TP53 codon 72 polymorphism as a risk factor for cardiovascular disease in a Brazilian population

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Abstract

TP53, a tumor suppressor gene, has a critical role in cell cycle, apoptosis and cell senescence and participates in many crucial physiological and pathological processes. Identification of TP53 polymorphism in older people and age-related diseases may provide an understanding of its physiology and pathophysiological role as well as risk factors for complex diseases. TP53 codon 72 (TP53:72) polymorphism was investigated in 383 individuals aged 66 to 97 years in a cohort from a Brazilian Elderly Longitudinal Study. We investigated allele frequency, genotype distribution and allele association with morbidities such as cardiovascular disease, type II diabetes, obesity, neoplasia, low cognitive level (dementia), and depression. We also determined the association of this polymorphism with serum lipid fractions and urea, creatinine, albumin, fasting glucose, and glycated hemoglobin levels. DNA was isolated from blood cells, amplified by PCR using sense 5'-TTGCGTCTCCAAGCAATGGATGA-3' and antisense 5'-TCTGGGAAGGGACAGAAGATGAC-3' primers and digested with the BstUI enzyme. This polymorphism is within exon 4 at nucleotide residue 347. Descriptive statistics, logistic regression analysis and Student t-test using the multiple comparison test were used. Allele frequencies, R (Arg) = 0.69 and P (Pro) = 0.31, were similar to other populations. Genotype distributions were within Hardy-Weinberg equilibrium. This polymorphism did not show significant association with any age-related disease or serum variables. However, R allele carriers showed lower HDL levels and a higher frequency of cardiovascular disease than P allele subjects. These findings may help to elucidate the physiopathological role of TP53:72 polymorphism in Brazilian elderly people.

Introduction

TP53, a tumor suppressor gene, has a critical role in cell cycle, apoptosis and cell senescence and participates in many crucial physiological and pathological processes (1). Also, TP53 is central in protecting against neoplastic diseases in humans and may affect survival (2). TP53 plays an important role in regulat-
ing vascular smooth muscle cell growth and may mediate an abnormal occurrence of apoptosis in atherosclerotic lesions by attenuating or accelerating the apoptotic death process (3). This protein has been observed in unstable atherosclerotic carotid plaque and the p53 pathway may be activated by lipid peroxidation products, as observed in human neuroblastoma cells (4). Furthermore, the involvement of p53 in some age-associated morbidities such as diabetes (5) and fatty liver diseases (6) has also been described.

Functional analysis of \( TP53 \) codon 72 (\( TP53:72 \)) variants showed differences in their transactivating activity, transforming capacity and apoptosis induction as well as in binding a variety of proteins. \( TP53 \) variant protein R72 (Arg) is significantly more efficient than P72 (Pro) in inducing apoptosis while P72 appears to induce a higher level of G1 arrest (1,7).

A remarkable activity of p53 has been described in mutant mice which display early aging-associated phenotypes (8,9). Among continental Italian and Sardinian centenarians, these variants did not have a sufficient impact on age-related mortality so as to alter gene frequency with age (2). On the other hand, a prospective study of 1226 people aged 85 years and over showed a 41% survival rate for Pro/Pro genotype carriers (10).

An association between RR genotype and minimum lumen diameter 3 months after angioplasty has been reported in Japanese patients (11). Zee et al. (12) demonstrated that \( TP53 \) haplotypes, including R72P polymorphism, have protective effects on restenosis after angioplasty mainly due to the P72 allele. Bonafe et al. (13) observed a higher extension of oxidative stress-induced apoptosis in isolated fibroblast and lymphocyte cells from Italian centenarians and sexagenarians with the RR genotype. The RR genotype modulates \textit{in vivo} ischemia-induced cell death in patients with acute coronary syndrome (13) and was also found to be associated with a poor outcome in patients with traumatic brain injury (14).

Investigation of this polymorphism in 109 Alzheimer disease patients did not show association and did not reveal an interactive effect with apolipoprotein E allele \( \epsilon4 \) (15).

