

A genetic polymorphism in the CYP1B1 gene in patients with squamous cell carcinoma of the esophagus: an Iranian Mashhad cohort study recruited over 10 years

Article (Accepted Version)

Moghadam, Azam Rastgar, Mehramiz, Mehramiz, Entezar, Malihe, Aboutalebi, Hamideh, Kohansal, Fatemeh, Dadjoo, Parisa, Fiuji, Hamid, Nasiri, Mohammadreza, Aledavood, Seyed Amir, Simab, Saeideh Ahmadi, Khorrami, Mohammad Sadegh, Moradi, Ali, Hassanian, Seyed Mahdi, Ferns, Gordon A, ShahidSales, Soodabeh et al. (2018) A genetic polymorphism in the CYP1B1 gene in patients with squamous cell carcinoma of the esophagus: an Iranian Mashhad cohort study recruited over 10 years. *The Pharmacogenomics Journal*, 19 (6). pp. 539-546. ISSN 1470-269X,

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/id/eprint/74278/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

A genetic polymorphism in the CYP1B1 gene in patients with squamous cell carcinoma of the esophagus: an Iranian Mashhad cohort study recruited over 10 years

Azam Rastgar Moghadam^{1,2,3}, Mehrane Mehramiz⁴, Malihe Entezari², Hamideh Aboutalebi⁴, Fatemeh Kohansal⁴, Parisa Dadjoo⁴, Hamid Fiuji⁴, Mohammadreza Nasiri⁵, Seyed Amir Aledavood³, Kazem Anvari³, Saeideh Ahmadi Simab², Mohammad Sadegh Khorrami⁶, Ali Moradi³, Seyed Mahdi Hassanian^{4,7}, Gordon A. Ferns⁸, Soodabeh ShahidSales^{3,#} Amir Avan^{4,5,#}

Affiliations:

- 1) *Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran*
- 2) *Department of Biology, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran*
- 3) *Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
- 4) *Metabolic syndrome Research center, Mashhad University of Medical Sciences, Mashhad, Iran*
- 5) *Recombinant proteins research group, The research institute of biotechnology, Ferdowsi University of Mashhad, Iran*
- 6) *Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
- 7) *Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
- 8) *Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.*

Corresponding Authors:

Amir Avan, PhD, Metabolic syndrome Research center, Mashhad University of Medical Sciences, Mashhad, Iran, Iran; E-mails: avana@mums.ac.ir; amir_avan@yahoo.com

SoodabehShahidSales, MD; Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. email: ShahidSaless@mums.ac.ir

Grant: this study was support by grant (nu# 951121; 951429, Amir Avan) from Mashhad University of Medical Sciences

Running title: CYP1B1 gene polymorphism in ESCC

Conflict of interest: The authors have no conflict of interest to disclose

Abstract

Background: Esophageal-cancer is the seventh most common-cause of cancer-related-deaths in men. Cytochrome-P450-family-1-subfamily-B-polypeptide-1 (CYP1B1) plays a role in the metabolism of xenobiotics, and is associated with several cancers. Here we investigated the association between a genetic-variant, CYP1B1-rs1056836, with the clinical-characteristics of patients with esophagus-squamous-cell-carcinoma (ESCC).

Method: 117-patients with ESCC and 208 healthy-subjects were recruited. DNA was extracted and genotyped. Kaplan-Meier curves were utilized to assess overall and progression-free survival. The relationship between clinicopathological-data, disease-prognosis, and survival, were evaluated with the genotypes.

Results: the genotypic frequency for GG, GC, and CC were 58.6%, 29.8%, 11.5% respectively in the healthy subjects and 51.8%, 36.14% and 12% in the ESCC group. An association between the GG genotype and stage of ESCC was found.

Conclusion: Our findings suggest a relationship between the CYP1B1-rs1056836 genetic polymorphism and clinical features of ESCC, supporting further studies in larger-populations in different-ethnic groups, taking into account potentially important environmental-factors.

