# Polymorphism of p53 gene in Jordanian population and possible associations with breast cancer and lung adenocarcinoma

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## ABSTRACT

**Objective:** To determine the prevalence of 3 polymorphisms in p53 gene in 3 healthy Jordanian groups and 2 cancer patient groups.

**Methods:** Genomic DNA was extracted from blood samples obtained from 84 cancer patients (breast and lung adenocarcinoma) and 136 healthy subjects (representing Jordanian general population, Bedouins and Charkas). Samples were collected from Al-Amal Hospital for Cancer, Amman and from health centers located in different regions of Jordan from March 2002 to October 2002. Polymerase chain reaction (PCR) was used to amplify intron 3, exon 4 and intron 6 and PCR products were analyzed using gel electrophoresis and *Bst*UI and *Msp*I analysis. Allele frequencies (A1) were estimated for the 3 polymorphisms and Chi-square ($\chi^2$) test was used to determine the significance of differences from the Hardy-Weinberg equilibrium.

**Results:** Differences in allele frequencies for all 3 polymorphisms were observed among the various groups. Analysis based on haplotype frequencies showed that *Msp*I A2 allele linked to *Bst*UI allele was associated with lung adenocarcinoma, whereas the loss of the 16-bp duplication allele in combination with *Msp*I A2 allele was associated with breast cancer. In the cancer patients, the most frequent extended haplotype was the absence of the 16-bp duplication in combination with the presence of the *Bst*UI A2 and *Msp*I restriction sites.

**Conclusion:** No significant difference was found with respect to the *Bst*UI polymorphism between cancer patients and healthy groups. However, a significant difference was found with respect to the *Msp*I polymorphism between lung adenocarcinoma patients and healthy Jordanian general population. Charkas have a higher cancer risk than Jordanian general population based on the (16bp A1-*Msp*I A2) for breast cancer and (*Msp*I A2-*Bst*UI A2) for lung adenocarcinoma.

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The p53 protein is a nuclear phosphoprotein consisting of 393 amino acids. p53 normally functions as a guardian of the genome, responding to genotoxic damage with a G1 block leading to either DNA repair or apoptosis, thus eliminating potentially carcinogenic cells.[1-3] The tumor suppressor gene p53 is one of the most frequently mutated genes in cancer of all types.[4] The majority of mutations are missense mutations which damage the DNA-binding properties and transactivation function of p53 protein.[5] Germ line mutations may also play a role in carcinogenesis, such as in the Li-Fraumeni syndrome, where there is a familial concentration of different types of tumors at a comparatively early age.[6] Breast cancer is the most common type of cancer among women in developed[7,8] and developing countries.[9] It has been suggested that 82% of breast cancer is sporadic, 5%

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autosomal dominant and 13% polygenically inherited.[10] Mutations in the p53 gene have been proposed to occur in 16-46% of breast cancers and appear to be a strong indicator of poor prognosis, independent of other risk factors.[11] Genetic polymorphisms in the p53 gene that are involved in tumorigenesis, may determine individual susceptibility to cancer such as breast, colorectal, lung, and nasopharyngeal cancers.[8,12,13] These include a 16-bp duplication polymorphism in intron 3, and *Bst*UI and *Msp*I RFLPs in exon 4 (codon 72) and intron 6.[12] For the *Bst*UI polymorphism, the presence of the restriction site at codon 72 (CG/CG) that code for arginine results in 2 fragments, the *Bst*UI A2 allele, where the substitution for a proline (CC/CG) leads to a single large fragment.[14] The *Bst*UI (Arg) allele in the absence of the 16-bp duplication has been associated with an increased risk for lung cancer.[15] Conversely, individuals homozygous for proline at codon 72 were found to be more susceptible to lung adenocarcinoma.[16] Although in breast cancer patients, the presence of 16-bp duplication linked to the absence of *Msp*I restriction site showed increased risk to breast cancer susceptibility.[17]

