 doi:10.20944/preprints201710.0050.v2

Article

Impact of Genetic Polymorphism of Sulpha Transferase Genes (SULT1A) Genes on the Risk of Females with Breast Cancer in Jordan

Mona Bustami [1], Abdel-Ellah Al-Shudifat [2], Nagham Hussein [1], Mohannad Yacoub [3], Eiad Atwa [4], Israr Sabri [5], Rania Abu-Hamdah [5], Walid Abu Rayyan [1], Tawfiq Arafat [1], Adnan Badran [1] and Luay Abu-Qatouseh [1,*]

[1] Faculty of Pharmacy, University of Petra, Amman, 11914 Jordan; Mbustami@uop.edu.jo (M.B.); naghamhussein93@gmail.com (N.H.); waburayyan@uop.edu.jo (W.A.R.); tarafat@uop.edu.jo (T.A.), abadran@uop.edu.jo (A.B.)
[2] Faculty of Medicine, The Hashemite University, Zarqa, 13115 Jordan; ashudaifat@hu.edu.jo
[3] Medical and Diagnostic Laboratories, specialty Hospital, Amman, 11914 Jordan; m.yacoub@gmail.com
[4] Al FAIHA/ Life Technologies Business Unit, Middle East Operation, Amman, 11914 Jordan; Eiad_Atwa@yahoo.com
[5] Faculty of Pharmacy, Nursing and Health Professions), Birzeit University, Birzeit, 627 Palestine; isabri@birzeit.edu (I.S.); dean.health@birzeit.edu (R.A.-H.)
* Correspondence: labuqatouseh@uop.edu.jo; Tel.: +962-79-701-2082

Abstract: Sulfotransferases (SULTs) family plays a significant role in the biotransformation of a variety of xenobiotics and endogenous compounds by which carcinogenesis and mutagenicity of different malignancies are increasingly affected. Recent data identified various genetically polymorphic SULTs enzymes with significant variations in the enzyme activity. This study aimed to investigate the impact of SULT1A1 gene polymorphism and and its potential risk on females with breast cancer in Jordan using a PCR-RFLP and Sanger Sequencing methods. The analysis showed that 24.7% of the patients and 25.3% of the controls were homozygous for the SULT1A1*1 allele (SULT1A1*1/SULT1A1*1) compared to 8.8% and 5.7% homozygous for the SULT1A1*2 allele (SULT1A1*2/SULT1A1*2) for patients and controls respectively. Most of the patients and controls were heterozygous for SULT1A1*1 allele (SULT1A1*1/SULT1A1*2) with rates of 66.5% and 69.0% in patients and controls respectively. In addition, the frequencies of the mutant SULT1A1*2 allele were 0.42 and 0.4 in the patient and control groups respectively. No significant difference in genotype and allele distribution was noted between the breast cancer and control groups. The risk of breast cancer in individuals carrying the SULT1A1*2 allele was determined by combining the SULT1A1*1/SULT1A1*2 and SULT1A1*2/SULT1A1*2 genotypes. No association was observed between SULT1A1 polymorphism and breast cancer incidence (P = 0.63; OR, 0.93; 95% CI, 0.68–1.26). However, SULT1A1*2 allele was found to increase the risk of breast cancer by 1.26-fold.

Keywords: polymorphism; risk for breast cancer; SULT1A allele

1. Introduction

In human physiology, sulfotransferases (SULTs) plays a key role in the conjugation of sulfate groups to a variety of endogenous and exogenous substrates, including many drugs, neurotransmitters, thyroid and steroid hormones and carcinogenic agents [1-2]. SULTs are genetically polymorphic and are expressed in a wide variety of tissues, such as the liver, lung, brain, kidney, and platelets [3].There are four major families of SULTs: SULT1, SULT2, SULT4 and SULT6 with a 13 human cytosolic SULT isoforms have been identified [4]. The gene encodes the SULTs alloenzymes; *SULT1A* (*SULT1A1*1* wild-type, *SULT1A1*2*, *SULT1A1*3* and *SULT1A1*4*) were mapped to chromosome 16p12.1-p11.2 with significant biochemical variations among their activities.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 9 November 2018 doi:10.20944/preprints201710.0050.v2

This genetic polymorphism is remarkably important in case of a mutation in exon 7 at the nucleotide of 638 (codon 213) resulted in a substitution of histidine by arginine (*SULT1A1*2* allele) which is associated with less enzymatic activity and thermal stability compared with the wild-type allele (*SULT1A1*1* allele) [5-6].

