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Impact of the $MDM2$ SNP309 and $TP53$ Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome

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ABSTRACT

The Li-Fraumeni syndrome, resulting from TP53 germline mutations, represents one of the most devastating genetic predispositions to cancer. Recently, the MDM2 SNP309 (T>G variation) was shown to be associated with accelerated tumour formation in TP53 mutation carriers. The impact of the common TP53 codon 72 polymorphism on cancer risk remains controversial. We have therefore investigated the effect of these two polymorphisms in 61 French germline TP53 mutation carriers. The mean age of tumour onset in MDM2 SNP309 G allele carriers (19.6 years) was significantly different from that observed in patients homozygous for the T allele (29.9 years, p<0.05). For the TP53 codon 72 polymorphism, the mean age of tumour onset in Arg allele carriers (21.8 years) was also different from that of Pro/Pro patients (34.4 years, p<0.05). We observed a cumulative effect of both polymorphisms since the mean ages of tumour onset in MDM2 G and TP53 Arg alleles carriers (16.9 years), and in patients with the MDM2 T/T and TP53 Pro/Pro genotype (43 years), were clearly different (p<0.02). Therefore, our results confirm the impact of the MDM2 SNP309 G allele on the age of tumour onset in germline TP53 mutation carriers, and suggest that this effect may be amplified by the TP53 72Arg allele. Polymorphisms affecting p53 degradation represent therefore one of the rare examples of modifier genetic factors identified so far in Mendelian predispositions to cancer.
The Li-Fraumeni syndrome (LFS, MIM 151623), which results from germline mutations of the TP53 gene (MIM 191170), represents one of the most devastating genetic predispositions to cancer and is characterized by a wide spectrum of early-onset malignancies including bone and soft-tissue sarcomas, brain tumours, adrenocortical tumours, leukaemia and pre-menopausal breast cancers (for review, see Chompret 2002[1] and Varley 2003[2]).

The wide spectrum of malignancies, the range of the age of tumour onset, and the incomplete penetrance in males complicate the genetic counselling and medical follow-up of affected families.

The recent report indicating that the SNP309 (T>G variation) [g.2580T>G (Genbank accession number AF527840); rs2279744], within the MDM2 gene (MIM 164785), was associated with accelerated tumour formation in TP53 mutation carriers[3][4] is of particular interest in this context of phenotypic variability. MDM2 is a key negative regulator of p53, which targets p53 towards proteasomal degradation, and the SNP309 T>G variation, located in the first intron of MDM2, has been found to increase Sp1 transcription factor binding and consequently MDM2 level. Since the 72Arg variant of the p53 protein has been shown to have a higher affinity towards MDM2 compared to the 72Pro variant,[5] we speculated that the p.Arg72Pro polymorphism might also influence the age of tumour onset in germline TP53 mutation carriers. The impact of this polymorphism, as a risk factor in sporadic cancers,[6] or as a modifier factor in Mendelian forms of cancers such as BRCA1-related breast cancers,[7] has already been analysed, but the results are controversial.

We have therefore investigated the effect on tumorigenesis of these 2 polymorphisms in 61 affected or unaffected germline TP53 mutation carriers collected by the French LFS network.

METHODS

Patients

Sixty-one affected or unaffected germline TP53 mutation carriers, from 41 unrelated LFS families collected by the French LFS network, were investigated. Clinical characteristics of the families and description of the mutations are available upon request. DNA analysis was performed after informed consent was obtained.
**MDM2 genotyping**

The first intron of MDM2 was PCR-amplified using primers 5'-AGGTCTCCGGAGTGTC-3' and 5'-CTGCCCACTGAACCGG-3'. PCR was performed in a 25 µL volume containing 100 ng of genomic DNA, 1.2 mM MgCl2, 160 µM dNTPs, 0.4 µM of each primer and 1 unit of Taq DNA polymerase (ABgene). After a denaturation step of 3 min at 95°C, the PCR consisted of 35 cycles of 10 s at 94°C, 10 s at 60°C, and 10 s at 72°C, and was followed by a final extension step of 7 min at 72°C. After purification of the PCR products using the QIAquick Gel Extraction Kit (Qiagen), sequencing analysis of the MDM2 SNP309 was performed using the PRISM Ampli Taq FS Ready Reaction Dye Terminators sequencing kit (PE Applied Biosystems) and a PE Applied Biosystems 377 automated DNA sequencer.

