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Chapter 4

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

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Abstract

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 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, i.e. 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 a 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 this allele contributes to cancer susceptibility in LFS and LFS-related families.

Introduction

Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome. The main tumour types of LFS include bone- and soft tissue sarcoma, 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 relative in the same lineage with any cancer under the age of 60 years [3]. Less stringent LFL criteria were formulated by Eeles et al. as two first- or second-degree relatives with typical LFS tumours at any age [4]. 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 a single nucleotide polymorphism 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 soft tissue sarcoma (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 a SNP309 G allele. Wilkening et al. showed that this polymorphism does not seem to play a role in familial breast cancer [11].

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 4 groups: 1) classic LFS [2], 2) LFL according to Birch [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. 36 *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 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, 4 stop mutations and 3 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'-TGTAACGACGCGCCAGTTCGGGAGTTCAGGGTAAAGGT-3', primer 2:

5'-CAGGAAACAGCTATGACCTCGGAACGTGTCTGAACTTG-3' in Amsterdam, primer 1: 5'-GTTTGTGGACTGGGGCTA-3', primer 2: 5'-CGGAACGTGTCTGAACTTGA-3' in Helsinki).

Table 1. SNP309 genotype in patients and controls

a)

| <i>(family history)</i> | <i>TP53 positive</i> SNP309 genotype | | | <i>TP53 negative</i> SNP309 genotype | | | | |
|---|---|-------------|-------------|---|-----------|-------------|-------------|-------------|
| | n | T/T | T/G | G/G | n | T/T | T/G | G/G |
| LFS | 11 | 2 | 8 | 1 | 4 | 1 | 3 | |
| LFL | 22 | 15 | 7 | | 29 | 15 | 8 | 6 |
| LFS-suggestive: | | | | | | | | |
| -Childhood sarcoma or brain tumour (<18 years) | 1 | | 1 | | 5 | 4 | 1 | |
| -At least 2 primary tumours | 1 | | 1 | | 17 | 10 | 2 | 5 |
| -2 first degree relatives with cancer (at least 1 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)

| <i>control cohorts</i> | Total (n) | <i>MDM2 SNP309 genotype</i> | | |
|--|-----------|-----------------------------|--------------|--------------|
| | | T/T n (%) | T/G n (%) | G/G 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 4 groups according to their (family) history.
 b) SNP309 genotype in Dutch and Finnish controls.

LFS = Li-Fraumeni syndrome fulfilling the classical criteria², LFL = Li-Fraumeni-like syndrome according to Birch³ or Eeles⁴

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 Chi-square test.

Results

The classification of the 108 LFS and LFS-related patients into 4 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 1 out of 36 (3%) patients. In *TP53* negative patients, T/G was found in 16 out of 72 (22%) patients, 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, $p = 0.02$), 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 1b, with breast cancer and soft tissue sarcoma (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 37.6 years 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, in the T/G and G/G group combined, the mean age was 33.3 years (Figure 1d, $p = 0.749$).

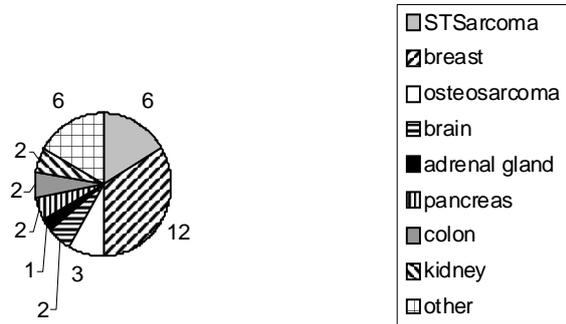
Discussion

We showed that the presence of a single nucleotide polymorphism 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 a SNP309 G allele as compared to the T/T

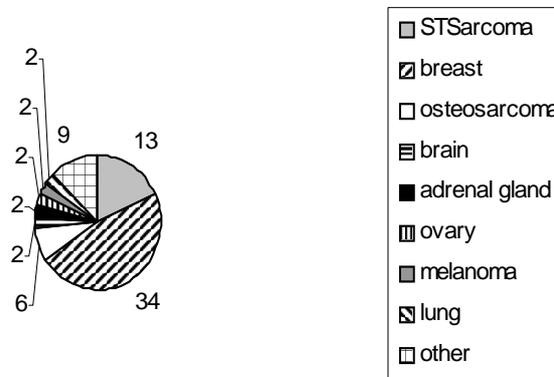
SNP 309 group (Figure 1c), confirming the conclusions of Bond et al. [9] and Bougeard et al.[10], who showed an age difference of 7 years and 10 years respectively. The SNP309 G polymorphism is proposed to act as a genetic modifying factor in *TP53* mutation carriers. Because of the small numbers, our analysis has been done 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.