Breast cancer constitutes the most frequent female malignancies worldwide, accounting for 1.7 million cases and 521,900 deaths in 2012 worldwide [7]. In the Middle East region, it has been shown that the incidence rate for breast cancer has increased progressively over the last decade especially in younger aged patients [8-9]. Epidemiological variation of breast cancer among different ethnic populations was reported suggesting that genetic and environmental factors influence the development of this type of malignancies [10-11]. The relationship between genetic polymorphisms of *SULT1A1* and several cancer types was investigated but results remain controversial [12-13]. This study investigated, for the first time, the relationship between the *SULT1A1* Arg213His polymorphism and female breast cancer susceptibility in Jordan.

2. Materials and Methods

2.1 Study population

During February 2015 to June 2017, a total of 340 females (182 patients and 158 controls) were enrolled in this study. The breast cancer patients were referred to the breast care unit, Prince Hamzah hospital (Amman). The patients were screened for breast cancer by mammograms and biochemical markers, and confirmed by histopathology. Next generation sequencing was performed to confirm the presence of *BRCA1*/*BRCA2* mutations by Specialty hospital laboratories (Amman). The control group consisted of females of ages above 20 years who are not presented with any clinical manifestation and who do not have a family history of breast cancer. Next generation sequencing was also used to confirm the wild type of *BRCA1*/*BRCA2* genes. Members of the study populations were informed with regards to the aims of the study. Epidemiological data were collected from the members using a designed standardized questionnaire. The study was approved by the Ethics Committee of Prince Hamzah hospital.

2.2 Sample processing and maintenance

Fresh blood samples were collected from each participant in EDTA tubes. A total of 0.3 ml of each sample was used while the remaining quantity was kept at -80^{o}C in case repeated testing is required. DNA extraction of the blood samples was performed using QIAamp Blood mini kit (Qiagen, Germany) according to the manufacturer's instructions. Whole blood was harvested and subsequently lysed by lysis solution. Treatment with proteinase K enzyme was performed for 30 minutes at 55^{o}C. Separation of DNA from proteins and cellular debris was performed using the spin columns and subsequently eluted in Elution buffer. The samples were stored at -80^{o}C until further analysis. Determination of DNA content was performed by measuring the optical density (OD) at 260 nm wavelength and the ratio between OD_{260} and OD_{280} using UV spectrophotometry (Biorad, Germany). The integrity of the extracted DNA was analyzed by 1% agarose gel electrophoresis stained by ethidium bromide [14].

2.3 Genotyping of *SULT1A1*

The presence of *SULTA1* gene in the DNA isolated samples was detected using conventional PCR method as described by Arslan *et al*. 2009 with some modifications [15]. Consensus oligonucleotide primers (1A1 forward: 5′-GTTGGCTCTGCAGGGTTTCTAGGA-3′ and 1A1 reverse 5′-CCCAAACCCCCTGCTGGCCAGCACCC-3′ were preliminary used to amplify *SULTA1* specific DNA of allele type. This was accomplished using oligonucleotide primers specific for the intron sequences flanking exon VII of the *SULT1A* (checked by Blastn). The amplified product was predicted to give a band of 333 bp in length, after analysis by gel electrophoresis, indicating positive results for

 doi:10.20944/preprints201710.0050.v2

SULTA1 gene. PCR reaction mixtures of 50µl were prepared by adding 2 µl DNA to 0.4 µM of each primer and 25 µl of 2× PCR Master Mix (New England Biolab, UK). Initial denaturation was performed at 95°C for 5 min, followed by 35 cycles of 94°C for 45 s, 55°C for 60 s, and 72°C for 90 s, with final extension at 72°C for 7 min.