A study of the codon 72 polymorphism in American lung cancer patients found significant differences between African Americans and Caucasians with respect to the frequencies of the *Bst*UI pro allele, where (pro) allele showed to be higher in African–Americans as compared to Caucasians.[18] Subsequently, significant differences in the allele frequencies and haplotypes for all 3 p53 polymorphisms were found in Europe, African, and certain Asian populations.[3] Ethnic and geographic variations in the incidence of cancer have been conventionally described to differences in socio-economic and environmental factors.[19] Carcinogenesis is a multistep process in which heredity and environment both play major roles.[20,21] In the presence of susceptible genotypes, exogenous environmental factors enhance the risk by interacting with "markers of inherited susceptibility".[22] Lung cancer is the leading cause of cancer death in Jordan and a low male-female ratio of lung cancer mortality is notably observed. Studies conducted in Jordan have shown that cigarette smoking is the principle risk factor for p53 molecular abnormalities and consequently to lung cancer prevalence.[23]

In the present study, the 3-biallelic p53 polymorphisms were studied in breast cancer patients, lung adenocarcinoma patients, 2 Jordanian ethnic groups (Bedouins and Charkas) and the Jordanian general population. The objectives of the study are to determine inherited cancer susceptibility due to p53 polymorphisms in the general population and Jordanian ethnic groups and to determine the frequency of the p53 polymorphisms and haplotypes in the Jordanian general population, 2 ethnic groups and 2 cancer patient groups.

**Methods.** ***Collection of samples.*** Blood samples were collected from 43 histopathologically confirmed breast cancer women and 41 lung adenocarcinoma patients (28 females and 13 males). Samples were collected from "Al-Amal Hospital for Cancer" in Amman in the period between March 2002 to October 2002. Moreover, blood samples were collected from 3 Jordanian groups: The Jordanian general population (47 subjects representing the general population, and 2 Jordanian ethnic groups: Bedouins (45), and Charkas (44). Samples were randomly collected from individuals who volunteered in different places and health centers located in different regions of Jordan. Prior to sample collection, written consent was obtained from patients and normal control individuals. The internal review committee on research using human subjects cleared the project after due deliberation.

***DNA extraction and polymerase chain reaction analysis.*** Genomic DNA from cancer samples was extracted from whole blood using the "Wizard Genomic DNA Purification" Kit (Promega, United States of America) according to the manufacturer instructions. A modified salting-out procedure[24] was used to extract genomic DNA from Jordanian general population and ethnic groups. The primer sequences and conditions used for the polymerase chain reaction (PCR) amplification of intron 3 and 6 and exon 4 of the p53 gene have been described previously.[7,12,14] Each PCR reaction was carried out in a in a total volume of 15µl, containing 25ng of genomic DNA, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 100 µM dNTPs, 4 pmol of each primer and 0.5 U *Taq* polymerase. The amplification fragments containing the 16-bp duplication polymorphism (intron 3) were separated on 4% LMP agarose gel for 3-hours at 90 V and stained with ethidium bromide. For the *Msp*I polymorphism, 5µl of the p53 intron 6 PCR products were digested in a total volume of 20 µl containing, 5 U *Msp*I (recognition site C-CGG), 2µl 10X restriction enzyme buffer, 0.2 µl BSA, at 37°C for 16 hours. *Bst*UI digestion was carried out in a total volume of 20µl including 5µl of p53 exon 4 PCR products, 10 U *Bst*UI (recognition site CG-CG), 2µl 10X restriction enzyme buffer at 60°C for 4 hours. The digested PCR-products were separated on 4% LMP agarose gel for 1.5 h at 90V and stained with ethidium bromide.

***Statistical analysis.*** Allele frequency (A1) was estimated for the 3 polymorphisms from each of the 5 studied Jordanian populations and the Chi-square ($\chi 2$) test was used to determine the significance of differences from the Hardy-Weinberg equilibrium.

