## Li-Fraumeni syndrome, clinical and molecular genetics

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Chapter 1

# General introduction and outline of the thesis

## 1.1 Li-Fraumeni syndrome

### 1.1.1. The clinical picture

More than forty years ago Frederic P. Li and Joseph F. Fraumeni studied the possible association between childhood-onset sarcoma and breast cancer. Their studies were based on the referral of two cousins who both developed rhabdomyosarcoma in childhood. Both children had one parent who developed cancer (acute myelocytic leukaemia at 24 years and female breast cancer at 28 years, respectively). Based on these probands a novel familial syndrome was suspected. Subsequently, the medical charts of 280 children treated for rhabdomyosarcoma and the records of 418 children who died of rhabdomyosarcoma were reviewed. The investigators found three additional families in which a sib pair had developed rhabdomyosarcoma. In these three additional families identified, two of the mothers developed breast cancer at 22 and 24 years respectively, whereas in the third family soft tissue sarcoma at 22 years of age was observed. The four families were described by Li and Fraumeni, as "Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?" in which they suggested the existence of a new familial cancer syndrome [1]. In 1982 this familial syndrome was named "Li-Fraumeni syndrome" [2].

**Figure 1.**

**Dr. Frederick P. Li**

**Dr. Joseph F. Fraumeni**

Li-Fraumeni syndrome (LFS) is now defined as a rare hereditary cancer predisposition syndrome (Online Mendelian Inheritance in Man #151623), characterised clinically by specific types of cancer, in particular early-onset bone and soft tissue sarcoma, breast cancer, brain tumours, adrenocortical carcinoma and leukaemia. Typically, patients with LFS develop multiple primary tumours, synchronously, or in the course of their lifetime [3]. The inheritance pattern of LFS is autosomal dominant. Since most tumours that are associated with LFS show somatic mutations in the tumour suppressor gene *TP53*, this gene was selected as a plausible candidate gene and in 1990 the involvement of *TP53* germline mutations in LFS families was established [4]. Besides the typical LFS tumours, other cancers also occur in *TP53*-positive families, according to Birch et al. the most notable being Wilms' tumour, phyllodes tumour, pancreatic cancer and neuroblastoma [5].

### 1.1.2 The role of *TP53* germline mutations

Before the identification of the *TP53* gene, diagnostic criteria for LFS were based on pedigree information. The criteria proposed by Li and Fraumeni [6] are given in Table 1a.
However, after the identification of *TP53,* germline mutations in this gene were also identified in families not meeting these LFS criteria. For this reason less restrictive criteria were proposed by Birch et al. [7] for Li-Fraumeni-like syndrome (LFL) (Table 1a). Currently, in about 75% of LFS and 40% of LFL families a causative germline *TP53* mutation is found [8].

**Table 1a.**
**Clinical criteria for LFS/LFL**

| | |
|---|---|
| Classical LFS criteria<br>Li et al. [6] | - a proband with sarcoma diagnosed under the age of 45 years<br>AND |
| | - a first-degree relative with any cancer under 45 years<br>AND |
| | - another first- or second-degree relative with either cancer under 45 years or a sarcoma at any age |
| LFL criteria<br>Birch et al. [7] | - a proband with any childhood cancer or sarcoma, brain tumour or adrenocortical tumour under the age of 45 years<br>AND |
| | - a first- or second degree relative with a typical LFS cancer at any age<br>AND |
| | - a first- or second degree relative in the same lineage with any cancer under 60 years |

**Table 1b.**
**Chompret criteria for *TP53* germline mutation testing**

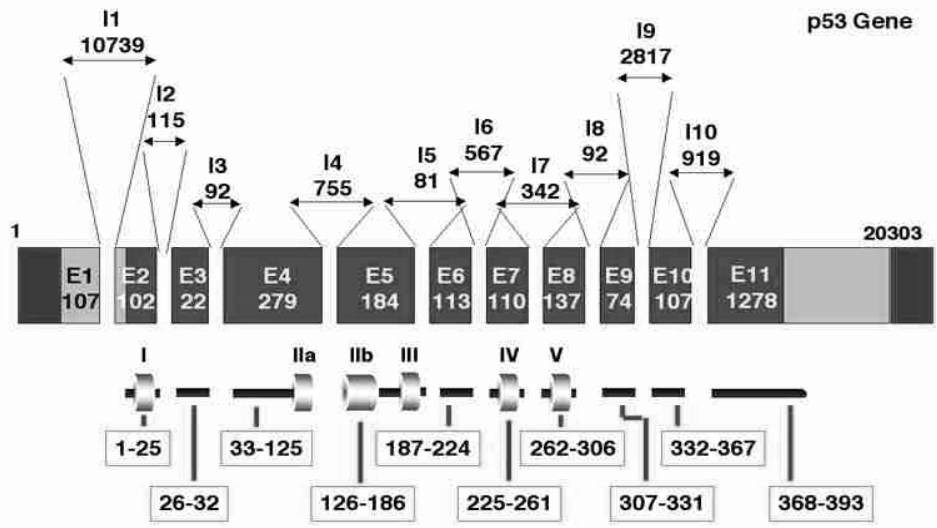
| | |
|---|---|
| Chompret criteria for LFS<br>Chompret et al. [9] | - a proband affected by a narrow spectrum cancer (sarcomas, brain tumours, breast cancer and adrenocortical carcinoma) before 36 years<br>and at least one first or second degree relative affected by a narrow spectrum tumour (other then breast cancer if the proband is affected by breast cancer) before 46 years or multiple primary tumours<br>OR |
| | - a proband with multiple primary tumours two of which belong to the narrow spectrum and the first of which occurred before 36 years<br>OR |
| | - a proband with adrenocortical carcinoma whatever the age of onset and family history |
| 2009 version of Chompret criteria for LFS<br>Tinat et al. [10] | - a proband with a tumour belonging to the LFS tumour spectrum (soft tissue sarcoma, osteosarcoma, brain tumours, pre-menopausal breast cancer, adrenocortical carcinoma, leukaemia, lung bronchoalveolar cancer) before 46 years<br>and at least one first or second degree relative with an LFS tumour (except breast cancer if the proband is affected by breast cancer) before 56 years or multiple primary tumours<br>OR |
| | - a proband with multiple primary tumours two of which belong to the LFS tumour spectrum and the first of which occurred before 46 years<br>OR |
| | - a proband with adrenocortical carcinoma or choroid plexus tumor, irrespective of family history |

LFS and LFL are defined by the occurrence of early-onset cancer in multiple family members. However, in addition to LFS and LFL families, *TP53* germline mutations were also detected in some "sporadic" patient with early-onset and/or multiple cancers and a negative family history for cancer. Therefore, in 2001, Chompret et al. developed novel criteria for *TP53* mutation testing [9] (Table 1b). These authors predicted that using these criteria *TP53* germline mutations would be detected in about 20% of cases. In 29% of the families selected on the Chompret criteria a germline *TP53* alteration could be detected in a series of French families [11]. Mutations were also found in some cases which did not fulfil these criteria, for example choroid plexus carcinoma. Therefore, the criteria for *TP53* mutation testing were updated in 2009 [10] by including lung bronchoalveolar cancer in the LFS tumour spectrum and sporadic adrenal cortical carcinoma irrespective of family history. In addition, the age limits were revised (see 2009 version of the Chompret criteria for LFS, Table 1b). Most LFS/LFL families will fulfil the Chompret criteria.

### 1.2 The *TP53* gene and its function

The *TP53* gene (Tumour Protein p53) is localised on the short arm of chromosome 17, 17p13.1 and approximately 20 kilobases (kb) in length, giving rise to a 2.8-kb mRNA transcript comprising 11 exons and encodes a 53-kD nuclear phosphoprotein of 393 amino acids. The protein was named p53 after its relative molecular weight. The gene has five highly conserved domains, I-V. Conserved domain I is responsible for transactivation properties, the remaining domains II-V make up the core DNA-binding domain, encoded by amino acids 100-293 (Figure 2).