PCR-RFLP assay to detect the G:A transition that results in the Arg:His replacement in *SULT1A1* was performed. And the incidence of SULT1A1 alleles was reported. PCR reaction products (10µl) were incubated with 1 unit of CutSmart *HaeII* (New England BioLab, UK) at 37°C for 20 min in a reaction mixture (total volume 50 µl) containing the appropriate enzyme reaction buffer supplied by the manufacturer. After digestion, heat inactivation of the enzyme was performed by incubating the reaction at 80°C for 20 min. Fragments were resolved on 3% (w/v) agarose gels containing ethidium bromide, and detected using UV transilluminator. The 333 bp fragment containing exon VII yielded two fragments of 168 and 165 bp after digestion with *HaeII*. Although these two fragments cannot be resolved on 3% (w/v) agarose gels, homozygotes for both alleles, (*SULT1A1*1*/*SULT1A1*1*, and *SULT1A1*2*/*SULT1A1*2*) and heterozygotes (*SULT1A1*1*/*SULT1A1*2*), can be readily identified by this method. Digestion does not take place with DNA fragments amplified from *SULT1A1* alleles harboring the CGC:CAC change at codon 213, because this alters the restriction site recognition sequence for *HaeII*. All samples were tested in triplicate for reproducibility. Confirmation of the presence of mutations was performed by Sanger sequencing according to the following protocol: 1 µL of the PCR products was mixed with 1 µM of the sequencing primer (forward primer) and 1 µL of the BigDye® Terminator (v 1.1/Sequencing Standard Kit), 3.5 µL 5× buffer, and 13.5 µL water in a total volume of 20 µL for 20 enzymatic primer extension/termination reaction cycles according to the instructions of the manufacturer (Applied Biosystems, USA). After dye-terminator cleanup with Dye Ex 2.0-Spin columns (Qiagen, Germany), the reaction mixture was loaded in an automated ABI 310 Genetic Analyzer for sequence analysis. Sequence alignments were performed against sequences stored in the GenBank database by on-line BLAST analysis. Controls for sample adequacy were included in the sequencing kit and were used for each run. Internal control for PCR polymerase inhibitors was used by amplification of human B globin gene.

2.4 Statistical analysis

Statistical Package for the Social Sciences (SPSS) release 20 (Chicago IL, USA) was used for the statistical analyses. Genotype and allele frequencies were tested by the Pearson's $\chi 2$ test. The statistical significance of the differences in *SULT1A1* among the cases and controls was determined by the $\chi 2$ test. Probability values <0.05 were regarded as statistically significant [16]. Odds ratios and 95% confidence intervals (CIs) for breast cancer were calculated to study the relationship between the allele type and the incidence of breast cancer in the studied population.

3. Results

Demographic characteristics of the patients and controls were summarized in Table 1. Mean ages of the patients and controls were 43.8±11.8 years (range, 26-55) and 36.6±8.4 years (range, 20-50), respectively. No significant relationship was found between the patients and controls in terms of smoking status (P=0.29) and history of hormone replacement therapy (p= 0.33).

 doi:10.20944/preprints201710.0050.v2

Table 1. Epidemiological data of the patients and controls

	Patients	Controls
Total number	182	158
Age		
Range	26-55	20-50
Mean ± SD	43.8±11.8	36.6±8.4
Smoking status n, (%)		
smoker	38 (20.9)	19 (12.0)
Non-smoker	144(79.1)	139 (88.0)
History of hormone replacement therapy n, (%)		
Users for more than one month	14(7.7)	8 (5.1)
Non-users	168(92.3)	150 (94.9)

SULT1A1 allele and genotype frequencies are indicated in Table 2. In the patient group, the frequencies of the homozygous wild-type genotypes (*SULT1A1*1*/*SULT1A1*1*), the heterozygous genotype (*SULT1A1*1*/*SULT1A1*2*) and the homozygous variant genotype (*SULT1A1*2*/*SULT1A1*2*) were 24.7, 66.5 and 8.8% respectively while in the control group, these frequencies were 25.3, 69.0 and 5.7% respectively.

