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The single-nucleotide polymorphism 309 in the *MDM2* gene contributes to the Li–Fraumeni syndrome and related phenotypes

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Li–Fraumeni syndrome (LFS) is an autosomal-dominant cancer predisposition syndrome of which the majority is caused by *TP53* germline mutations and is characterised by different tumour types occurring at relatively young age. Recently, it was shown that a single-nucleotide polymorphism (SNP) in the *MDM2* gene, SNP309 (T > G variation), was associated with accelerated tumour formation in LFS patients who carry a *TP53* germline mutation. To confirm this finding in different populations, we screened 25 Dutch and 11 Finnish *TP53* mutation carriers for the presence of the SNP309 G allele in the *MDM2* gene. Additionally, we investigated whether the SNP309 G allele plays a role in 72 Dutch *TP53*-negative LFS and LFS-related patients. In the *TP53* germline mutation carriers, a significant difference was seen in the mean age of tumour onset for the SNP309 G allele group, that is, 29.7 years as compared to the SNP309 homozygous T group 45.5 years ($P = 0.005$). In patients of LFS and LFS-related *TP53*-negative families, no difference was seen in the mean age of tumour onset. However, this *TP53*-negative group did show a significantly higher percentage of SNP309 homozygotes (G/G) compared to the general population ($P = 0.02$). In conclusion, *TP53* germline mutation carriers who have an SNP309 G allele have an earlier onset of tumour formation. The higher prevalence of *MDM2* SNP309 homozygous G/G carriers in the *TP53*-negative group suggests that this allele contributes to cancer susceptibility in LFS and LFS-related families. *European Journal of Human Genetics* (2007) 15, 110–114. doi:10.1038/sj.ejhg.5201715; published online 27 September 2006

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Introduction

Li–Fraumeni syndrome (LFS) is an autosomal-dominant cancer predisposition syndrome. The main tumour types of LFS include bone- and soft-tissue sarcoma (STS), breast

cancer, brain tumour, adrenocortical carcinoma and leukaemia.¹ The classical LFS criteria are a proband with a sarcoma aged under 45 years and a first-degree relative with any cancer aged under 45 years, plus a first- or second-degree relative in the same lineage with any cancer aged under 45 years or a sarcoma at any age.² In addition, Li–Fraumeni-like syndrome (LFL) criteria were formulated as a proband with any childhood tumour, or a sarcoma, brain tumour or adrenocortical tumour aged under 45 years and a first or second degree in the same lineage with a typical LFS tumour at any age, plus a first- or second-degree

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relative in the same lineage with any cancer under the age of 60 years.³ Less stringent LFL criteria were formulated by Eeles⁴ as two first- or second-degree relatives with typical LFS tumours at any age. In 1990, germline mutations in the *TP53* gene were found to be associated with LFS.⁵ At present, *TP53* germline mutations are detected in approximately 75% of LFS and 40% of LFL families.⁶ Because still 25–60% of LFS/LFL families do not carry a germline *TP53* mutation, alternative LFS susceptibility genes have been proposed.⁷ Moreover, single-nucleotide polymorphisms (SNP) may contribute to predisposition to cancer.⁸ Recently, it was suggested that an SNP in the promoter region of the *MDM2* gene is associated with a significantly earlier age of onset of tumours in both *TP53* mutation carriers and sporadic *TP53*-negative STS.⁹ The *MDM2* gene is an important negative regulator of *TP53*. Bond *et al*⁹ showed that the *MDM2* SNP309 G creates an improved SP1 site, leading to higher basal levels of *MDM2* in cells, thereby attenuating the p53 pathway. The impact of the *MDM2* SNP309 G on the age of tumour onset in germline *TP53* mutation carriers was confirmed by Bougeard *et al*,¹⁰ by screening 41 affected *TP53* mutation carriers for the presence of an SNP309 G allele. Wilkening *et al*¹¹ showed that this polymorphism does not seem to play a role in familial breast cancer.

We investigated the presence of the SNP309 G allele in Dutch and Finnish *TP53* mutation carriers to look at the

effect on age of onset. Furthermore, we tested 72 family members of 68 *TP53*-negative LFS and LFS-related families from the Netherlands to determine the possible effect of SNP309 G on cancer susceptibility.

