Li-Fraumeni syndrome, clinical and molecular genetics

Ruijs, M.W.G.

2010

document version
Publisher's PDF, also known as Version of record

Chapter 5

TP53 in clinical practice

Chapter 5.1
Late-onset common cancers in a kindred with an Arg213Gln *TP53* germline mutation

Chapter 5.2
Two *TP53* germline mutations in a classical Li-Fraumeni Syndrome family

Chapter 5.1

Late-onset common cancers in a kindred with an Arg213Gln *TP53* germline mutation

Mariëlle W.G. Ruijs[1,2], Senno Verhoef[1], Gea Wigbout[1], Roelof Pruntel[1], Arno N. Floore[1], Daphne de Jong[1], Laura J. van ´t Veer[1], Fred H. Menko[2]

[1]Family Cancer Clinic, The Netherlands Cancer Institute, Amsterdam; The Netherlands
[2] Department of Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam; The Netherlands

Familial Cancer 2006;5(2):169-74

Abstract
Li-Fraumeni syndrome (LFS) is an autosomal-dominant condition characterized by early-onset sarcoma, breast cancer and other specific tumour types. In most LFS kindreds germline *TP53* mutations have been identified. In general, *TP53* germline mutations are not associated with late-onset common cancers. We encountered a large kindred in which a wide spectrum of tumour types occurred, including melanoma, breast, ovarian, colorectal, stomach and renal cell cancer, without clear-cut early ages at onset of disease. An Arg213Gln *TP53* germline mutation was detected in 12 out of 15 affected family members whereas testing for other cancer susceptibility genes in selected patients was negative. *In vitro* testing indicated that the specific *TP53* mutation inactivates the protein transcriptionally. Our findings suggest that this *TP53* germline mutation is a causative factor in this family and that specific *TP53* germline mutations can be associated with relatively late-onset common cancers.

Introduction

Tumour susceptibility syndromes were defined, until recently, by their clinical phenotypes. Classical diagnostic criteria for Li-Fraumeni syndrome (LFS) are: a proband diagnosed with sarcoma before 45 years of age, a first-degree relative with cancer before 45 years of age, and another first- or second-degree relative in the same lineage with any cancer diagnosed under 45 or sarcoma occurring at any age [1]. Criteria for Li-Fraumeni-like syndrome (LFL) as defined by Birch et al. [2] are: a proband with any childhood cancer or sarcoma, brain tumour, or adrenocortical carcinoma diagnosed before 45 years of age, with one first- or second-degree relative with a typical LFS cancer (sarcoma, breast cancer, brain tumour, leukaemia, or adrenocortical carcinoma) diagnosed at any age, plus one first- or second-degree relative in the same lineage with any cancer diagnosed under age 60.

In 1990 germline mutations in the *TP53* tumour suppressor gene were shown to be causative of both LFS and LFL syndrome [3, 4]. Since then pathogenic *TP53* mutations – most of them missense mutations - have been found in up to 75% of LFS families and 40% of LFL kindreds [5-7].

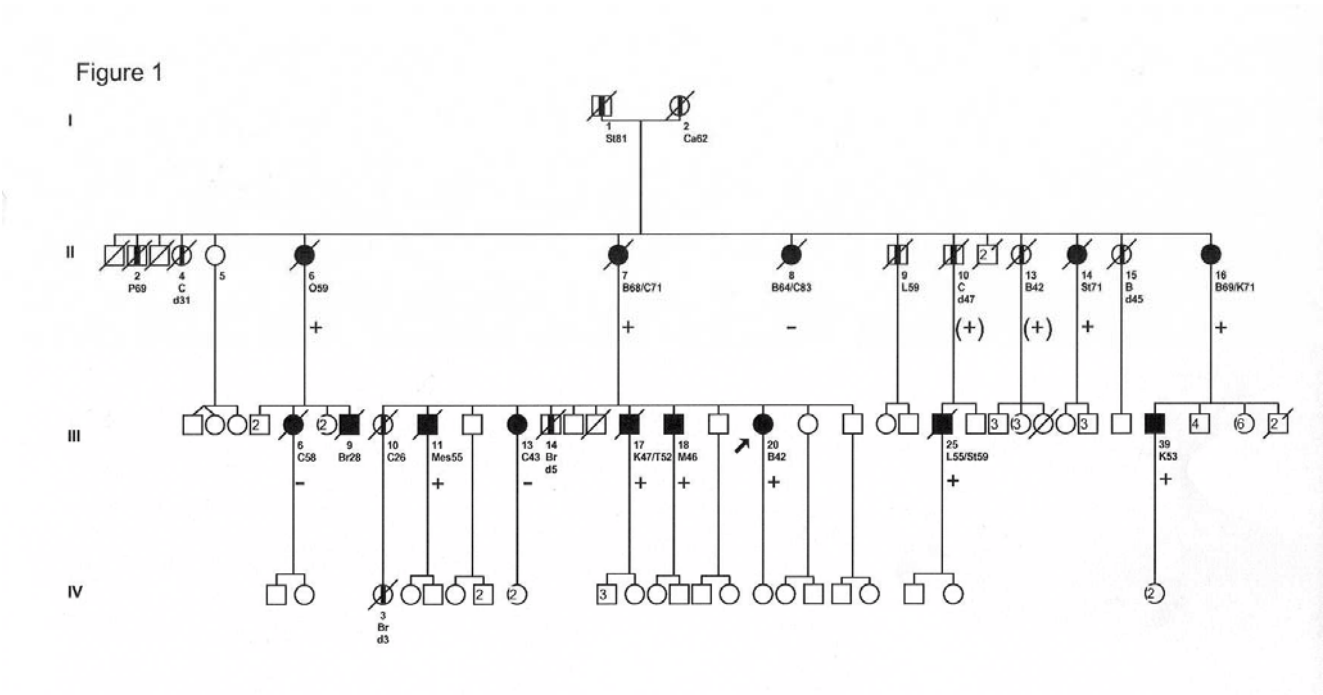
Recently, the spectrum of tumours in 28 LFS/LFL families with *TP53* mutations was evaluated by Birch et al. [8]. These authors found the following malignancies to be associated with germline *TP53* mutations: female breast cancer, tumours of the brain and spinal cord, soft tissue sarcoma, osteosarcoma (except Ewing´s sarcoma), adrenocortical carcinoma and Wilms´ tumour. To a lesser degree, carcinoma of the pancreas seemed related. Noticeably, there was no association with the majority of other common carcinomas (apart from breast cancer). In general, the ages at diagnosis of cancer in *TP53* mutation carriers are lower than for sporadic cancer. However, it has been suggested that the types of malignancies and ages at onset might be more variable than assumed thus far [9-11].