Table 2. Frequencies and risk estimation of SULT1A1 genotypes in patients and controls

	Breast cancer (n=182)	Controls (n=158)	P value	χ^2
Genotype frequency, n (%)				
*SULT1A1*1*/*SULT1A1*1*	45 (24.7)	40 (25.3)	0.55	1.192
*SULT1A1*1*/*SULT1A1*2*	121 (66.5)	109 (69.0)		
*SULT1A1*2*/*SULT1A1*2*	16 (8.8)	9 (5.7)		
Allele frequency, n (%)				
*SULT1A1*1*	211 (58)	189 (59.8)	0.63	0.237
*SULT1A1*2*	153 (42)	127 (40.2)		

The risk of breast cancer in individuals carrying the *SULT1A1*2* allele was determined by combining the *SULT1A1*1*/*SULT1A1*2* and *SULT1A1*2*/*SULT1A1*2* genotypes. No statistically significant difference was observed between the patients and controls in comparison of the genotype combination (P=0.63; OR, 0.93; 95% CI, 0.68-1.26). Similar results were observed when the smoker and non-smoker, and history of hormone replacement therapy populations were compared for genotype combinations.

4. Discussion

Breast cancer is so far, the most frequent type of malignancies among women worldwide. The rates of breast cancer have been shown to be variable among ethnic groups [17]. Recent data showed that the incidence is increasing in the Middle East [18, 19]. *SULT1A* is considered as a phase II enzyme it also bioactivates various carcinogenic agents which are related to the development of different types of cancers [20]. Recent evidence suggests a potential role of the genetic polymorphisms in xenobiotic metabolizing enzymes in increasing the susceptibility of individuals to cancer [21, 22]. In this context, different genetic background in the *SULT1A1* would importantly affects the risk estimates associated with the development and prognosis of breast cancer. It is believed that the development of breast cancer depends not only on germ line mutations of *BRCA1*/*BRCA2* genes, but also on several factors including age, tobacco use, hormone therapy and ethnicity [23, 24].

The frequencies of the *SULT1A1*2* allele differ ranging from 5 to 32% among ethnic populations [4, 25]. In the present study, the frequency of this allele in the control group was 40.2% which is higher than the frequency in Turkish, Chinese, Taiwanese and Koreans while close to the frequency reported

 doi:10.20944/preprints201710.0050.v2

for Caucasians [4, 26]. In regards to the *SULT1A1* genotypes and alleles, no statistically significant difference between the patients and controls was observed in this study.

The presented study showed clearly that the polymorphism of *SULT1A1* gene did not have a significant relationship with female breast cancer although the presence of *SULT1A1**2 allele increased the risk in the patient' group. This is in agreement with a study conducted in Turkey in cases of prostate cancer which also suggested no role of age and smoking as factors in the allelic polymorphism of *SULT1A* gene [16]. In contrast, Palli *et al*., 2013 revealed that *BRACA2* male breast cancer is highly associated with SULT1A1 low enzymatic activity and accordingly to environmental exposure variations [27]. Roupreˆt *et al*., 2007 also found a significant association between the *SULT1A1**2 allele and risks for cancers of the urinary tract [28]. In addition, the risk of smoking and use of hormone replacement in the patients with breast cancer was not associated with the allele variation and the risk of cancer development in the present study. Since ethnicity cannot be excluded as a factor for these controversial results, other related factors including the exposure to geographical variations might be less involved in the development of breast cancer and more concern should be placed on the genetic variation of *BRCA1*/*BRCA2* in patients with breast cancer.

Although the results of our study supports the fact that the *SULT1A1* polymorphism does not play a role in breast cancer susceptibility, the current study is the first to report the allele frequencies of *SULT1A* gene in Jordan population with breast cancer. Hence, more work is needed for better understanding of the relationship between the *SULT1A1* polymorphism and breast cancer especially with regards to the environmental exposure to specific carcinogens in larger population studies.