**Determination of the phase of the TP53 codon 72 polymorphism**

The phase (cis or trans) of the TP53 codon 72 polymorphism, relative to the germline mutation, was determined in the Arg/Pro heterozygotes, by cloning either PCR-amplified genomic DNA in bacteria, using primers encompassing exons 4 to 5, 7 or 8 or PCR-amplified cDNA in yeast, as previously described.[8]

**Statistical analysis**

Analysis of variance was used to compare the mean age of onset between the different groups and a $\chi^2$ was used to compare the distribution of tumour types between the different genotypes;

**RESULTS and DISCUSSION**

For MDM2, the observed frequencies in our series for the SNP309 T/T, T/G, and G/G genotypes were 37%, 46%, and 17%, respectively (table 1). Among the 41 affected carriers for whom age of onset was known, comparison of the mean age of first tumour onset between the three MDM2 genotypes, using variance analysis, revealed no significant difference. When we stratified the patients according to the presence of the G allele, we observed a difference between G allele carriers (n=27) and patients homozygous for the T allele (n=14) (19.6 versus 29.9 years, p<0.05, table 2). This result is in agreement with the results published by Bond et al. (2004). We detected no dosage effect for the G allele, since the mean age of tumour onset in T/G patients was...
17.8 years, whereas it was 23.3 years in G/G subjects. Among the 34 G allele carriers, 50% had developed a tumour before 20 years of age, whereas only 21% of the subjects homozygous for the T allele were affected before this age (fig 1A).

For the TP53 codon 72 polymorphism, the distribution of the Arg/Arg, Arg/Pro, and Pro/Pro genotypes was 41%, 46%, and 13%, respectively (table 1). We observed a difference in the mean age of tumour onset only when the patients were stratified according to the presence of the Arg allele. Indeed, the mean age of tumour onset in Arg allele affected carriers (n=38) was 21.8 years and in Pro/Pro patients (n=8) 34.4 years (p<0.05, table 2). Among the 52 Arg allele carriers, 40% had developed a tumour before the age of 20 years, whereas only 12% of the 8 subjects homozygous for the Pro allele were affected before this age (fig 1B).

Since the potential effect of the codon 72 polymorphism may depend on its cis or trans position relative to the germline mutation, we determined the phase in the Arg/Pro heterozygotes, but we did not detect any significant effect of the phase on the age of tumour onset.

Since both the MDM2 SNP309 and TP53 codon 72 polymorphism might exert their effect through the degradation of p53, we analysed the combined effect of the MDM2 G and TP53 Arg alleles. Despite a limited number of subjects, we observed a clear difference in the mean age of tumour onset of TP53 mutation carriers according to their combined genotype for the MDM2 and TP53 polymorphisms. The mean age of onset was the lowest (16.9 years) for the 23 individuals with putative at risk genotypes at both loci (T/G or G/G and Arg/Pro or Arg/Arg). The mean age of onset was the highest (43.0 years) for the 2 individuals who did not carry risk alleles at both loci (T/T and Pro/Pro), and intermediate for the 16 individuals with a genotype at risk at only one locus (28.5 years) (table 3). The difference between these three groups was highly significant (p<0.001), suggesting a cumulative effect of both polymorphisms.

We did not detect any effect of these polymorphisms on the tumour type in our series. Indeed, the comparison of the tumour type, stratified into three categories (breast, sarcoma, other cancer), between individuals with at risk genotypes at both loci and individuals with other genotypes was not significant (X^2=4.50). In each family, we obtaines DNA samples only from a limited number of affected individuals (1-3 people), which hampered the analysis of the impact of the polymorphisms on the tumour type within each family. Neither the MDM2 SNP309, nor the TP53 codon 72 polymorphism could explain the incomplete penetrance in males of germline TP53 mutations that we had previously estimated at 41% at age 45 (versus 84% in females).[9]
Therefore, our results confirm the impact of the MDM2 SNP309 G allele on the age of tumour onset in germline TP53 mutation carriers, and suggest that this effect may be amplified by the TP53 72Arg allele, although this latter observation required to be confirmed on a larger series of germline TP53 mutation carriers. Polymorphisms affecting p53 degradation represent therefore one of the rare examples of modifier genetic factors identified so far in Mendelian predispositions to cancer.