**Figure 2. Organisation of the *TP53* gene.**



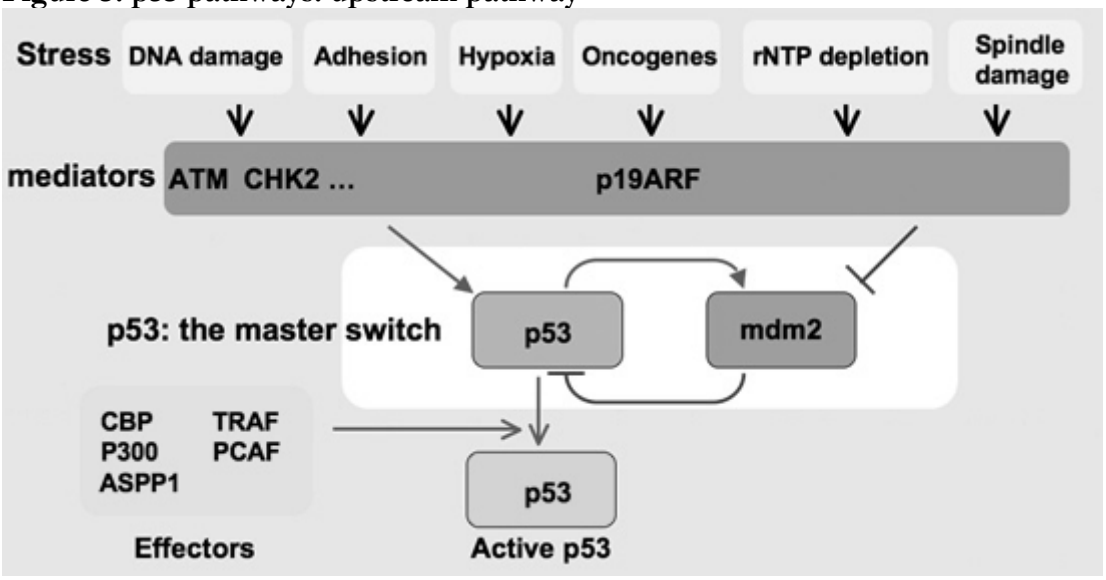

**The TP53 Web Site, last updated October 2008, http://p53.free.fr/p53_info/p53_gene.html**
**E=exon, I-V=domain I-V**

Wild type p53 has a half life of 6-20 minutes in normal cells, the mutant protein is more resistant to proteolysis and its half life is therefore longer (about 2-12 hours). However, these 'stable' mutant proteins are functionally inactive and they can even capture the wild-type p53 into the mutant conformation [12]. The *MDM2* gene product forms a complex with the p53 protein and inhibits p53-mediated transactivation [13].
*MDM2* is the key regulator of the level of p53 by acting as an ubiquitin ligase ensuring that p53 is a short-lived protein and masking its trans-activation domain. The stabilisation of p53 requires reduced *MDM2* binding for example through the activation of *CHEK2* or *ATM* in response to DNA damage.

At first, the *TP53* gene was thought to be a cellular oncogene, because of its ability to immortalize cells and to transform primary fibroblast cultures in cooperation with the *RAS* gene, another oncogene. Subsequently, it was observed that the presumed 'wild type' *TP53* proteins, acting as an oncogene, were in fact mutant. In addition, loss of the wild type allele has been associated with mutation of the other allele in many cancers [14]. The *TP53* gene was therefore reclassified as a tumour suppressor gene. Furthermore, *TP53* was shown to be a frequently mutated gene in human tumours, *TP53* somatic mutations are found in most types of sporadic human cancers at various frequencies (5-70%) [15]. The *TP53* gene has been termed the 'guardian of the genome' [16]. *TP53* is a checkpoint control gene functioning in response to genotoxic stresses including DNA damage (for example by radiation, see Figure 3) nucleotide depletion and hypoxia. Under those circumstances, the p53 protein accumulates in the nucleus and is activated as a transcription factor [17] leading to either a delay in cell cycle progression in G1 close to S

phase or in G2 before mitosis to allow time for DNA repair or initiation of apoptosis, a program of cell death that also depends on *TP53*.

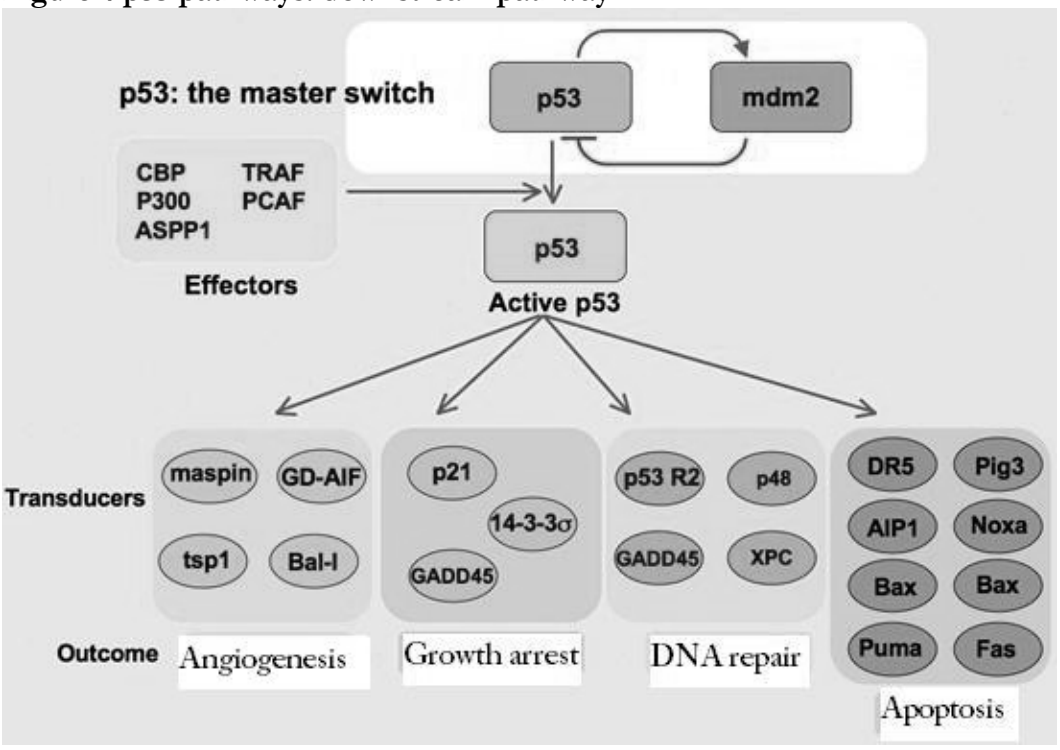
**Figure 3. p53 pathways: upstream pathway**




**The TP53 Web Site, last updated October 2008, http://p53.free.fr/p53_info/p53_Pathways.html**

Activated p53 protein influences (positively or negatively) the expression of more than 150 genes that mediate arrest of cell cycle progression or apoptosis. By this mechanism the *TP53* gene protects against accumulation of genetic alterations, induced by various stress signals. As a transcription factor, *TP53* may regulate the expression of many genes, for example see figure 4.