Keywords: cytochrome p450, esophagus squamous cell carcinoma

Introduction

More than 450000 people worldwide are affected by esophageal carcinoma, and there has been a rapidly rising incidence. Esophageal cancer has been reported to be the eighth most common cancer globally (1) and the seventh most common cause of cancer-related death in men (2). A large sex difference has been reported in esophageal carcinoma and morbidity and mortality rates are more than 4 times higher in men in comparison to women(2). Despite recent advances in diagnosis and treatment, the five-year survival rate for esophageal cancer patients ranges from 15-25%; this may be related to the late diagnosis of the disease; its aggressiveness, or the lack of efficient treatment strategies (3). Further studies are required to understand the underlying disease mechanisms for earlier diagnosis and predicting prognosis. Several polymorphisms in a number of genes have reported to have an association with the development of esophageal cancer (4). There are also several genetic predisposing factors that contribute to ESCC such as genes involved in the cell cycle or differentiation (5), genes involved in folate metabolism (6) Epidermal Growth Factor Receptor Signaling Pathway, Epigenetic Factors, Somatic Mutational Signature and polymorphisms(7) (e.g., cytochrome P450).

Recent studies have revealed that the cytochrome P450, family 1, subfamily B, polypeptide 1 is associated with several cancer types. Cytochrome P450 family 1, a member of cytochrome P superfamily, includes three members: CYP1A1, CYP1A2, and CYP1B1 (8,9). The CYP1B1 gene is a 12 kilobase sequence located on 2p22-21 with 3 exons and 2 introns and gives rise to a 5.2-kb mRNA. CYP1B1 has a high expression rate in some tissues such as uterus, breast, and prostate but barely detectable in the liver(10). Several studies have shown that CYP1B1 metabolizes various potential human carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic amines and also overexpression has been reported in different tumor tissues (11). CYP1B1 accelerates estrogen hydroxylation reactions such as 4-hydroxylation of 17 β estradiol (E2), resulting in the production of 4-hydroxyestradiol which has a reduced activity. This conversion can result in tumor initiation (12). Hence, it appears that CYP1B1 may play an important role in the development and progression of tumors and has the potential to be a tumor biomarker and a target for anticancer drugs (13). More than 50 genetic variations have been identified affecting the CYP1B1 protein. The Rs1056836 polymorphism, also known as 4326C/G, is one of these variations. It is located in the third exon of CYP1B1 and it can result in a change in amino acid from Leu to Val. This polymorphism is associated with estrogen and progesterone receptor activity in breast cancer (14). It also increases the K_m of the 4-hydroxylation of estradiol by 4 fold which would change the estradiol binding status of CYP1B1 (15). This polymorphism is associated with a higher risk of cancers of the prostate (16), lung (17,18), larynx (19) and endometrium (20) but not with breast (21) or colorectal cancer (22). Despite the lack of expression in hepatic tissue CYP1B1- rs1056836 has been found to be associated with elevated risk of hepatocellular carcinoma among HBsAg-positive individuals, in whom the risk increases by 13.9 fold among smokers and also induces a high risk of cirrhosis in patients with

hepatocellular carcinoma (23). The variation is also associated with concentrations of T4, FT3, and FT4 in the serum of polycystic ovary syndrome patients. Another study has reported a strong association between CYP1B1 – rs9341266 and ESCC risk (24). With regards to these findings, we designed this study to determine whether there is an association between the CYP1B1-rs1056836 polymorphism with clinical outcomes and features of esophagus squamous cell carcinoma patients.

Materials and methods

Ethics statement

This study is approved by Mashhad University of Medical science. (MUMS, Mashhad, I.R.Iran)

Patients and controls

One hundred and seventeen patients who had been diagnosed with esophageal squamous cell carcinoma in Omid Hospital of Mashhad Medical University from May 2006 to August 2014 were selected, for the case group in the study. The inclusion criteria were: locally advanced or metastatic ESCC. The 224 subjects in the control group were healthy people who had took part in the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study as previously described (25). The inclusion criteria for the control group included a lack of history in diseases such as myocardial infarction, cancer, infectious disease, or family backgrounds for similar conditions such as stroke and type II diabetes. Samples were got collected from paraffin embedded blocks fixed in formalin, and Nine-mm slices were processed serially.

