Brief Communication

Screening for *hOGG1* S326C variant in normal Saudi population

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The *hOGG1* (MIM 601982) gene is the human homologue of *Escherichia coli* (*E.coli*), DNA repair protein MutM, which excises 8-oxo-7,8-dihydro 2'deoxyguanosine (8-oxoG) from oxidatively damaged DNA. It is a DNA glycosylase-apurinic (AP) lyase and a member of helix-hairpin-helix (HhH-GDP)-superfamily that nicks the DNA and releases 8-oxoG. It removes 8-oxoG through its glycosylase activity and cleaves the DNA sugar backbone through its lyase activity. The *hOGG1* gene is located on 3p26.2 and comprises 7 coding exons and an 8th alternatively spliced exon.[1] The *hOGG1* gene has been studied in a number of cancer and normal subjects; several genetic variants have been identified in different populations. The most common polymorphism of *hOGG1* is S326C (C>G substitution at position 977 in exon 7). This polymorphism has a different allele frequency among different ethnic groups and may play a role in various types of cancer.[2,3]

An association between the S326C polymorphism in *hOGG1* and increased risk of lung cancer has been reported. Homozygotes for the 326C allele exhibited an increased risk of developing squamous cell carcinoma and non-adenocarcinoma of the lungs compared to heterozygotes for S326C and homozygotes for 326S combined. Furthermore, population-based studies on the association of S326C with lung cancer revealed that the 326C allele confers a 2-fold increased risk of lung cancer.[2] Candidate genetic markers that are indicators for cancer susceptibility may vary in frequency among different ethnic groups. That is why it is important to study the prevalence of these markers in different populations to determine the significance of these markers in increasing cancer susceptibility in different ethnic groups. Polymorphisms of the *hOGG1* gene in Saudi individuals have not been studied so far. Assay for these DNA changes in the normal population is an important step towards the documentation of the prevalence of different DNA changes, which can be used as a reference for further studies. The aim of this study was to determine the frequency of S326C in a representative sample of the normal Saudi population. One hundred and fifty blood samples from Saudi individuals were randomly collected from Prince Salman Hospital, Riyadh, Saudi Arabia (consent forms were obtained). The DNA was extracted from whole blood using the QIAamp Blood Maxi Kit (Qiagen) according to the manufacturer instructions. Exon 7 of *hOGG1* gene was amplified with the following primers; forward 5' ACTGTCACTAGTCTCACCAG 3' and the reverse 5' TGAATTCGGAAGGTGCTTGGGGAAT 3' to yield a 207 bp product.[3] Polymerase chain reaction (PCR), was performed in a 25 µl reaction using ready-to-go PCR beads (puReTaq, Amersham Biosciences) and 50 ng genomic DNA. Cycling parameters were: 94°C for 3 minutes, 40 cycles of (94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds) followed by a final elongation step at 72°C for 1 minute. The PCR products were assayed for S326C polymorphism using *Ita* I restriction enzyme; 10 µl of PCR product was mixed with *Ita* I (10 U) and incubated at 37°C for 3 hours. The digestion products were analyzed by electrophoresis using pre-cast polyacrylamide gels. Bands were visualized by silver staining. The enzyme cuts the mutant allele (G allele) to yield a 100 bp and 107 bp products, while the wild type allele (C allele) remains uncut.

In this study, the frequency of the wild type mutant allele was approximately 0.74:0.26. The results were almost identical to the reported frequencies in the single nucleotide polymorphism (SNP) database of a multiracial group of 657 individuals (frequencies of 0.724:0.276; National Center for Biotechnology Information (NCBI), rs1052133) *p*-value of 0.5 (χ^2 value of 0.454 and 95% CI: 0.671-1.209). Moreover, the allele frequencies of the different genotypes in this study (**Table 1**) were almost identical with those reported in the NCBI database (reported values were 54.3%, 36.3% and 9.6%). In comparison with 5 different ethnic groups, the Saudi group (present study) showed similarities with Caucasians (105 individuals, *p*-value of 0.45; χ^2 value of 0.56 and a 95% CI: 0.79 – 1.82)[4] and Hispanic population (23 individuals, *p*-value of 0.464 using Fisher's exact test, CI: 0.65-3.08, NCBI rs1052133), but not with the Japanese population (197 individuals, *p*-value of 0.001; χ^2 value of 16.1 and a 95% CI: 0.359-0.696) and the Chinese

Table 1 - Genotype for the S326C polymorphism in the Saudi population.

