

Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome.

Gaëlle Bougeard, Stéphanie Baert-Desurmont, Isabelle Tournier, Sophie Vasseur, Cosette Martin, Laurence Brugières, Agnès Chompret, Brigitte Bressac-de Paillerets, Dominique Stoppa-Lyonnet, Catherine Bonaïti-Pellié, et al.

► **To cite this version:**

Gaëlle Bougeard, Stéphanie Baert-Desurmont, Isabelle Tournier, Sophie Vasseur, Cosette Martin, et al.. Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome.. *Journal of Medical Genetics*, BMJ Publishing Group, 2006, 43 (6), pp.531-3. 10.1136/jmg.2005.037952 . inserm-00128004

HAL Id: inserm-00128004

<https://www.hal.inserm.fr/inserm-00128004>

Submitted on 30 Jan 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

G Bougeard, S Baert-Desurmont, I Tournier, S Vasseur, C Martin, L Brugieres, A Chompret, B Bressac-de Paillerets, D Stoppa-Lyonnet, C Bonaïti-Pellié, T Frebourg

Authors' affiliations

G Bougeard, S Baert-Desurmont, I Tournier, S Vasseur, C Martin, T Frebourg, Inserm U614, Faculty of Medicine, and Department of Genetics, Rouen University Hospital, Rouen, France

L Brugieres, Department of Pediatric Oncology, Institut Gustave Roussy, Villejuif, France

A Chompret, Department of Medicine, Institut Gustave Roussy, Villejuif, France

B Bressac-de Paillerets, Department of Genetics, Institut Gustave Roussy, Villejuif, France

D Stoppa-Lyonnet, Department of Genetics, Institut Curie, Paris, France

C Bonaïti-Pellié, Inserm U535, Villejuif, France

Address for correspondence and reprints: Dr. Thierry Frebourg, Inserm U614, Faculty of Medicine, 22 Boulevard Gambetta, 76183 Rouen, France. Phone: 33 2 32 88 81 82; Fax number: 33 2 32 88 80 80; E-mail: Frebourg@chu-rouen.fr

The corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its licensees, to permit this article (if accepted) to be published in JMG and any other BMJPG products and to exploit all subsidiary rights, as set out in the licence.

Running title: *MDM2* and *TP53* polymorphisms in LFS

Keywords: Li-Fraumeni syndrome, *TP53*, *MDM2*, polymorphism

Word count: 2012

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'-AGGTCTCCGCGGGAGTTC-3' and 5'-CTGCCCACTGAACCGGC-3'. PCR was performed in a 25 μ L volume containing 100 ng of genomic DNA, 1.2 mM MgCl₂, 160 μ M dNTPs, 0.4 μ M 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 χ^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 ($\chi^2=4.50$). In each family, we obtained 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

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>.
CHIP Bioinformatics, <http://snpper.chip.org/bio/snpper-enter/>.

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

- 1 Chompret A. The Li-Fraumeni syndrome. *Biochimie* 2002;**84**:75-82.
- 2 Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat* 2003;**21**:313-20.
- 3 Bond GL, Hu W, Bond EE, *et al.* A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004;**119**:591-602.
- 4 Bond GL, Hu W, Levine A. A single nucleotide polymorphism in the MDM2 gene: from a molecular and cellular explanation to clinical effect. *Cancer Res* 2005;**65**:5481-4.
- 5 Dumont P, Leu JI, Della Pietra AC, 3rd, *et al.* The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 2003;**33**:357-65.
- 6 Donehower LA. p53: guardian AND suppressor of longevity? *Exp Gerontol* 2005;**40**:7-9.
- 7 Martin AM, Kanetsky PA, Amirimani B, *et al.* Germline TP53 mutations in breast cancer families with multiple primary cancers: is TP53 a modifier of BRCA1? *J Med Genet* 2003;**40**:e34.
- 8 Flaman JM, Frebourg T, Moreau V, *et al.* A simple p53 functional assay for screening cell

lines, blood, and tumors. *Proc Natl Acad Sci U S A* 1995;**92**:3963-7.

9 Chompret A, Brugieres L, Ronsin M, *et al.* P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br J Cancer* 2000;**82**:1932-7.

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

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

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

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

*Of first tumour onset

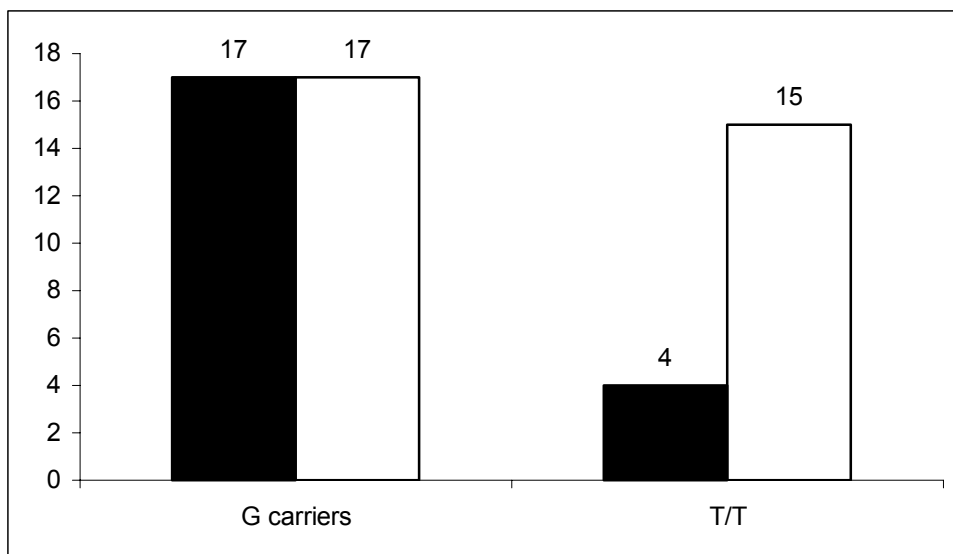
Table 3 Mean age of first tumour onset in *TP53* mutation carriers according to the combined *MDM2* SNP309 and *TP53* codon 72 genotypes

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

*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



B

