Li-Fraumeni syndrome, clinical and molecular genetics

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Chapter 7.1

Summary

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Chapter 1 provides a general introduction to the Li-Fraumeni syndrome. Frederic P. Li and Joseph F. Fraumeni studied the possible association between childhood-onset sarcoma and breast cancer, after the referral of two cousins who both developed rhabdomyosarcoma in childhood. Subsequently, Li and Fraumeni suggested the existence of a new familial cancer syndrome with a predisposition to sarcoma, breast cancer, brain tumour, adrenal cortical carcinoma and leukaemia. In literature, various clinical criteria for Li-Fraumeni Syndrome (LFS) have been proposed, as listed in Table 1. LFS patients are at risk for multiple primary tumours: about 27% - 50% of LFS patients develop a second primary tumour.
In 1990 germline *TP53* mutations were found in LFS kindreds and DNA analysis became available. Because germline *TP53* mutations were also detected in families not fulfilling the LFS criteria, less stringent criteria for "Li-Fraumeni-like" (LFL) syndrome were defined. The LFL criteria were also based on the familial occurrence of cancer and included three affected family members. Subsequently, in 2001, Chompret et al. defined a novel set of criteria, updated in 2009, that included indications for *TP53* analysis in sporadic cancer patients. Table 1 gives an overview of the LFS, LFL, Chompret en revised Chompret criteria.
According to the literature, about 75% of LFS families, 40% of LFL families and 30% of families fulfilling the 2001 Chompret criteria carried pathogenic *TP53* germline mutations. Currently, 423 *TP53* germline mutations have been identified in the IARC mutation database (http://www-p53.iarc.fr/). The proportion of *de novo TP53* germline mutations is between 7 and 24%. Since not all LFS or LFL families carry a *TP53* germline mutation, other LFS candidate genes have been considered, but at present no alternative LFS genes have been identified.
In *TP53* mutation carriers the life time cancer risk is estimated to be 68%-100%; for women the risk is higher than for men. Management of *TP53* mutation carriers remains a difficult issue, due to the different tumour sites and types of cancer involved and the variable ages of onset. In addition, for most LFS/LFL tumour types early detection and treatment are not available. Breast cancer surveillance is recommended for all female mutation carriers. It is still controversial whether mammography should be avoided due to increased radiosensitivity of *TP53* germline mutation carriers. An annual clinical review and abdominal ultrasound during childhood are advised by some authors, surveillance can be recommended according to the familial phenotype (Chapter 5.1).
The aims of this thesis are highlighted in Chapter 1.9. The main objective of this study was to define recommendations for genetic counselling of Li-Fraumeni syndrome families by collecting all families tested for *TP53* germline mutations in the Netherlands and determine which families carry a *TP53* germline mutation (Chapter 2). In addition, the *CHEK2* gene was screened for mutations as a possible candidate gene for *TP53*-negative Li-Fraumeni syndrome (Chapter 3). The SNP309 (T>G variation) in the *MDM2* gene was assessed in both *TP53*-positive and *TP53*-negative families to study its effect on age of tumour onset in *TP53*-positive families and to investigate whether it plays a role in *TP53*-negative families (Chapter 4). The complexities of counselling are addressed in Chapter 5 by describing two *TP53* mutation families. Finally, the collected data led to a guideline for genetic counselling and recommendations for Li-Fraumeni syndrome families (Addendum).

Table 1. Different criteria for *TP53* germline mutation testing

Classical LFS criteria	- a proband with sarcoma diagnosed under the age of 45 years AND - a first-degree relative with any cancer under 45 years AND - another first- or second-degree relative with either cancer under 45 years or a sarcoma at any age
LFL criteria	- a proband with any childhood cancer or sarcoma, brain tumour or adrenal cortical tumour under the age of 45 years AND - a first- or second degree relative with a typical LFS cancer at any age AND - a first- or second degree relative in the same lineage with any cancer under 60 years
2001 Chompret criteria	- a proband affected by a narrow spectrum cancer (sarcomas, brain tumours, breast cancer and adrenal cortical carcinoma) before 36 years 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 OR - a proband with multiple primary tumours two of which belong to the narrow spectrum and the first of which occurred before 36 years OR - a proband with adrenal cortical carcinoma whatever the age of onset and family history
2009 Chompret criteria	- a proband with a tumour belonging to the LFS tumour spectrum (soft tissue sarcoma, osteosarcoma, brain tumours, pre-menopausal breast cancer, adrenal cortical carcinoma, leukaemia, lung bronchoalveolar cancer) before 46 years 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 OR - a proband with multiple primary tumours (except multiple breast tumours), two of which belong to LFS tumour spectrum and the first of which occurred before 46 years OR - a proband with adrenal cortical carcinoma or choroid plexus tumour, irrespective of the family history

