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# TP53 germline mutation testing in 180 families suspected of Li–Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes

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## ABSTRACT

**Background** Li–Fraumeni syndrome (LFS) is a rare autosomal dominant cancer predisposition syndrome. Most families fulfilling the classical diagnostic criteria harbour *TP53* germline mutations. However, *TP53* germline mutations may also occur in less obvious phenotypes. As a result, different criteria are in use to decide which patients qualify for *TP53* mutation analysis, including the LFS, Li–Fraumeni-like (LFL) and Chompret criteria. We investigated which criteria for *TP53* mutation analysis resulted in the highest mutation detection rate and sensitivity in Dutch families. We describe the tumour spectrum in *TP53*-positive families and calculated tumour type specific relative risks.

**Method** A total of 180 Dutch families referred for *TP53* mutation analysis were evaluated. Tumour phenotypes were verified by pathology reports or clinical records.

**Results** A *TP53* germline mutation was identified in 24 families. When the Chompret criteria were used 22/24 mutations were detected (sensitivity 92%, mutation detection rate 21%). In LFS and LFL families 18/24 mutations were found (sensitivity 75%). The two mutations detected outside the 'Chompret group' were found in a child with rhabdomyosarcoma and a young woman with breast cancer. In the mutation carriers, in addition to the classical LFS tumour types, colon and pancreatic cancer were also found significantly more often than in the general population.

**Conclusion** We suggest *TP53* mutation testing for all families fulfilling the Chompret criteria. In addition, *TP53* mutation testing can be considered in the event of childhood sarcoma and breast cancer before 30 years. In addition to the risk for established LFS tumour types, *TP53*-positive individuals may also have an elevated risk for pancreatic and colon cancer.

## INTRODUCTION

In 1969 Li and Fraumeni described a novel autosomal dominant cancer syndrome with a predisposition to bone and soft tissue sarcoma, breast cancer, brain tumour, adrenal cortical carcinoma and leukaemia.<sup>1</sup> Patients with this syndrome are at increased risk for multiple primary tumours.<sup>2</sup> Clinical criteria for classical Li–Fraumeni syndrome (LFS)<sup>3</sup> and Li–Frau-

meni-like syndrome (LFL) have been established<sup>4</sup> whereby at least three family members were affected (table 1). Different LFL criteria were formulated by Eeles<sup>5</sup> who defined only two family members as being affected. In 1990 DNA analysis for LFS became available when germline mutations in the *TP53* gene were found in LFS kindreds.<sup>8</sup> All germline mutations that have been detected and published are collected in the International Agency for Research on Cancer (IARC) mutation database.<sup>9</sup> Currently 423 mutations have been described in 419 families (<http://www-p53.iarc.fr/>). In a study by Varley *TP53* germline mutations were seen in approximately 75% of LFS and 40% of LFL families.<sup>10</sup> Because *TP53* germline mutations were also identified in families not fulfilling the LFS/LFL criteria due to their alternative tumour spectrum, age at diagnosis or sporadic occurrence of cancer, Chompret *et al* proposed different criteria for *TP53* germline mutation testing,<sup>6</sup> which were updated in 2009<sup>7</sup> (table 1). A *TP53* germline mutation was found in 29% and 35% of French and American families, respectively, who fulfilled the 2001 Chompret criteria.<sup>11 12</sup> Table 1 summarises the different criteria used to categorise families suspected of having LFS. The classical LFS criteria,<sup>3</sup> the LFL criteria according to Birch *et al*<sup>4</sup> and the revised Chompret criteria by Tinat *et al*<sup>7</sup> are used for this article.

In *TP53* mutation carriers the tumour spectrum is wider than the classical tumour types used to recognise LFS (or LFL) defined in 1988 as breast cancer, sarcoma, brain tumour, adrenal cortical cancer and leukaemia.<sup>3</sup> Roughly, 20–30% of the tumours in *TP53* mutation-positive families do not belong to the classical LFS tumour spectrum.<sup>13–15</sup>

Besides the classical tumour spectrum, Birch *et al*<sup>15</sup> found Wilms' tumour and phyllodes tumours to be strongly associated, pancreatic cancer moderately associated, and neuroblastoma weakly associated in *TP53* mutation carriers.

In this study we evaluated the clinical spectrum of families undergoing *TP53* mutation analysis in the Netherlands, in order to help improve the guidelines specifying when *TP53* mutation analysis should be performed. We chose a probability of more than 10% to detect a germline mutation in order to be eligible for DNA analysis. We also

**Table 1** Criteria used for *TP53* germline mutation testing

|                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Classical Li–Fraumeni syndrome (LFS) criteria<br>Li <i>et al</i> <sup>3</sup> | A proband with sarcoma diagnosed under the age of 45 years<br>AND<br>A first degree relative with any cancer under 45 years<br>AND<br>Another first or second degree relative with either cancer under 45 years or a sarcoma at any age                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Li–Fraumeni-like syndrome (LFL) criteria<br>Birch <i>et al</i> <sup>4</sup>   | A proband with any childhood cancer or sarcoma, brain tumour or adrenal cortical tumour under the age of 45 years<br>AND<br>A first or second degree relative with a typical LFS cancer at any age<br>AND<br>A first or second degree relative in the same lineage with any cancer under 60 years                                                                                                                                                                                                                                                                                                                                                                                                                      |
| LFL criteria<br>Eeles <sup>5</sup>                                            | Two different tumours that are part of extended LFS in first or second degree relatives at any age (sarcoma, breast cancer, brain tumour, leukaemia, adrenal cortical tumour, melanoma, prostate cancer and pancreatic cancer)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| LFS Chompret criteria<br>Chompret <i>et al</i> <sup>6</sup>                   | 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 than breast cancer if the proband is affected by breast cancer) before 46 years or multiple primary tumours<br>OR<br>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<br>A proband with adrenal cortical carcinoma whatever the age of onset and family history                                                                                                                            |
| LFS Chompret criteria revised<br>Tinat <i>et al</i> <sup>7</sup>              | 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<br>OR<br>A proband with multiple primary tumours (except multiple breast tumours), two of which belong to the LFS tumour spectrum and the first of which occurred before 46 years<br>OR<br>A proband with adrenal cortical carcinoma or choroid plexus tumour, irrespective of the family history |