**Figure 4 p53 pathways: downstream pathway**




**The TP53 Web Site, last updated October 2008, http://p53.free.fr/p53_info/p53_Pathways.html**

The various stress signals that increase the level of p53 include temperature change and DNA damage by UV radiation. Shi et al. [18] show that population differences in the frequencies of two polymorphisms in the p53 tumour suppressor pathway, p53 codon 72 (proline or arginine) and *MDM2* SNP309 (T>G), are linked to the environmental stresses, winter temperature and UV radiation, in East Asian populations. Previous studies showed that these two polymorphisms are differentially distributed in different ethnic populations. The p53 codon 72 Arg allele and the *MDM2* SNP309 G allele are more common in northern Europeans than in Africans or African-Americans [19-21]. It was hypothesised that these alleles were latitude dependent. Shi et al. [18] confirmed that the frequency of p53 Arg72 was associated with latitude. This latitude dependency was shown to be tightly associated with winter temperature. Although no latitude dependency of the *MDM2* SNP309 locus could be demonstrated in these East Asian populations, high frequency of the G/G genotype was associated with low UV radiation strength.
The occurrence of these two polymorphisms in *TP53* germline mutation carriers was shown to be related to an earlier age of tumour onset [22, 23]

Two homologues of p53 have been identified, p73 [24] and p63 [25]. All three genes regulate cell cycle and apoptosis after DNA damage [26]. In cancer p53 is frequently mutated and mice lacking p53 (p53-/-) are prone to spontaneous tumours within 6 months [27]. Neither p73 nor p63 are frequently mutated in cancer, and mice lacking p73 or p63 are not prone to spontaneous tumours [28]. In contrast, Flores et al. [29] did show that p63+/- and p73+/- mice develop spontaneous tumours and mice heterozygous for mutations in both p53 and p63 or p53 and p73 displayed an higher tumour burden compared to p53+/- mice.
Heterozygous mutations in the p63 gene cause at least six different syndromes with various combinations of ectodermal dysplasia, orofacial clefting and limb malformations [30].

## 1.3 *TP53* mutations

### *1.3.1 Mutation detection methods*

In 1990, using a candidate gene approach, germline *TP53* mutations were found in five LFS families [4]. Because subsequently many mutations were found in exons 5-8, these were the exons mostly screened. In 1995 it was shown that mutations can be found scattered throughout the entire gene [31]. Mutation detection is now mostly performed by sequencing all coding exons and flanking intron-exon boundaries. In 2003 it was recommended to include the search for larger deletions or duplications [32], at least in classical LFS families, although only two larger rearrangements were described so far; a deletion of exon 10 and a deletion of the entire *TP53* gene [32].

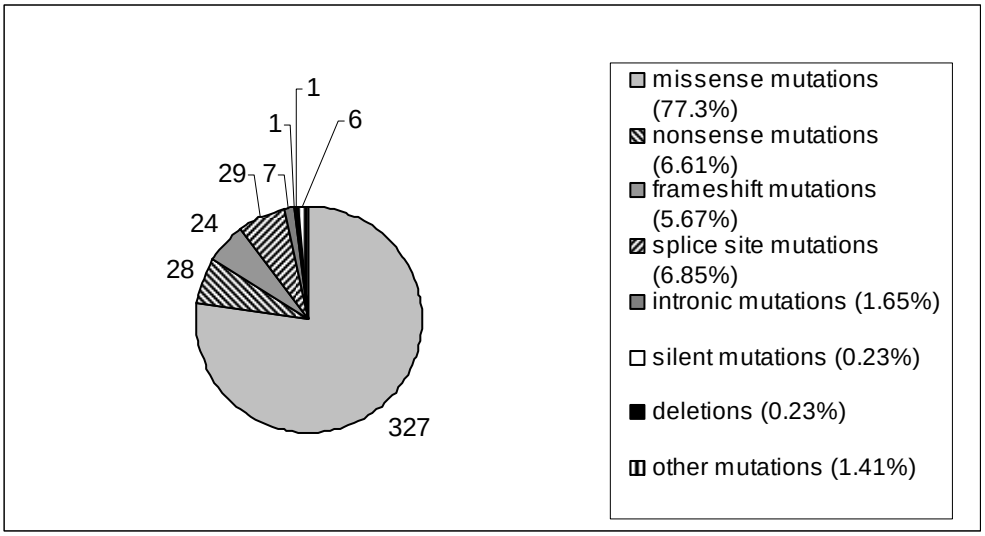
### *1.3.2 De novo mutation rate*

It is difficult to determine the de novo mutation rate of the *TP53* gene, because DNA analysis is mostly performed in pedigrees with a strong family history for cancer. Chompret et al. [33] tested 268 children with any solid tumour for *TP53* germline mutations and found 17 mutation carriers. In all of them, both parents could be tested, and 4 out of 17 *TP53* germline mutations (24%) were found de novo, none of these four families fulfilled the LFS or LFL criteria. Gonzalez et al. [34] published their results of 341 patients with early onset cancer in which 75 *TP53* mutation carriers were detected. Five de novo mutations were detected, an additional ten *TP53* germline mutations were likely to be de novo by family history. The de novo rate in *TP53* germline mutations would be 7-20%.

### *1.3.3 Pathogenic TP53 germline mutation spectrum*

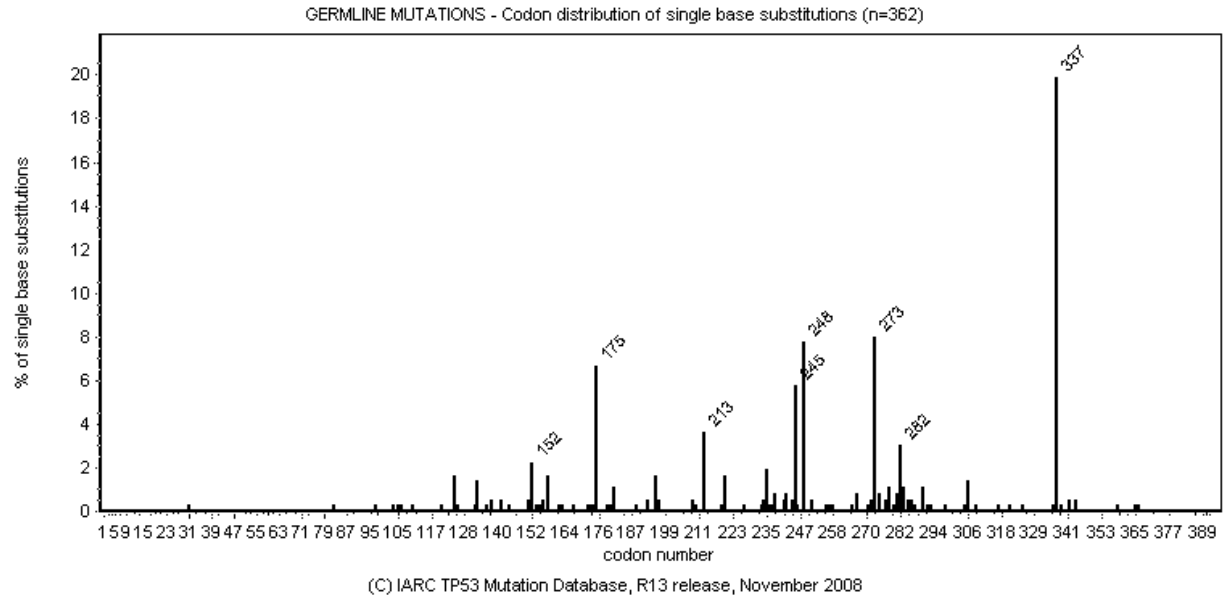
Currently, 423 germline *TP53* mutations in 419 families have been identified, most frequently missense mutations (77.3%), followed by nonsense (6.61%) and frameshift (5.67%) mutations (Figure 5, IARC Mutation Database, R13 release, November 2008, [35]). The high proportion of missense mutations indicates that the p53 mutant protein in cells has some selective advantage in carcinogenesis. It was shown that the mutant p53 can inhibit the function of the wild type p53 in a dominant-negative manner by forming a tetrameric complex of which the DNA binding capacity is abolished [12]. Besides a dominant-negative function, some mutant p53 proteins have acquired a gain of function, defined as the ability to augment cell proliferation in the absence of endogenous wild-type p53. For example, by transactivation of p53-independent target genes that mediate various oncogenic effects [36].