Number of chromosomes screened	Genotype Frequency (%)	
	Genotype	Frequency (%)
300	S326S	(54.79)
	S326C	(39.73)
	C326C	(5.48)

population (98 individuals, *p*-value of 0.001; χ^2 value of 58.6 and a 95% CI: 0.678-1.2).[2] The Japanese and Chinese populations had a higher prevalence of the 326C mutant allele than any other population. The Saudi population also showed a marked difference with African American ethnic group (24 individuals, *p*-value of 0.0262 using Fisher's exact test, CI 1.135-7.85, NCBI, rs1052133). The prevalence of the 326C allele in the African American sample was very low and the 326C/326C allele frequency was 0%, a variation of what was reported in other populations, but the small number of samples assayed might contribute to the lack of identifying the mutant homozygous allele.

The Kingdom of Saudi Arabia is a vast and an ethnically diverse country. Although, there are pockets of ethnically homogenous populations where there is very little population drift, inter-population differences have been reported for several other genetic loci including sickle cell gene and ß-thalassemias.[5] This study showed a significant difference in the genotype allele frequency of *hOGG1* S326C in Saudis compared to Chinese and Japanese populations. Further studies are required to genotype the *hOGG1* S326C in different regions of Saudi Arabia to document any regional variations and the association of this polymorphism and increased risk of different types of cancer in Saudi Arabia remain to be investigated.

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Is Saudi Arabia a fertile land for exchanging infectious diseases?

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Over 2 million people from around the world visit Saudi Arabia every year to perform the Muslim Pilgrimage, Hajj. All of these people congregate at the 2 Holy Mosques in the cities of Mecca and Madina, remaining in crowded environments for up to 3 weeks. A further 2 million people visit the Kingdom to perform Omra in the 2 Holy Mosques. In addition, the country is the home of more than 6 million working expatriates. Most of these visitors, pilgrims and expatriate laborers come from impoverished third world countries where tuberculosis (TB) is endemic.[1] This huge number of expatriates and pilgrims associated with Hajj means that there is a lot of contact between people; more than enough to transfer, spread and exchange communicable diseases. In the past, several outbreaks of meningitis and cholera have occurred, demonstrating transmission of infectious diseases via human-to-human contact.[2] During these visits, a number of people suffer from minor upper respiratory tract infections but there is little consideration for the involvement of serious contagious diseases.[3] One isolated report highlighted the fact that *Mycobacterium Tuberculosis*, a re-emerging communicable disease, was responsible for cases of pneumonia during Hajj.[4] This report was strengthened by Wilder-Smith et al,[5] when they measured the immune response to TB antigen prior to departure and 3 months after return from Hajj pilgrimage. At the end of Hajj, Pilgrims return to their home countries taking with them any contagious disease they may have acquired. These observations suggest that Saudi Arabia is a fertile environment for the spread and exchange of several indigenous and imported diseases. Previous observations have been reinforced by recent results during an on-going nationwide epidemiological study. For the last 2 years, we have been able to focus our research efforts on finger-printing *M. Tuberculosis* in Saudi Arabia. More than 1,400 isolates have been collected and typing is in the final stage. Preliminary data shows many imported clades in Saudi Arabia (previously identified in other parts of the world) such as Beijing, Manila, Latin-America-Mediterranean, Delhi and many others. The presence of several of these families in one country is a strong evidence that the crowdness of Hajj and Omra is facilitating the exchange of communicable diseases. There is a paucity of information regarding other diseases but it is unlikely that TB is unique. Further data is required in order to study other communicable