the general population [4]. Genetically and epigenetically mediated gene silencing is also important during lung carcinogenesis [6].The development of gene mutation or promoter methylation was also associated with functional polymorphisms of genes leading to increased activation or decreased detoxification of carcinogen [7,8], which has been advanced as a possible mechanism of LC susceptibility.

Cytochrome P450 (*CYP450*) enzymes are crucial phase I xenobiotic metabolizing enzymes (XMEs). Most *CYP450s* are polymorphic, because of gene deletions, single nucleotide polymorphisms (SNPs), gene duplications and mutated alleles [9,11] *CYP1A1* and *CYP2E1* are two of the main CYP450 isoforms involved in the metabolism of endogenous compounds and xenobiotics considered to be responsible for the development of several human diseases. Phase I xenobiotic enzymes metabolically activate carcinogens, such as PAHs and N-nitrosamines, to reactive intermediates [9,11]. These intermediates are capable of binding covalently to DNA to form DNA adducts, potentially initiating the carcinogenic process. Both biological and biochemical evidence indicates that genetic polymorphisms of *CYP450* genes can influence the balance between metabolic activation and detoxication of some toxicants, such as benzo[a] pyrene, and thus they are relevant to individual susceptibility to LC.

Polymorphisms in genes encoding the drug metabolizing enzymes (e.g. *CYP1A1*) contribute to the variability in susceptibility to various cancers [11,12]. However, exact mechanisms explaining the occurrence of LC remain unclear. Several studies have been since performed examining the potential association between the *CYP1A1* variants and the cancer susceptibility [13,17].The human *CYP1A1* gene, located on the long arm of chromosome 15 (15q22–q24), is 5,987 base pairs long and encodes a 512-amino acid protein. It comprises 7 exons and 6 introns. Polyaromatic hydrocarbons and other chemicals serve as inducers as well as substrate in the regulation of gene expression [12]. *CYP1A1* is a good candidate gene, because they are modifiers of risk of LC due to their allelic variants that alter the inducibility of the enzyme by the inducers. The effect of metabolic polymorphisms on LC risk has been shown to depend on the level of exposure to xenobiotics in some subgroups of individuals. For example, it is suggested that the effect of the *CYP1A1* polymorphisms is greater in non-smokers than in smokers and in women than in men [18,19]. It is conceivable that individuals who have inherited specific variants in these genes, such as *CYP1A1*, may become

susceptible to chemical carcinogens and thus at a high risk of developing LC. Genetic polymorphisms in *CYP1A1* gene contributing to inter-individual susceptibility in the cigarette smoking-induced cancer may modify the risk of myocardial infarction, particularly among smokers [20]. However, to the best of our knowledge, the role of *CYP1A1* genetic polymorphisms in the risk, clinical features and biological characteristics of LC remain unknown at least in Libya. Thus, this study was designed, for the first time, to investigate the possible association between the *CYP1A1* genetic polymorphisms (exon 7 and 3' non-coding region) and LC susceptibility using polymerase chain reaction (PCR) and direct DNA sequencing. The potential effect of the smoking habit on this association was also evaluated. Also, clarifying the effect of these genetic polymorphisms on clinical parameters was of particular interest in our study.

Materials and Methods

Study Population

The case–control study consisted of 20 controls and 32 LC patients. General population (unrelated) controls were recruited from healthy population. LC patients attending the Department of Oncology at Tripoli Medical Cancer, Tripoli, Libya, were included in the present study. For sample preservation and genetic analysis, local institutional review board approval and informed consent from all subjects were obtained by the Ethics Committee of the First Affiliated Tripoli Medical Cancer at Tripoli, Libya. LC was confirmed by histological examination after lung biopsy. The median age of the case series was 58 years (range, 46-75 years), all were males (100%), and all were Libyans (undefined ethnic group). After adjustment, there were no difference of age and cigarette smoking between the group and control case group.