Genotyping

QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) has been used for genomic DNA extraction from peripheral blood, according to manufacturer's approved protocol. NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA) was used to determine the DNA's concentration and purity. In order to analyze CYP1B1- rs1056836 genotype polymorphism, we used Taqman®-probes-based assay. Polymerase chain reaction amplification was performed in a 12.5 µl final reaction volume containing 10-20 ng/µl of DNA, and TaqMan® Universal Master Mix with specific primers and probes (C_3099976_30; Applied Biosystems Foster City, CA). To assess samples allelic content, ABIPRISM-instrument and SDS version-2.0 software were applied (26, 27). More than 10% of the samples were randomly selected for the double check and the results were concordant.

Statistical analysis

All the analyses were accomplished two-sided with statistical significance set to 0.05 ($P < 0.05$). Data analysis conducted using SPSS-20 software (SPSS Inc., IL, USA). To report ESCC patients Descriptive statistics, mean and standard deviations(SD) were assessed in terms of

the continuous variables and for categorical variables, frequencies and percentages were used. We used Pearson χ distribution In order to evaluate Hardy–Weinberg equilibrium (HWE) aberrations, and the assessed genotype and allele frequencies of CYP1B1 – rs1056836 polymorphism. Logistic regression (adjusted for age, sex and BMI) was used to evaluate whether there's an association between risk of ESCC for CC and CG genotype relative to GG genotype under recessive genetic model. For ESCC risk assessment, odd ratio (OR) and its corresponding 95 % confidence interval (CI) has been applied. To assess the relationship between CYP1B1- rs1056836 and clinicopathological characteristics Pearson's chi-square χ^2 test were applied for categorical variables while Student's t tests utilized for continuous variables. Calculation of overall survival was performed using the Kaplan-Meier method and subjects were observed from the day of treatment until the end point (which has been defined as death or censoring) and the data has been compared by Wilcoxon tests and log-rank. In Cox's proportional hazard model univariate analysis of significant prognostic variables was included. In order to evaluate the orientation and significance of the effect, the Hazard ratio was utilized.

Results

clinicopathological characteristics of population

The clinicopathological characteristics of patients are summarized in table 1. The median age of the participants was 58 ± 11 and BMI was 19.9 ± 5.2 . 45.3% of patients were female and 54.7% were male. In 1.9% of patients, cancer cells spread into the lining of the esophagus (T1 status), in 5.6% of cases, invaded cells spread into although not through the muscle wall of the esophagus while in about 21.5% of them, cancer cells spread through the muscle wall of the esophagus into surrounding tissues. Finally, 71% of participants were in T4 status, where cancer cells penetrate into surrounding tissues. In 63% of cases, tumor cells were absent from regional lymph nodes and nodal status determined as N0 while in 27% of the cases lymph node metastasis was present, defined as N1 to N3. In 15.4% distant metastasis was observed. To assess the influence of patients features on clinical outcome, the progression-free survival (PFS) and overall survival (OS) were analyzed and we found an association between this polymorphism and stage of the disease

Genetic variant association with ESCC

We conducted DNA extraction from peripheral blood and ESCC tissue samples and genotyped all the samples with minimum errors. The duplicate check was performed on randomly selected samples and no deviations were found. Overall 291 age and sex matched subjects (208 healthy controls and 83 ESCC) were included in our study (table 2). The distribution of CYP1B1-rs1056836 in the studied population was in agreement with Hardy-Weinberg equilibrium (HWE) ($P > 0.05$) which is shown in table 2 in addition to genotype frequencies. The frequency of the minor allele (C) was 0.3. the genotypic frequency for GG, GC, and CC are respectively 58.6% , 29.8%, 11.5% in the control group and 51.8%, 36.14%