**Table 1 -** Genotype distribution and $A_1$ allele frequencies of 3 p53 polymorphisms in Jordanian ethnic groups and cancer patients.

| Population | Polymorphism | p53 genotype N | 1-1 | 1-2 | 2-2 | A1 allele | $\chi^2$ HW |
|---|---|---|---|---|---|---|---|
| JGP | 16bp | 47 | 30 | 14 | 3 | 0.787 | 0.59 |
| BdP | | 45 | 28 | 13 | 4 | 0.767 | 1.687 |
| CkP | | 44 | 36 | 6 | 2 | 0.886 | 4.589* |
| BrCP | | 43 | 29 | 12 | 2 | 0.814 | 0.249 |
| LaCP | | 41 | 32 | 8 | 1 | 0.878 | 0.305 |
| JGP | *Msp*I | 47 | 2 | 11 | 34 | 0.159 | 0.779 |
| BdP | | 45 | 7 | 13 | 25 | 0.300 | 4.385* |
| CkP | | 44 | 4 | 7 | 33 | 0.171 | 8.447** |
| BrCP | | 43 | 2 | 7 | 34 | 0.128 | 3.239 |
| LaCP | | 41 | 2 | 5 | 34 | 0.109 | 3.417 |
| JGP | *Bst*UI | 47 | 5 | 26 | 16 | 0.383 | 1.371 |
| BdP | | 45 | 18 | 13 | 14 | 0.544 | 7.836** |
| CkP | | 44 | 6 | 12 | 26 | 0.273 | 4.239* |
| BrCP | | 43 | 8 | 19 | 16 | 0.407 | 0.303 |
| LaCP | | 41 | 6 | 15 | 20 | 0.329 | 1.224 |

JGP - Jordanian general population, BdP - bedouins population,
CkP - charkas population, BrCP - breast cancer patients,
LaCP - lung adenocarcinoma patients, HW - Hardy-Weinberg equilibrium
*$p$<0.05 when $\chi^2$>3.841
**$p$<0.01 when $\chi^2$>6.635

**Table 2 -** Significance ($\chi^2$ values) of p53 allele differences between different Jordanian ethnic groups and cancer patients.

| Polymorphism Population | 16bp | *Msp*I | *Bst*UI |
|---|---|---|---|
| JGP-BdP | $0.115^{NS}$ | $2.69^{NS}$ | $2.163^{NS}$ |
| JGP-CkP | $0.089^{NS}$ | 6.244* | $2.775^{NS}$ |
| BdP-CkP | $0.15^{NS}$ | 7.842* | 3.899* |
| JGP-BrCP | $0.627^{NS}$ | $2.93^{NS}$ | $0.036^{NS}$ |
| JGP-LaCP | $1.067^{NS}$ | 5.25* | $0.032^{NS}$ |

JGP - Jordanians general population, BdP - bedouins population
CkP - charkas population, BrCP - breast cancer patients
LaCP - lung adenocarcinoma patients
*p<0.05
NS - not significant at 5% level

**Table 3 -** Estimated pairwise haplotype frequencies and percent of linkage disequilibrium between p53 polymorphisms in Jordanian ethnic groups and cancer patients.