Chapter 2 gives an overview of all known families suspected of harbouring a germline *TP53* mutation in the Netherlands (180 families tested) and the mutation detection rate and sensitivity of different selection criteria applied to these families (Table 2). A total of 180 families was screened for *TP53* germline mutations in the period 1995 to 2008; 24 mutation families were detected. In total 105 families fulfilled the revised Chompret criteria, 22 families carried a *TP53* germline mutation (mutation detection rate 21%, sensitivity 92%). Of the 11 classical LFS families 8 families carried a *TP53* germline mutation (73%), of the 36 families fulfilling the LFL criteria 10 carried a *TP53* germline mutation (28%). The sensitivity of the combined LFS/LFL criteria was 75% (18/24).

Table 2.

(family) history	**Number of families (n=180)***	***TP53* positive families (n=24)**
Revised Chompret (including 10 LFS and 31 LFL)	105	22 (21%)
LFS	11	8 (73%)
LFS not fulfilling revised Chompret	1	0 (0%)
LFL	36	10 (28%)
LFL not fulfilling revised Chompret	4	0 (0%)
LFS-suspected (not fulfilling revised Chompret, LFS, LFL)	70	2 (2.9%)

*Total number of families tested for *TP53* germline mutations: 105 + 1 + 4 + 70 = 180

The mutation detection rate for families fulfilling the revised Chompret criteria was 21% with a high sensitivity (22 out of 24 mutations would have been detected, 92%). Therefore, we recommend performing *TP53* mutation analysis in all families fulfilling the revised Chompret criteria. The 2 mutations that would not have been found, using these criteria, were detected in a child with a rhabdomyosarcoma and a woman who developed breast cancer at 24 years of age. Therefore, *TP53* germline mutation testing may be considered also for childhood sarcoma and breast cancer before 30 years of age without a *BRCA1/2* mutation. In the second part of the study the different tumour types with their associated elevated risks are described. The relative risks to develop pancreatic cancer, colon cancer and liver cancer are significantly increased in *TP53* mutation carriers. Because metastatic liver disease could not be excluded, only the pancreatic and colon cancer might be LFS-component tumours.

The mutation detection rate in families with suspected LFS is not 100%, which has led to the hypothesis of additional candidate genes. In **Chapter 3** we present the results of screening for *CHEK2* germline mutations in our *TP53*-negative families. We were able to screen 65 Dutch *TP53*-negative candidate patients out of families with suspected LFS (1 LFS family, 35 LFL families and 29 LFS-suggestive families) for *CHEK2* germline mutations to determine their contribution to the LFS/LFL phenotype. Six index patients were identified with a *CHEK2* sequence variant, four with the c.1100delC variant and two sequence variants of unknown significance, p.Phe328Ser and c.1096-?_1629+?del. In all four of the c.1100delC families, this sequence variant seemed to be associated with breast cancer or breast and colorectal cancer, there is no evidence that the sequence variants found caused the complete LFS phenotype in these families. In our sample the frequency of the *CHEK2* c.1100delC variant was 6.2% (4/65), significantly different from that for healthy controls (p=0.006). Our data illustrate that *CHEK2* is not a major LFS susceptibility gene in the Dutch population, although the *CHEK2* gene might be a factor contributing to individual tumour development. Therefore, these families with *CHEK2* mutation carriers may subsequently be recognised as having a Li-Fraumeni phenotype. In

addition to the *CHEK2* gene, many more modifiers or low penetrance susceptibility genes might occur in families showing a Li-Fraumeni phenotype.