Sample collection and DNA Extraction

Blood samples (five-mL) were taken from all participants by peripheral antecubital venous puncture, drawn into sodium EDTA tube and was then stored at −20°C until analysis. Genomic DNA was isolated from the blood samples of controls and LC patients by a stand aid procedure (standard phenol and chloroform) [21,22]. The integrity of extracted genomic DNA and its concentration were measured by UV-spectrophotometry (BioPhotometer, eppendorf), with absorbance A260/A280 nm ratios at pH 8.0 between 1.7 and 2 for all samples, and ethidium-bromide fluorescence of DNA separated by 1.5% AGE (agarose gel electrophoresis). The quality was checked by amplifying β-actin housekeeping gene. Purified DNA was stored at -70 °C till are being used.

Molecular Characterization of LC Patient Samples

CYP1A1 mutations were analyzed by PCR amplification of *CYP1A1* exon 7 and 3' non-coding region and followed by direct bidirectional DNA sequencing]. PCR amplification was carried out using a Perkin-Elmer 480 thermocycler (Applied Biosystems) in a 25 µl reaction mixture containing 10X PCR buffer, 1.5 mM $MgCl^{2+}$, 200 µM of dNTP's (AB Gene), 1 U of Hotstart Taq Polymerase (AB Gene), 10 pmol of forward and reverse primers with 100 ng of genomic DNA (Table 1). The reaction was heated at 95°C for 5 min, then amplified for 30 cycles [95°C /30 sec, annealing/30 sec (60°C for exon 7 and 65°C for 3' non-coding region) and 72°C/30 sec] followed by 5 min final extension at 72°C. PCR amplification and the absence of primer dimers were confirmed by analysis of cycling and melting curves. Negative controls (water instead of DNA) were included in all PCR experiments. Linearity and specificity of PCR amplification were validated before quantification. *CYP1A1* exon 7 and 3' non-coding region amplified products (204 bp and 238 bp, respectively) were visualized by electrophoresing on a 1.5 % AGE. All PCR amplifications resulted in a single and specific product of the expected size (Figure 1). The banding pattern of extracted genomic DNA did not show any evidence of DNA degradation (Figure 1).

Qualified PCR products (amplicons) were cleaned using exonuclease I and shrimp alkaline phosphatase (SAP). Sequencing reaction was performed using Big Dye Terminator Cycle Sequencing Ready Reaction kit v3.0 (Applied Biosystems) at 5′ → 3′ direction, , as indicated by the manufacturer. The nucleotide sequence detection was performed in the ABI Prism 310 Genetic Analyzer (Applied Biosystems) using standard protocols. Finally, the sequencing results were compared with the standard sequence of *CYP1A1* gene (exon 7 and 3' non-coding region). Reproducibility of the results was confirmed by randomly repeating the mutation analysis for 5% of all DNA samples. The sequencing was carried out in the Biochemistry Department, Hospital of Infants, Tunisia.

Table 1. Primers used to amplify exon 7 and 3' non-coding end of the *CYP1A1* gene.

Exon	Primer sequence	Amplicon size (bp)	Annealing temperature
Exon 7	FP: 5'CTGTCTCCCTCTGGTTACAGGAAGC3' RP: 5'TTCCACCCGTTGCAGCAGGATAGCC3'	204	60 °C
3' non-coding end	FP: 5'AAGAGGTGTAGCCGCTGCACT 3' RP: 5'TAGGAGTCTTGTCTCATGCCT3'	338	65 °C

FP: forward primer; RP: reverse primer; bp: base pair.

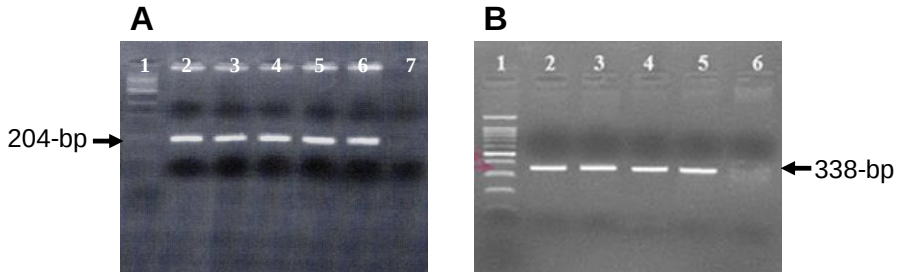

Figure 1. Gel electrophoresis of *CYP1A1* exon 7 and 3' non-coding region PCR products using allele-specific primers. (**A**) Lane 1 represents the 500 bp DNA marker; lanes 2 to 6 show amplified products of *CYP1A1* exon 7 (204 bp); lane 7 correspond to the negative control. (**B**) Lane 1 correspond to the molecular size marker 500 bp; lanes 2 to 5 show amplified products of 3' non-coding region of the *CYP1A1* gene (338 bp); lane 6 correspond to the negative control. *CYP1A1* gene: cytochrome P450, family 1, subfamily A, polypeptide 1 gene. PCR: polymerase chain reaction; bp: base pair.