In any family with a novel *TP53* germline mutation the question arises whether the mutation is pathogenic or represents a non-pathogenic polymorphism. Arguments in favour of pathogenicity include segregation of the mutation with the cancer phenotype in the kindred, the type of mutation [12] and *in vitro* assays to determine its functional effect [13, 14]. Several authors have studied tumours from *TP53* mutation carriers for loss of the wild-type allele (loss of heterozygosity, LOH) and accumulation of *TP53* protein, with variable patterns of LOH and immunohistochemical staining of *TP53* protein in target organs [15, 16]. In general, the presence of LOH and accumulation of *TP53* protein strengthens the belief of a mutation being pathogenic, albeit the absence of LOH and *TP53* staining can not exclude pathogenicity.

We describe a cancer family in which a germline Arg213Gln *TP53* mutation was identified. Twelve out of 15 affected family members were mutation carriers. The carriers exhibited a variety of common cancers without a clear-cut early onset of disease.
We evaluated the tumour spectrum, the functional effect of the germline *TP53* mutation, somatic genetic alterations and the possible contribution of other genetic defects in this family.

Patients and methods

The index patient (Fig.1, III-20) developed unilateral breast cancer at the age of 42 years. Her maternal family history was positive for various types of malignancy. Medical data on affected family members, including surgical and pathology reports, was collected. Tumours for which tissue samples were available were reviewed. Informed consent for DNA-analysis of the *TP53* gene, and when applicable other genes, was obtained after *BRCA1, BRCA2* testing showed no mutation in the index patient. After the identification of a germline *TP53* mutation in the proband, almost all other cancer patients were tested for this mutation.



Pedigree of the family with the germline Arg213Gln *TP53* mutation

→ index patient; ■: male with documented malignancy; □: male unaffected; ●: female with documented malignancy; ○: female unaffected; ∅: deceased; Φ: diagnosis based on family history
Abd: abdominal cancer, B: breast cancer, Br: brain tumour, C: colorectal cancer, K: kidney (renal cell) cancer, L: Lung cancer, M: melanoma, Mes: mesothelioma, O: ovarian cancer, P: prostate cancer, St: stomach cancer, T: thyroid cancer; C42: age at diagnosis, d…: age at death
+: mutation carrier; (+): obligate mutation carrier; -: non-carrier

Selected family members were also tested for germline mutations in *CHEK2, MLH1, MSH2* and *MSH6*. DNA was isolated from peripheral lymphocytes according to standard procedures. From four deceased affected family members DNA was isolated from archival tissue samples. 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. Multiplex ligation-dependent probe amplification (MLPA) was performed on 5 samples, to exclude additional *TP53* deletions or duplications (*TP53* MLPA KIT of MRC-Holland) [17]. Details on primers and PCR conditions are available on request. The frequency of the identified *TP53* germline mutation was assessed in a control group of 135 anonymous blood donors, using denaturing gradient gel electrophoresis (DGGE).

The functional effect of the *TP53* germline mutation was evaluated in four family members – three *TP53* mutation carriers and one affected family member without the *TP53* germline mutation - by functional analysis of separated alleles in yeast (FASAY) using RNA isolated from blood lymphocytes [13, 14].

LOH (loss of heterozygosity) studies were performed on tumour tissue from five affected mutation-carriers. The presence or absence of the wild-type *TP53* allele was determined by DNA sequencing of *TP53* exon 6, the allele ratio of the normal versus mutated allele in the tumour compared to normal DNA. To determine whether the other *TP53* allele in the tumour was mutated by a different independent mutation, other codons were sequenced as well in one tumour showing LOH.

Immunohistochemical staining of the *TP53* protein was performed in tumour and normal tissues from nine affected mutation carriers, using the mouse monoclonal antibody DO7, according to standard procedures. (Dako, Glostrup, Denmark). Staining of the *TP53* protein stands for the presence of aberrant protein, in a normal situation immunohistochemical staining of the *TP53* protein is absent.

Microsatellite instability was evaluated in tumour tissue of 4 individuals affected by colorectal cancer using 5 microsatellite markers as described [18] and immunohistochemical staining of the *MLH1, MSH2* and *MSH6* proteins was performed in these 4 individuals.

Results

The pedigree data fulfil the criteria of Li-Fraumeni-like syndrome as defined by Birch et al. [2]. The pedigree of the LFL family is depicted in Figure 1, with a summary of the clinical data of affected family members given in Table 1. 10 out of 13 tested family members affected by cancer had a germline mutation in codon 213 of *TP53*, exon 6: nt 638 G>A, Arg213Gln, two additional affected family members were obligate carriers. The three patients who did not exhibit the gene defect on repeated examination were patient III-13, who had colorectal cancer at the age of 43 years, patient II-8, who had breast cancer at 64 and colorectal cancer at 83 years of age and patient III-6, who developed rectal cancer at the age of 58 years. The father of the index patient (partner of II-7) died without a previous history of cancer, there was no history of cancer in his family. No mutations were found in specific cancer susceptibility genes (*BRCA1*, *BRCA2*, *CHEK2, MLH1, MSH2* and *MSH6*) tested in selected family members (Table 1).

The mean age at diagnosis of *TP53* mutation carriers with a documented malignancy was 58.4 years (range 42-71 years). When we include the ages at diagnosis of patients for whom only family history data are available (I-1, I-2, II-2, II-4, II-9, II-10, II-13, II-15, III-10, III-14 and IV-3) or mutation analysis could not be performed (III-9), the mean age was 50,6 years (range 3-81 years). The mean age at diagnosis of the three affected non-carriers was 62 years (range 43-83 years). Of 36 unaffected family members tested, 10 mutation carriers were identified at a mean age of 41,4 years (range 21 to 53 years).