examined specific tumour type relative cancer risks in the *TP53* mutation families. In addition, cancer risks for *TP53*-positive and *TP53*-negative LFL families were compared to identify tumour types, apart from the typical LFS tumours, that are more specifically associated with a *TP53* mutation.

## PATIENTS AND METHODS

### Patients

In the Netherlands, *TP53* mutation analysis was introduced in a clinical setting in 1995. The clinical use of *TP53* mutation testing, including exon sequencing and genomic analysis, is started after indication according to the LFS and LFL criteria and was influenced by the limited preventive strategies. In 2005 the second version of the Dutch national guidelines recommended *TP53* mutation testing for LFS, LFL, patients with multiple LFS associated tumours and children with adrenal cortical carcinoma. In addition, *TP53* analysis should be considered for breast cancer under 30 years of age.<sup>16</sup> From 1995 until 2008, Dutch clinicians referred 180 families for counselling and *TP53* germline mutation

testing predominantly because of the occurrence of cancer types possibly related to LFS. The families were classified into four groups: (1) families fulfilling the revised Chompret criteria<sup>7</sup>; (2) classic LFS<sup>3</sup> (excluding the revised Chompret criteria); (3) LFL according to Birch *et al*<sup>4</sup> (excluding the revised Chompret criteria and LFS); and (4) other families considered to be suggestive of a germline *TP53* defect (LFS suspected: two or more primary tumours at any age, two first degree relatives with a tumour at any age, of which at least one tumour is a typical LFS tumours (sarcoma, brain tumour, breast cancer, leukaemia and adrenal cortical cancer), early onset (before 21 years of age) sarcoma or brain tumours and breast cancer before 35 years of age (without detectable *BRCA1* or *BRCA2* mutation) (table 2a). In addition, in table 2b the total number of families fulfilling the LFS or LFL criteria regardless of Chompret criteria are summarised.

The sensitivity and specificity were calculated for the revised Chompret, LFS and LFL criteria. The sensitivity was calculated as the number of *TP53*-positive families meeting a classification divided by the total number of *TP53*-positive families. The specificity was calculated as the number of *TP53*-negative families not meeting the specific classification divided by the total number of *TP53*-negative families.

Family members of proven *TP53* mutation carriers were counselled and offered *TP53* mutation testing.

### Molecular analysis

DNA analysis was performed in two laboratories, in Amsterdam (161 families) and Groningen (19 families). DNA was isolated from peripheral blood lymphocytes according to standard procedures. In Amsterdam mutation analysis of the *TP53* gene in the index patient, affected by cancer, was performed by sequence analysis of all coding exons (2–11) and the flanking intron–exon boundaries of these exons using standard procedures and multiplex ligation dependent probe amplification (MLPA) to screen for large *TP53* deletions or duplications (*TP53* MLPA kit P056 produced by MRC-Holland).<sup>17</sup> In Groningen the *TP53* gene was analysed by denaturing gradient gel electrophoresis (DGGE).<sup>18</sup> All possible candidate variants, identified as aberrant DGGE fragments, were confirmed with sequence analysis. Details on primers and polymerase chain reaction (PCR) conditions are available upon request. To assess the possibly pathogenic status of a mutation the frequency of each identified *TP53* germline mutations was determined in a control group of 150 anonymous blood donors, using denaturing gradient gel electrophoresis (DGGE). All possible candidate aberrant fragments were confirmed with sequence analysis.

### Cancer risk

To estimate the relative cancer risk in the *TP53* positive families a study cohort of tested and untested family members was selected with at least a 50% prior probability of being a carrier.

Pedigrees of all LFS counselled families were available through the clinical genetic centres, including age at cancer diagnosis and tumour types. From the pedigrees of the *TP53* positive families, all mutation carriers and their first degree relatives were included. In the *TP53* negative LFL (non-LFS) families, patients who were tested for *TP53* germline mutations and their first degree relatives were included. Subsequently, the obligate carriers (father or mother) with their siblings were selected from the pedigree and included. We chose not to adjust for ascertainment bias because of the multiple case definitions of LFS and LFL. We present the results for the typical LFS tumours separately from the other tumours.