Acknowledgments: This study was supported by the Deanship of scientific research (project number13/4/2018) – University of Petra-Jordan. The authors wish to thank Deanship of Academic Research and the Faculty of Pharmacy, University of Petra, Jordan for providing necessary facilities and funds for conducting this research. The authors also wish to thank Prince Hamzah Hospital and its staff for preparing the subjects for the study. In addition, the authors are thankful for Specialty Hospital and Al-Faiha Company for their technical support

Author Contributions: Mona Bustami, Tawfiq Arafat, and Luay Abu-Qatouseh conceived and designed the study. Israr Sabri, Eiad Atwa, and Mohannad Yacoub and Luay Abu-Qatouseh designed and performed the experiments. Abdel-Elah Shudaifat and Nagham Hussein followed up the patients and designed the questionnaire. Abdel-Elah Shudaifat was also responsible for sample collection. Mona Bustami, Walid Abu Rayyan, Rania Abu-Hamdah and Adnan Badran analyzed the data. Mona Bustami, Nagham Hussein and Luay Abu-Qatouseh wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. Falany, CN. Enzymology of human cytosolic sulfotransferases. *FASEB J* **1997**, *11*, 206-16.
2. Glatt, H. Sulfotransferases in the bioactivation of xenobiotics. *Chem Biol Interact* **2000,** *129*, 141–70.
3. Pereira, WO.; Paiva, AS.; Queiroz, JW.; Toma, L.; Dietrich, CP.; Nader, HB.; Jerônimo, SM. Genetic polymorphism in the sulfotransferase SULT1A1 gene in cancer. *Cancer Genet Cytogenet* **2005**, *60*,160:55.
4. Glatt, H.; Engelke, CE.; Pabel, U.; Teubner, W.; Jones, AL.; Coughtrie, MW.; Andrae, U.; Falany, CN.; Meinl, W. Sulfotransferases: genetics and role in toxicology. *Toxicol Lett*. **2000**, *11*, 341-48.
5. Raftogianis, RB.; Wood, TC.; Otterness, DM.; Van Loon, JA.; Weinshilboum, RM. Phenol sulfotransferase pharmacogenetics in human: association of common SULT1A1 alleles with TS PST phenotype. *Biochem Biophys Res Commun* **1997**, *239*, 298-304.
6. Nowell, S.; Ambrosone, CB.; Ozawa, S.; MacLeod, SL.; Mrackova, G.; Williams, S.; Plaxco, J.; Kadlubar, FF.; Lang, NP. Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfo-transferase phenotype in platelet cytosol. *Pharmacogenetics* **2000**, *10*, 789-97.
7. Torre, LA.; Bray, F.; Siegel, RL.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA Cancer J Clin* **2015**, *65*, 87–108.

8. Missaoui, N.; Jaidene, L.; Abdelkrim, SB.; Abdelkader, AB.; Beizig, N.; Yaacoub, LB.; Hmissa, S. Breast cancer in Tunisia: clinical and pathological findings. *APJCP* **2011**, *12*,169–72.

9. Laraqui, A.; Uhrhammer, N.; El Rhaffouli, H.; Sekhsokh, Y.; Lahlou-Amine, I.; Bajjou, T.; Amzazi, S. BRCA genetic screening in Middle Eastern and North African: mutational spectrum and founder BRCA1 mutation (c. 798_799delTT) in North African. *Dis Markers* 2015, 194293-301.

10. Imyanitov, EN.; Hanson, KP. Mechanisms of breast cancer. *Drug Discov Today Dis Mech* **2004**,*1*, 235–45.

11. Narod, SA.; Foulkes, WD**.** BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer* **2004,** *4*, 665–76.

12. Hung, R J.; Boffetta, P.; Brennan, P.; Malaveille, C.; Hautefeuille, A.; Donato, F.; Scotto di Carlo, A. GST, NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. *Int J Cancer* **2004**, *110*, 598 – 604.