Patients and methods

For this study, 108 selected cancer patients were screened for *TP53* germline mutations, 97 at the Family Cancer Clinic of the Netherlands Cancer Institute and 11 at the Helsinki University Central Hospital. These patients were divided into four groups: (1) classic LFS,² (2) LFL according to Birch *et al*³ or Eeles,⁴ (3) LFS-suggestive, including childhood onset (under 18 years) sarcoma or brain tumours, two or more primary tumours at any age, or two first-degree relatives with a tumour at any age, of which at least one is a typical LFS tumour and (4) breast cancer before 30 years of age (without detectable *BRCA1* or *BRCA2* mutation) (Table 1). Groups 2–4 combined are further referred to as LFS-related families. Thirty-six *TP53*-positive and 72 *TP53*-negative family members out of 87 families (19 *TP53*-positive families, 68 *TP53*-negative families, respectively) were tested for the presence of SNP309 G in the *MDM2* gene. The 68 *TP53*-negative families were all Dutch; in Finland, *TP53*-negative families were not tested for the presence of SNP309 G in the *MDM2* gene. DNA from peripheral blood lymphocytes was isolated

Table 1 SNP309 genotype in patients and controls

(Family) history	n	<i>TP53</i> -positive SNP309 genotype			n	<i>TP53</i> -negative SNP309 genotype		
		T/T	T/G	G/G		T/T	T/G	G/G
LFS	11	2	8	1	4	1	3	
LFL	22	15	7		29	15	8	6
<i>LFS suggestive</i>								
Childhood sarcoma or brain tumour (<18 years)	1		1		5	4	1	
At least two primary tumours	1		1		17	10	2	5
Two first-degree relatives with cancer (at least one typical LFS tumour)					12	7	3	2
Breast cancer before 30 years (<i>BRCA1/BRCA2</i> negative)	1	1			5	3	1	1
	36	18 (50%)	17 (47%)	1 (3%)	72	39 (54%)	16 (22%)	17 (24%)
(b)								
Control cohorts	Total (n)	<i>MDM2</i> SNP309 genotype						
		T/T	T/G	G/G				
		n (%)	n (%)	n (%)				
Dutch population controls	230	94 (41%)	109 (47%)	27 (12%)				
Finnish population controls	551	185 (34%)	259 (47%)	107 (19%)				
Combined Dutch/Finnish controls for 36 <i>TP53</i> -positive patients	Adjusted	39%	47%	14%				

(a) SNP309 genotype in *TP53*-positive and *TP53*-negative patients. Patients were divided into four groups according to their (family) history.

(b) SNP309 genotype in Dutch and Finnish controls.

LFS = Li–Fraumeni syndrome fulfilling the classical criteria,² LFL = Li–Fraumeni-like syndrome according to Birch³ or Eeles.⁴

according to standard procedures. Mutation analysis of the *TP53* gene was performed by sequence analysis of all coding exons (2–11) and flanking intron–exon boundaries of these exons using standard procedures. The 19 *TP53* germline mutations in the *TP53*-positive families included 12 missense mutations, four stop mutations and three splice site mutations. DNA was sequenced for the presence of SNP309 G in the *MDM2* promoter (Different primer sets were used in the Netherlands and Finland; primer 1: 5'-TGTAACGACGGCCAGTCGGGAGTTCAGGGTAAAGGT-3', primer 2: 5'-CAGGAAACAGCTATGACCTCGGAACGTGTCTGAACTTG-3' in Amsterdam, primer 1: 5'-GTTTTGTTGGACTGGGGCTA-3', primer 2: 5'-CGGAACGTGTCTGAAC TTGA-3' in Helsinki).

A control group of 230 Dutch healthy blood bank controls, males and females and 551 Finnish healthy female control subjects (from the same geographical region) were screened for the presence of SNP309 G. Proportions of haplotype frequencies in the combined control group were adjusted for the numbers of Dutch and Finnish patients. The Dutch controls were genotyped as described above, the Finnish controls were genotyped using restriction fragment length polymorphisms: PCR products were digested with *MspA1* I (New England Biolabs) and run on a 3% agarose gel; the PCR product with a T allele is cut once and the PCR product with a G allele is cut twice.

A *t*-test was used to determine the statistical significance for the age of onset of cancer between the groups with and without SNP309 G. Proportions were tested by a χ^2 test.