and 12% in the case group. In table 3 genotype frequencies were evaluated with respect to clinicopathological characteristics of ESCC patients under the recessive genetic model. 84% of women had a GG genotype. With respect to the recessive genetic inheritance model, we found an association between the GG genotype and stage of ESCC. Statistically significant results were not found for this variation and risk of ESCC. According to Kaplan-Mayer curves, patients with GC/CC genotype had a significantly shorter overall survival with mean range of 51.6 ± 7.5 months in comparison with GG patients with mean range of 68 ± 8.89 months (figure 1- a). Furthermore, the PFS of cases with GC/CC genotype was 34.9 ± 5.9 vs GG genotypes with PFS of 46.3 ± 8.9 months. (figure 1-b).

Discussion

To the best of our knowledge this is the first study demonstrates that CYP1B1 – rs1056836 was associated with ESCC patients. Devlin et.al in a study on CYP1B1 protein expression in the rat model of esophageal tumorigenesis showed that CYP1B1 protein has an extremely low expression levels in esophageal tissues of the normal rat while expressed in high levels in esophageal hyperplasia and squamous cell carcinoma. This study claimed that expression of CYP1B1 might be an early event in rat tumor formation and together with its regulatory mechanisms of expression, might play a role in the development of high-grade dysplasia to carcinoma (28), which is in agreement with our findings.

Similar to these findings another study reported elevated levels of CYP1B1 in the tongue, esophagus, lung and colon of the mice with short-term exposure to tobacco smoke. They investigated the impact of tobacco smoke condensate and benzo[a]pyrene on the expression of CYP1B1 *in vitro* and *in vivo*. The *in vitro* phase conducted on H2122, SCC450 and MSKLeuk1 cell lines, derived from human non-small cell lung adenocarcinoma, esophageal squamous cell carcinoma and dysplastic oral leukoplakia lesion respectively. The results showed that both tobacco smoke condensate and benzo[a]pyrene result in elevated levels of CYP1B1 protein and mRNA in cell lines. Also, treatment with tobacco smoke-induced AhR signaling leads to CYP1B1 induction (29). We did not find any statistically significant association between risk of ESCC and rs1056836 which is in agreement with previously published studies. An etiological case-control study on ESCC found no significant difference between allelic frequencies of CYP1A1 3'-UTR, CYP1B1, CYP2E1, CYP1A1 exon7, and, CYP2A6 (del) in case and control groups (30). Also in another case-control study conducted in Kashmir India on the association of Leu432Val Polymorphism of CYP1B1 on ESCC, the frequency of both Val432Val and Leu432Val genotypes reported equal in both case and control group (31). The association of CYP1B1 – rs1056836 with different cancers have been studied intensively. Li et.al in a study of over 724 SCCHN (Squamous Cell Carcinoma of the Head and Neck) cases and 1226 cancer-free controls, found no significant effect of CYP1B1-rs1056836 in SCCHN (32). Similar to these findings, Yon Ko et.al did not find any important impact for CYP1B1 – rs1056836 in SCCHN but reported it as a susceptibility factor in smokers (33). CYP1B1–rs1056836 polymorphism has been shown to

be associated with progression-free survival and response rate of non-small cell lung cancer (NSCLC) patients treated with docetaxel, and the GG genotype has been reported to result in shorter progression-free survival in comparison to other genotypes (17). Also in a meta-analysis on 6501 subjects with lung cancer CYP1B1-rs1056836 were found to be associated with the risk of lung cancer and Val allele of CYP1B1 codon 432 is reported as a low penetrance risk factor in this cancer which is more common in lung adenocarcinoma, Caucasians or in smokers (18). Peng-Ju Yu et.al in a case-control study in 2015 on 600 subjects including healthy and laryngeal cancer patients, reported a significant difference in frequencies of rs1056827 and rs1056836 genotypes between case and control group and the G allele carriers of rs106836 had a lower risk of laryngeal cancer development in comparison to C allele carriers (19). In approval of these findings Diljit Kaur-Knudsen et.al reported decreasing risk of tobacco-related cancer with CG and GG genotypes of CYP1B1-rs1056836 versus CC genotype but they did not found any association between CYP1B1-rs1056836 and ischemic heart disease (IHD), chronic obstructive pulmonary disease (COPD), ischemic cerebrovascular disease (ICVD), cancer overall and female cancers (34). A meta-analysis on Estrogen-Related Pathway Genes polymorphisms has reported associations between CYP1B1 and CYP1A1 genotypes and risk of prostate cancer (16) which is supported by several other studies (16,35-38).