All cancers were coded according to the International Classification of Disease, revision 9. We attempted to confirm all

**Table 2** Families classified according to familial phenotype

| (Family) history                                                                                                                                                                                                                 | Number of families (180)                                                 | TP53-positive families (%) |         |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------|---------|
| a. Number of families (n=180) presented for TP53 mutation analysis divided into 4 groups of different familial phenotypes (LFS-Chompret revised, LFS-Li, LFL-Birch and LFS-suspected) including the TP53-positive results (n=24) |                                                                          |                            |         |
| LFS-Chompret revised                                                                                                                                                                                                             | Including 10 LFS and 32 LFL                                              | 105                        | 22 (21) |
| LFS-Li (not fulfilling revised Chompret)                                                                                                                                                                                         |                                                                          | 1                          | 0       |
| LFL-Birch (not fulfilling revised Chompret)                                                                                                                                                                                      |                                                                          | 4                          | 0       |
| LFS-suspected                                                                                                                                                                                                                    |                                                                          | 70                         | 2 (2.9) |
|                                                                                                                                                                                                                                  | At least two primary tumours                                             | 30                         | 0       |
|                                                                                                                                                                                                                                  | Two first degree relatives with cancer (at least one typical LFS tumour) | 19                         | 0       |
|                                                                                                                                                                                                                                  | Early onset sarcoma or brain tumour (<21 years)                          | 9                          | 1       |
|                                                                                                                                                                                                                                  | Breast cancer before 35 years                                            | 12                         | 1       |
|                                                                                                                                                                                                                                  |                                                                          |                            | (%)     |
| b. Families fulfilling the LFS or LFL criteria                                                                                                                                                                                   |                                                                          |                            |         |
| LFS                                                                                                                                                                                                                              |                                                                          | 11                         | 8 (73)  |
| LFL*                                                                                                                                                                                                                             |                                                                          | 36                         | 10 (28) |

LFS-Chompret revised = the 2009 revised Chompret criteria for Li–Fraumeni syndrome,<sup>7</sup> LFS-Li = Li–Fraumeni syndrome according to Li *et al*,<sup>3</sup> LFL-Birch = Li–Fraumeni-like syndrome according to Birch *et al*,<sup>4</sup> LFS-suspected = LFS suspected families that do not fulfil the revised Chompret, LFS or LFL criteria.

LFL, Li–Fraumeni-like syndrome; LFS, Li–Fraumeni syndrome.

\*=Excluding LFS.

cancer diagnoses by collecting clinical records and pathology reports. Follow-up started on 1 January 1960 (because reference data were available from that time onwards) or date of birth, whichever occurred later, and ended at the date of first cancer diagnosis, date of death or the date of last contact.

Since 36% of the birth dates were missing, we introduced unknown birth dates based on the known birth dates in each generation of the family and the pedigree structure. The dates of death were used for missing dates of diagnosis for individuals who died from cancer at a known site. In addition, when the dates of diagnosis of affected individuals who did not die from cancer were missing, site specific mean ages at diagnosis of cancer in the general population were used, based on data of the Comprehensive Cancer Centre Amsterdam and the Netherlands Cancer Registries. Cancer risk for all TP53 positive and TP53 negative LFL (non LFS) family members was assessed with the standardised incidence ratio relative to the risk in the general population (relative risk (RR)), which is the ratio of observed to expected cases in the cohort. The expected number of cases was calculated by multiplying person years at risk by the age, sex, calendar period, and site specific cancer incidence rates in the general population, with the use of a computer program developed at the Netherlands Cancer Institute.<sup>19</sup> Incidence rates were obtained from the Eindhoven Cancer Registry up to 1990 and from the Netherlands Cancer Registry from 1990 onwards.

The comparison of RRs for TP53-negative and TP53-positive families was performed within the LFL group (excluding LFS families).

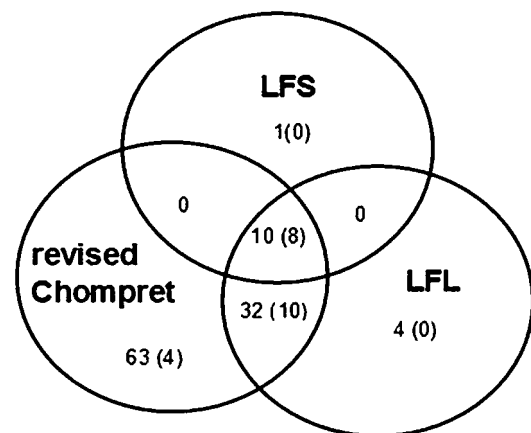
## RESULTS

Among the 180 patients analysed for TP53 mutations, 24 TP53-positive families were diagnosed (detection rate 13%). One of the 24 mutations was a large TP53 rearrangement, a deletion of exons 2–11. None of the identified TP53 germline mutations was identified in a control group of 150 anonymous blood donors. The 180 families were subdivided into four groups as described in 'Patients and methods'. In these subgroups, 22 out of 105 (21%) families fulfilling the revised Chompret criteria carried a TP53 germline mutation (table 2a), eight out of 11 (73%) classical LFS families carried a TP53 germline mutation (table 2b), 10 out of 36 (28%) LFL families (table 2b) and two out of 70 (2.9%) in the remaining LFS suspected group (table 2a).

The two mutations in the non-revised Chompret/LFS/LFL group occurred in a child with rhabdomyosarcoma at 4 years of age and a woman with breast cancer at 24 years of age.

The overlap in families fulfilling the revised Chompret, LFS and LFL criteria is shown in figure 1. No mutation was found in the five families that fulfilled the LFS or LFL criteria but did not fulfil the revised Chompret criteria (table 2a). The LFS family that did not fulfil the revised Chompret criteria showed one sarcoma, no other typical LFS tumours, and the other tumours did develop at young ages. The LFL families that did not fulfil the Chompret criteria did have two typical LFS tumours, but one of the tumours did not occur before 56 years of age. On average 3.7 individuals were tested in the TP53-positive families, revealing 2.8 individuals as mutation carriers; in the TP53-negative families only the index patient was tested.

The sensitivity for the revised Chompret criteria was 92% (22/24 TP53 mutation families), and the specificity was 47% (73/156). Eighteen out of 24 TP53 mutation families fulfilled the LFS or LFL criteria (sensitivity 75%). The specificity for the



**Figure 1** Venn diagram showing the overlap of families fulfilling the revised Chompret criteria, Li–Fraumeni syndrome (LFS) and Li–Fraumeni-like syndrome (LFL) families. Number of families are given, in brackets are the mutation positive families (n=22). Two TP53 germline mutations are not included in the figure because they were found in patients who did not fulfil the criteria that are shown here.