Statistics

All calculations were performed using the SPSS software package (version 20.0). Data were expressed as mean ± S.E.M. The patients' characteristics were analyzed by the chi-squared (χ^2) or Fisher's exact tests for univariate analysis. Potential confounders, such as age, sex, cigarette smoking, and biochemical parameters were also studied. Differences between controls and cases were compared by either Student's t-test or one way ANOVA, as appropriate. *P* values less than 0.05 were deemed statistically significant.

Results

Molecular Analysis of CYP1A1 Mutations

CYP1A1 mutations were identified in 11.5 % (n=52) adult male subjects and cases analyzed. Overall, 11 *CYP1A1* mutations were documented in this study (Table 2), involving exon 7 and 3' non-coding region. Among these, nonsense, missense, and frame-shift mutations accounted for, respectively, 27.3 % (3/11), 63.6 % (7/11) and 9.1 % (4/11) of all *CYP1A1* mutations (Table 2). Three missense mutations (*m2, m4*, and *m1*) have already been described [23,24]. The remaining mutations have not been reported previously.

The frequency of specific *CYP1A1* mutations identified in the present study is listed in Table 2 and graphically depicted in Figure 2. The mutations *CYP1A1*2B/m2 (*rs1048943*), CYP1A1*4/m4 (*rs1799814) and *CYP1A1*2A/m1*

*(*rs4646903) accounted for, respectively, 18.2 % (2/11), 9.1 % (1/11) and 9.1 % (1/11) of all *CYP1A1* mutations.

Table 2. Characteristics of LC patients with a *CYP1A1* gene mutation.

Case no.	Sex	Age	Mutation (s)	Region	Mutation Type	Protein Level	Status
1 (S-Ctrl)	M	55	4889A>G CYP1A1*2B/m2	Exon 7	Missense	462Ile/Val	R
2 (S-Ctrl)	M	32	4889A>G CYP1A1*2B/m2	Exon 7	Missense	462Ile/Val	R
3 (LCP)	M	63	4887C>A CYP1A1*4/m4	Exon 7	Missense	461Asp/Asp	R
4 (S-Ctrl)	M	55	6235T/C CYP1A1*2A/m1	3' non-coding	Missense	-	R
5 (S-Ctrl)	M	55	4861delG	Exon7	Nonsense	Meth452X	N
6 (S-Ctrl)	M	32	4861delG	Exon7	Nonsense	Met452X	N
7 (LCP)	M	55	4861T>G	Exon7	Missense	Met452Arg	N
8 (S-Ctrl)	M	45	4793insC	Exon7	Frame-shift	Glu431X	N
9 (LCP)	M	55	4793insA	Exon7	Nonsense	Glu431X	N
10 (NS-Ctrl)	M	27	4842A>C	Exon7	Missense	Lys446Thr	N
11 (S-Ctrl)	M	45	4842A>C	Exon7	Missense	Lys446Thr	N

CYP1A1 gene: cytochrome P450, family 1, subfamily A, polypeptide 1 gene*; M: male; m: mutation; S-Ctrl: smoking control; LC: lung cancer; S-Ctrl: smoking control; NS-Ctrl: non-smoking control; >: substitution; ins: insertion; del: deletion; R: reported; N: novel.*

The Genotype Frequency of CYP1A1 Gene

The frequency distribution of specific genotypes of *CYP1A1* is shown in Tables 3. Specifically, there were two control subjects (smokers) carried heterozygotic genotypes containing mutation *m2* (4889A/G [*462Ile/Val*]) (Table 2 and Figure 2A-B). However, we observed only one LC subject (smoker) carried heterozygotic genotype containing mutation *m4* (4887C/A [461Asn/Asn]). Interestingly, heterozygotic genotypes containing m2 or m1 were not represented in cancer groups. In addition, we detected one subject (smoker) carried the heterozygotic

genotypes containing mutation *m1* (6235T/C). Furthermore, no homozygotic genotypes composed of mutated alleles were found.