Figure 1.
Prevalence of different tumour types and mean age of tumour onset

a)



b)



c)

| | | Mean age of onset | Median age of onset | Age range |
|------|------------|-------------------|---------------------|-----------|
| n=18 | T/T | 45.5 yr | 46.5 yr | 22-71 yr |
| n=18 | T/G or G/G | 29.7 yr | 29 yr | 1-59 yr |

d)

| | | Mean age of onset | Median age of onset | Age range |
|------|------------|-------------------|---------------------|-----------|
| n=39 | T/T | 32.1 yr | 36 yr | 0.5-64 yr |
| n=33 | T/G or G/G | 33.3 yr | 32 yr | 0.5-63 yr |

- a) First tumours in *TP53* mutation carriers (n=36)
 Other = stomach cancer, melanoma, mesothelioma, ovary cancer, lung and thyroid cancer
- b) First tumours in Dutch *TP53*-negative LFS and LFS-related patients (n=72)
 Other = bladder cancer, Hodgkin lymphoma, kidney cancer, thyroid cancer, basal cell carcinoma, acute myeloid leukemia, non-Hodgkin lymphoma, colon 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

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, though not related to 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 age of tumour onset in the homozygous SNP309 G carriers as compared to the T/T and T/G carriers. Although 3 out of 4 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 soft tissue sarcoma (STS), specifically for the homozygotes (G/G). In our *TP53* negative patients, 13 STS developed as first tumour, 6 patients showed a T/T genotype, 5 a T/G genotype and 2 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 a 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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Reference List

- (1) Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 1969 Oct;**71**(4):747-52.
- (2) Li FP, Fraumeni JF, Jr., Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW. A cancer family syndrome in twenty-four kindreds. *Cancer Res* 1988 Sep 15;**48**(18):5358-62.
- (3) Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Jones PH, Binchy A, Crowther D, Craft AW, Eden OB, Evans DGR, Thompson E, Mann JR, Martin J, Mitchell ELD, Santibanez-Koref MF. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res* 1994 Mar 1;**54**(5):1298-304.
- (4) Eeles RA. Germline mutations in the TP53 gene. *Cancer Surv* 1995;**25**:101-24.
- (5) Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990 Nov 30;**250**(4985):1233-8.
- (6) Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat* 2003 Mar;**21**(3):313-20.
- (7) Bachinski LL, Olufemi SE, Zhou X, Wu CC, Yip L, Shete S, Lozano G, Amos CI, Strong LC, Krahe R. Genetic mapping of a third Li-Fraumeni syndrome predisposition locus to human chromosome 1q23. *Cancer Res* 2005 Jan 15;**65**(2):427-31.
- (8) Houlston RS, Peto J. The search for low-penetrance cancer susceptibility alleles. *Oncogene* 2004 Aug 23;**23**(38):6471-6.
- (9) Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004 Nov 24;**119**(5):591-602.
- (10) Bougeard G, Baert-Desurmont S, Tournier I, Vasseur S, Martin C, Brugieres L, Chompret A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonaiti-Pellie C, Frebourg T. Impact of the MDM2 SNP309 and TP53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. *J Med Genet* 2005 Oct 28.
- (11) Wilkening S, Bermejo JL, Burwinkel B, Klaes R, Bartram CR, Meindl A, Bugert P, Schmutzler RK, Wappenschmidt B, Untch M, Hemminki K, Forsti A. The single nucleotide polymorphism IVS1+309 in mouse double minute 2 does not affect risk of familial breast cancer. *Cancer Res* 2006 Jan 15;**66**(2):646-8.