| Population | Haplotype | Estimated pairwise frequencies 1-1 | 1-2 | 2-1 | 2-2 | N of alleles | D | $D_{rel}$ |
|---|---|---|---|---|---|---|---|---|
| JGP | | 0.0585 | 0.7287 | 0.1011 | 0.1117 | 94 | -0.07 | -1.97* |
| BdP | | 0.1389 | 0.6278 | 0.1611 | 0.0722 | 90 | -0.09 | -1.30* |
| CkP | 16bp-*Msp*I | 0.0909 | 0.7345 | 0.0194 | 0.0341 | 88 | -0.06 | -3.10* |
| BrCP | | 0.0413 | 0.7727 | 0.0864 | 0.0993 | 86 | -0.06 | -2.64* |
| LaCP | | 0.0422 | 0.8359 | 0.0676 | 0.0543 | 82 | -0.05 | -4.06* |
| JGP | | 0.2414 | 0.5458 | 0.2018 | 0.0712 | 94 | -0.06 | -0.74* |
| BdP | | 0.3223 | 0.4445 | 0.2222 | 0.0111 | 90 | -0.09 | -0.75* |
| CkP | 16bp-*Bst*UI | 0.1875 | 0.6989 | 0.0852 | 0.0282 | 88 | -0.05 | -1.75* |
| BrCP | | 0.2558 | 0.5582 | 0.1512 | 0.0348 | 86 | -0.08 | -0.99* |
| LaCP | | 0.2257 | 0.6524 | 0.1036 | 0.0183 | 82 | -0.06 | -1.58* |
| JGP | | 0.1011 | 0.0585 | 0.2819 | 0.5584 | 94 | 0.04 | 0.41** |
| BdP | | 0.2882 | 0.0118 | 0.2558 | 0.444 | 90 | 0.13 | 0.91* |
| CkP | *Msp*I-BstUI | 0.1427 | 0.0283 | 0.1303 | 0.6987 | 88 | 0.09 | 0.77* |
| BrCP | | 0.0931 | 0.0349 | 0.3139 | 0.5581 | 86 | 0.04 | 0.54** |
| LaCP | | 0.0915 | 0.0183 | 0.2378 | 0.6524 | 82 | 0.06 | 0.75* |

JGP -Jordanian general population, BdP - bedouins population,
CkP - charkas population, BrCP - breast cancer patients,
LaCP - lung adenocarcinoma patients, HW - Hardy-Weinberg equilibrium
$D_{rel}=D/D_{max}$ - significance of the maximal linkage disequilibrium
*$p$>0.01 when $\chi^2$>3.841
**$p$<0.05 when $\chi^2$>6.635

**Table 4 -** Significance ($X^2$ values) of differences in pairwise haplotype distribution between Jordanian ethnic groups and cancer patients.

| Haplotype Population | 16bp-*Msp*I | 16bp-*BstU*I | *Msp*I-*BstU*I |
|---|---|---|---|
| JGP-BdP | $6.589^{NS}$ | $3.364^{NS}$ | 11.202* |
| JGP-CkP | $5.468^{NS}$ | $2.492^{NS}$ | 19.635* |
| BdP-CkP | $5.387^{NS}$ | $3.023^{NS}$ | 25.856* |
| JGP-BrCP | 6.662* | $2.895^{NS}$ | $5.257^{NS}$ |
| JGP-LaCP | $5.744^{NS}$ | $2.552^{NS}$ | 9.558* |

JGP - Jordanians general population, BdP - bedouins population
CkP - charkas population, BrCP - breast cancer patients
LaCP - lung adenocarcinoma patients
*p<0.01
NS - non significant at 1% level

**Table 5 -** Estimated frequencies (%) of p53 extended haplotypes (16bp duplication-*Msp*I RFLP-*Bst*UI RFLP) in Jordanian ethnic groups and cancer patients.

| Population | N of alleles | 1-1-1 | 1-1-2 | 1-2-1 | 2-1-1 | 1-2-2 | 2-1-2 | 2-2-1 | 2-2-2 |
|---|---|---|---|---|---|---|---|---|---|
| JGP | 94 | 2.925 | 2.925 | 20.500 | 7.225** | 52.350 | 2.925 | 7.700 | 3.450* |
| BdP | 90 | 13.350 | 0.550** | 18.850 | 15.550** | 43.850** | 0.550 | 6.650 | 0.550* |
| CkP | 88 | 7.750 | 1.425** | 11.100 | 6.600** | 68.475* | 1.425 | 2.000 | 1.425* |
| BrCP | 86 | 2.235 | 1.750 | 23.225 | 6.025** | 54.050 | 1.750 | 8.125 | 1.750* |
| LaCP | 82 | 3.338 | 0.915** | 19.238 | 5.738** | 64.363 | 0.9125 | 4.588 | 0.9125* |