**Figure 5. Germline mutations – mutation type / 423 mutations**



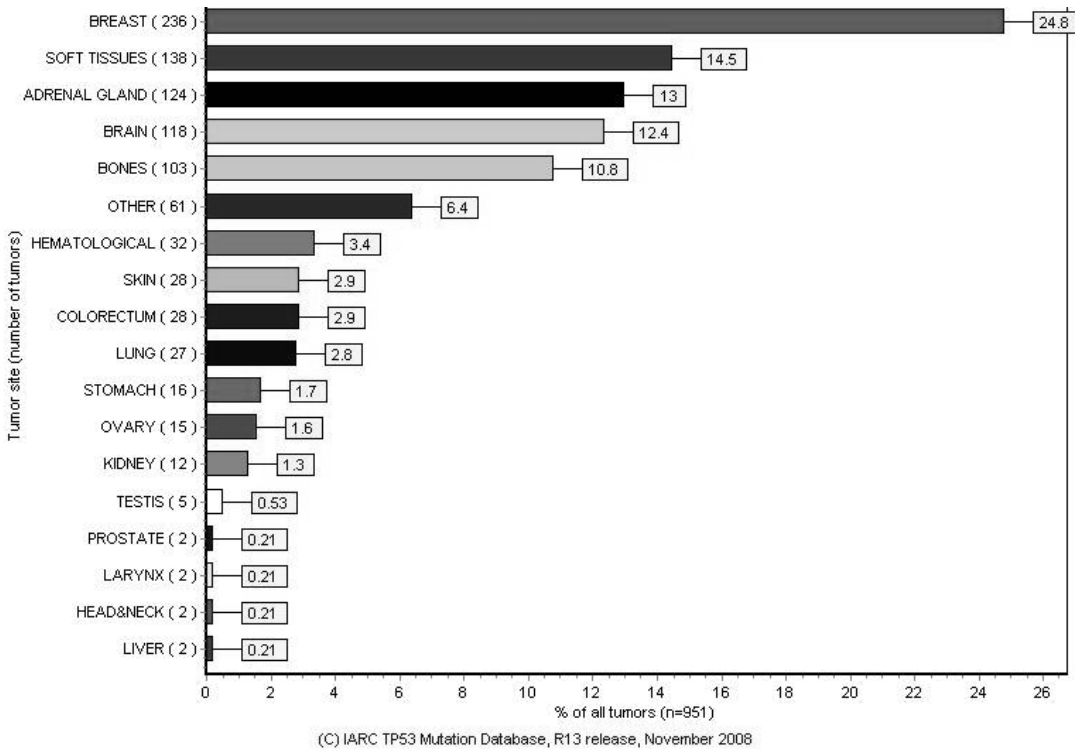
The mutations are detected throughout the entire gene, although some codons are more frequently affected than others (Figure 6, IARC Mutation Database, R13 release, November 2008, [35]).

**Figure 6. Germline mutations – codon distribution**




The tumours that are associated with *TP53* germline mutations and their relative frequencies are shown in Figure 7 (IARC Mutation Database, R13 release, November 2008, [35]). The most frequently developed tumours in *TP53* mutation carriers are breast cancer, adrenal gland tumours, sarcoma (soft tissue and bone) and brain tumours.

**Figure 7. Types and relative frequencies of tumours associated with *TP53* germline mutations**



### *1.3.4 Genotype-phenotype correlations*

In Li-Fraumeni syndrome patients many types of mutations are scattered throughout the *TP53* gene. If the type of mutation or the position of the mutation within the gene sequence has influence on cancer risks, this might be of importance in genetic counselling. Nearly 250 distinct germline *TP53* mutations have been described in the literature [8]. A database of *TP53* mutations has been established and can be accessed at www.iarc.fr/p53 [35]. So far, 423 *TP53* germline mutations have been identified, most frequently missense mutations (77.3%) and located in the DNA binding domain (about 90%). Studies on genotype-phenotype correlations have focussed on differences in age of onset, (predominant) tumour type and penetrance of different *TP53* germline mutations.

In a series of individuals with childhood adrenal cortical carcinoma (ACC) and no significant cancer family histories, 35 of 36 children were found to have the identical R337H germline mutation. Only one of the 126 first-degree relatives was affected with cancer (lung cancer), in 50 first-degree relatives *TP53* mutation analysis was performed, 24 mutation carriers were detected. Differences in intragenic polymorphic markers demonstrated that at least some mutant alleles arose independently. Since no evidence was found for a founder effect and a cancer family history was absent, the authors suggested that the R337H mutation is a low-penetrance *TP53* allele [37]. Figueiredo et al. [38] showed a penetrance of ACC associated with the *TP53* R337H of 10%, by testing 40 children with ACC and their family members. The R337H mutation acting as a low-penetrance *TP53* allele was challenged in 2006 by Achatz et al. [39] who described 6 Brazilian families that fulfilled the LFS or LFL criteria and presented with a wide tumour spectrum including breast cancer, brain tumours, STS and ACC. Other potential low-penetrance *TP53* mutations, Pro152Leu and Arg158His, have been reported among individuals with childhood ACC with non-contributory family histories [40]. Again this can be challenged by finding these mutations in LFL families as mentioned in the IARC mutation database, www.iarc.fr/p53. The latter mutation is found in our series in a LFS and LFL family, see chapter 2.

Birch et al. [41] investigated cancer incidence in 34 Li-Fraumeni families, in 19 of these families a germline *TP53* mutations were found. Families with germline missense mutations in the core DNA binding domain were characterised by a higher cancer incidence and earlier age at diagnosis, especially of breast cancer and brain tumours, compared with families carrying protein truncating or other inactivating mutations.

Olivier et al. [42] collected 265 LFS/LFL families, 223 of these families carried a *TP53* germline mutation. In the mutation carriers, brain tumours were associated with missense *TP53* mutations located in the DNA binding loop that contact the minor groove of DNA (codons 164 to 194 and 237 to 250). Adrenal gland carcinomas were associated with missense mutations located in the non DNA binding domain. Differences in age at onset were seen for breast cancer and brain tumours. For breast cancer significant difference was seen between missense mutations in the DNA binding domain and missense mutations outside of the DNA binding domain. The mean age of tumour onset was 32 years and 42 years, respectively. For brain tumours a significant difference was seen between null and non-null mutations (mean age of tumour onset 9 years and 25.5 years, respectively).

Bougeard et al. [11] collected 82 *TP53* positive families and found a 9 year earlier tumour onset in patients harbouring *TP53* missense mutations as compared to other types of alterations.

In conclusion, the most consistent genotype-phenotype correlation is found for carriers of missense mutations in the *TP53* gene showing an earlier age of onset of tumours and a higher cancer incidence compared to other types of mutations. For brain tumours an earlier age of onset was seen for null mutations compared to non-null mutations. Also the position of the mutation can be relevant, missense mutations in the non DNA binding domain were associated with the development of ACC. Missense mutations in the DNA binding domain showed an earlier age of onset for breast cancer than missense mutations outside the DNA binding domain.

### *1.3.5 Polymorphisms in the TP53 gene.*

An identified *TP53* germline mutation can be classified as a pathogenic mutation or a variant of unknown significance. Although polymorphisms are expected to be phenotypically silent, some studies investigated *TP53* variants that might affect cancer risk, as discussed below.

The 13964G>C mutation in intron 6 of the *TP53* gene was defined as a polymorphism of unclear significance by Buller et al. [43]. In a series of individuals with breast cancer, the 13964G>C mutation was identified in three of 42 breast cancer patients with a strong family history of breast cancer, making it yet another possible low-penetrance mutation [44].

