عنوان المشروع باللغة العربية	العلاقة بين مورثات إصلاح المادة الوراثية (OGG1 و APE1) و الولادة المبكرة في عينة من النساء السعوديات
عنوان المشروع باللغة الإنجليزية	Association of DNA repair genes OGG1 and APE1 with preterm birth risk in a sample of Saudi women
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التخصص الدقيق للمشرف الرئيس	Molecular Biology
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	12 months
Abstract or synopsis of the proposal (200 words or less):	Pre-term birth (PTB) remains the leading cause of infant mortality and morbidity. Its etiology is multifactorial, with a strong genetic component. Preterm birth (PTB) incidence estimated between 5–12%, depending on the geographic regions. Spontaneous preterm births (PTB) are documen ted to be associated with oxidative stress (OS), and imbalances in the redox system have been reported in the maternal–fetal intrauterine compartments. Oxidative stress has been reported to cause DNA damage which is playing a major role in preterm birth pathology. Damaged base residues in DNA can be removed by one of two separate excision repair processes. Lesions generated endogenously by hydrolysis or exposure to active oxygen are corrected by base excision repair (BER), with release of the altered base in free form by a DNA glycosylase and formation of an abasic site (apurinic/apyrimidinic site, AP site) as a key intermediate. Defects in key activities of the BER process, such as AP endonuclease, OGG1, DNA polymerase β, or the XRCC1-DNA ligase III heterodimer, lead to embryonic lethal phenotypes, indicating that repair of endogenous DNA lesions is essential during development. In the present study we are intended to evaluate the association of BER pathway genes (OGG1 a nd APE1) association with preterm birth risk.
Hypothesis or scientific justification of the proposal	The majority of preterm deliveries are idiopathic preterm births and preterm births due to preterm premature rupture of fetal membranes. In both cases, much of the etiology is unknown. While many environmental contributors to preterm birth such as stress, smoking, and inflammation, are identified, a large body of research suggests that genetic predisposition plays an important role. Recent studies revealed

	that premature aging of the placenta due to oxidative stress is the cause of many preterm births. Oxidative stress has been reported to cause DNA damage in preterm birth cases. DNA damage is a regular event during growth, and it is precisely fixed or repaired by multitudes of DNA repair mechanisms. Failure to resolve these lesions through one or mo re DNA-repair processes is associated with genome instability, emphasizing the importance of proteins involved in the repair of oxidative DNA damage. Maintaining the chemical integrity of DNA in the face of assault by oxidizing agents is a constant challenge for living organisms. Base-excision repair has an important role in preventing mutations associated with a common product of oxidative damage to DNA, 8-oxoguanine. Recent structural studies have shown that 8-oxoguanine DNA glycosylases use an intricate series of steps to locate and excise 8-oxoguanine lesions efficiently against a high background of undamaged bases. In the present study for the first time we are evaluating the role of DNA base excision repair genes (OGG1 and APE1) and their association with preterm birth risk using genotypic assays and gene expression studies.
Specific objectives	1. Evaluation of OGG1 and APE1 SNP's association with preterm birth risk in Saudi population. 2. Evaluation of expression levels of BER pathway genes OGG1 and APE1 in normal versus preterm birth samples using Q-RT PCR.
Methodology & Major Techniques to be used	**Patient samples:** Preterm birth and normal samples will be obtained from the collaborators and clinicians as per the guidelines of IRB. Fifty mothers and preterm infants will be enrolled for this study. Fifty blood specimens will be collected from mothers of full-term neonates and 50 samples from mothers of preterm neonates in EDTA tubes (for DNA extraction). The plasma will be stored frozen till required for analysis. Placentas will be collected from 10 mothers with PTB and 10 from the mother of the full-term delivery at King Khalid University Hospital, Riyadh and will be placed in RNAlater solution until ready for analysis of gene expression studies. **Nucleic acid Isolation:** High–molecular-weight DNA/RNA will be obtained from freshly collected placenta samples and blood samples (DNA) using Qiagen DNA/RNA mini prep kit and Qiagen nucleic acid extraction kits according to the manufacturer's protocol. **Genotyping:** Genotyping of SNPs in OGG1, APE1 will be performed using

	TaqMan genotyping assays. The genotype data will be scored for further analysis. Deviation of the genotype frequencies of each SNP in the control subjects from those expected under the Hardy- Weinberg equilibrium (HWE) will be assessed by $\chi 2$ test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (95% CI). **Gene Expression:** Isolated RNA concentration and quality will be analyzed using Agilent 2100 Bio-analyzer (Agilent Technologies, Palo Alto, CA). cDNA from RNA will be synthesized using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. The resulting cDNA will be then subjected to realtime quantitative PCR for evaluation of the relative mRNA levels of OGG1, APE1 and GAPDH. Gene-specific amplification will be performed using an ABI 7500 fast real-time PCR system (Life Technologies, Carlsbad, California, USA). Data will be analyzed using the comparative threshold cycle (2-ΔCT) method.
Availability of Samples	YES
Availability of Chemicals	YES
Availability of Instruments	YES
Availability of Ethical Approval (if needed)	YES
Recent References	1. Rani Vibha and Umesh Chand Singh Yadav, eds. Free Radicals in Human Health and Disease. Springer, 201 4. 2. Ramkumar Menon, Istvan Boldogh, Hal K. Hawkins, Michael Woodson, Jossimara Polettini, Tariq Ali Syed, Stephen J. Fortunato, George R. Saade, John Papaconstantinou, Robert N. Taylor. Histological Evidence of Oxidative Stress and Premature Senescence in Preterm Premature Rupture of the Human Fetal Membranes Recapitulated in Vitro. The American Journal of Pathology, 2014 3. Dolan SM: Genetic and environmental contributions to racial disparities in preterm birth. Mt Sinai J Med 2010, 77(2):160-165. 4. Menon, R. (2014). Oxidative stress damage as a detrimental factor in preterm birth pathology. Frontiers in immunology, 5.