The influence of modifier genes might also be an explanation for the variation in clinical expression in *TP53* mutation families. In 2004 it was shown that a single nucleotide polymorphism in the *MDM2* gene, SNP309 (T>G variation), was associated with accelerated tumour formation in LFS patients who carry a *TP53* germline mutation. In **Chapter 4** we evaluated this finding in our patient population. Furthermore, 11 Finnish *TP53* mutation carriers were also included to enlarge our study population. Our results confirm the findings. Moreover, even a 16-year earlier age of tumour onset was shown for *TP53* mutation carriers with a SNP309 G allele (G/G and G/T) compared to the T/T SNP 309 group. In addition, we investigated whether the SNP309 G allele plays a role in the Dutch *TP53*-negative LFS and LFS-suspected patients. The age of tumour onset was not significantly different for SNP 309 G allele patients compared to T/T patients in our *TP53*-negative LFS and LFS-related groups. We did find a higher prevalence of *MDM2* SNP309 homozygous G/G carriers in the *TP53*-negative LFS and LFS-related patients than in the general population. These data suggest that homozygosity for SNP309 (G/G) contributes to the LFS phenotype, but further confirmation is needed.

In **Chapter 5** the complexities of counselling *TP53* mutation families are addressed by describing two *TP53* mutation families. In **Chapter 5.1** a kindred is described with late-onset common cancers and a p.Arg213Gln *TP53* germline mutation. Although the family fulfils the LFL criteria, the carriers developed a variety of common cancers without a clear-cut early onset of disease. In addition, 10 mutation carriers were without any malignancy (age 21-53 years) and 3 out of 15 affected family members did not carry the *TP53* germline mutation. Therefore, we evaluated the functional effect of the germline *TP53* mutation and the possible contribution of other genetic defects in this family. A functional test (FASAY) showed that the mutated allele lacks biological transcriptional activity and no mutations were found in *BRCA1*, *BRCA1*, *CHEK2*, *MLH1*, *MSH2* and *MSH6* in selected family members. In addition, this specific mutation was previously found in a LFS family and has been reported as a somatic mutation; the mutation is located in the DNA-binding domain and was absent in healthy controls. On the basis of these results we concluded that this p.Arg213Gln *TP53* mutation is a causative factor in this family and that specific *TP53* germline mutations can show reduced penetrance and later average age of onset of cancer. In ***chapter 5.2*** a classical LFS family is described in which two *TP53* germline mutations were detected, an intron 5 splice site mutation and the exon 7 p.Asn235Ser mutation. The latter mutation was detected through pre-symptomatic DNA testing in a healthy family member and had been reported repeatedly in the literature as a pathogenic mutation. Because the mutation did not segregate in our family, the functional test (FASAY) showed normal transcriptional activity, the mutation was found once in 300 controls, splice site prediction programs predicted no cryptic splice site and the 5 studies reported in the literature did not include functional tests, did not test controls and did not have classical LFS families, we conclude that p.Asn235Ser is a rare neutral variant or at best a low penetrance allele rather than a pathogenic mutation for LFS. When germline sequence variants with uncertain functional effects are detected, additional tests should be performed to confirm the pathogenicity of the mutation.

The general discussion in ***chapter 6*** addresses the mutation detection rate and sensitivity of different criteria applied to Dutch families suspected of harbouring a *TP53* germline mutation. We recommend using the revised Chompret criteria because a high sensitivity with a mutation detection rate of 21% is achieved, the mutation detection rate for women who develop breast cancer before 30 years of age is discussed. In addition, the psychological consequences of *TP53* germline mutation testing are considered. Also, the role of modifiers and low penetrance genes in families with a LFS phenotype is evaluated. In addition to the *CHEK2* gene and SNP309 in the

MDM2 gene, other genes might be identified which influence the familial phenotype of *TP53*-negative families and the phenotype of *TP53* mutation carriers. Finally, perspectives on future research are presented.

The addendum provides a guideline for recommendations and management of LFS. This is a translation of a Dutch guideline that was drawn up in cooperation with the Dutch foundation of detecting hereditary tumours and the Dutch committee of clinical oncogenetics (STOET, WKO).