Whibley et al. [45] provide an overview of *TP53* polymorphisms in 2009. 90% of the polymorphisms in *TP53* occur in the non-coding sequences. Only one intronic polymorphism, a 16 bp insertion in intron 3, has been associated with an increase in the risk of ovarian cancer [46] breast cancer [47], lung cancer [48] and colorectal cancer [49]. However, the close proximity of this polymorphism to the well known codon 72 polymorphism in exon 4, which will be discussed later, might partly explain the proposed association of this allele with cancer. 19 exonic polymorphisms have been reported, 8 are synonymous polymorphisms (without amino-acid change), 11 are non-synonymous (with amino-acid change). R72P is a frequently found non-synonymous *TP53* polymorphism that is worth discussing, specifically because many studies have looked at its effect. Codon 72 has either the CCC sequence, which is the ancestral form and encodes proline (p53-P72), or CGC, which encodes arginine (p53-R72). Several meta-analyses have been published of breast [50], gastric [51] and lung cancer [52], and these do not support a role for this polymorphism in the risk of developing these cancers. A meta-analysis of studies investigating the R72P polymorphism in cervical cancer reported some evidence of association. Women who carried two copies of arginine were at increased risk compared to those carrying one copy [53]. However, there was no difference in the risk between women who carry two arginine (Arg) alleles and those who carry two proline (Pro) alleles.

The effect of the codon 72 variant in conjunction with deleterious mutations in the p53 DNA binding domain has been investigated by Bougeard et al.[23] in 61 French carriers of the *TP53* germline mutation. The mean age of tumour onset in Arg allele carriers (21.8 years) was different from that of Pro/Pro patients (34.4 years, $p<0.05$). They even observed a cumulative effect of The *TP53* codon 72 and *MDM2* SNP309 (described in chapter 1.5) polymorphisms because the mean ages of tumour onset in carriers of the MDM2G and p53Arg alleles (16.9 years) and those with the MDM2T/T and p53Pro/Pro genotypes (43 years) were clearly different ($p<0.02$).

In conclusion, it is difficult to differentiate between pathogenic germline mutations, mutations of unknown significance and polymorphisms and even known polymorphisms might have cancer-related phenotypical manifestations.

### *1.3.6 Somatic TP53 mutation spectrum*

The *TP53* gene is the most frequently mutated gene in various tumour types, mutated in up to 50% of tumours. As seen with germline *TP53* mutations, somatic *TP53* mutations are most frequently missense mutations as well (Figure 8, IARC Mutation Database, R13 release, November 2008, [35]). The codon distribution is shown in Figure 9 (IARC Mutation Database, R13 release, November 2008, [35]).

**Figure 8. Somatic *TP53* mutations – mutation type (n = 24785)**

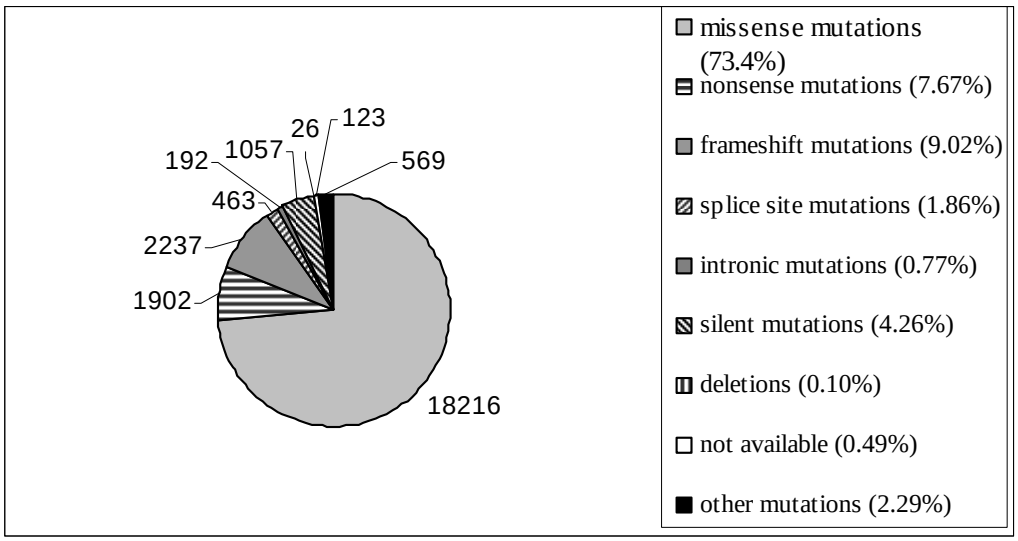
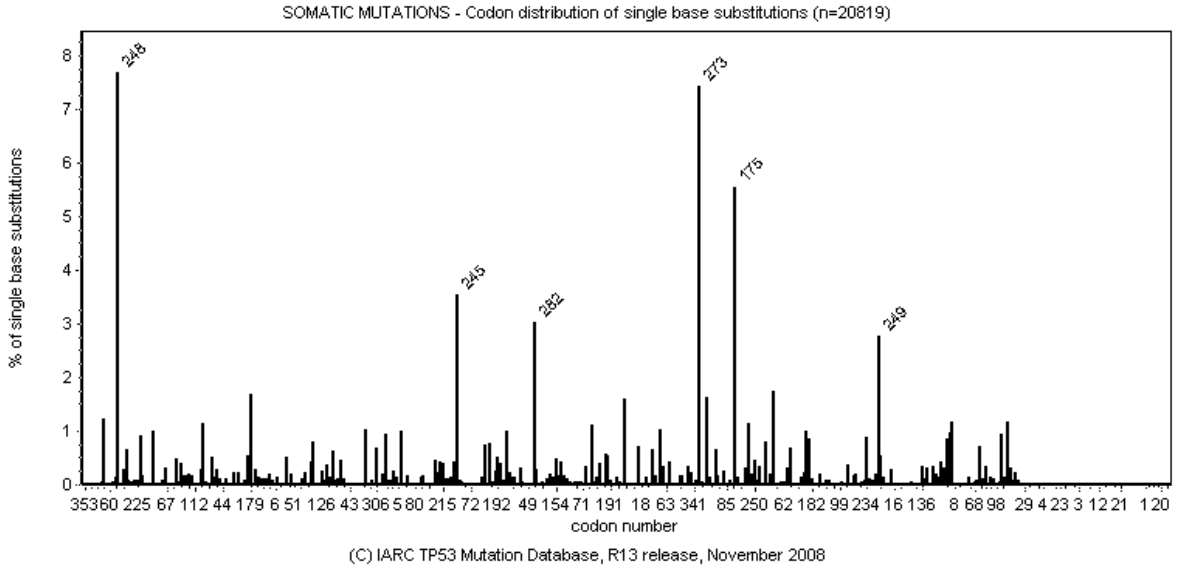
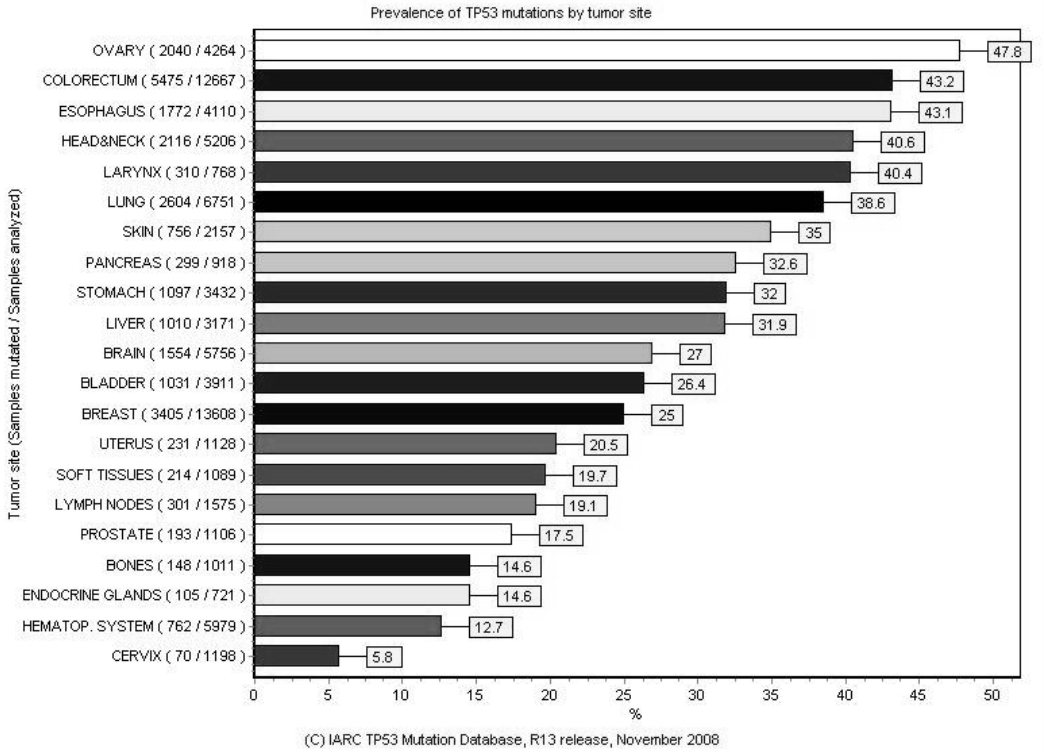