JGP - Jordanians general population, BdP - bedouins population
CkP - charkas population, BrCP - breast cancer patients
LaCP - lung adenocarcinoma patients
*$p$<0.05 when $\chi 2$>3.84
**$P$<0.01 when $\chi 2$>6.635

Haplotype frequencies, pairwise haplotype frequencies and extended haplotypes frequencies were estimated according to the principle outlined by Hill.[25]

**Results.** The codon 72 *Bst*UI A1 allele and the intron 6 *Msp*I A1 allele are defined as absence of the restriction site[26,27] and the intron 3, 16-bp duplication A1 allele is defined as absence of the duplication.[28] The genotype distribution and the A1 allele frequencies of the 3 p53 gene biallelic polymorphisms are shown in **Table 1**. The genotypic distribution values at each locus for the 5 studied groups were in a good fit to Hardy-Weinberg equilibrium except in the case of the Charkas for 16-bp duplication, Bedouin and Charkas for the *Bst*UI and *Msp*I polymorphisms. Differences in the A1 allele frequency for all 3 polymorphisms were observed in all the groups studied **(Table 1)**. The A1 allele frequency for the 16-bp duplication was highest in Charkas (0.886) and the least in Bedouins (0.767). The Msp I RFLP A1 allele frequency was the most frequent in the Bedouins (0.3) and least common in the Jordanian general population (0.159), whereas the A1 allele frequency for BstU I RFLP ranged in frequency from 0.544 in the Bedouins to 0.273 in Charkas. In the Jordanian breast cancer and lung adenocarcinoma cases, the absence of the 16-bp duplication was the most frequent (0.886, 0.878, respectively), followed by BstU I (0.407, 0.329, respectively) and Msp I polymorphisms (0.128, 0.109).

In general, no differences were observed between the studied groups **(Table 2)** with the exception of the Jordanian general population-Charkas for the *Msp*I polymorphism, Bedouin-Charkas for the *Msp*I and *Bst*UI polymorphisms and Jordanian general population-lung adenocarcinoma patients for the *Msp*I polymorphism. The estimated pairwise haplotype frequencies, as well as linkage disequilibrium (D) and Drel (D/Dmax) values obtained for the studied groups are shown in **Table 3**. The $X^2$ goodness of fit showed that the loci being in linkage equilibrium could confidently be rejected at the 5% level for all of the pairwise haplotype combinations in all the studied groups. The pairwise haplotypes combinations for the 3-p53 biallelic polymorphisms showed unequal distribution in the Jordanian studied groups **(Table 3)**. For the 16 bp-*Msp*I haplotype combination, 1-2 was the most frequent, followed by the 2-1, and the 1-1 and 2-2. For the 16 bp-*Bst*UI, 1-2 was the most frequent haplotype followed by the haplotypes 1-1, 2-1, and

2-2. While for the *Msp*I-*Bst*UI haplotype combination, the 2-2 haplotype had the highest frequency, followed by 1-2, 1-1 and 1-2. At 1% significance level, the *Msp*I-*Bst*UI combination showed a significant population differences between all the Jordanian studied groups with the exception of the Jordanian general population when compared to breast cancer patients group that differed significantly in the 16bp-*Msp*I haplotypic combination **(Table 4). Table 5** shows the estimated frequencies of the extended p53 haplotypes (16 bp-*Msp*I-*Bst*UI) in the 5 Jordanian groups. Generally, a pronounced diversity of haplotypes was observed among different populations. The most common haplotype (1-2-2) represented an absence of the 16-bp duplication in combination with the A2 alleles for the *Msp*I and *Bst*UI polymorphisms. This haplotype was common in 64.4% and 54% of the lung adenocarcinoma and breast cancer patients,. Also, it was common in 68.5% of Charkas, 52.4% of Jordanian general population and 43.9% of Bedouins. The second most extended haplotype (1-2-1) was nearly the same in all groups except in Charkas (11.1%). The remaining haplotypes occurred in low frequencies with the exception of (1-1-1) which was found in 13.4% of Bedouins.