TABLE 1. CLINICAL DATA, *TP53* GERMLINE MUTATION ANALYSIS, *TP53* FUNCTIONAL TESTING (FASAY), MLPA AND INVESTIGATION OF ADDITIONAL GENES IN FAMILY MEMBERS WITH DOCUMENTED MALIGNANCIES

Pedigree number (Fig.1)	Tumour type (age at diagnosis, yrs)	Arg213Gln *TP53* germline mutation	FASAY	MLPA	Additional DNA mutation testing			MSI/ IHC* : *MLH1/ MSH2/ MSH6*
					BRCA 1/2	*CHEK2*	*MLH1/ MSH2/ MSH6*	
II-6	Ovarian carcinoma (59)	Carrier§						
II-7	Breast carcinoma(68) Colon carcinoma(71)	Carrier§						Stable/ + / + / +
II-8	Breast carcinoma(64) Colon carcinoma (83)	Non-Carrier§						Stable/ + / + / +
II-14	Stomach carcinoma cardia (71)	Carrier	Pos	Neg		Neg		
II-16	Breast mucinous carcinoma (69) Renal cell carcinoma (mixed papillary/clear cell) (71)	Carrier		Neg		Neg		
III-6	Rectal carcinoma (58)	Non-carrier					Neg	Stable/ - / + / +
III-9	Astrocytoma (28)	Not tested						
III-11	Mesothelioma (55)	Carrier		Neg		Neg		
III-13	Colon carcinoma (43)	Non-carrier	Neg	Neg		Neg	Neg	Stable/ + / - / -
III-17	Renal cell (papillary) carcinoma (47) Thyroid carcinoma (52)	Carrier	Pos					
III-18	Melanoma (46)	Carrier	Pos					
III-20	Breast carcinoma (42)	Carrier		Neg	Neg	Neg		
III-25	Lung carcinoma (55) Stomach carcinoma (59)	Carrier					Neg	
III-39	Renal cell (clear cell) carcinoma (53)	Carrier						

TABLE 2. LOSS OF HETEROZYGOSITY AND IMMUNOHISTOCHEMICAL STAINING OF TP53 PROTEIN IN TISSUES

Pedigree number (Fig.1)	Tumour type	Loss of wild-type *TP53* allele	Other somatic mutations	*TP53* staining pattern	
				Tumour tissue	Normal tissue
II-6	Ovarian cancer			Positive	± (sporadic cells)
II-7	Colon cancer	Yes		Positive	±
II-14	Stomach cancer			Positive	±
II-16	Breast cancer	No		Positive	Positive
III-11	Mesothelioma	No		Positive	Positive
III-17	Kidney cancer	No		Positive	±
III-18	Melanoma	Yes	No	Positive	±
III-20	Breast cancer			Positive	Positive
III-39	Kidney cancer			Positive	±

No *TP53* deletions or duplications were demonstrated by MLPA in 5 family members tested (Table 1). The Arg213Gln *TP53* mutation was absent in 135 control individuals. Six controls carried a known silent polymorphism, Arg213Arg, in the *TP53* gene.

The yeast-based assay (FASAY), which can distinguish inactivating mutations from functionally silent mutations in exons 4 to 10, showed that the mutated allele lacks biological transcriptional activity (Table 1).

Loss of the *TP53* wild type allele was found for 2/5 tumours from mutation-carriers (Table 2). In the two tumours showing LOH, all *TP53* exons were sequenced. Only in one sample, DNA was of sufficient quality to evaluate, no (additional) somatic mutation was found.

Immunohistochemical *TP53* staining was found for all 9 tumours from mutation-carriers, 3 of the 9 adjacent normal tissues stained positive, the other six showed sporadic positive cells.

There was no microsatellite instability in the 4 colorectal tumours tested, in 2 colon tumours all 3 MMR proteins stained positive, in 1 colon tumour *MSH2* and *MSH6* staining was negative and in the rectal tumour, *MLH1* staining was negative.

Discussion

In the family reported here a wide variety of tumours occurred. The pedigree data fulfil the criteria of Li-Fraumeni-like syndrome [2]. Several results suggest that the germline Arg213Gln *TP53* mutation is a causative factor for the cancer phenotype in this family: 1) this specific mutation was found previously in an LFS family [5] and has also been reported as somatic mutation in malignancies [5], 2) in the kindred under investigation the mutation was found in 12/15 cancer patients, 3) the specific mutation is located within codon 213, which is one of the 6 codons harbouring 46% of all *TP53* missense mutations [12], 4) an *in vitro* test demonstrated that the mutation has a functional effect, 5) the mutation was absent in healthy controls and 6) tests for other cancer susceptibility genes in selected patients were negative.

Many of the tumour types in the family we investigated are common cancers including melanoma, breast, ovarian, colorectal, stomach and renal cell carcinoma. There was no clear-cut early age at onset of disease. In fact, 79% of all documented malignancies of the *TP53* mutation carriers occurred after 50 years of age and 36% after 60 years of age. Some of the cancers may simple be sporadic due to environmental influences and chance, especially those malignancies which developed above the population median ages. The picture is somewhat biased towards adult tumours due to the fact that we were not able to confirm the two brain tumours occurring in childhood and the two colorectal tumours occurring in early adulthood. Additionally, no mutation analysis could be performed in the brain tumour occurring at 28 years of age (III-9). The complete pedigree data is suggestive for a predisposition not only for adult-onset but also for childhood-onset malignancies. The association of a *TP53* germline mutation with relatively late-onset common cancers has not been documented before [8, 11]. It has been suggested, however, that in some families with *TP53* mutations there may be a lower cancer risk and later onset of tumours suggesting that deleterious *TP53* mutations may be more frequent in the population than generally assumed [10].