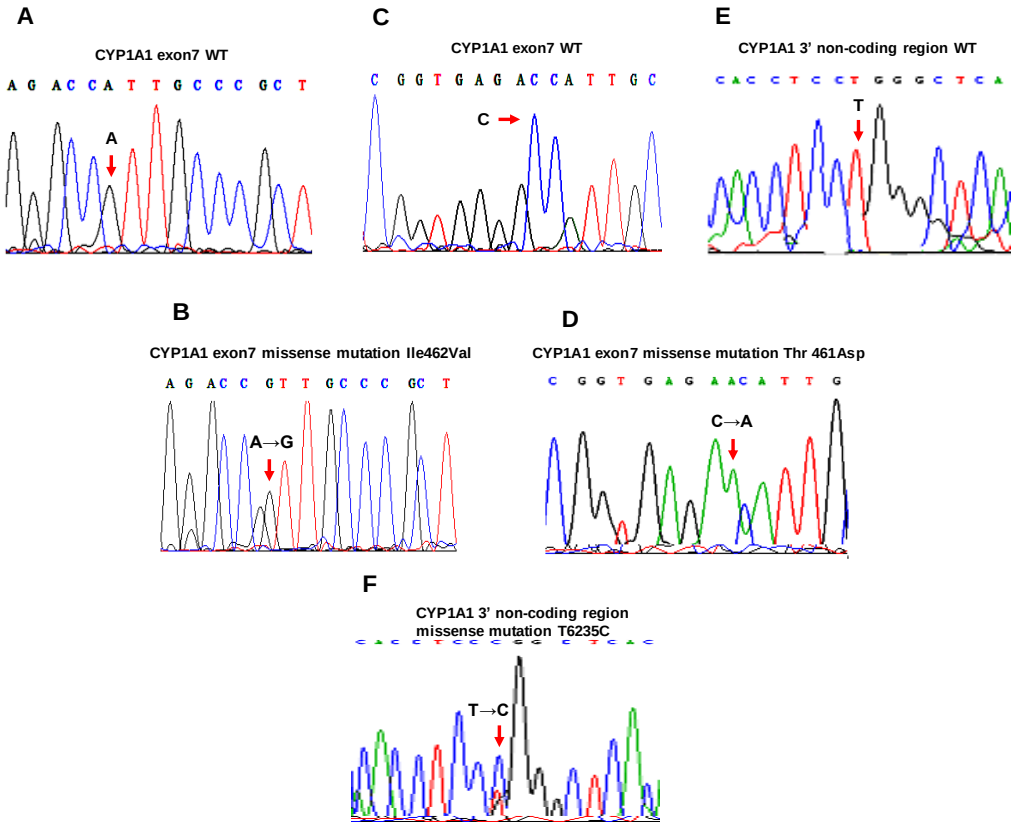

Figure 2. *CYP1A1* gene mutations in controls and LC patients. (A-F) Representative DNA sequencing chromatograms of the control and patient genomic DNA samples showing mutations in exon 7 and 3' non-coding region of *CYP1A1* gene. *CYP1A1* gene: cytochrome P450, family 1, subfamily A, polypeptide 1 gene; WT: wild type.

Association of CYP1A1 Gene Mutations with Controls and LC Patient Clinical Features

The clinical features of *CYP1A1* wild type and *CYP1A1* mutant controls and LC cases are compared in Table 5. LC cases, but not control subjects, with *CYP1A1* mutations had lower PLT counts (P=0.739) and lower WBC counts (P=0.066 and P=0.254, respectively), than the controls and cases without *CYP1A1* mutations. Furthermore, the average age of the controls and cases with *CYP1A1* gene mutations was lower than the average age of the controls and cases without *CYP1A1* mutations (P=0.646 and P=0.794, respectively). The continued analysis

Table 3. Frequency distribution of *CYP1A1* gene polymorphisms in controls and LC patients

Genotype	Controls, N (%)	Cases, N (%)	Adjusted OR (95% CI)	*P*-value
CYP1A1*2B/ m2				
462Ile/Ile (4889A/A)- WT homozygous	18 (90)	32 (100)	1.21 (0.67-2.21)	0.357
462Ile/Val (4889A/G)- Heterozygous	2 (10)	0 (00)	1.34 (0.60–2.9)	0.674
CYP1A1*4/m4				
461Thr/ Thr (4887C/C)- WT	20 (100)	31 (96.9)	1.12 (1.23–3.02	0.565
homozygous	0 (00)	1 (3.1)	2.39 (1.45-2.3)	0.198
461Thr/ Asp (4887C/A)- Heterozygous				
CYP1A1*2A/m1				
(6235T/T)- WT homozygous	19 (95)	32 (100)	1.21 (0.67-2.21)	0.418
(6235T/C)- Heterozygous	1 (5)	0 (00)	1.34 (0.60–2.9)	0.239

LC: lung cancer; *CYP1A1* gene: cytochrome P450, family 1, subfamily A, polypeptide 1 gene. WT: wild-type, OR Odds ratio, 95% CI 95% confidence interval.

showed that no significant differences between the controls with *CYP1A1* mutations and cases with *CYP1A1* mutations were found for PLT and WBC counts or for average age (data not shown). In addition, there was significant differences between the controls and cases without *CYP1A1* mutations and controls and cases with *CYP1A1* mutations were found for PLT and WBC counts or for average age (data not shown).

Table 5. Controls and LC Patients' characteristics and *CYP1A1* gene mutations.

Subject' characteristics	*CYP1A1* wild type	*CYP1A1* mutated	*P value*
Controls			
PLT, $\times 10^9$/L(range)	223 ± 25.33	250 ± 22.98	0.503
WBC count, 10^9/L (range)	7.71 ± 0.61	5.75 ± 0.63	0.066
Age *(*years*)*	43.72 ± 5.14	39.75 ± 6.34	0.646
LC patients			
PLT, $\times 10^9$/L(range)	314 ± 69.55	249 ± 236	0.739
WBC count, 10^9/L (range)	12.5 ± 1.81	7 ± 1	0.254
Age *(years)*	61.17 ± 3.17	59 ± 4	0.794

PLT: platelets; WBC: white blood cell; LC: lung cancer; *CYP1A1* gene: cytochrome P450, family 1, subfamily A, polypeptide 1 gene.

Discussion

Worldwide, lung cancer (LC) remains a leading cause of morbidity and mortality. Several risk factors for LC are already recognised (e.g. tobacco smoke, radon, asbestos, metals including arsenic, chromium, nickel, etc.) [3]. Moreover,

in recent years, the genetic background was shown to be involved in the etiology of this cancer [23-24].

The human *CYP450* family is particularly well studied. The *CYP450* enzyme activity influenced by genetic polymorphisms and their association with development of different disorders was widely studied. The best studied cytochromes are: *CYP1A1*, *CYP2D6*, *CYP2C9* and *CYP2C19*. Among them *CYP1A1* is of special interest because it metabolises and activates mainly carcinogens [12,14; 23,24]. In the human *CYP1A1* gene, four base substitutions are known, namely *m1*, *m2*, *m3*, and *m4* (*25*). The presence of point mutations could result in higher *CYP1A1* induction degree, which would accelerate the metabolic activation of procarcinogens [12,14*;* 23,24; 26]. Since we know that *CYP1A1* is active in many tissues; we assumed that *CYP1A1* gene mutations could be susceptibility factor to lung carcinogenesis. The *CYP1A1* mutations have been postulated as a risk factor for cancer susceptibility [27]. In molecular–epidemiological studies functional consequences of *m1* and *m2* point mutation of *CYP1A1* gene have been assumed to be a risk factor for LC development [23,24*;* 28). In Japan, the frequency distribution of *m1* and *m2* mutation was higher in the subjects with LC [29,30]. This finding was not confirmed in Europe [14; 31,32]. However, the frequency distribution of *CYP1A1* point mutations is different in the many populations, from small number in Caucasians to higher frequency in The Far East. It was also reported that *CYP1A1* gene polymorphism is closely correlated with bladder cancer occurrence. However, it was not confirmed by other study [33]. The role of *CYP1A1* was also studied as a susceptibility factor for other diseases. It was shown that *CYP1A1* gene polymorphism could be implicated in the pathogenesis of cardiovascular diseases and atherosclerosis [34].

In the present study, we have investigated the potential association between the *CYP1A1* gene polymorphisms and the LC susceptibility among a population of Libyan males. We have also studied whether these polymorphisms are associated with the clinical features of LC patients. In our study, *CYP1A1* mutations were identified in 11.5 % adult subjects and cases analysed. Overall, 11 *CYP1A1* mutations were documented in this study involving exon 7 and 3' non-coding region. Three mutations (m2 [rs1048943], *m4* [rs1799814], and *m1* [rs4646903]) have already been described (*23-24, 35*). The remaining variants/ mutations have not been reported previously. The *CYP1A1*2B/m2* (rs1048943) and *CYP1A1*4/m4* (rs1799814) are variations located at exon 7 of the *CYP1A1* gene. *CYP1A1*2B/m2* corresponds to a substitution of an adenine (A) by a guanine (G) at position 4889 (4889A/G) of the nucleotide sequence resulting in an isoleucine (Ile)- valine (Val) substitution in codon 462 (462IIe/Val) in the mutant form of the protein (35)., the *CYP1A1*4/m4* corresponds to a substitution of a cytosine (C) by

an adenine (A) at position 4887 (4887C/A) of the nucleotide sequence resulting in a tryptophan (Thr)- asparagine (Asn) substitution in codon 461 (461 Thr/Asn) in the mutant form of the protein [14]. However, the CYP1A1*2A/*m1* mutation (rs4646903) is a variation located at 3′-flanking region of *CYP1A1* gene. In a molecular epidemiological study, mutation *m2* was shown to increase the risk for LC in their carriers [23]. It was also shown that mutation *m1* can increase the risk for LC in their carriers [23]. Previously, it has been reported that the mutation *m4* may not represent a susceptibility factor for LC [14]. We present the genotype frequencies of only one *CYP1A1* mutation *m4* [rs1799814] in relation to LC risk and two additional *CYP1A1* mutations, namely m2 (rs1048943) and *m1* (rs4646903) associated with cigarette smoking.

Previously, several studies of *CYP1A1* gene polymorphism in different ethnic groups were documented. A broad interethnic diversity was reported. In Caucasians, the highest frequency of *m1* point mutation was found in USA (13.4%) and Norway (11.5%), and *m4* mutation was not detected [32, 36]. The higher frequency of *m4* point mutation was identified in Turkish healthy population (5.7%) (*37*). Contrary, in Japan the highest frequency of *m1* and *m2* mutation was found (*32, 36*). In the Polish population, the frequency of *m1* point mutation was 6.6%, of *m2* 2.2% and of *m4* 2.0% (*38*). Motykiewicz *et al*. investigated the healthy population in Silesian region [39] and found higher frequency of *m2* point mutation. In the present study, we have observed two apparently heterozygous carriers of mutation *m2* (*CYP1A1* 4889A/G [462Ile/Val]) from our own study. We also detected one heterozygous carrier of mutation *m4* (*CYP1A1* 4887C/A [461Thr/Asp]) and one heterozygous carrier of mutation *m1* (6235T/C) in controls. In spite of the absence of any statistical significance, it seems that the presence of only one *m4* mutation is sufficient to develop higher LC risk.

Collectively, this study did not show any evidence of a significant association between *CYP1A1* polymorphisms and increased risk of LC. Although this study is the first study to investigate LC in Libya at molecular level, the large sample size provides more reliable conclusions regarding LC among Libyans. In this study, all controls and LC cases showing *CYP1A1* mutation were males. It needs to be verified that *CYP1A1* mutation is more associated with male gender or occurred by chance due to the preponderance of males in Libyan LC patients. The polymorphisms of the other genes should be taken into consideration, and we would suggest that *CYP1A1* polymorphism could be a susceptibility factor for LC carcinogenesis.

Authors' Contributions

Najah A. Faras, Othman A. El-Ansari and Mohamed A. Al-Griw substantially contributed to the conception and design of the study, acquisition, analysis and interpretation of data; all authors drafted the article and made critical revisions related to the intellectual content of the manuscript and approved the final version of the article to be published.

Conflict of Interest

To the best of our knowledge, no conflict of interest exists.

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