A strength of the current study is that it was performed in a well-characterized cohort of individuals, with or without ESCC in a cohort of 10 years cases with clinical outcome (OS and PFS). The main limitations were the cross-sectional design and modest sample size. Additionally, it is possible that other lifestyle characteristics such as diet have an influence on the outcome.

This study indicates that CYP1B1-rs1056836 can be as a potential stage biomarker for ESCC patients, but further studies with larger samples sizes in different ethnic groups are required to approve our findings in other populations.

References

- 1) Pennathur A, Gibson M, Jobe B, Luketich J. Oesophageal carcinoma. *The Lancet*. 2013;381(9864):400-412.
- 2) Siegel R, Miller K, Jemal A. Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians*. 2017;67(1):7-30.
- 3) Pennathur A, Farkas A, Krasinskas AM, et al. Esophagectomy for T1 esophageal cancer: outcomes in 100 patients and implications for endoscopic therapy. *Ann Thorac Surg* 2009; 87: 1048–55.
- 4) Hiyama T, Yoshihara M, Tanaka S, Chayama K. Genetic polymorphisms and esophageal cancer risk. *International Journal of Cancer*. 2007;121(8):1643-58
- 5) Meseure D, Vacher S, Alsibai KD, Nicolas A, Chemlali W, Caly M, et al. Expression of ANRIL-Polycomb Complexes-CDKN2A/B/ARF Genes in Breast Tumors: Identification of a Two-gene (EZH2/CBX7) Signature with Independent Prognostic Value. *Molecular Cancer Research*. 2016:molcanres. 0418.2015.
- 6) Song C, Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Research*. 2001;61(8):3272-5.
- 7) Ohashi S, Miyamoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent Advances From Basic and Clinical Studies of Esophageal Squamous Cell Carcinoma. *Gastroenterology*. 2015;149(7):1700-1715.
- 8) S.R. Beedanagari, I. Bebenek, P. Bui, O. Hankinson, Resveratrol inhibits dioxin-induced expression of human CYP1A1 and CYP1B1 by inhibiting recruitment of the aryl hydrocarbon receptor complex and RNA polymerase II to the regulatory regions of the corresponding genes, *Toxicol. Sci.* 110 (2009)61–67
- 9) R. Mikstacka, W. Baer-Dubowska, M. Wieczorek, S. Sobiak, Thiomethylstilbenes as inhibitors of CYP1A1, CYP1A2 and CYP1B1 activities, *Mol. Nutr. Food Res.* 52 (Suppl. 1) (2008) S77–83.
- 10) M. Sankhwar, S.N. Sankhwar, Variations in CYP isoforms and bladder cancer: a superfamily paradigm, *Urol. Oncol.* 32 (2014) e33–40
- 11) Murray GI, Taylor MC, McFadyen MC, McKay JA, Greenlee WF, et al. 1997. Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res.* 57:3026–31
- 12) 4. Liehr J. Is Estradiol a Genotoxic Mutagenic Carcinogen?. *Endocrine Reviews*. 2000;21(1):40-54.
- 13) Murray G, Melvin W, Greenlee W, Burke M. REGULATION, FUNCTION, AND TISSUE-SPECIFIC EXPRESSION OF CYTOCHROME P450 CYP1B1. *Annual Review of Pharmacology and Toxicology*. 2001;41(1):297-316.
- 14) Bailey LR, Roodi N, Dupont WD, Parl FF. 1998. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid hormone receptor status in breast cancer. *Cancer Res.* 58:5038–41