13. Arslan, S.; Silig, Y.; Pinarbasi, H**.** Sulfotransferase 1A1 Arg213His polymorphism and prostate cancer risk. *Exp Ther Med* **2011,** *2*, 1159-62.

14. Pachouri, SS.; Sobti, RC.; Kaur, P.; Singh, J.; Gupta, SK**.** Impact of polymorphism in sulfotransferase gene on the risk of lung cancer. *Cancer Genet Cytogenet* **2006**, *171*, 39 – 43.

15. Qatouseh, LA.; Sabri, I.; Alkhatib, I.; Atwa, E.; Arafat, T. Detection of High-Risk Human Papillomavirus Genotypes 16 and 18 in Head and Neck Squamous Cell Carcinomas in Jordan. *APJCP* **2017**, *18*, 1337-41.

16. Arslan, S.; Silig, Y.; Pinarbasi, H**.** An investigation of the relationship between SULT1A1 Arg213His polymorphism and lung cancer susceptibility in a Turkish population. *Cell biochem and funct* **2009**, *27*, 211-5.

17. Tao, Z.; Shi, A.; Lu, C**.** Breast cancer: epidemiology and etiology. *Cell biochem and biophysics* **2015,** *72*, 333-8.

18. Azim, HA.; Ibrahim, AS**.** Breast cancer in Egypt, China and Chinese: statistics and beyond. *J Thorac Dis* **2014**, *6*, 864-6.

19. Saggu, S.; Rehman, H.; Abbas, ZK. Recent incidence and descriptive epidemiological survey of breast cancer in Saudi Arabia. *Saudi Med J* 2015, *36*, 1176.

20. James, MO.; Ambadapadi, S**.** Interactions of cytosolic sulfotransferases with xenobiotics. *Drug Metab Rev* **2013**, *45*, 401-14.

21. Umamaheswaran, G.; Kumar, DK.; Adithan, C. Distribution of genetic polymorphisms of genes encoding drug metabolizing enzymes & drug transporters-a review with Indian perspective. *IJMR* **2014**, *139*, 27.

22. Mota, P.; Silva, HC.; Soares, MJ**.** Genetic polymorphisms of phase I and phase II metabolic enzymes as modulators of lung cancer susceptibility. *J Cancer Res Clin Oncol* **2015,** *141*, 851-60.

23. Donenberg, T.; Ahmed, H.; Royer, RA. Survey of BRCA1, BRCA2, and PALB2 mutations in women with breast cancer in Trinidad and Tobago. *Breast Cancer Res Treat* **2016**, *159*, 131-8.

24. Lai, KN.; Ho, WK.; Kang, IN**.** Characterization of BRCA1 and BRCA2 variants in multi-ethnic Asian cohort from a Malaysian case-control study. *BMC cancer* **2017,** *17*, 149**.**

25. Coughtrie, MW.; Gilissen, RA.; Shek, B**.** Phenol sulphotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. *Biochem J* **1999,** *337*, 45-9.

26. Lee, SJ.; Kim, WY.; Jarrar, YB**.** Single Nucleotide Polymorphisms in SULT1A1 and SULT1A2 in a Korean Population. *Drug Metab Pharmacokinet* **2013**, *28*, 372-7.

27. Palli, D.; Rizzolo, P.; Zanna, I.; Silvestri, V.; Saieva, C.; Falchetti, M.; Russo**, A.** SULT1A1 gene deletion in BRCA2-associated male breast cancer: a link between genes and environmental exposures? *J Cell Mol Med* **2013**; *17*, 605–7.

28. Rouprêt, M.; Cancel-Tassin, G.; Comperat, E.; Fromont, G.; Sibony, M; Molinié, V.; Larré, S. Phenol sulfotransferase SULT1A1* 2 allele and enhanced risk of upper urinary tract urothelial cell carcinoma. *Cancer Epidemiol Biomarkers Prev* **2007,** *16*, 2500-3.