Germline TP53 Mutations and Li-Fraumeni Syndrome

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For the p53 Special Issue

There are now reports of nearly 250 independent germline TP53 (p53) mutations in over 100 publications. Such mutations are typically associated with Li-Fraumeni or Li-Fraumeni-like syndrome, although many have been identified in cohorts of patients with tumors considered to be typical of LFS. In general, the spectrum of mutations that has been detected in the germline reflects that found in tumors, although there are some notable exceptions in certain tumor types. Detailed knowledge of the pedigrees allows a comprehensive analysis of genotype–phenotype correlations and an understanding of the tumors that are associated with germline TP53 mutations. This review will discuss the spectrum of mutations and the methods for mutation detection, the tumors associated with inheritance of a germline mutation, and some of the ethical and clinical problems in patients with a germline TP53 mutation. Hum Mutat 21:313–320, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: germline; p53; TP53; cancer; tumor; Li-Fraumeni syndrome; LFS; LFL; germline mutation; adrenocortical carcinoma; ACC; mutation detection

DATABASES:
TP53 – OMIM: 191170, 151623 (LFS); GenBank: X54156
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/p53 (The IARC p53 Mutation Database)

INTRODUCTION

Many cancer predisposition syndromes have been identified due to the occurrence of a non-random aggregation of cancers in families. Careful epidemiological studies are needed to distinguish between environmental and genetic causes, and in many cases this has confirmed the existence of inherited predisposition. One such example is Li-Fraumeni syndrome (LFS; MIM# 151623), which was initially proposed in 1969 and subsequently confirmed by a number of epidemiological studies [Li and Fraumeni, 1969a,b; Lynch et al., 1973, 1978; Li and Fraumeni, 1982; Birch et al., 1984, 1990; Strong et al., 1987; Li et al., 1988]. While many cancer predisposition syndromes are characterized predominantly by site-specific cancers such as breast cancer, colorectal carcinoma, or melanoma, Li-Fraumeni syndrome is associated with a variety of different tumor types occurring over a wide age range, including childhood. A definition of LFS originated from Li and Fraumeni’s work [Li et al., 1988] as a proband with a sarcoma aged under 45 years with a first-degree relative aged under 45 years with any cancer, plus an additional first- or second-degree relative in the same lineage with any cancer aged under 45 years or a sarcoma at any age. Birch et al. [1994a] subsequently formulated a definition for Li-Fraumeni-like syndrome (LFL) based on more extensive and updated information of the types of tumors and the ages of onset in families. LFL is defined as a proband with any childhood tumor, or a sarcoma, brain tumor, or adrenocortical tumor aged under 45 years plus a first- or second-degree relative in the same lineage with a typical LFS tumor at any age, and an additional first- or second-degree relative in the same lineage with any cancer under the age of 60 years.

GERMLINE TP53 MUTATIONS AND LI-FRAUMENI SYNDROME

The underlying genetic defect in many Li-Fraumeni families is a germline mutation in the TP53 gene (MIM# 191170) as first described by Malkin et al. [1990]. The first five mutations to be described in this
study were all clustered in a short region of exon 7 leading to optimism that all causative mutations could be clustered. Subsequently this was found not to be the case, and mutations have been found throughout the gene. Methods reported for the detection of germline TP53 mutations vary considerably. Some groups still only screen exons 5 to 8 using SSCP, and often this analysis does not include splice sites. Other groups carry out more detailed studies, for example by analyzing the entire coding region of the gene by SSCP, by using the yeast functional assay, or by sequencing the coding regions. However many studies focus on analysis of the so-called hot-spots in the TP53 gene, namely regions II to V encoded by exons 5 to 8 of the gene [Soussi et al., 1990].

The above raises an extremely important issue with respect to testing for mutations within TP53 in patients or families suspected of having a germline mutation. The "gold standard" for mutation detection in TP53 that has been used by our group is direct sequencing from genomic DNA of the entire coding region plus the non-coding first exon, the promoter, the 3'-untranslated region, and all splice junctions. If no mutation is found, the TP53 transcripts are analyzed to see if there are any of abnormal size (indicating, for example, a mutation within an intron leading to aberrant splicing), and if both alleles are expressed; the latter only being possible if the patient has an expressed polymorphism such as that at codon 72. Using this rigorous approach, our group has found germline mutations in 77% (23/30) of LFS and 40% (12/30) of LFL families [Birch et al., 1994a; Varley et al., 1995, 1996a, 1996b, 1997b, 1998a, 1998b].