The fact that we detected the *TP53* germline mutation in cancer patients is not sufficient proof that the cancer is in fact caused by the mutation. Therefore, we studied tumours of mutation carriers to investigate this issue. We found loss of heterozygosity for some tumours, immunohistochemical *TP53* staining for all cancers and *TP53* staining of normal tissue in three mutation carriers. Although these results should be interpreted with caution, they are in tune with the *TP53* germline mutation being an underlying cause of disease in this kindred (e.g., it is consistent with LOH found in about 50% of tumours of pathogenic *TP53* mutation carriers [19]). Family members who carried the *TP53* germline mutation, underwent screening procedures for occult malignancies, including renal ultrasound. This led to the diagnosis of renal cell cancer

in II-16 and III-39. So far, no other malignancies have been found by screening of mutation carriers.

The specific missense mutation in this family leads to relatively late onset malignancies, confirmed by the fact that 10 mutation carriers are without any malignancy. It must be noted however, that the average age of these healthy mutation carriers is significantly lower (independent samples t-test, p=0,013) than the average age at diagnosis of cancer is this family. If the penetrance of this mutation is reduced, the mutant protein product might still have some residual functional activity, although the functional test showed a lack of transcriptional activity. However, the only other family reported in the *TP53* mutation database [5] with the same mutation had, according to the limited information provided, early-onset malignancies and fulfilled the classic criteria for LFS. The fact that this specific mutation is only rarely found in published high penetrance LFS/LFL mutation families, could be explained by a low penetrance phenotype in other families not examined thus far. Our data are not in agreement with the studied genotype-phenotype correlations in LFS/ LFL families by Birch et al. [20], who found a higher penetrance and earlier age at onset for missense point mutations in the core DNA binding domain of *TP53* in comparison with families harbouring mutations leading to truncated or non-functional proteins.

The effect of the Arg213Gln *TP53* mutation in the family presented here may be modified by other genetic and/or environmental factors. Alternatively, the picture might be complicated by the existence of mutations in other cancer susceptibility genes. Due to the occurrence of breast, ovarian and colorectal cancer in the family we indeed tested selected patients for mutations in other susceptibility genes known to be associated with these tumour types: *BRCA1, BRCA2, CHEK2* and the DNA-mismatch repair (MMR) genes *MLH1, MSH2* and *MSH6*. *BRCA1* and *BRCA2* are associated primarily with breast and ovarian cancer, *MLH1, MSH2* and *MSH6* with HNPCC and *CHEK2* with breast and colorectal cancer [21]. No germline alterations in these genes were found. The colorectal cancers showed no microsatellite instability, in 2 tumours the immunohistochemical staining was abnormal but in three patients tested (including the two patients with abnormal staining in tumour tissue), no *MLH1, MSH2* or *MSH6* mutations were found. Therefore, there is no positive evidence for involvement of the MMR genes in these cancers [22]. *BRCA1* and *BRCA2* was only tested in the youngest breast cancer patient. The other breast cancer patients developed breast cancer at an older age in combination with a different malignancy, not typical for *BRCA1* or *BRCA2*. Renal malignancies reported in multiple family members have been associated with many syndromes including Von Hippel-Lindau syndrome and Birt-Hogg-Dubé syndrome. In this family there are no clinical signs which would suggest one of these syndromes.

On the basis of our observations, we propose that the detected mutation contributes considerably to the cancer risk in this large family, although not as severely as previously reported for Li-Fraumeni families or other Li-Fraumeni-like families. If more such families with a reduced penetrance and later average age of onset exist, they might remain unnoticed due to the generally applied restrictive policies for *TP53* mutation testing.
More research is needed to confirm the existence of (other) low penetrance *TP53* germline mutations.

Reference List

(1) 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.

(2) 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.

(3) 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.

(4) Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 1990 Dec 20;**348**(6303):747-9.

(5) Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 2002 Jun;**19**(6):607-14.

(6) Bougeard G, Brugieres L, Chompret A, Gesta P, Charbonnier F, Valent A, Martin C, Raux G, Feunteun J, Bressac-de Paillerets B, Frebourg T. Screening for TP53 rearrangements in families with the Li-Fraumeni syndrome reveals a complete deletion of the TP53 gene. Oncogene 2003 Feb 13;**22**(6):840-6.

(7) Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 2003 Mar;**21**(3):313-20.

(8) Birch JM, Alston RD, McNally RJ, Evans DG, Kelsey AM, Harris M, Eden OB, Varley JM. Relative frequency and morphology of cancers in carriers of germline TP53 mutations. Oncogene 2001 Aug 2;**20**(34):4621-8.

(9) Kleihues P, Schauble B, zur Hausen A, Esteve J, Ohgaki H. Tumors associated with p53 germline mutations: a synopsis of 91 families. Am J Pathol 1997 Jan;**150**(1):1-13.

(10) Varley JM, McGown G, Thorncroft M, James LA, Margison GP, Forster G, Evans DG, Harris M, Kelsey AM, Birch JM. Are there low-penetrance TP53 Alleles? evidence from childhood adrenocortical tumors. Am J Hum Genet 1999 Oct;**65**(4):995-1006.

(11) Nichols KE, Malkin D, Garber JE, Fraumeni JF, Jr., Li FP. Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomarkers Prev 2001 Feb;**10**(2):83-7.

(12) Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P, Eeles RA. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res 2003 Oct 15;**63**(20):6643-50.

(13) Ishioka C, Frebourg T, Yan YX, Vidal M, Friend SH, Schmidt S, Iggo R. Screening patients for heterozygous p53 mutations using a functional assay in yeast. Nat Genet 1993 Oct;**5**(2):124-9.

(14) Flaman JM, Frebourg T, Moreau V, Charbonnier F, Martin C, Chappuis P, Sappino AP, Limacher IM, Bron L, Benhattar J, Tada M, Van Meir EG, Estreicher A, Iggo RD. A simple p53 functional assay for screening cell lines, blood, and tumors. Proc Natl Acad Sci U S A 1995 Apr 25;**92**(9):3963-7.

(15) Sedlacek Z, Kodet R, Seemanova E, Vodvarka P, Wilgenbus P, Mares J, Poustka A, Goetz P. Two Li-Fraumeni syndrome families with novel germline p53 mutations: loss of the wild-type p53 allele in only 50% of tumours. Br J Cancer 1998 Apr;**77**(7):1034-9.