Results

The classification of the 108 LFS and LFS-related patients into four groups (see Patients and methods) is shown in Table 1a. The presence of the SNP309 G in *TP53*-positive patients was found in heterozygous state, T/G, in 17 out of 36 (47%) patients and in the homozygous state, G/G, in one out of 36 (3%) patients. In *TP53*-negative patients, T/G was found in 16 out of 72 (22%) patients, and G/G was found in 17 out of 72 patients (24%, Table 1a). The Dutch and Finnish population controls, including the ratio-adjusted Dutch/Finnish controls are listed in Table 1b. In the *TP53*-negative LFS and LFS-related patients, the G/G genotype was twice the percentage in the general population (24 vs 12%, respectively, $P=0.02$), and the T/G genotype proportion was significantly lower as compared to the general population (22 vs 47%, $P<0.01$).

The prevalence of the different first tumour types in both *TP53*-positive and *TP53*-negative patients is shown in Figure 1a and b, with breast cancer and STS being the most frequent cancers. The average age of onset of all first cancers was 34.3 years. When the group is divided in *TP53*-positive and *TP53*-negative patients, the average age was

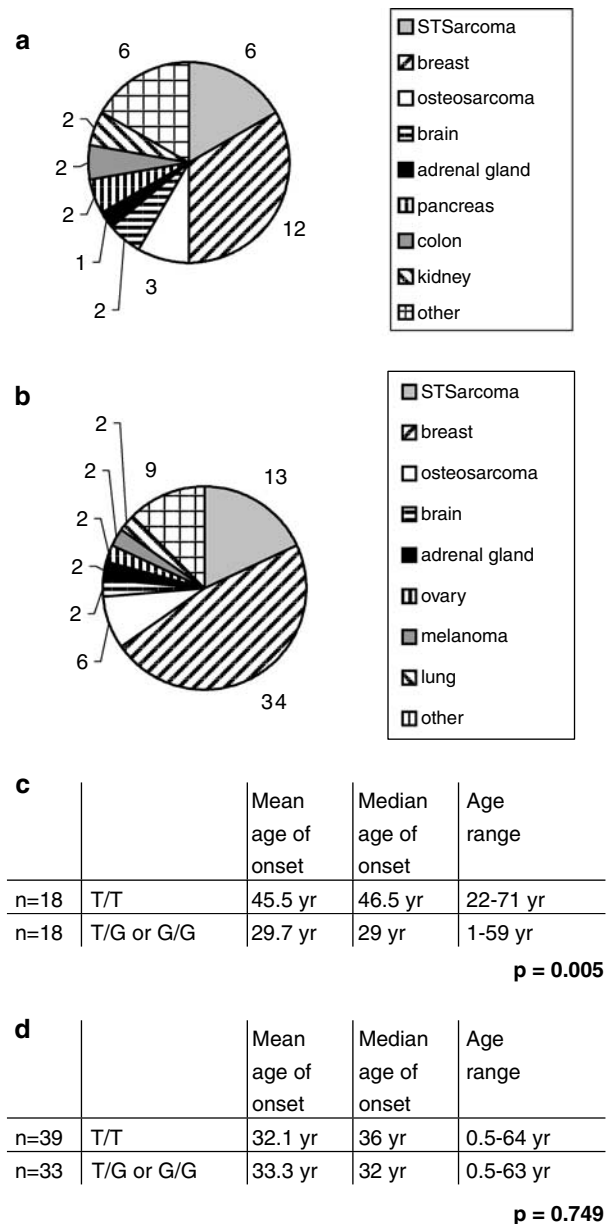


Figure 1 Prevalence of different tumour types and mean age of tumour onset. (a) First tumours in *TP53* mutation carriers ($n=36$). Other = stomach cancer, melanoma, mesothelioma, ovary cancer, lung cancer and thyroid cancer. (b) First tumours in *TP53*-negative LFS and LFS-related patients ($n=72$). Other = bladder cancer, Hodgkin's lymphoma, kidney cancer, thyroid cancer, basal cell carcinoma, acute myeloid leukaemia, colon cancer, non-Hodgkin's lymphoma and stomach cancer. (c) Mean age of tumour onset in *TP53* mutation carriers according to their *MDM2* SNP309 genotype. (d) Mean age of tumour onset in *TP53*-negative LFS and LFS-related patients according to their *MDM2* SNP309 genotype.