**Table 3** Clinical and molecular data on the 24 *TP53* germline mutation families

| Exon/intron | Base pair change | Protein effect | Family-nr | Criteria        | Index case age (age of death) | Family history: tumour type, age (age of death)                                                                                                                              |
|-------------|------------------|----------------|-----------|-----------------|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4           | c.374C→G         | Thr125Arg†     | 1302      | C-revised /LFL  | Br-20 (25)                    | <i>B-30/Br-43/Br-46 (46), Br-? (5), Li-? (28)</i>                                                                                                                            |
| 4           | c.375G→A         | Thr125Thr      | 1860      | C-revised / LFL | B-40                          | Leu-35 (36), Br-24 (24), Br-5 (5), <i>B-41 (43)</i>                                                                                                                          |
| 5           | c.422G→A         | Cys141Tyr      | 1856      | LFS-susp        | B-24 (24)                     | <i>B-37 (37)</i>                                                                                                                                                             |
| 5           | c.451C→T         | Pro151Ser      | 6569      | C-revised /LFL  | Br-24                         | <i>STS-5 (?), ACC-52 (?)</i>                                                                                                                                                 |
| 5           | c.473G→A         | Arg158His      | 93        | C-revised /LFL  | Pa-49 (50)                    | <i>B-34/B-50 (53), STS-36/Lu-36 (38), Bl-61 (61)</i>                                                                                                                         |
| 5           | c.473G→A         | Arg158His      | 706       | C-revised /LFS  | LMS-44                        | St-41/B-43, ChS-30 (35), <i>Br-16 (17), Schw-58, Scc-25</i>                                                                                                                  |
| Intr 5      | 560-1G→A         | IVS5-1G>A      | 1141      | C-revised /LFS  | LMS-36/ La-48 (49)            | OS-22 (23), Pa-52 (52), <i>LS-2 (2), Br-41 (42), RMS-11 (12), Lu-51 (52)</i>                                                                                                 |
| 6           | c.637C→T         | Arg213stop     | 1308      | C-revised /LFS  | B-27/Pa-45 (47)               | <i>STS-38/Pa-41 (41), Ly-9 (10), Br-2 (?)</i>                                                                                                                                |
| 6           | c.638G→A         | Arg213Gln      | 934       | C-revised /LFL  | B-42                          | Ki-48/Th-52 (52), <i>Br-? (5), Mes-55 (55), C-26 (27), Mel-46, B-68/ C-71 (?), O-59 (62), Br-28 (32), B-42 (47), St-71 (?), C-? (47), Lu-55/St-59 (?), B-69/Ki-71, Ki-53</i> |
| 6           | c.641A→G         | His214Arg†     | 108       | C-revised /LFL  | B-35/Br-40/ ACC-41 (41)       | B-30/O-30 (33), Leu-36 (37), <i>B-39/Lu-56 (56)</i>                                                                                                                          |
| 6           | c.665C→T         | Pro222Leu†     | 3838      | LFS-susp        | RMS-4                         | <i>Ca-? (68)</i>                                                                                                                                                             |
| 7           | c.695T→C         | Ile232Thr†     | 3070      | C-revised /LFL  | RMS-54                        | <i>B-37 (37), Br-? (28), Lu-? (22)</i>                                                                                                                                       |
| 7           | c.733G→A         | Gly245Ser      | 3843      | C-revised /LFL  | B-28 (?)                      | B-34 (35), <i>Li-10, Bl-40/Lu-50 (52)</i>                                                                                                                                    |
| 7           | c.733G→A         | Gly245Ser      | 3901      | C-revised /LFL  | Br-27/Oes-37 (37)             | B-28, B-34 (38), Ce-43, B-44                                                                                                                                                 |
| 7           | c.742C→T         | Arg248Trp      | 1375      | C-revised /LFS  | OS-13/B-26                    | B-39 (41), <i>B-? (28)</i>                                                                                                                                                   |
| 7           | c.742C→T         | Arg248Trp      | 324       | C-revised /LFL  | B-32/LMS-42                   | <i>RMS-2/OS-18 (?), Ca-? (40)</i>                                                                                                                                            |
| 8           | c.794T→C         | Leu265Pro      | 3075      | C-revised /LFS  | RMS-2/OS-16 (?)               | RMS-2 (3), Br-7/Oes-44 (45), B-40                                                                                                                                            |
| 8           | c.818G→A         | Arg273His      | 4057      | C-revised       | Br-0                          | <i>OS-33 (39)</i>                                                                                                                                                            |
| 8           | c.916C→T         | Arg306stop     | 816       | C-revised /LFS  | B-34/RMS-48                   | <i>RMS-14 (?), RMS-? (29)</i>                                                                                                                                                |
| 8           | c.916C→T         | Arg306stop     | 16        | C-revised       | RMS-4/B-22 (?)                | <i>father Pr-?, mother B-?</i>                                                                                                                                               |
| Intr 9      | c.994-1 G→T      | IVS9-1G>T†     | 3603      | C-revised /LFS  | C-28/STS-37/Br-51             | <i>B-34/Br-36 (41), Lu-61 (61), Ca-? (35), Ca-? (35)</i>                                                                                                                     |
| 10          | c.1027G→T        | Glu343stop†*   | 5838      | C-revised       | ACC-5                         |                                                                                                                                                                              |
| 10          | c.1031T→C        | Leu344Pro      | 37        | C-revised /LFS  | B-32/B-33/Scc-34              | OS-18 (20), <i>Lu-55 (57), OS-? (40), OS-? (40), B-? (?), OS-? (?), Br-? (?)</i>                                                                                             |
| 2–11        | c.1-?_1182 +?del | Del exon 2-11  | 2556      | C-revised       | ChS-13/LMS-45 /C-48/OS-49 (?) | <i>B-29 (29), C-69</i>                                                                                                                                                       |

LFL, Li–Fraumeni-like syndrome criteria according to Birch; C-revised, Chompret criteria revised in 2009; LFS, classical Li–Fraumeni syndrome criteria; LFS-susp, suspected for LFS but not fulfilling LFS/LFL/revised Chompret criteria.