(16) Trkova M, Foretova L, Kodet R, Hedvicakova P, Sedlacek Z. A Li-Fraumeni syndrome family with retained heterozygosity for a germline TP53 mutation in two tumors. Cancer Genet Cytogenet 2003 Aug;**145**(1):60-4.

(17) Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002 Jun 15;**30**(12):e57.

(18) Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004 Feb 18;**96**(4):261-8.

(19) Varley JM, Thorncroft M, McGown G, Appleby J, Kelsey AM, Tricker KJ, Evans DG, Birch JM. A detailed study of loss of heterozygosity on chromosome 17 in tumours from Li-Fraumeni patients carrying a mutation to the TP53 gene. Oncogene 1997 Feb 20;**14**(7):865-71.

(20) Birch JM, Blair V, Kelsey AM, Evans DG, Harris M, Tricker KJ, Varley JM. Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. Oncogene 1998 Sep 3;**17**(9):1061-8.

(21) Meijers-Heijboer H, Wijnen J, Vasen H, Wasielewski M, Wagner A, Hollestelle A, Elstrodt F, Van Den Bos R, de Snoo A, Tjon AF, Brekelmans C, Jagmohan S, Franken P, Verkuijlen P, van den Ouweland A, Chapman P, Tops C, Moslein G, Burn J, Lynch H, Klijn J, Fodde R, Schutte M. The CHEK2 1100delC

Mutation Identifies Families with a Hereditary Breast and Colorectal Cancer Phenotype. Am J Hum Genet 2003 May;**72**(5):1308-14.

(22) Hendriks Y, Franken P, Dierssen JW, De Leeuw W, Wijnen J, Dreef E, Tops C, Breuning M, Brocker-Vriends A, Vasen H, Fodde R, Morreau H. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. Am J Pathol 2003 Feb;**162**(2):469-77.

Chapter 5.2

Two *TP53* germline mutations in a classical Li-Fraumeni Syndrome family

Mariëlle W.G. Ruijs[1,3,4], Liselotte P. van Hest[2,4], Anja Wagner[2], Conny A. van der Meer[2], Senno Verhoef[1], Laura J. van 't Veer[1], Hanne Meijers-Heijboer[2]

[1]Family Cancer Clinic, The Netherlands Cancer Institute, Amsterdam, The Netherlands
[2]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands
[3]Department of Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam, The Netherlands
[4]These authors contributed equally to this work.

Familial Cancer 2007;6(3):311-6

Abstract
Li-Fraumeni syndrome (LFS) is an autosomal dominantly inherited cancer predisposition syndrome characterized by a combination of tumors including sarcoma, breast cancer, brain tumors, adrenocortical carcinoma and leukemia. Germline mutations in the tumor suppressor gene *TP53* are associated with LFS. We present a family with LFS in which initially a novel germline *TP53* intron 5 splice site mutation was found. A second germline *TP53* mutation, the exon 7 Asn235Ser (704A→G) mutation, was detected in this family through pre-symptomatic DNA testing. This latter mutation has been reported repeatedly in the literature as a pathogenic mutation involved in LFS. We provide evidence for pathogenicity of the novel intron 5 splice site mutation, whereas this evidence is lacking for the exon 7 Asn235Ser (704A→G) mutation. Our findings emphasize the importance of performing additional tests in case of germline sequence variants with uncertain functional effects.

Introduction

Li-Fraumeni syndrome (LFS) was first described in 1969 [1] as a hereditary cancer predisposition syndrome characterized by the occurrence of bone and soft tissue sarcoma, breast cancer, brain tumors, adrenocortical carcinoma and leukemia. Germline mutations in the TP53 tumor suppressor gene on chromosome 17p13 were associated with LFS in 1990 [2]. About 75% of clinical LFS-families carry a TP53 germline mutation, and 40% of families with the less stringent criteria of Li-Fraumeni-like syndrome (LFL) [3]. So far, 283 different pathogenic germline mutations have been reported of which 74% are missense mutations and 4% splice site mutations [4].

LFS is a very rare disease, and therefore it is not surprising that thus far only one family with more than one germline mutation has been reported [5]. Quesnel et al. described a child with a rhabdomyosarcoma at two years of age and a brain tumor at age 10 who carried three different TP53 germline alterations (R290H on one allele and R156H/R267Q on the other allele). Individual analysis of each mutant indicated that they separately have either a weak mutant phenotype or no mutant phenotype at all. However, the R156H/R267Q double mutant had a strong mutant behavior [5, 6].

TP53 knock out mice are viable and are usually born without any observable gross defects, but then rapidly develop a variety of tumors, including sarcomas and other tumors commonly seen in LFS [7-9].

We here describe a classical LFS family with two germline *TP53* mutations; one novel splice site mutation, and one missense mutation that had been classified as a pathogenic germline mutation before.

Patients and methods

The family (Figure 1) was of Dutch ancestry and presented at the department of Clinical Genetics at Erasmus MC, Rotterdam. Family history data were confirmed through medical records and pathology reports. Informed consent was obtained from the patients or from a first degree relative in case the patient was deceased. DNA was isolated from peripheral lymphocytes according to standard procedures.

From deceased affected family members DNA was extracted from paraffin embedded blocks of the tumor. Screening for *TP53* germline mutations was performed by sequence analysis of all coding exons (2-11) and flanking intron-exon boundaries.

The functional effect of the germline mutations was examined by FASAY, a yeast-based assay studying the biological transcriptional ability of p53 [10].

In a control group of 150 anonymous blood donors the presence of both mutations was analyzed by denaturing gel electrophoresis (DGGE).