**Figure 9 Somatic *TP53* mutations – codon distribution**




Somatic *TP53* mutations are most prevalent in cancer of the ovary, colorectum, esophagus and head & neck (Figure 10, IARC Mutation Database, R13 release, November 2008, [35]). In the typical LFS tumours, somatic *TP53* mutations are prevalent in 13.2 to 26.7%.

**Figure 10 Prevalence of somatic *TP53* mutations by tumour site**



## 1.4 *TP53* germline mutations in "sporadic" cancer patients.

In addition to LFS/LFL families, *TP53* germline mutation analysis could also be considered in patients with a LFS-related tumour without a striking family history. The proportion of "sporadic" typical LFS-tumour patients or patients with multiple tumours who carry a *TP53* germline mutation described in the literature is summarised in table 2.

**Table 2. LFS-related tumours and germline *TP53* mutations**

| Tumour type | Number of patients investigated | | | *TP53*-positive (%) | Additional information | Ref |
|---|---|---|---|---|---|---|
| | Unse-lected | No FH | Pos FH | | | |
| Adrenocor-tical carcinoma (ACC) | 6 | | | 3/6 (50%) | ACC <18 yr, no LFS | [54] |
| | 11 | | | 9/11 (82%) | exons 2-11 analysed, ACC <15 yr, no LFL/ LFS | [40] |
| | 36 | | | 35/36 (97.2%) | exons 2-11 analysed, ACC <14 yr, no LFS/LFL | [37] |
| | 21 | | | 14/21 (67%) | exons 2-11 analysed, 15/21<18 yr, 2/14 mutation carriers →LFS | [34] |
| Brain tumour | 47 | | | 1/47 (2%) Pediatric brain tumours | exons 4-8 analysed | [55] |
| | 80 | | | 1/80 (1.3%) gliomas | exons 5-8 analysed | [56] |
| | | | 15 | 3/15 (20%) gliomas | exons 5-8 analysed, 0/3 mutation carriers →LFS | [56] |
| | 38 | | | 2/38 (5.3%) Astrocytoma (22 adult, 16 childhood) | exons 5-8 analysed, (2/22 adults carried a *TP53* mutation, 9%) | [57] |
| | | | 44 | 6/44 (13.6%) astrocytoma | 2/6 mutation carriers →LFS | [58] |
| | 8 | | | 8/8 (100%) CPC | exons 2-11 analysed, 1/8 mutation carriers → LFS | [34] |
| Breast cancer | 167 | 40 | 30 | 1/167 (0.6%) unselected 1/40 (2.5%) B<35 yr | Both mutation carriers had family history suggestive of LFS | [59] |
| | 136 | | | 1/136 (0.7%) | exons 5-9 analysed | [60] |
| | 126 | | | 1/126 (0.8%) B<40 yr | exons 5-8 analysed | [61] |
| | | 61 | 22 | 4/83 (4.8%) 2/61 no FH (3.3%) 2/22 pos FH (9.1%) | Breast cancer <30 years 4 mutations: 1 LFS, 1 LFL, 1 de novo, 1 mother bladder cancer | [62] |
| Childhood sarcoma | 235 | | | 7/235 (3%) osteosarcoma | exons 5-8 analysed, in some patients also exon 2 and 9 1/7 mutation carriers →LFS | [63] |
| | | 33 | | 3/33 (9%) RMS | Exons 2-11 analysed, all mutation carriers <3 yr | [64] |
| | 107 | | | 7/107 (6.6%) STS | exons 2-11 analysed, 6/7 mutation carriers→ LFS | [65] |
| Leukaemia | | | 10 | 0/10 (0%) fam leukemia | Exons 4-8 analysed | [66] |
| | 25 | | | 1/25 (4%) paediatric ALL | Exons 4-8 analysed Mutation carrier→ LFS | [67] |
| Multiple primary tumours | 59 | | | 4/59 (6.8%) two primaries | 59 children/young adults no Li-Fraumeni families | [68] |
| | 5 | | | 1/5 (20%) triple primaries | Mutation carrier→LFS | [69] |

Ref=reference, Unselected=patients not selected for family history, FH=family history, Pos=positive, LFL=Li-Fraumeni-like syndrome, LFS= Li-Fraumeni syndrome, CPC=choroid plexus carcinoma, B=breast cancer, RMS=rhabdomyosarcoma, STS=soft tissue sarcoma, fam leukaemia=familial leukaemia, ALL=acute lymphoblastic leukaemia, yr=years, →LFS=fulfilled the LFS criteria

In summary, the largest proportion of *TP53* germline mutations is found in patients with adrenal cortical carcinoma. For brain tumours, the strongest association is with familial gliomas, although only a small cohort was tested, and choroid plexus carcinoma (CPC). Krutilkova et al. [70] and Gonzalez et al. [34] detected 13 new families with childhood CPC and *TP53* germline mutations, strengthening the association of choroids plexus carcinoma with LFS or with *TP53* germline mutations as mentioned by Garber et al. in 1990 [71]. Two of the patients in the cohort of Gonzalez et al. [34] carried proven de novo mutations. Although no large group of unselected CPC patients has been tested for *TP53* germline mutations, CPC seems to be associated to *TP53* germline mutations.

Only two small cohorts of multiple primary cancers patients have been tested for *TP53* germline mutations. Many studies have addressed the percentage of *TP53* germline mutation carriers that develop more than one primary tumour. Hisada et al. (Hisada, 1998) investigated cancer development after the first primary tumour in 24 LFS kindreds, of which 8 carried a *TP53* germline mutation. Thirty of 200 family members with cancer developed multiple primary cancers (15%). Studying 107 children with soft tissue sarcoma (STS) 7 were found to carry a *TP53* germline mutation [72]. In these 7 families, 63 mutation carriers were detected, of which 17 developed multiple primary neoplasms (17/63, 27%). Gonzalez et al. [34] found 91 *TP53* mutations carriers of 525 patients tested. Of these mutation carriers, 50% had more than one primary cancer.

In conclusion, the largest proportion of *TP53* germline mutations in "sporadic" patients is found in adrenal cortical carcinoma (50-97%), also CPC seems to be associated to *TP53* germline mutations. 15-50% of *TP53* mutation carriers develop multiple primary tumours.