**ELECTRONIC-DATABASE INFORMATION**


**ACKNOWLEDGEMENTS**

We are grateful to Mario Tosi for critical review of the manuscript. This work was supported by funds from the French Ministry of Health, and the Ligue Nationale Contre le Cancer.

**REFERENCES**

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8 Flaman JM, Frebourg T, Moreau V, *et al.* A simple p53 functional assay for screening cell

Table 1 Allele and genotype distributions of the MDM2 SNP309 and TP53 codon 72 polymorphism

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency (%)</th>
<th>Genotype</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM2 SNP309</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>60</td>
<td>T/T</td>
<td>37</td>
</tr>
<tr>
<td>G</td>
<td>40</td>
<td>T/G</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>17</td>
</tr>
<tr>
<td>TP53 codon 72 polymorphism</td>
<td>Arg 64</td>
<td>Arg/Arg</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Pro 36</td>
<td>Arg/Pro</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pro/Pro</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2 Mean age of first tumour onset in TP53 mutation carriers according to the MDM2 SNP309 or TP53 codon 72 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of patients</th>
<th>Mean age (years)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM2 SNP309</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>14</td>
<td>29.9</td>
</tr>
<tr>
<td>T/G</td>
<td>19</td>
<td>17.8</td>
</tr>
<tr>
<td>G/G</td>
<td>8</td>
<td>23.3</td>
</tr>
<tr>
<td>T/G+G/G</td>
<td>27</td>
<td>19.6</td>
</tr>
<tr>
<td>TP53 codon 72 polymorphism</td>
<td>Arg/Arg 18</td>
<td>21.1</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>20</td>
<td>22.5</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>8</td>
<td>34.4</td>
</tr>
<tr>
<td>Arg/Pro+Arg/Arg</td>
<td>38</td>
<td>21.8</td>
</tr>
</tbody>
</table>

*Of first tumour onset
Table 3 Mean age of first tumour onset in TP53 mutation carriers according to the combined MDM2 SNP309 and TP53 codon 72 genotypes

<table>
<thead>
<tr>
<th>Number of at risk loci</th>
<th>Corresponding genotypes</th>
<th>No. of patients</th>
<th>Mean age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>T/T + Pro/Pro</td>
<td>2</td>
<td>43.0</td>
</tr>
<tr>
<td>1</td>
<td>(T/G or G/G + Pro/Pro) or (T/T + Arg/Arg or Arg/Pro)</td>
<td>16</td>
<td>28.5</td>
</tr>
<tr>
<td>2</td>
<td>T/G or G/G + Arg/Arg or Arg/Pro</td>
<td>23</td>
<td>16.9</td>
</tr>
</tbody>
</table>

*Of first tumour onset
Figure 1. Numbers of TP53 mutation carriers who developed the first tumour before and after 20 years of age, according to the MDM2 SNP309 (A) and TP53 codon 72 polymorphism (B). The black columns indicate the numbers of individuals who developed their first tumour before 20 years of age and the white columns indicate the numbers of individuals either who developed their first tumour after 20 years of age or who were unaffected by the time they were 20 years old.

A

![Bar chart showing the numbers of TP53 mutation carriers who developed the first tumour before and after 20 years of age, according to the MDM2 SNP309. The black columns indicate the numbers of individuals who developed their first tumour before 20 years of age and the white columns indicate the numbers of individuals either who developed their first tumour after 20 years of age or who were unaffected by the time they were 20 years old.](chart1a.png)

B

![Bar chart showing the numbers of TP53 mutation carriers who developed the first tumour before and after 20 years of age, according to the TP53 codon 72 polymorphism. The black columns indicate the numbers of individuals who developed their first tumour before 20 years of age and the white columns indicate the numbers of individuals either who developed their first tumour after 20 years of age or who were unaffected by the time they were 20 years old.](chart1b.png)