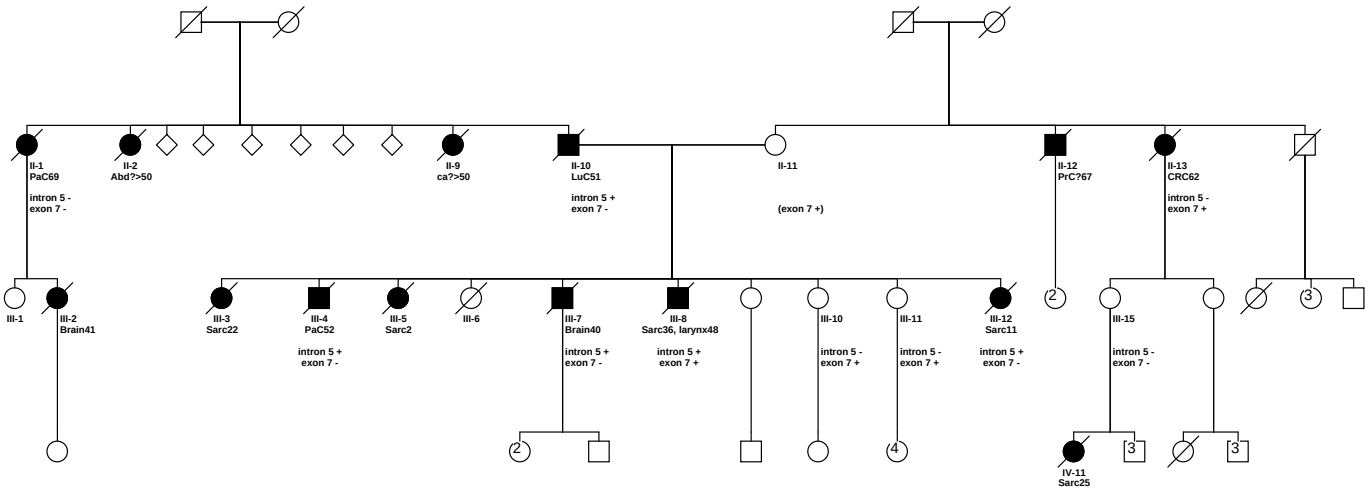
Immunohistochemical staining was performed in 7 affected family members to assess the presence of the p53 protein, using the mouse monoclonal antibody DO7, according to standard procedures. (Dako, Glostrup, Denmark).

Two splice site prediction programs were applied to examine the two mutations (NetGene2 Server and BDGP Splice Site Prediction by Neural Network [11, 12]).

The conservation throughout evolution of the mutational spots and the polarity status of the normal and mutated amino acids were studied.

Two *TP53* mutation-databases were checked: the IARQ *TP53* database [4] and the p53 Soussi mutation database cancer [4, 13].

Figure 1



Pedigree of the studied family

Square symbols indicate males, round symbols indicate females, diamond symbols indicate individuals of unknown sex, line across symbol means deceased individual. Tumor type and age at diagnosis of the tumors are indicated below the individual identifiers. When a question mark follows the diagnosis, this indicates affected individuals with diagnosis by family history, all other diagnosis are confirmed by pathology reports. PaC=pancreatic cancer, Abd=abdominal cancer, ca=cancer of unknown origin, LuC=lung cancer, PrC=prostate cancer, CRC=colorectal cancer, Brain=brain tumor, Sarc=sarcoma, Larynx=laryngeal carcinoma, intron 5 and exon 7 are the two different mutations tested for, + = mutation present, - = mutation absent.

Results

The pedigree is depicted in Figure 1 and further clinical details are outlined in Table 1. The family was clinically diagnosed with Li-Fraumeni syndrome. Based on the family history, it seemed likely that individual II-10 might have had a de novo *TP53* mutation, which he subsequently had passed to the majority of his offspring (individual II-10 and his offspring, further called core-family). Another hypothesis would be the presence of a germline mosaicism in individual II-10 or his spouse.

A novel intron 5 splice site mutation (IVS5-1 G→A) was found in the index patient (pedigree number III-4). This *TP53* mutation segregated with the disease; the 5 affected family members of the core family of whom DNA was available were carriers (pedigree numbers II-10, III-4, III-7, III-8 and III-12). The mutation was inherited paternally. The sister of the father who developed pancreatic cancer at 69 years of age (pedigree number II-1) did not carry the intron 5 mutation. One healthy individual was shown to be a carrier at the age of 29 years.

FASAY analysis showed that the intron 5 mutation lacks biological transcriptional activity (photo of data not shown).

In 300 control alleles the intron 5 germline mutation was not found. Immunohistochemical staining for p53 was negative in all tumors of the carriers of the intron 5 mutation. A phenomenon more frequently observed for *TP53* splice site mutations.

Two splice site prediction programs confirmed that the effect of this mutation is splicing out of exon 6, leading to a frameshift with a transcriptional stop early in exon 7. The IVS5-1 affects a 100% conserved splice acceptor site.

The exon 7 missense mutation, Asn235Ser (704A→G), was initially detected in a presymptomatic test of relative III-10. The mutation did not segregate with the disease as 4 out of 5 cancer patients from the core-family did not carry this mutation, while three healthy women were found carriers at the ages of 47, 51 and 83 years (pedigree numbers III-10, III-11 and II-11, the last one being an obligate carrier). The single affected carrier in the core family (pedigree number III-8) developed a sarcoma at 36 years and laryngeal carcinoma at 48 years. The Asn235Ser mutation turned out to be maternally transmitted as individual II-13 carried this mutation. She developed colorectal cancer at 62 years of age. Interestingly, the healthy daughter of this woman (pedigree number III-15), who's daughter died of a leiomyosarcoma of the face, tested negative for the exon 7 mutation.

FASAY analysis showed that the Asn235Ser mutation had normal transcriptional activity (photo of data not shown). In 300 control alleles this mutation was found once. Immunohistochemical staining for p53 was negative in tumor material of both affected mutation carriers, including the tumor of the patient with both germline mutations (pedigree number III-8).

Two different splice site prediction programs were unanimous in their prediction that the Asn235Ser mutation did not create a cryptic splice site. Orthologous, the Asparagine on this spot is conserved in mouse and rat but not in certain fish. Of note, some fish have a Serine instead of Asparagine at this spot. Paralogous, the Asparagine is conserved in *TP51*, *TP63* and *TP73*. The polarity of the amino acids Asparagine and Serine is similar.

The Asn235Ser mutation was reported 5 times in the *TP53* germline mutation databases screened.