### 1.5 Are other genes involved in LFS?

In general, in 75% of classical LFS families, 40% of LFL-Birch, and 20%-30% of LFL-Chompret families a *TP53* germline mutation is identified, comparable to 73%, 28% and 21% respectively, in the Netherlands (see chapter 2). Since not all families suspected for LFS carry a *TP53* germline mutation, other LFS candidate genes have been considered as summarised in table 3.
Most candidate genes tested did not show any mutations.

**Table 3. Other genes and LFS**

| Gene | Number of patients | | | mutation-positive (%) | Reference |
|---|---|---|---|---|---|
| | LFS-variant | LFS | LFL | | |
| *BAX* | | 37 | 36 | 0 | [73] |
| *Bcl10* | | 7 | 20 | 0 | [74] |
| *CDKN2:* | | | | | |
| *p16* | | 6 | 10 | 0 | [75] |
| | 14 | 4 | 40 | 0 | [76] |
| $p19^{ARF}$ | | 6 | 10 | 0 | [75] |
| *CHEK1* | 18 | | 4 | 0 | [77] |
| | 32 | 1 | 6 | 0 | [78] |
| *PTEN* | | 6 | 10 | 0 | [75] |
| | | 10 | 16 | 0 | [79] |
| *TP63* | 5 | | 1 | 0 | [80] |
| *CHEK2* | 67 | 25 | 71 | 9/163 (5.5%) Mainly 1100delC, leading to breast cancer risk ↑ (see chapter 3) | [77, 78, 80-85] |
| *MDM2* | | | | SNP309, leading to earlier age of onset in *TP53* mutation carriers (see chapter 4) | [22, 23] |
| Locus 1q23 | | 4 | | Linkage 1q23 | [86] |

LFS-variant=families suggestive of LFS, not fulfilling the LFS/LFL criteria, LFS=Li-Fraumeni syndrome, LFL=Li-Fraumeni-like syndrome

In 1999 the *CHEK2* gene was proposed to be an alternative LFS gene [77]. Subsequent studies and this thesis collected increasing evidence that germline mutations in *CHEK2* might not cause LFS [84, 87], although the *CHEK2* gene might be a factor contributing to individual tumour development in families that are subsequently recognised as having a Li-Fraumeni phenotype (chapter 3). In 2002 the specific *CHEK2* 1100delC mutation was found to be a low penetrance breast cancer susceptibility allele with a relative risk of 2 for women and 10 for men to develop breast cancer [88].
Bond et al. [22] showed that a single nucleotide polymorphism in the *MDM2* gene influenced the age of tumour onset in *TP53* mutation carriers, which was confirmed by Bougeard et al. [23] and this thesis.
Linkage was identified to a region of approximately 4 cM on chromosome 1q23 in four LFS families [86], while no linkage was identified to the 17p13.1 region containing the *TP53* gene in these families. So far, the gene and its mutations have not been identified and characterised.
In conclusion, no alternative LFS gene has been identified until now.

### 1.6 Cancer risks in LFS/LFL family members

Many researches evaluated cancer risks in Li-Fraumeni syndrome families.
By the follow up of 24 LFS kindreds, the relative risk of cancer was calculated for different age groups [89], *TP53* mutation analysis was not performed in these families yet. At ages 0-19 years relative cancer risk was 21.1, 6,2 in the 20-44 year age group and 2.4 for the 45-59 age group. No excess cancer occurrence was seen above 60 years of age [89].
LFS/LFL families are rare and family members usually develop tumours at a young age. Cancer risk for LFS family members was estimated at 50% by age 40 and 90% by age 60 by Lustbader et al.[90]. This was based on data of 12 families, of which only two families had confirmed *TP53* germline mutations. LeBihan et al. [91] estimated age-specific cancer risks, based on five families. Risks were 42% between age 0 and 16 years, 38% between 17 and 45 years and 63% after age 45 years, life time risk was estimated to be 85%.
In 2000 cancer risks were estimated based on 13 *TP53* positive families, collected through probands with childhood cancer [33]. A program based on a survival analysis approach was used in which the event considered was age at onset of cancer. By age 16, 45 and 85 years the cancer risks for *TP53* mutation carriers were 12%, 84% and 100% respectively for females and 19%, 41% and 73% respectively for men. This sex difference was almost entirely explained by breast cancer occurrence, which represents 80% of all cancers in the age-class 16-45 years.
By screening 107 probands with childhood STS, 7 *TP53* positive families were diagnosed [72], including 63 mutation carriers. Again a sex difference was found in cancer risk: the cumulative risks for the development of cancer were 18%, 49%, 77% and 93% for female carriers by ages 20, 30, 40 and 50 years respectively. For male carriers the cumulative risks were 10%, 21%, 33% and 68%. In contrast to the explanation above, this female-male difference remained when the sex related tumours, breast-, ovarian- and prostate cancer, were excluded.
The presence of the SNP309 G polymorphism in the *MDM2* gene is associated with a significantly earlier age of cancer onset in *TP53* mutation carriers of 7 to 10 years, which we confirmed in our dataset [22, 23].
In conclusion, a life time cancer risk of 68%-100% is found for *TP53* mutation carriers. The cancer risk for women seems to be higher then for men, which might not be explained all together by sex related tumours. In addition, a polymorphism in the *MDM2* gene is related with an earlier age of tumour onset in *TP53* mutation carriers.

## 1.7 *TP53* and radiation

In chapter 1.2 we mentioned the role of the *TP53* protein in the response to DNA damage induced by radiation. Before the functions of *TP53* were clarified, clinical studies in LFS families had shown that second primary tumours in LFS patients frequently occurred in sites previously exposed to radiotherapy.

*Mouse models*

Mice lacking p53 (p53 -/-) are prone to spontaneous tumours within 6 months [27]. Heterozygous *TP53*-deficient mice spontaneously develop tumours, mainly lymphomas and sarcomas [92]. When heterozygous *TP53* mice are irradiated with 4 Gy, tumour development is accelerated as compared to the non-irradiated heterozygous *TP53* mice [93].

*Therapeutic irradiation*

In general, therapeutic irradiation leads to an increased risk of second primary tumours in the radiation field [94]. For *TP53* mutation carriers this has been suggested as well and this assumption is based on the following clinical studies:

Thirty out of 200 (15%) affected LFS family members from 24 families developed a second cancer [3]. Treatment records were available of 27 of these family members, which showed that 9 patients received radiotherapy for their first cancer; 6 of these 9 patients developed a tumour in the radiation field 3-22 years after treatment.

In a second study, 3 out of 14 children with LFS-associated adrenal cortical tumours developed a second cancer [40]. They were the only 3 children who received radiotherapy and survived >2 years. The second primary tumours all developed within the radiation field.

A *TP53* mutation carrier was reported who developed 17 primary tumours [95]. The incidence of new tumours rose steeply after adjuvant vaginal vault radiotherapy for endometrial cancer and adjuvant tamoxifen treatment for breast cancer.

A woman from an LFL family underwent mastectomy for breast cancer at 25 years of age [96]. After surgery she was treated with adjuvant chemotherapy combining adriamycin, cyclophosphamide, and 5-fluorouracil followed by locoregional radiotherapy (46 Gy) and hormonal therapy with tamoxifen. When a tumour developed in a supraclavicular lymph node at 29 years of age, the tumour was excised and the supraclavicular area was irradiated (30 Gy). The ovaries were also irradiated through a pelvic field (20 Gy) for ovariolysis. Subsequently, the patient developed a small cell lung cancer in the chest wall irradiation field and a sigmoid carcinoma in the pelvic irradiation field at 33 years of age, she died at 34 years of age.