ACC, adrenal cortical carcinoma; B, breast cancer; Bl, bladder cancer; Br, brain tumour; C, colorectal cancer; Ca, cancer unspecified; Ce, cervical cancer; ChS, chondrosarcoma; Ki, kidney cancer; La, laryngeal carcinoma; Leu, leukaemia; Li, liver cancer; LMS, leiomyosarcoma; LS, lymphosarcoma; Lu, lung cancer; Ly, lymphoma; Mel, melanoma; Mes, mesothelioma; O, ovarian cancer; Oes, oesophageal cancer; OS, osteosarcoma; Pa, pancreatic cancer; Pr, prostate cancer; RMS, rhabdomyosarcoma; Schw, schwannoma; Scc, squamous cell carcinoma; St, stomach cancer; STS, soft tissue sarcoma; Th, thyroid cancer; -, age of diagnosis unknown; (?), age of death unknown; Intr, intron.

†Not included in IARC database.

\*de novo mutation. In italics=tumours of patients for which the date of birth or diagnosis are unknown.

combined LFS/LFL criteria was 81% (127/156). The sensitivity and specificity for the LFS criteria was 33% (8/24) and 98% (153/156), and for the LFL criteria (excluding LFS) 42% (10/24) and 83% (130/156), respectively.

### Tumour spectrum and age of onset

In 52 affected family members (out of 24 families) a germline *TP53* mutation was identified; the mutations, tumour types and ages of onset are given in table 3. The affected mutation carriers developed their first tumour at a mean age of 34.2 years (first tumour age: range 11 months–69 years). When one large family with relative late onset cancers<sup>20</sup> was excluded, the first tumour developed at a mean age of 28.2 years. Twenty-two of the 52 mutation carriers developed at least two primary tumours (42.3%). The mean age of onset of all 77 tumours in *TP53* mutation carriers was 37.4 years (all tumours age: range 11 months–71 years). The tumour type most frequently detected was breast cancer (21/77 tumours; 27%), followed by soft tissue sarcoma (13/77; 17%) and brain tumours (12/77; 16%); eight astrocytoma/glioblastoma, a choroid plexus carcinoma, a malignant plexus papilloma, a medulloblastoma, and a ependyoma). In total 31% of tumours (24/77) were not part of the typical LFS tumour spectrum (breast cancer, soft tissue sarcoma, osteosarcoma, brain tumours, adrenal cortical

tumours and leukaemia). Most tumours were symptomatic and detected before the diagnosis LFS/LFL or LFS suspected was made; two kidney tumours (both renal cell carcinoma) were detected through ultrasound screening. In the 156 *TP53* negative families, the index patient was the only patient tested for *TP53* mutations. The first tumour in these 156 index patients was diagnosed at a mean age of 32.7 years (range 0.5–65 years, data not shown). In total 53/156 index patients developed at least two primary tumours (34%). The mean age at diagnosis of all tumours (236) was 38.2 years. In this group also, the most frequently seen tumour type was breast cancer (89/236, 38%), followed by soft tissue sarcoma (31/236, 13%) and melanoma (22/236, 9.3%); 38% of the tumours diagnosed in the index patients were not typical LFS tumours.

### Cancer risk in *TP53*-positive families

Compared with the expected risks in the general population, the risks for colon, liver and pancreatic cancer were significantly increased in this *TP53*-positive group (RR 2.8, 18 and 7.3, respectively, table 4a), in addition to those for the main LFS tumour types (RR ranging from 6.4 for breast cancer to 107 for bone cancer, table 4). The overall relative cancer risk was increased four times (RR 4, 95% CI 3.3 to 4.8). On average 65%

**Table 4** Tumour type specific cancer risks in *TP53* mutation families, *TP53*-positive LFL families and *TP53*-negative Li–Fraumeni-like syndrome (LFL) families