In the present study, we determined allele and genotype frequencies and the association of \( TP53:72 \) polymorphism with morbidities such as cardiovascular disease, type II diabetes, obesity, neoplasia, low cognitive level (dementia), and depression, as well as with serum lipid and protein levels, in a cohort of elderly subjects followed in longitudinal study in São Paulo, Brazil. To our knowledge, there are no literature reports investigating the association of \( TP53:72 \) with all of these morbidities. Lipids, proteins, urea, creatinine, fasting glucose, and glycated hemoglobin were also investigated concerning this polymorphism.

Material and Methods

Population

The study population consisted of 383 participants from an Elderly Longitudinal Study (16). This study began in 1991 and originally involved 1667 people over the age of 66 years living in a São Paulo community, Brazil. Subjects were clinically evaluated every two years and a subsample of 383 in wave 4 (2000-2001) were invited to participate in the present study. This population was composed of individuals of European (89.2%), Japanese (3.3%), Middle Eastern (1.8%), and mixed and/or other origin (5.7%). The mean age of this population cohort was 79.80 ± 5.32 years (range: 66-97 years).

Clinical inquiries were performed to obtain information about medical history, current medication use, lifestyle, and anthropometric measurements. Physicians performed a physical exam and blood samples were collected for laboratory procedures. The
Research Ethics Committee of UNIFESP approved this study and all participants gave written informed consent.

Subjects were considered positive for cardiovascular disease when they self-reported previous myocardial infarction and/or coronary heart disease, cerebrovascular disease and/or transitory ischemic attacks and were also taking specific medication prescribed by physicians. Those currently taking insulin or oral medication and those with fasting glucose equal to or above 126 mg/dL were considered positive for type II diabetes (17). Subjects were considered to be positive for neoplasia when they self-reported a previous diagnosis with confirmation by the results of histological exams in their medical records. Subjects with a body mass index above 27 kg/m² were considered to be obese (18,19). Cognitive function was evaluated by the Mini-Mental State Examination screening instrument (20) validated for the Brazilian population (21). A Mini-Mental State Examination score of less than 24 (of 30) has 80-90% sensitivity and 80% specificity for discriminating subjects with low cognition level, roughly classified as dementia, from normal subjects (21,22). Depression was characterized by a score above 5 in a validated Brazilian version of the instrument from Older Americans Resources and Services (23).

Although some studies have shown that self-reported past history and medical records are usually concordant for selected medical conditions in the elderly (24), past histories were only accepted when there was also evidence in physical examinations, ECG, CT-scan or physician reports.

TP53:72 polymorphism was also investigated in 56 elderly healthy controls, ranging in age from 56 to 95 years (mean age: 73.07 ± 8.18 years) and 59 young healthy controls ranging in age from 7 to 23 years (mean age: 20.3 ± 1.5 years). The elderly control sample was composed of 89.3% European, 5.4% Japanese, 1.8% Afro-Brazilian, and 3.5% mixed origin subjects. The young control sample was composed of 89.8% European, 8.5% Japanese, and 1.6% mixed origin subjects.

**Laboratory exams**

Lipid and lipid fraction measurements were performed by routine enzymatic tests. Creatinine, albumin, urea, and fasting serum glucose levels were investigated by usual colorimetric, kinetic and UV tests. Glycated hemoglobin levels were analyzed by high-performance liquid chromatography (25).

**DNA extraction**

Blood was collected into tubes containing 0.1% EDTA and genomic DNA was isolated using procedures modified from Lahiri and Nurnberger (26).

**Genotyping**

TP53 R72P polymorphism was analyzed using procedures modified from Helland et al. (27) and Ara et al. (28). A 199-bp sequence containing the polymorphic site was amplified by PCR using sense 5′-TTGCC GTCCCAAGCAATGGATGA-3′ and anti-sense 5′-TCTGGGAAGGGAGAAGAT GAC-3′ primers.

Each PCR mixture contained 50 ng genomic DNA and PCR buffer, MgCl₂, dNTPs, Taq polymerase, and primers. The mixture was heated for 4 min at 95°C and underwent 35 cycles of amplification: annealing (55°C for 30 s), extension (72°C for 45 s) and denaturation (94°C for 45 s). The PCR product was digested with BrsUI for 4 h at 60°C, producing two fragments of 113 and 86 bp in relation to the R allele and a fragment with 199 bp in relation to the P allele.