Table 1. Clinical data and mutation analysis

Patient	Diagnosis	Age of onset	Intron 5: IVS 5-1G>A	Exon 7: Asn235Ser (704 A→G)
II-1	pancreatic adenocarcinoma	69	- (t)	- (t)
II-2	abdominal cancer, not verified	>50		
II-9	cancer, not verified	>50		
II-10	small cell lung carcinoma	51	+ (t)	- (t)
II-11	healthy, 83 years of age		not tested	not tested, obligate carrier
II-12	prostate cancer, not verified	67		
II-13	adenocarcinoma of colon transversum	62	- (t)	+ (t)
III-2	oligodendroglioma	41		
III-3	osteosarcoma of the humerus	22	material not suitable for analysis	material not suitable for analysis, suspicion wild type
III-4	pancreatic adenocarcinoma	52	+ (b)	- (b)
III-5	lymphosarcoma of the kidney	2		
III-6	this girl died at the age of twelve months, reason unknown			
III-7	anaplastic oligodendroglioma	40	+ (t)	- (t)
III-8	leiomyosarcoma right leg	36	+ (t)	+ (t)
	laryngeal carcinoma	48		
III-10	healthy, 51 years of age		- (b)	+ (b)
III-11	healthy, 47 years of age		- (b)	+ (b)
III-12	rabdomyosarcoma, retroperitoneal	11	+ (t)	- (t)
III-15	healthy, 67 years of age		- (b)	- (b)
IV-x	healthy, 29 years of age		+ (b)	- (b)
IV-11	leiomyosarcoma of the face	25	not tested	not tested

Not verified = diagnosis by family history, >50 = age of onset over 50 years, t = tested on DNA isolated from tissue, b = tested on DNA isolated from blood, + = mutation present, - = mutation absent, not tested = mutation analysis not performed

Discussion

We here present a LFS family with, at first sight, two pathogenic germline *TP53* mutations. Additional tests, however, showed that one of them was highly unlikely to be causative to the disease phenotype.

The novel *TP53* intron 5 splice site mutation (IVS5-1 G>A) was first detected. We considered this mutation causative to LFS in view of its co-segregation with the 5 affected cases in the core-family, its functional consequence (stop of transcription early in exon 7), and the 100% conservation of this splice acceptor site throughout evolution. We therefore offered the family presymptomatic testing for this mutation. In the process, surprisingly, a missense mutation in exon 7 Asn235Ser (704A→G) was detected, which had been classified before as a pathogenic germline mutation in multiple reports (see table 2)[14-18]. In order to provide meaningful diagnostic genetic testing within this family, we defined the predicted contribution of each of the mutations to the disease phenotype in more detail.

In summary, all data obtained on the novel intron 5 mutation pointed towards a causative association of this mutation with LFS within the core-family.

In contrast, the exon 7 Asn235Ser mutation did not segregate with disease in the core-family as only 1 out of 5 affected cases carried this mutation while three healthy individuals were found carriers at ages 47, 51 and 83 years. Of note, a third-degree relative of the core-family who died of leiomyosarcoma at age 25 years was also excluded as a carrier. The immunohistochemical staining of the two tumors of carriers of the Asn235Ser showed no expression of p53, while positive staining is commonly seen for a pathogenic missense mutation [19]. Although generally codon 100 to 300 is called the DNA binding domain, codon 235 is not directly involved in DNA-binding; it is located in between two domains that interact extensively to provide DNA contacts [20]. Therefore, this mutation was likely not to affect the DNA-binding properties of p53. Indeed, normal results, DNA-binding properties and transcription activation, were obtained by FASAY.

The Asn235Ser mutation has been reported in the germline five times before (table 2) [14-18]. None of the authors of these reports found the mutation in a classical LFS family. The authors unfortunately performed no functional assays, or determined its prevalence in healthy controls. Still, three out of these five reports classified the mutation as pathogenic. Diller et al. [14] and Auer et al. [17] based their conclusion on the fact that the mutation had been described as a somatic mutation in cancer before. Huusko et al. [18] described a LFL family with this mutation. The predominant cancer type in this family was breast cancer and no *BRCA1* or *BRCA2* mutation was identified. They claimed the mutation to be pathogenic on the basis of results in the tumors regarding loss of heterozygosity of the *TP53* locus and p53 immunohistochemistry, and on the fact that the mutation had been associated with cancer predisposition before by Diller et al.[14] and Cornelis et al.[15]. To note, the mutation did co-segregate; two out of three patients in this family were tested. Both were carriers, however, also two healthy adults were carrier. Ponten et al. [16] concluded the mutation to be a rare polymorphism. He found the Asn235Ser in a 72 year old male with two basal cell carcinoma's in which he found also two somatic *TP53* mutations. Cornelis et al. [15] recommended functional assays to determine the pathogenic nature of this mutation.

Soussi et al. [6] studied all somatic *TP53* mutations of the *TP53* mutation database (http://p53.curie.fr) by using very extended functional assays. They analyzed the transactivation activity of the mutations with respect to eight promotors and compared the activity to p53 wild type (wt) activity. The Asn235Ser mutation was described 14 times as a somatic mutation, 6 times in combination with another somatic mutation. The mean activity of Asn235Ser on 8 promotors was 86% of wt activity, well above their cut off point for pathogenic mutations (<20% of wt activity).

Table 2. Literature overview germline *TP53* exon 7 Asn235Ser (704 A→G) mutation

Author	Familial diagnosis	Index/ age at diagnosis	Family history	Segregation	LOH	IHC	*BRCA1* *BRCA2*	Remarks
Diller 1995	Single case	RMS 19 months	neg	-	ND	ND	-	follow up 19 yr
Cornelis 1997	HBOC	BrC 26 yr, recurrence 30 yr	mother OvC, sister BrC	ND	first tumor neg, recurrence pos	pos	ND	second somatic *TP53* mutation (G245V)
Ponten 1997	Single case	BCC 72 yr	neg	-	pos	neg	-	follow up 8 yr 2 other somatic mutations
Auer 1999	LuC family	LuC 39 yr	2 brothers LuC at 42 yr and 65 yr	ND	ND	ND	-	smoking 60 pack/yr
Huusko 1999	LFL-family	bil BrC 57 yr	sister BrC 43 yr, nephew Ep 19 yr	nephew Ep 19 yr: carrier two healthy adults: carrier	BrC: neg Ep: pos	BrC: pos Ep: neg	neg	

HBOC= hereditary breast and ovarian cancer, LuC= lung cancer, LFL-family= Li-Fraumeni-like family, RMS= rhabdomyosarcoma, BrC= breast cancer, yr= years, BCC= basal cell carcinoma, bil= bilateral, neg= negative, OvC= ovarian cancer, Ep= ependyoma, - = not applicable, ND= not done, LOH= loss of heterozygosity, IHC= immunohistochemical staining, pos= positive, *BRCA1*/*BRCA2*= *BRCA1* and *BRCA2* mutation analysis

Besides the fact that Asparagine is conserved in paralogs, all data provide evidence that Asn235Ser is a rare polymorphism or at best a low penetrance allele rather than a pathogenic mutation for LFS. It is remarkable that this mutation is found often in combination with another (either somatic or germline) mutation.
Our case report illustrates potential pitfalls in clinical genetic testing for cancer susceptibility. In order to provide optimal accurate risk assessment in cancer susceptibility testing, critical literature study is a prerequisite. We showed the importance of confirmation of carrier status of all affected family members once a pathogenic mutation within the family is found. Also, in case of sequence variants with uncertain functional consequences, additional tests are mandatory before genetic testing is offered in clinical settings.

Electronic-database information

NetGene2 server (splice site finder), www.cbs.dtu.dk./services/NetGene2/

BDGP splice site prediction by Neural Network, www.fruitfly.org/seq_tools/splice.html

IARQ *TP53* database, http://www-p53.iarc.fr/

p53 Soussi mutation database cancer, http://p53.free.fr/

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) 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.

(3) Varley JM. Germline TP53 mutations and Li-Fraumeni syndrome. Hum Mutat 2003 Mar;**21**(3):313-20.

(4) Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 2002 Jun;**19**(6):607-14.

(5) Quesnel S, Verselis S, Portwine C, Garber J, White M, Feunteun J, Malkin D, Li FP. p53 compound heterozygosity in a severely affected child with Li- Fraumeni syndrome. Oncogene 1999 Jul 8;**18**(27):3970-8.

(6) Soussi T, Kato S, Levy PP, Ishioka C. Reassessment of the TP53 mutation database in human disease by data mining with a library of TP53 missense mutations. Hum Mutat 2005 Jan;**25**(1):6-17.

(7) Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992 Mar 19;**356**(6366):215-21.

(8) Purdie CA, Harrison DJ, Peter A, Dobbie L, White S, Howie SE, Salter DM, Bird CC, Wyllie AH, Hooper ML, . Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. Oncogene 1994 Feb;**9**(2):603-9.

(9) Sah VP, Attardi LD, Mulligan GJ, Williams BO, Bronson RT, Jacks T. A subset of p53-deficient embryos exhibit exencephaly. Nat Genet 1995 Jun;**10**(2):175-80.

(10) Flaman JM, Frebourg T, Moreau V, Charbonnier F, Martin C, Chappuis P, Sappino AP, Limacher IM, Bron L, Benhattar J, Tada M, Van Meir EG, Estreicher A, Iggo RD. A simple p53 functional assay for screening cell lines, blood, and tumors. Proc Natl Acad Sci U S A 1995 Apr 25;**92**(9):3963-7.

(11) Hebsgaard SM, Korning PG, Tolstrup N, Engelbrecht J, Rouze P, Brunak S. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. Nucleic Acids Res 1996 Sep 1;**24**(17):3439-52.

(12) Celniker SE, Wheeler DA, Kronmiller B, Carlson JW, Halpern A, Patel S, Adams M, Champe M, Dugan SP, Frise E, Hodgson A, George RA, Hoskins RA, Laverty T, Muzny DM, Nelson CR, Pacleb JM, Park S, Pfeiffer BD, Richards S, Sodergren EJ, Svirskas R, Tabor PE, Wan K, Stapleton M, Sutton GG, Venter C, Weinstock G, Scherer SE, Myers EW, Gibbs RA, Rubin GM. Finishing a whole-genome shotgun: release 3 of the Drosophila melanogaster euchromatic genome sequence. Genome Biol 2002;**3**(12):RESEARCH0079.

(13) Beroud C, Soussi T. The UMD-p53 database: new mutations and analysis tools. Hum Mutat 2003 Mar;**21**(3):176-81.

(14) Diller L, Sexsmith E, Gottlieb A, Li FP, Malkin D. Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma. J Clin Invest 1995 Apr;**95**(4):1606-11.

(15) Cornelis RS, van Vliet M, van de Vijver MJ, Vasen HF, Voute PA, Top B, Khan PM, Devilee P, Cornelisse CJ. Three germline mutations in the TP53 gene. Hum Mutat 1997;**9**(2):157-63.

(16) Ponten F, Berg C, Ahmadian A, Ren ZP, Nister M, Lundeberg J, Uhlen M, Ponten J. Molecular pathology in basal cell cancer with p53 as a genetic marker. Oncogene 1997 Aug 28;**15**(9):1059-67.

(17) Auer H, Warncke K, Nowak D, Koops F, Jaeger U, Kurzbauer R, Ruediger HW. Variations of p53 in cultured fibroblasts of patients with lung cancer who have a presumed genetic predisposition. Am J Clin Oncol 1999 Jun;**22**(3):278-82.

(18) Huusko P, Castren K, Launonen V, Soini Y, Paakkonen K, Leisti J, Vahakangas K, Winqvist R. Germ-line TP53 mutations in Finnish cancer families exhibiting features of the Li-Fraumeni syndrome and negative for BRCA1 and BRCA2. Cancer Genet Cytogenet 1999 Jul 1;**112**(1):9-14.

(19) Lane DP. On the expression of the p53 protein in human cancer. Mol Biol Rep 1994 Jan;**19**(1):23-9.

(20) Cho Y, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 1994 Jul 15;**265**(5170):346-55.