- 15) Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg T. 2000. Polymorphisms in P450 CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. *Pharmacogenetics* 10:343–53
- 16) Holt S, Kwon E, Fu R, Kolb S, Feng Z, Ostrander E et al. Association of variants in estrogen-related pathway genes with prostate cancer risk. *The Prostate*. 2012;73(1):1-10
- 17) Vasile E, Tibaldi C, Leon G, D’Incecco A, Giovannetti E. Cytochrome P450 1B1 (CYP1B1) polymorphisms are associated with clinical outcome of docetaxel in non-small cell lung cancer (NSCLC) patients. *Journal of Cancer Research and Clinical Oncology*. 2014;141(7):1189-1194.
- 18) Chen B, Qiu L, Li Y, Xu W, Wang X, Zhao W et al. The CYP1B1 Leu432Val polymorphism contributes to lung cancer risk: Evidence from 6501 subjects. *Lung Cancer*. 2010;70(3):247-252.
- 19) Li Z. Association between CYP1B1 Gene Polymorphisms and Risk Factors and Susceptibility to Laryngeal Cancer. *Medical Science Monitor*. 2015;21:239-245
- 20) Wang F, Zou YF, Sun GP, Su H, Huang F (2011) Association of CYP1B1 gene polymorphisms with susceptibility to endometrial cancer: a meta-analysis. *Eur J Cancer Prev* 20(2):112–120. doi: 10.1097/CEJ.0b013e3283410193
- 21) Yao L, Fang F, Wu Q, Zhong Y, Yu L (2010) No association between CYP1B1 Val432Leu polymorphism and breast cancer risk: a meta-analysis involving 40,303 subjects. *Breast Cancer Res Treat* 122(1):237–242. doi:10.1007/s10549-009-0689-2
- 22) Xie Y, Liu GQ, Miao XY, Liu Y, Zhou W, Zhong DW (2011) CYP1B1 Leu432Val polymorphism and colorectal cancer risk among Caucasians: a meta-analysis. *Tumour Biol*. doi:10.1007/s13277-011-0298-7
- 23) Liu F, Luo L, Wei Y, Wang W, Yan L, Wen T et al. P0162 CYP1B1 polymorphisms and hepatocellular cancer risk in a Chinese population. *European Journal of Cancer*. 2014;50:e54-e55
- 24) Hyland P, Freedman N, Hu N, Tang Z, Wang L, Wang C et al. Genetic variants in sex hormone metabolic pathway genes and risk of esophageal squamous cell carcinoma. *Carcinogenesis*. 2013;34(5):1062-1068
- 25) Mehramiz M, Ghasemi F, Esmaily H, Tayefi M, Hassanian SM, Sadeghzade M, Sadabadi F, Moohebbati M, Azarpazhooh MR, Parizadeh SM, Heidari-Bakavoli A, Safarian M, Nematy M, Ebrahimi M, Ryzhikov M, Ferns GA, Ghayour-Mobarhan M, Avan A. Interaction between a variant of CDKN2A/B-gene with lifestyle factors in determining dyslipidemia and estimated cardiovascular risk: A step toward personalized nutrition. *Clin Nutr*. 2016 Dec 28. pii: S0261-5614(16)31359-0. doi: 10.1016/j.clnu.2016.12.018
- 26) Mehramiz M, Ghasemi F, Esmaily H, Tayefi M, Hassanian SM, Sadeghzade M, Sadabadi F, Moohebbati M, Azarpazhooh MR, Parizadeh SM, Heidari-Bakavoli A, Safarian M, Nematy M, Ebrahimi M, Ryzhikov M, Ferns GA, Ghayour-Mobarhan M, Avan A. Interaction between a variant of CDKN2A/B-gene with lifestyle factors in determining dyslipidemia and estimated cardiovascular risk: A step toward personalized nutrition. *Clin Nutr*. 2016 Dec 28. pii: S0261-5614(16)31359-0. doi: 10.1016/j.clnu.2016.12.018