37.6 and 32.6 years, respectively. When the *TP53*-positive group was further divided by the presence or absence of SNP309 G, the T/T group revealed a mean age of 45.5 years, as compared to the group where SNP309 G was present in

heterozygous or homozygous state (T/G or G/G) where the mean age was significantly earlier, 29.7 years ($P=0.005$, Figure 1c). In the T/T group of *TP53*-negative patients, the mean age was 32.1 years, and in the T/G and G/G group combined, the mean age was 33.3 years ($P=0.749$; Figure 1d).

Discussion

We showed that the presence of an SNP in the *MDM2* gene, SNP309 (T>G variation) in Dutch and Finnish *TP53* germline mutation carriers was associated with accelerated tumour formation. Our results show a 16 years earlier age of tumour onset in *TP53* mutation carriers with an SNP309 G allele as compared to the T/T SNP 309 group (Figure 1c), confirming the conclusions of Bond *et al*⁹ and Bougeard *et al*,¹⁰ who showed an age difference of 7 and 10 years, respectively. The SNP309 G polymorphism is proposed to act as a genetic modifying factor in *TP53* mutation carriers. Because of the small numbers, our analysis has been carried out in a joint sample of Dutch and Finnish origin. Although the allele frequencies and genotype frequencies differ between the Dutch and Finnish controls, as shown in Table 1b, the genotype frequencies in the *TP53*-positive group did not significantly differ from the ratio-adjusted Dutch/Finnish control group, allowing a combined analysis.

Neither Bond *et al*⁹ nor Bougeard *et al*¹⁰ screened LFS and LFS-related patients without a *TP53* germline mutation. In our *TP53*-negative LFS and LFS-related group, the age of tumour onset for SNP 309 G allele patients and T/T patients was not significantly different. The presence of the SNP 309 G allele was therefore not shown to have a tumour accelerating effect in our *TP53*-negative LFS-related patients. However, it was striking to see that the percentage of the G/G genotype in the *TP53*-negative LFS and LFS-related patients is twice the percentage in the general population. These data indicate an association between homozygosity for SNP309 (G/G) and the occurrence of cancer, although not related to the age of onset. The fact that these patients were considered for counselling might well be due, in part, to the presence of the homozygous G allele, under the assumption of the SNP309 G polymorphism acting as a modifier or (additional) disease-causing factor. Within the *TP53*-negative group, there is no difference in the age of tumour onset in the homozygous SNP309 G carriers as compared to the T/T and T/G carriers. Although three out of four classical LFS family members show a G/G genotype, the family history does not seem to be specific for the homozygous SNP309 G carriers (Table 1).

Bond *et al*⁹ found the presence of SNP309 to be associated with earlier age of onset for sporadic STS, specifically for the homozygotes (G/G). In our *TP53*-negative patients, 13 patients developed STS as first

tumour, six patients showed a T/T genotype, five a T/G genotype and two a G/G genotype, the mean ages did not significantly differ between the G/G and T/G or T/T groups. Although both groups (our group of *TP53*-negative LFS and LFS-related STS patients and Bond's group of sporadic STS) are *TP53* negative, the difference in proportion of G/G and in family history of patients, in addition to the small numbers in both studies, could explain the discordance in age of onset.

In conclusion, our results confirm the association of the presence of SNP309 G with a significantly earlier age of onset of the first tumour in *TP53* germline mutation carriers. As such, the *TP53* combined *MDM2* SNP309 G germline mutation now becomes an established example of how a gene–gene interaction synergistically acts in cancer susceptibility. This association raises the question whether *MDM2* SNP309 G analysis should be embedded in clinical diagnosis of *TP53* mutation carriers. This remains controversial, especially because for many tumour types no early detection procedures are available.

Although the presence of an SNP309 G allele in *TP53*-negative LFS and LFS-related patients was not shown to be correlated with an earlier age of onset of tumours, the higher prevalence of *MDM2* SNP309 homozygous G/G carriers in the *TP53*-negative group may suggest that this allele contributes to the LFS phenotype. Before clinical genetic implementation, these observations need to be confirmed in a larger series of *TP53*-negative LFS and LFS-related patients.

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