| ICD code                                               | Location                                        | Obs | Exp  | RR  | 95% CI      | p Value      | Pa n (%)                  | No of fam |
|--------------------------------------------------------|-------------------------------------------------|-----|------|-----|-------------|--------------|---------------------------|-----------|
| a. Cancer risks for <i>TP53</i> mutation families      |                                                 |     |      |     |             |              |                           |           |
| —                                                      | All                                             | 106 | 26   | 4   | 3.3 to 4.8  | <b>0.000</b> | 69 (65)                   | 24        |
| Typical Li–Fraumeni syndrome (LFS) tumours             |                                                 |     |      |     |             |              |                           |           |
| 170                                                    | Bones                                           | 9   | 0.08 | 107 | 49 to 203   | <b>0.000</b> | 5 (56)                    | 6         |
| 171                                                    | Connective tissues                              | 14  | 0.23 | 61  | 33 to 102   | <b>0.000</b> | 10 (71)                   | 11        |
| 174                                                    | Breast                                          | 28  | 4.4  | 6.4 | 4.3 to 9.3  | <b>0.000</b> | 22 (79)                   | 16        |
| 191                                                    | Brain                                           | 13  | 0.37 | 35  | 19 to 60    | <b>0.000</b> | 9 (69)                    | 10        |
| 194                                                    | Adrenal cortical                                | 2   | *    |     |             |              | 1 (50)                    | 2         |
| 205                                                    | Leukaemia                                       | 2   | 0.6  | 3.2 | 0.4 to 12   | 0.258        | 2 (100)                   | 2         |
| Other tumours                                          |                                                 |     |      |     |             |              |                           |           |
| 153                                                    | Colon                                           | 6   | 2.2  | 2.8 | 1 to 6      | <b>0.049</b> | 3 (50)                    | 3         |
| 155                                                    | Liver                                           | 2   | 0.1  | 18  | 2.1 to 64   | <b>0.017</b> | 1 (50)                    | 2         |
| 157                                                    | Pancreas                                        | 4   | 0.54 | 7.3 | 2 to 19     | <b>0.006</b> | 3 (75)                    | 4         |
|                                                        | Other tumours                                   | 20  | 11   | 1.8 | 1.1 to 2.8  |              |                           |           |
|                                                        | Stomach                                         | 3   | 1.1  | 2.6 | 0.5 to 7.7  | 0.215        | 2 (67)                    | 3         |
|                                                        | Lung                                            | 6   | 3.9  | 1.5 | 0.6 to 3.4  | 0.398        | 2 (33)                    | 5         |
|                                                        | Pleura/<br>mesothelium                          | 1   | 0.1  | 9.6 | 0.2 to 53   | 0.21         | 1 (100)                   | 1         |
|                                                        | Melanoma                                        | 1   | 0.7  | 1.4 | 0.03 to 7.6 | 1.000        | 1 (100)                   | 1         |
|                                                        | Cervix uteri                                    | 1   | 0.4  | 2.6 | 0.07 to 15  | 0.622        | 1 (100)                   | 1         |
|                                                        | Ovarium                                         | 1   | 0.6  | 1.6 | 0.04 to 8.7 | 0.933        | 1 (100)                   | 1         |
|                                                        | Prostate                                        | 1   | 1.9  | 0.5 | 0.01 to 3   | 0.896        | 0                         | 1         |
|                                                        | Bladder                                         | 1   | 0.9  | 1.1 | 0.03 to 6.1 | 1.000        | 0                         | 1         |
|                                                        | Kidney                                          | 3   | 0.7  | 4.4 | 0.9 to 13   | 0.067        | 3 (100)                   | 2         |
|                                                        | Other/unspecified<br>parts of nervous<br>system | 1   | 0.03 | 39  | 1 to 218    | 0.075        | 1 (100) (schwan-<br>noma) | 1         |
|                                                        | Non-Hodgkin<br>lymphoma                         | 1   | 0.8  | 1.3 | 0.03 to 7.1 | 1.000        | 1 (100)                   | 1         |
|                                                        | Unknown                                         | 6   |      |     |             |              |                           |           |
| b. Cancer risks for <i>TP53</i> -positive LFL families |                                                 |     |      |     |             |              |                           |           |
| —                                                      | All                                             | 55  | 8.6  | 6.4 | 4.8 to 8.3  | <b>0.000</b> | 38 (69)                   | 10        |
| Typical LFS tumours                                    |                                                 |     |      |     |             |              |                           |           |
| 170                                                    | Bones                                           | —   |      |     |             |              |                           |           |
| 171                                                    | Connective tissues                              | 4   | 0.1  | 41  | 11 to 104   | <b>0.000</b> | 2 (50)                    | 4         |
| 174                                                    | Breast                                          | 20  | 2    | 10  | 6.1 to 16   | <b>0.000</b> | 16 (80)                   | 9         |
| 191                                                    | Brain                                           | 9   | 0.16 | 57  | 26 to 108   | <b>0.000</b> | 6 (67)                    | 6         |
| 194                                                    | Adrenal cortical                                | 1   | *    |     |             |              | 0                         | 1         |
| 205                                                    | Leukaemia                                       | 2   | 0.23 | 8.7 | 1.1 to 31   | 0.052        | 2 (100)                   | 2         |
| Other tumours                                          |                                                 |     |      |     |             |              |                           |           |
| 153                                                    | Colon                                           | 4   | 0.7  | 5.6 | 1.5 to 14   | <b>0.014</b> | 2 (50)                    | 1         |
| 155                                                    | Liver                                           | 2   | 0.04 | 57  | 6.9 to 205  | <b>0.004</b> | 1 (50)                    | 2         |
| 189                                                    | Kidney                                          | 2   | 0.2  | 9.6 | 1.2 to 35   | <b>0.045</b> | 2 (100)                   | 1         |
|                                                        | Other tumours                                   | 11  | 2.6  | 4.3 | 2.5 to 8.1  |              | 9 (69)                    |           |
|                                                        | Stomach                                         | 1   | 0.33 | 3.0 | 0.1 to 17   | 0.555        | 1 (100)                   | 1         |
|                                                        | Pancreas                                        | 1   | 0.17 | 5.8 | 0.2 to 32   | 0.321        | 1 (100)                   | 1         |
|                                                        | Lung                                            | 3   | 0.8  | 3.7 | 0.8 to 11   | 0.103        | 1 (33)                    | 2         |
|                                                        | Pleura/<br>mesothelium                          | 1   | 0.02 | 42  | 1.1 to 232  | 0.072        | 1 (100)                   | 1         |
|                                                        | Melanoma                                        | 1   | 0.33 | 3   | 0.1 to 17   | 0.558        | 1 (100)                   | 1         |
|                                                        | Cervix uteri                                    | 1   | 0.2  | 5.4 | 0.1 to 30   | 0.339        | 1 (100)                   | 1         |
|                                                        | Ovarium                                         | 1   | 0.3  | 3.4 | 0.1 to 19   | 0.503        | 1 (100)                   | 1         |
|                                                        | Prostate                                        | 1   | 0.2  | 4.9 | 0.1 to 27   | 0.369        | 0                         | 1         |
|                                                        | Bladder                                         | 1   | 0.2  | 5   | 0.1 to 28   | 0.364        | 0                         | 1         |

Continued

Table 4 Continued

| ICD code                                               | Location             | Obs | Exp  | RR  | 95% CI      | p Value      | Pa n (%) | No of fam |
|--------------------------------------------------------|----------------------|-----|------|-----|-------------|--------------|----------|-----------|
| c. Cancer risks for <i>TP53</i> -negative LFL families |                      |     |      |     |             |              |          |           |
| —                                                      | All                  | 112 | 25   | 4.5 | 3.7 to 5.4  | <b>0.000</b> | 63 (56)  | 26        |
| Typical LFS tumours                                    |                      |     |      |     |             |              |          |           |
| 170                                                    | Bones                | 4   | 0.07 | 60  | 16 to 154   | <b>0.000</b> | 2 (50)   | 3         |
| 171                                                    | Connective tissues   | 12  | 0.21 | 58  | 30 to 102   | <b>0.000</b> | 10 (83)  | 11        |
| 174                                                    | Breast               | 35  | 3.41 | 10  | 7.1 to 14.3 | <b>0.000</b> | 25 (71)  | 24        |
| 191                                                    | Brain                | 11  | 0.34 | 33  | 16 to 58    | <b>0.000</b> | 6 (55)   | 7         |
| 194                                                    | Adrenal cortical     | 1   | *    |     |             |              | 1 (100)  | 1         |
| 205                                                    | Leukaemia            | 4   | 0.55 | 7.3 | 2 to 19     | <b>0.006</b> | 1 (25)   | 4         |
| Other tumours                                          |                      |     |      |     |             |              |          |           |
| 153                                                    | Colon                | 6   | 2    | 3.1 | 1.1 to 6.7  | <b>0.032</b> | 5 (83)   | 4         |
| 162                                                    | Lung                 | 10  | 4.5  | 2.3 | 1.1 to 4.1  | <b>0.033</b> | 2 (20)   | 8         |
| 172                                                    | Melanoma             | 8   | 0.7  | 12  | 5.3 to 24   | <b>0.000</b> | 5 (63)   | 7         |
|                                                        | Other tumours        | 17  | 7    | 2.4 | 1.4 to 3.9  |              | 8 (47)   |           |
|                                                        | Salivary gland       | 1   | 0.05 | 20  | 0.5 to 109  | 0.12         | 1 (100)  | 1         |
|                                                        | Pharynx              | 1   | 0.16 | 6.4 | 0.2 to 36   | 0.292        | 1 (100)  | 1         |
|                                                        | Stomach              | 4   | 1.15 | 3.5 | 1 to 9      | 0.06         | 2 (50)   | 2         |
|                                                        | Pancreas             | 1   | 0.51 | 2   | 0.1 to 11   | 0.786        | 0        | 1         |
|                                                        | Cervix uteri         | 1   | 0.31 | 3.3 | 0.1 to 18   | 0.52         | 0        | 1         |
|                                                        | Uterus               | 1   | 0.46 | 2.2 | 0.1 to 12   | 0.732        | 0        | 1         |
|                                                        | Prostate             | 2   | 2.07 | 1   | 0.1 to 3.5  | 1.000        | 2 (100)  | 2         |
|                                                        | Bladder              | 1   | 0.98 | 1.0 | 0.0 to 5.7  | 1.000        | 0        | 1         |
|                                                        | Kidney               | 1   | 0.69 | 1.5 | 0.0 to 8.1  | 0.983        | 0        | 1         |
|                                                        | Thyroid              | 1   | 0.14 | 7.3 | 0.2 to 40   | 0.264        | 1 (100)  | 1         |
|                                                        | Non-Hodgkin lymphoma | 3   | 0.44 | 4.6 | 0.6 to 17   | 0.146        | 1 (33)   | 3         |
|                                                        | Unknown              | 4   |      |     |             |              |          |           |

See methods section for estimating relative risk.

ICD code, International Classification of Disease code; Obs, observed; Exp, expected; RR, relative risk; Pa, confirmed by pathology report or clinical records; No of fam, number of families in which the specific tumor type occurred.

\*No data were available on occurrence in the general population.

were confirmed by pathology reports (table 4). Leukaemia was the only typical LFS cancer which showed a non-significantly elevated RR. RRs for all tumour types are shown in table 4a.

#### Comparison of *TP53*-positive and *TP53*-negative LFL (non LFS) families

In the *TP53*-positive LFL families (overall cancer RR 6.4), the RRs for colon, liver and kidney cancer were significantly increased in addition to the RRs for the classical LFS tumour types (table 4b). For the *TP53*-negative LFL families the risks for melanoma and lung and colon cancer were higher than in the general population (overall cancer RR 4.5, table 4c).

#### DISCUSSION

Should *TP53* germline mutation testing only be performed in LFS or LFL families, or do other families or sporadic cases need to be included as well? In the Netherlands, the general advice in 2001 was to be cautious about offering *TP53* mutation testing because present preventive strategies are limited<sup>21</sup>; in 2005 *TP53* mutation testing was recommended for LFS families, LFL families, patients with multiple LFS associated tumours, children with adrenal cortical carcinoma, and possibly for those with breast cancer under 30 years of age.<sup>16</sup>

We tried to determine which familial phenotypes carried a *TP53* germline mutation. In 180 Dutch families, the highest proportion with *TP53* mutations is found in classical LFS families (8/11, 73%). The revised Chompret criteria achieved

the highest sensitivity (92%) with a mutation detection rate of 21%.

Six *TP53*-positive families did not fulfil the LFS or LFL criteria (table 2). Four of these six *TP53*-positive families did fulfil the revised Chompret criteria; of these four, two mutations were detected in patients with at least two typical LFS tumours, one in a child with a choroid plexus carcinoma and one in a child with an adrenal cortical carcinoma. In addition, for the *TP53*-positive LFS suspected cases, the family of the child with a rhabdomyosarcoma at 4 years of age and the family of the patient with breast cancer at 24 years of age did not fulfil the revised Chompret criteria. However, 5 years after detecting the *TP53* germline mutation in the patient who developed breast cancer at 24 years of age, the mother of the patient developed a sarcoma at 61 years of age and turned out to be a carrier of the *TP53* germline mutation as well.

Many authors have investigated the prevalence of *TP53* germline mutations in patients with typical LFS tumours without a striking family history. Patients with sporadic childhood soft tissue sarcomata (STS) carry a *TP53* germline mutation in 6.6–9% of cases, although some of these cases turn out to be familial.<sup>22–23</sup> In the Dutch families described here, only seven patients with STS were included and one *TP53* germline mutation was found (14%). In breast cancer under 30 years of age, *TP53* germline mutations were found in 3.3% of sporadic cases, and 9% (2/22) of familial cases.<sup>24</sup> In three large study cohorts of breast cancer, 0.8% (4/499) carried a *TP53* germline

mutation, and all four mutation carriers showed a family history of cancer.<sup>25–27</sup> In our cohort of 12 patients with breast cancer under 35 years of age, one *TP53* germline mutation was found (8%). Breast cancer at young age was not included in the 2009 revised Chompret criteria because they concluded that the mutation detection rate for patients with early onset breast cancer without a family history of cancer or multiple primary tumours was <5%.<sup>7</sup> Ginsburg *et al* did not find any *TP53* mutations in 95 sporadic breast cancer cases under 30 years of age.<sup>28</sup> Testing adrenal cortical carcinoma patients for germline *TP53* mutations revealed a 50–97% chance of finding a mutation.<sup>29–32</sup> The only sporadic adrenal cortical carcinoma in our cohort carried a de novo *TP53* germline mutation. Garber *et al*<sup>33</sup> mentioned the association of choroid plexus carcinoma (CPC) with LFS or with *TP53* germline mutations; this was confirmed by Krutilkova *et al*.<sup>34</sup> Gonzalez *et al*<sup>12</sup> also reported a strong link between individuals with a CPC and germline *TP53* mutations. All eight patients in their series with CPC referred for sequencing of the *TP53* gene were found to carry a *TP53* germline mutation. In our cohort two patients who developed childhood CPC were included; one of them carried a germline *TP53* mutation.

In the family subtype group ‘two first degree relatives with a tumour at any age’ and the family subtype group ‘at least two primary tumours at any age’ (table 2), no germline *TP53* mutations were detected in 19 and 30 index patients, respectively. Therefore, we concluded that it is not advisable to perform *TP53* germline mutation analysis for families with two cancer affected first degree relatives, when only one of these tumours is typical for LFS, and not for individuals with two or more primary tumours, when their families do not fulfil the revised Chompret criteria.

Our series does of course have some ascertainment bias, because only families who were referred to a clinical genetics centre and were offered and had chosen for *TP53* mutation testing were included. The number of families who did not seek, had not been offered, or had refused genetic counselling and *TP53* mutation testing was not known.

The second part of our study dealt with assessment of the RR of developing cancer based on our survey of different tumour types and ages of onset in LFS/LFL families, with or without *TP53* germline mutation, compared to the general population.

In all *TP53*-positive families, in addition to the main LFS tumour types the RRs for colon, pancreatic and liver cancer were significantly increased. This means that *TP53* mutation carriers might exhibit higher risks for these tumour types (table 4a). Pancreatic cancer as an LFS component tumour has been mentioned before,<sup>15</sup> but is not part of either the Chompret or Dutch recommendations for *TP53* mutation testing. Liver cancer occurred twice in our study sample—one was confirmed by pathology report, the other might very well have been a metastatic disease.

Focusing on the *TP53*-positive LFL families only (n=10, table 4b), in addition to the classical LFS tumour types, the RRs for colon, liver and kidney cancer were significantly increased. In the *TP53*-negative LFL families (n=26, table 4c) the RRs for melanoma and lung and colon cancer were significantly increased. So the occurrence of melanoma and lung cancer in LFL families might not be a very good predictor at present for finding a *TP53* germline mutation.

In conclusion, we recommend *TP53* germline DNA analysis for all families that fulfil the revised Chompret criteria because it has the highest sensitivity for finding a *TP53* germline mutation and a mutation detection rate above 20%. In addition, *TP53* germline DNA analysis could be considered in childhood sarco-

mata and breast cancer before the age of 30 years (without detectable *BRCA1* or *BRCA2* mutation). Pancreatic and colon cancer might be LFS component tumours because of their increased RRs in *TP53* mutation carriers.

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## ***TP53* germline mutation testing in 180 families suspected of Li –Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes**

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