Salmon et al. [97] described a *TP53* mutation carrier who developed bilateral breast cancer at 27 years of age and three years later a sarcoma of the right clavicle and another primary breast cancer, both in the radiation field applied for the treatment of the first breast cancer.

*Diagnostic irradiation*

In general, exposure to mammographic X-rays confers a risk of radiation-induced breast cancer, which is greater the younger the women were when exposed [98]. Berrington de Gonzales et al. [99] investigated mammographic screening before age 50 years in the UK and suggested that a decade of annual mammographic screening before age 40 years would result in a net increase in breast cancer deaths of 0.37-0.86 breast cancer deaths per 1000 women screened. Ronckers et al. [100] studied women monitored in the past by radiography at young ages for scoliosis. Based on 78 cases of invasive breast cancer out of 3010 exposed women the authors found that the subjects who had >60 X-rays had been exposed to a 3-fold risk of breast cancer compared with subjects who had <10 X-rays.

The possible effect of low doses of ionizing radiation (<0.1Gy) on the risk of breast cancer among *TP53* mutation carriers has not been evaluated so far. Both *TP53* and *BRCA1/2* are involved in DNA repair after DNA damage through ionizing radiation so similar effects can be

anticipated. In addition, *BRCA1* associates with p53 and stimulates its transcriptional activation. Because studies usually combine *BRCA1* and *BRCA2*, we will discuss the effect of low doses of ionizing radiation among *BRCA1/2* mutation carriers.
In 2006 Andrieu et al. [101] suggested a potentially important association between radiation exposure from chest X-rays and breast cancer risk in *BRCA1/2* carriers. The hazard ratio was mostly elevated in the group exposed before 20 years and the group exposed to at least 5 X-rays (HR 1.76 and 1.92 respectively). In contrast, Goldfrank et al.[102] did not find a significant association between cancer status and mammography exposure in 213 *BRCA* mutation carriers, Narod et al.[103] compared 1600 cases of breast cancer and 1600 controls, all *BRCA* mutation carriers, and found no association between ever having screening mammography and risk of breast cancer. Gronwald et al.[104] concluded that women with a *BRCA1* mutation may be more sensitive than non-carriers to the effect of ionizing radiation because 138 *BRCA1* mutation carriers with breast cancer reported more frequent chest X-ray exposure before the age of 30 years compared to 158 non-*BRCA1* carriers with breast cancer. Berrington de Gonzales et al. [105] estimated the risk of radiation-induced breast cancer from mammographic screening for young *BRCA* mutation carriers. They concluded that the reduction in breast cancer mortality from screening among women with *BRCA* mutations is not substantially greater than the risk of radiation-induced breast cancer mortality when screening before age 34 years.

*Conclusion*
Several authors reported that *TP53* mutation carriers developed second primary tumours in the radiation field applied for the treatment of the first primary tumour. In heterozygous *TP53* mice, tumour development accelerated after irradiation with 4 Gy. No mouse model, however, has dealt with radiation doses comparable to chest X rays (about 0.5 mGy) used for screening.
A systemic evaluation of radiation-related breast cancer or other LFS tumour types has not yet been possible due to lack of cases[106]. The literature on *TP53* and radiation is all based on relatively high doses of radiation. In *BRCA* mutation carriers an association between radiation exposure from chest X-rays and mammography and breast cancer was seen, especially in young patients and multiple exposures [101, 104, 105], which might be applicable for *TP5*3 germline mutation carriers. In contrast, two case-control studies of *BRCA1* and *BRCA2* mutation carriers showed no hazardous effect of mammography [102, 103].

### 1.8 Management of *TP53* mutation carriers

Management of patients with germline *TP53* mutations is still a difficult issue. Surveillance is complicated by the different sites and types of cancer associated with LFS and the variable age of onset. In addition, early detection and treatment is not available for all tumour types.

In 2003 Varley et al. [8] reported that there are no national UK guidelines for *TP53* germline mutation testing. There are no widely accepted procedures for surveillance of high-risk individuals in LFS families or those with verified germline *TP53* mutations. They recommended annual clinical review and access to informed clinicians. MRI can be offered to women at risk of developing breast cancer, and abdominal ultrasound can be performed in childhood. There is a strong indication that radiotherapy should be avoided if possible [8].

In 2006 Moule et al. [107] discuss the implications for management when a *TP53* germline mutation is detected. Because of the theoretical risk of inducing malignancy in *TP53* mutation carriers by exposure to radiation during mammography, the use of magnetic resonance imaging (MRI) should be considered. Additionally, tumours in *TP53* mutation carriers that lack functional p53 might demonstrate resistance to DNA damaging agents, chemotherapy and radiotherapy, used for treatment [108, 109]. Moreover, *TP53* mutation carriers are at a higher risk for developing treatment induced second cancers. It was recommended that radiotherapy as a treatment modality should be avoided if there are other feasible treatment modalities. For example, breast cancer with onset before 30 years of age would be an indication for urgent germline *TP53* diagnostic testing since if a germline mutation is found, mastectomy rather than conservative surgery combined with radiotherapy would be preferred.

Bougeard et al. [11] updated the Chompret criteria in 2008, because identification of a germline *TP53* mutation in a patient will allow to confirm the diagnosis of LFS, to ensure a regular clinical review by an informed clinician in order to avoid a delay in the diagnosis of a second tumour, to offer to women breast imaging screening program, to avoid radiation, whenever possible and finally to offer prenatal diagnosis to the families.

Overall screening benefit for LFS individuals has not been evaluated, partly because there is currently no international agreement on optimal surveillance strategies and the number of individuals is low.

In conclusion, most authors recommend performing *TP53* germline mutation analysis in LFS/ LFL families or families that fulfil the Chompret criteria which include childhood adrenal cortical cancer and choroid plexus tumour and multiple primary cancers. For surveillance, breast cancer screening is recommended, whether mammography should be avoided is still unclear. An annual clinical review and abdominal ultrasound during childhood is mentioned. Surveillance can be recommended according to family phenotype (chapter 5.1). For treatment, radiotherapy should be avoided whenever possible.

**1.9 Aims and outline of this thesis**

The main objective of this thesis was to evaluate Li-Fraumeni syndrome in the Netherlands and propose recommendations for *TP53* mutation analysis and surveillance of *TP53* mutation carriers.

One of the aims of this thesis was to collect all families that were tested for *TP53* germline mutations in the Netherlands to evaluate the families to whom *TP53* germline mutation analysis was offered and the families in which a *TP53* germline mutation was identified. On the basis of these results recommendations for*TP53* mutation analysis are proposed.
Chapter 2 gives an overview of all families suspected to harbour a germline *TP53* mutation in the Netherlands and the mutation detection rate in these kindreds. The different tumour types with their associated elevated risks are described.

Since the mutation detection rate is not 100%, the second aim of this thesis was to look for possible candidate genes for Li-Fraumeni syndrome without a detectable *TP53* mutation. In Chapter 3 we describe the *CHEK2* gene as possible candidate gene for Li-Fraumeni syndrome and results of screening for *CHEK2* germline mutations in our *TP53*-negative families.

*TP53* mutation families show a variation in clinical expression. A possible explanation is the involvement of modifier genes. Because it was recently 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, we evaluated this finding in our patient population (Chapter 4). To enlarge our study population, 11 Finnish *TP53* mutation carriers were also included. Additionally, we investigated whether the SNP309 G allele plays a role in the Dutch *TP53*-negative LFS and LFS-suspected patients.

Chapter 5 addresses the difficulties in counselling *TP53* mutation families. In this context a family with relatively late-onset common cancers and a classical LFS family with two *TP53* germline mutations are described.

The collected information led to a guideline for genetic counselling in Li-Fraumeni syndrome families including recommendations for *TP53* germline mutation testing and considerations for surveillance and treatment (Addendum).

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