Restriction fragment length polymorphism products were analyzed on 4% GTG agarose gel and stained with ethidium bromide. Figure 1 shows the TP53 R72P alleles.
Statistical analysis

Descriptive statistics, logistic regression analysis, chi-square test, and t-test were performed using SPSS 10.0. Genotype and allele frequencies were calculated by allele counting as described by Emery (29). Genotype distribution was investigated in terms of Hardy-Weinberg equilibrium. Two allele groups were considered for statistical analysis: one with a P allele (PP + PR genotypes together) and the other with a non-P allele (RR genotype). The mean age of subjects with the P allele was 79.57 ± 5.40 years and the mean age of subjects with the non-P allele was 80.06 ± 5.23 years. Age comparison between allele groups did not show a significant difference (t = 0.894; d.f. = 381; P = ns). Gender association with morbidity was also evaluated in the entire sample using the chi-square test (α = 0.05).

Logistic regression analysis for the investigation of polymorphism association with morbidity was performed considering the allele as a dependent variable and morbidity, age and sex as co-variables in the model. Odds ratios and 95% confidence interval were calculated using the SPSS 10.0 software. The Student t-test was used to compare laboratory findings for P and non-P allele carriers (α = 0.05).

Results

The allele frequencies observed in our population were 0.31 for the P allele and 0.69 for the R allele. Observed genotype frequencies were 0.47 for RR, 0.44 for PR, and 0.09 for PP. Genotype distributions were within Hardy-Weinberg equilibrium for the whole sample, elderly and young controls and for the groups with cardiovascular disease, type II diabetes, obesity, and neoplasia (data not shown). Genotype distribution and allele frequency for the whole sample and the subsamples are shown in Table 1.

Logistic regression analysis revealed a tendency of the R allele (non-P allele) to associate with cardiovascular disease (Table 2). Table 3 shows descriptive statistics and Student t-test values for the two allele groups concerning laboratory findings. We detected an association of the R allele with lower
Table 1. Number and distribution of TP53:72 genotypes and alleles in a Brazilian elderly cohort and in elderly and young healthy controls.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>PP</th>
<th>PR</th>
<th>RR</th>
<th>Total</th>
<th>P allele frequencies</th>
<th>R allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole sample</td>
<td>33</td>
<td>169</td>
<td>181</td>
<td>383</td>
<td>0.3068</td>
<td>0.6932</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>55</td>
<td>51</td>
<td>121</td>
<td>0.3512</td>
<td>0.6488</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>114</td>
<td>130</td>
<td>262</td>
<td>0.2863</td>
<td>0.7137</td>
</tr>
<tr>
<td>Elderly controls</td>
<td>3</td>
<td>21</td>
<td>32</td>
<td>56</td>
<td>0.2411</td>
<td>0.7589</td>
</tr>
<tr>
<td>Young controls</td>
<td>3</td>
<td>31</td>
<td>35</td>
<td>59</td>
<td>0.2681</td>
<td>0.7319</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Affected subjects</th>
<th>Total</th>
<th>P allele frequencies</th>
<th>R allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>5</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>26</td>
<td>102</td>
<td>114</td>
</tr>
<tr>
<td>Obesity</td>
<td>12</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Depression</td>
<td>4</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>Low cognitive level</td>
<td>0</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>2</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2. Logistic regression results concerning association between morbidities and TP53:72 polymorphism in a cohort from a Brazilian elderly population.

<table>
<thead>
<tr>
<th>Variables</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>0.071</td>
<td>0.638</td>
<td>0.389</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>0.865</td>
<td>1.000</td>
<td>0.629</td>
</tr>
<tr>
<td>Obesity</td>
<td>0.662</td>
<td>0.901</td>
<td>0.565</td>
</tr>
<tr>
<td>Depression</td>
<td>0.075</td>
<td>1.634</td>
<td>0.953</td>
</tr>
<tr>
<td>Low cognitive level</td>
<td>0.599</td>
<td>1.226</td>
<td>0.574</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>0.952</td>
<td>1.020</td>
<td>0.531</td>
</tr>
</tbody>
</table>

Table 3. Descriptive statistics concerning P allele and non-P allele carriers and laboratory findings.