- 27) Oladi M, Nohtani M, Avan A, Mirhafez SR, Tajbakhsh A, Ghasemi F, et al. Impact of the C1431T polymorphism of the peroxisome proliferator activated receptor-gamma (PPAR- γ) gene on fasted serum lipid levels in patients with coronary artery disease. *Annals of Nutrition and Metabolism*. 2015;66(2-3):149-54.
- 28) Devlin A, McIlroy M, McKeen H, Bonde P, Menezes A, Swarbrick C et al. Cytochrome P450 1B1 expression in rat esophageal tumorigenesis promoted by gastric and duodenal reflux. *Molecular Carcinogenesis*. 2009;48(2):110-117.29)8. Port J, Yamaguchi K, Du B, De Lorenzo M, Chang M, M.Heerdt P et al. Tobacco smoke induces CYP1B1 in the aerodigestive tract. *Carcinogenesis*. 2004;25(11):2275-2281.
- 30) Wang Z, Tang L, Sun G, Tang Y, Xie Y, Wang S et al. Etiological study of esophageal squamous cell carcinoma in an endemic region: a population-based case control study in Huaian, China. *BMC Cancer*. 2006;6(1).
- 31) Shah I, Mehta P, Lone M, Dar N. Leu432Val Polymorphism of CYP1B1 is Not Associated with Squamous Cell Carcinoma of Esophagus - a Case-Control Study from Kashmir, India. *Asian Pacific Journal of Cancer Prevention*. 2015;16(13):5337-5341.
- 32) Li G1, Liu Z, Sturgis EM, Chamberlain RM, Spitz MR, Wei Q. CYP2E1 G1532C, NQO1 Pro187Ser, and CYP1B1 Val432Leu Polymorphisms Are Not Associated with Risk of Squamous Cell Carcinoma of the Head and Neck. *Cancer Epidemiology Biomarkers & Prevention*. 2005;14(4):1034-1036.
- 33) Ko Y, Abel J, Harth V, et al. Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res* 2001;61:4398 – 404
- 34) Kaur-Knudsen D, Nordestgaard B, Tybjaerg-Hansen A, Bojesen S. CYP1B1 genotype and risk of cardiovascular disease, pulmonary disease, and cancer in 50 000 individuals. *Pharmacogenetics and Genomics*. 2009;19(9):685-694.
- 35) Beuten J, Gelfond JA, Franke JL, Weldon KS, Crandall AC, Johnson-Pais TL, Thompson IM, Leach RJ. Single and multigenic analysis of the association between variants in 12 steroid hormone metabolism genes and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2009;18(6):1869–1880. [PubMed: 19505920]
- 36) Chang BL, Zheng SL, Isaacs SD, Turner A, Hawkins GA, Wiley KE, Bleecker ER, Walsh PC, Meyers DA, Isaacs WB, Xu J. Polymorphisms in the CYP1B1 gene are associated with increased risk of prostate cancer. *Br J Cancer*. 2003; 89(8):1524–1529. [PubMed: 14562027]
- 37) Cunningham JM, Hebring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN. Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2007; 16(5):969–978. [PubMed: 17507624]
- 38) Cussenot O, Azzouzi AR, Nicolaiew N, Fromont G, Mangin P, Cormier L, Fournier G, Valeri A, Larre S, Thibault F, Giordanella JP, Pouchard M, Zheng Y, Hamdy FC, Cox A, Cancel-Tassin G. Combination of polymorphisms from genes related to estrogen metabolism and risk of

prostate cancers: the hidden face of estrogens. *J Clin Oncol.* 2007; 25(24):3596–3602.
[PubMed:17704407]

Figure 1. Kaplan–Meier survival curves. (a) Overall survival (OS) and (b) Disease-free survival (DFS) based on different genotypes of CYP1B1 rs1056836 polymorphism. P-values were calculated with the log-rank test.