**Discussion.** Only few investigations have been published so far considering these three polymorphisms simultaneously.[8,12,29] To our knowledge, this is the first study carried out in the Middle East and included people of Middle-Eastern origin. The frequencies of 3 biallelic polymorphisms in the p53 gene were determined in 3 ethnic groups and 2 cancer groups; lung adenocarcinoma and breast cancer. Bedouins showed the highest A1 allele frequency among the five studied groups for *Msp*I and *Bst*UI polymorphisms, 0.300 and 0.544. With few exceptions, the studied populations were in Hardy-Weinberg equilibrium. The deviation from Hardy-Weinberg equilibrium in the Charkas for the 3 polymorphisms and for the Bedouin BstU I and Msp I polymorphisms is possibly a result of inbreeding. The Charkas in Jordan are originated from Europe where few families migrated from Russia in the early twentieth century and established themselves in Jordan where they live and marry from each other. On the other hand, Bedouins live in the isolated harsh desert environment in Eastern Jordan. The absence of the 16-bp duplication was the most common polymorphism in the 5 studied groups, ranging in frequency from 0.886 in the Charkas to 0.767 in the Bedouins. The very high A1 allele frequency for the 16-bp duplication in the Charkas is comparable with the high frequency reported for this allele in the Hazara-Pakistani (0.90), Swedes (0.850), Chinese Singapore (0.950), Chinese Guizhou (0.981), Amerindian-Brazilians (1.00), Euro-Brazilians (0.879), Finns (0.890) and Swedish-Saamis (0.905).[8,9,12] The most common extended haplotype (1-2-2) was found in all 5 studied groups and represented an absence of the 16-bp duplication combined with the *Msp*I A2 and *Bst*UI Arg allele. This result is similar to that obtained for 6 Pakistani ethnic groups; Baloch, Brahui, Burusho, Hazara, Kalash and Pathans[9] and for 3 Brazilian ethnic groups; Amerindians, Euro-Brazilians and Afro-Brazilians.[28] The second most common extended haplotype (1-2-1) was found in all Jordanian groups and represented an absence of the 16-bp duplication combined with the *Msp*I A2 and *Bst*UI pro allele. Similar results were presented for Pakistani and Brazilian ethnic groups.[9,29]

In the Jordanian breast cancer group, the absence of 16-bp duplication was most frequent (0.814), followed by *Bst*UI and *Msp*I polymorphisms (0.407 and 0.128). The same pattern was observed for the Pakistani breast cancer cases.[9] Similar results were obtained for the Jordanian lung adenocarcinoma group where the absence of 16-bp duplication was most frequent (0.878), followed by *Bst*UI and *Msp*I polymorphisms (0.329 and 0.109). We found no significant difference with respect to the *Bst*UI polymorphism between cancer patients and healthy groups. This is in contrast to what has been found by Sjalander et al[12] where *Bst*UI A1 allele was associated with lung cancer and in agreement with results presented by Birgander et al.[7] However, we did find a significant difference with respect to the *Msp*I polymorphism between lung adenocarcinoma patients and healthy Jordanian General Population. A more pronounced difference between cancer groups and healthy groups was seen when comparisons were based on haplotype frequencies. Analysis showed that *Msp*I A2 allele linked to *Bst*UI allele was associated with lung adenocarcinoma, whereas the loss of the 16-bp duplication allele in combination with *Msp*I A2 allele was associated with breast cancer. It is known that the Haplotype structure of a population is reminiscent of its evolutionary history, which is influenced by genetic mechanisms such as selection, mutation, genetic drift and admixture. Thus, it is not surprising that different haplotypes are associated with disease in different ethnic populations. The present results support the previous proposition that extended haplotypes may be more informative than single polymorphism in studies of ethnic differences and associations between p53 germline mutations and cancer.

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