<table>
<thead>
<tr>
<th></th>
<th>P allele carriers</th>
<th>Non-P allele carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean ± SD</td>
<td>N</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>135</td>
<td>147.53 ± 77.75</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>135</td>
<td>216.74 ± 41.45</td>
</tr>
<tr>
<td>HDL</td>
<td>135</td>
<td>55.96 ± 15.27</td>
</tr>
<tr>
<td>LDL</td>
<td>130</td>
<td>131.06 ± 33.88</td>
</tr>
<tr>
<td>VLDL</td>
<td>131</td>
<td>27.62 ± 11.45</td>
</tr>
<tr>
<td>Creatinine</td>
<td>130</td>
<td>0.9669 ± 0.2501</td>
</tr>
<tr>
<td>Urea</td>
<td>131</td>
<td>40.95 ± 13.31</td>
</tr>
<tr>
<td>Albumin</td>
<td>130</td>
<td>4.0669 ± 0.3099</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>180</td>
<td>98.07 ± 35.32</td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>105</td>
<td>4.8857 ± 1.3273</td>
</tr>
</tbody>
</table>
HDL levels ($P = 0.047$). Serum triglyceride, total cholesterol, VLDL, LDL, urea, creatinine, albumin, fasting glucose, and glycated hemoglobin levels did not show significant differences between P allele carriers and non-P allele carriers (Student $t$-test).

**Discussion**

Studies conducted on older populations have the advantage of demonstrating specific aspects of the aging process. Our sample was mostly composed of European descendants and our results concerning allele and genotype frequencies are similar to those reported in other studies on populations of the same ethnic composition. Another study on a Brazilian adult population reported the following genotype frequencies: $RR = 0.518$, $PR = 0.405$, and $PP = 0.078$ (30). A study on a Chilean adult population sample reported similar frequencies: $RR = 0.472$, $PR = 0.453$, and $PP = 0.075$ (31), as also reported for a Mexican sample ($RR = 0.45$, $PR = 0.62$, and $PP = 0.08$) (32).

Female gender was associated with obesity in our sample ($P = 0.011$), confirming the findings of a Brazilian epidemiological study involving residents over 60 years in the Bambuí community, Minas Gerais State (33). Another epidemiological investigation in the Northeast and Southeast regions of Brazil also showed higher a prevalence of obesity among women older than 50 years (34).

Genotype distribution was within Hardy-Weinberg equilibrium in the whole sample and in all the subsamples, except for the depression and low cognitive level groups. $TP53$ variants did not have a sufficient impact on age-related mortality so as to alter gene frequency in comparison with the young control group ($P = 0.081$) in this sample.

$TP53:72$ polymorphism did not show a significant association with any age-related morbidity or serum variable. Our population is mainly composed of European descendants, especially from Portugal, Spain, and Italy. In Spanish and Italian populations, the R allele has been associated with restenosis after angioplasty and with a greater extent of cells with oxidative stress in centenarians and sexagenarians (2,12). This ethnic composition may account for the observed tendency of the R allele to associate with cardiovascular disease and lower HDL levels.

The relationship between $TP53$ and lipid metabolism is not well known but many reports support correlations. Lower levels of HDL lipoprotein have been considered to be an independent risk factor for cardiovascular disease and type II diabetes (35). Moreover, modification of low-density lipoprotein by endothelial cells has been associated with lipid peroxidation and with degradation of low-density lipoprotein phospholipids (36). In addition, the lipid peroxidation product has also been considered a potential trigger of the p53 pathway in human neuroblastoma cells. Patients with acute myocardial infarction showed four times higher levels of serum oxidized LDL compared to control (37).

Our findings also did not show an association of the polymorphism with type II diabetes or with some serum variables associated with impaired renal function, although the p53 pathway contributes to an altered neovascularization process in diabetes (38).

These findings may help to elucidate the physiopathological role of $TP53:72$ polymorphism in elderly Brazilian people.
TP53 polymorphism as a cardiovascular risk factor

References


36. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells
