عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	العلاقة المحتملة بين التغيرات في تعدد أشكال الجينات المكونة لمسار نظام إصلاح استئصال النوكليوتيدات (NER) نتيجة تدخين السجائر
Title of the proposed project in English	Potential Association between variation of nucleotide excision repair (NER) pathway gene polymorphisms and cigarette smoking
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التخصص الدقيق للمشرف الرئيس - Specialty of PI	Molecular and cellular biology
المشرف المساعد - Co-PI	Dr Mohammad Alanazi
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	10
Abstract of the proposal (No more than 200 words)	Cigarette smoking accounts for at least 30% of all cancer deaths in developed countries (1,2). Many hypotheses have suggested evidence that cigarette smoking induces more genetic changes in genes involved in the development of many cigarette-related diseases (3,4,5). This alteration may be from single-nucleotide polymorphisms (SNPs), particularly the genes involved in DNA repair system. Defects in the DNA repair system, particularly in the nucleotide excision (NER) pathway and hence, NER activity is likely critical to the prevention of carcinogen-induced mutation that contributes to neoplasia associated with smoking. This pathway is known to be the versatile pathway involved on remove various DNA lesions including the lesions caused by chemicals carcinogens which make up the different compounds of the cigarette smoke (6). In mammals, more than 20 protein factors constitute this critical pathway, and is comprised of two sub-pathways: in the first, DNA damage recognition is accomplished by XPC, wich is stabilized by its binding partners RAD23B (7). In the second sub-pathway, DNA damage is recognized by the stalling of the RNA polymerase complex at the site of damage (8). Several studies have reported the molecular mechanisms of lung cancer and others cancers development caused by tabacco smoke (9-10). Many type of genetic alteration are found in different type of cancer are associated with smoking in lung cancer as well as , the mutation in KRAS and TP53 genes but still now any association between DNA repair gene variation arte associated with cigarette. The aim of this study was to investigate the polymorphisms in NER pathway associated with cigarette smoking in the Saudi population. Also, to determine the structural function along with the effect of these SNPs Blood samples were already collected from 192 smokers and 192 healthy controls (non-smokers). Genotype frequencies from seven single nucleotide polymorphisms (SNPs) (Three from

	XPC gene and four SNP's from XPA gene) will be determined using TaqMan® genotyping. Genetic variants of NER pathways can affect susceptibility to smoking-related diseases and Investigating DNA repair system polymorphisms is crucial for elucidating the mechanisms underlying tobacco-induced diseases and developing novel therapeutic approaches.
Hypothesis of the proposal	Many type of genetic alteration are found in different type of cancer are associated with smoking in lung cancer as well as , the mutation in KRAS and TP53 genes but still now any association between DNA repair gene variation arte associated with cigarette.
Specific objectives	The aim of this study is 1- To investigate the polymorphisms in key genes in the NER pathway that are associated with the repair of cigarette smoke-induced DNA damage in smokers versus nonsmokers subjects 2- To correlate the level 8-oxoguanine as an indicator of oxidative DNA stress, to the smoking status of the study subjects and to the polymorphism data generated from aim (1). 3- To determine the structural function relation of the effects of the selected SNPs Blood samples were already collected from 192 smokers and 192 healthy controls (non-smokers). Genotype frequencies from seven single nucleotide polymorphisms (SNPs) (Three from XPC gene and four SNP's from XPA gene) will be determined using TaqMan® genotyping. Genetic variants of NER pathways can affect susceptibility to smoking-related diseases and Investigating DNA repair system polymorphisms is crucial for elucidating the mechanisms underlying tobacco-induced diseases and developing novel therapeutic approaches.
	1- Ethics statement and blood collection Written ethical consent for this study was already reviewed and obtained by the Research Ethics Committee of the College of Applied Medical Sciences at King Saud University (KSU), in Riyadh, Saudi Arabia (Approval Number: CAMS 13/3536). A total of 384 Participants (192smokers and 192 non-smokers). Each participant provided informed consent and completed a written survey. Data included in the survey comprised age, number of cigarettes smoked per day, years of smoking, and body mass index (BMI). were obtained through a self-completed questionnaire. 2- DNA extraction DNA from the blood samples will be extracted by Qiagen Genomic DNA Mini Kit, as per the manufacturer's instructions. The DNA concentration will be quantitated using a NanoDrop 8000 (Thermo Fisher Scientific, Waltham, MA, USA). The purity of each DNA sample will be then determined by calculating the ratio of A260/A280 nm and A260/A230 nm. 3- Elisa: The levels of 8-OHdG (ng/ml) were measured using Quantikine sandwich enzyme linked immunosorbent assays (ELISA; Cell Biolabs Inc, San Diego, CA, USA). The assays were conducted according to the manufacturer's guidelines. The kit has an 8-OHdG detection sensitivity range of 100 pg/mL to 20

Methodology & Major Techniques to be used	ng/mL. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4951340/ 4- TaqMan genotyping assay Genomic DNA of each blood sample will be prepared before genotyping. Four SNPs in the XPA gene named rs104894131 , rs104894132 , rs104894133 and rs 104894134 and three SNPs in the XPC gene called rs74737358 , rs121965088 and rs767569346 will be evaluated by genotyping assay. 5- In silico analysis will be used with collaboration with Dr Nouf S. Al-Numair from King Faisal Specialist Hospital and Research Center to determine the structural function along with the effect of these SNPs. For 3D structure, a homology model will be built up using SWISS-MODEL template library (SMTL version 2017-10-23, PDB release 2017-10-13). 6- Data analysis As described in our previous work [41,42], the deviation of the computed genotypic and allelic frequencies of each SNP will be checked using the Hardy–Weinberg equilibrium; genetic comparisons will be performed with the aid of the $\chi 2$ test and allelic odds ratios (ORs). In addition, by using Fisher's exact test (two-tailed), 95% confidence intervals (CIs) will be measured. The Statistical Package for the Social Sciences (SPSS) version 16.0 statistical software (SPSS, Chicago, USA) will be used to perform statistical analysis. P values of less than 0.05 will be considered as evidence of statistical significance. Homology modeling of the 3D structure of the human NER pathway genes will be performed on the SWISS-MODEL server.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
Recent References	1. Organization WH. WHO global report: mortality attributable to tobacco.2012. 2. Organization WH. Global status report on non-communicable diseases 2010; Description of the global burden of NCDs, their risk factors and determinants2011. 3. Inc ACS. Cancer Facts & Figures 2014. Atlanta: 2014. 4. Menotti A, Puddu PE, Maiani G, Catasta G. Lifestyle behaviour and lifetime incidence of heart diseases. Int J Cardiol. 2015;201:293-9. Epub 2015/08/25. 5. Gutierrez A, Suh R, Abtin F, Genshaft S, Brown K. Lung cancer screening. Semin Intervent Radiol. 2013;30(2):114-20. Epub 2014/01/18. 6. Scharer OD, Nucleotide excision repair in eukarotes. Gold Spring Harbor perspectives in biology.2013 7. Sugasawa K, Ng JM. Masutani C et al. Xeroderma

pigmentosum group C protein complex is the inhibitor of Global genome nucleotide excision repair. Molecular cell . 1998
8. Vermeulen W, Fousteri M. Mammalian transcription- coupled excision repair. Vold Spring Harbor perspectives in biology. 2013
9. Coggins CR. A futher review of inhalation studies with cigarette smoke and lung cancer in experimental animals, including transgenic mice. Inha Toxicolo.2010
10. Hecht SS. Carcinogenicity studiesa of inhaled cigarette smoke in laboratory animals: olp and new. Carcinogenisis.2005