**DISTRIBUTION OF GERMLINE TP53 MUTATIONS**

Of the 48 germline mutations we have found, 13 (27%) are located outside the coding regions of exons 5 to 8, and include one large deletion, one complex insertion-deletion, and seven splicing mutations [Birch et al., 1994a; Varley et al., 1997b, 2001]. These mutations would have been undetected using the methods employed by some groups. Similar conclusions about a bias in detection of somatic TP53 mutations have been reached by Soussi and Beroud [2002] in a detailed analysis of the Institut Curie database (www.p53.curie.fr) [for recent review see Béroud and Soussi, 2003]. These authors estimate that 13.6% of somatic mutations are located outside exons 5 to 8, leading to an unacceptable bias when estimating the clinical value of TP53 mutation status as a prognostic or predictive marker [Soussi and Beroud, 2002].

Given the range of techniques used and regions of the gene screened, it can be difficult to come to firm conclusions about the sites and types of mutations in the germline. With the corollary that there is a bias in mutation detection in the germline, but that a similar bias exists in the somatic mutation databases, in general it seems that the mutation spectrum in the germline does reflect that seen in tumors. Mutations in codons 248, 273, 245, 175, and 282 are the most common in both sporadic tumors and the germline, although their ranking is somewhat different sporadic tumors and the germline (Table 1). Note, however, that this figure does not include the hot-spot mutation at codon 337 found at high frequency in children with adrenocortical carcinoma from Brazil, which is discussed in more detail below. However there is the same frequency of germline mutations affecting codon 213 as codon 282 in the germline, which is higher than in tumors. Other sites that are reported more frequently as germline mutations include codons 235, 133, and 181. The reasons for this are unclear at present, but are not due to either a bias in detection methods (all are within exons 5 to 8) or to the occurrence of founder mutations. Splicing

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**TABLE 1. Details of the Amino Acids Encoded by the 11 Exons of TP53**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amino acids</th>
<th>Conserved domains</th>
<th>Somatic hot spot mutations</th>
<th>Germline hot spot mutations</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Non-coding</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Start–26</td>
<td></td>
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<td></td>
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<tr>
<td>3</td>
<td>27–32</td>
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<td></td>
<td></td>
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<tr>
<td>4</td>
<td>33–125</td>
<td>Ila 117–125</td>
<td>158, 175(1), 183</td>
<td>175(4)</td>
</tr>
<tr>
<td>5</td>
<td>126–186</td>
<td>IIb 126–142</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III 171–181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>187–224</td>
<td></td>
<td>198, 213</td>
<td>213</td>
</tr>
<tr>
<td>7</td>
<td>225–261</td>
<td>IV 234–258</td>
<td>234, 245(4), 248(2)</td>
<td>235, 245(5), 248(2)</td>
</tr>
<tr>
<td>9</td>
<td>307–331</td>
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<tr>
<td>10</td>
<td>332–367</td>
<td></td>
<td></td>
<td>337(1*)</td>
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<td>11</td>
<td>368–393</td>
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The positions of the five highly conserved residues are shown, and the most frequently detected mutations in both the germline and in sporadic tumors are indicated. The ranking of the mutations in decreasing frequency are given for the four most common somatic and the five most common germline mutations.

Note that the most frequent mutation reported in the germline affects codon 337, but this has been detected primarily in children in southern Brazil with adrenocortical carcinoma.
mutations have also been detected more frequently in the germline than in tumors. Studies from our own group have detected seven splicing mutations in a total of 48 cases in which germline TP53 mutations have been found (15%) [Varley et al., 2001; unpublished data] and in all 19 splicing mutations have been reported in the literature, representing around 8% of all mutations. Whether this does indeed reflect a genuinely higher frequency of splicing mutations in the germline remains open, as most of the somatic splicing mutations may not have been detected using methods described by many groups. However in some studies where a comprehensive analysis of somatic mutations has been carried out, a significant proportion of splicing mutations has been found [e.g., Casey et al., 1996].

**FREQUENCY AND MORPHOLOGY OF CANCERS IN LI-FRAUMENI FAMILIES**

A number of studies initially identified component tumors of LFS as bone and soft-tissue sarcomas, premenopausal breast carcinoma, brain tumors, adrenocortical carcinoma, and leukemias [Li and Fraumeni, 1969a; Lynch et al., 1978; Li et al., 1988]. Other studies have indicated that a wider range of tumors such as melanoma, Wilms’ tumor, and lung, gastric, and pancreatic carcinoma could occur at increased frequency in LFS families [Strong et al., 1987; Hartley et al., 1993; Varley et al., 1995, 1997b]. However most of the studies have not systematically analyzed families that are well characterized and documented, with verified cancers and accurate ages of affected and unaffected family members.

We have examined a cohort of 28 families with germline TP53 mutations in which all cancers had been verified, and the ages of all family members, affected and unaffected, were known. The incidence of cancer in these families was analyzed by comparison to national data for the same time periods giving an accurate picture of the component tumors associated with a germline TP53 mutation. Proband and support cancers were excluded from the analysis because, as they are prescribed, they would introduce a bias into the analysis. These data have been reported [Birch et al., 2001], but in summary the distribution of cancers in carriers of a germline TP53 mutation is highly significantly different from the expected cancer distribution in the general population. The tumors originally identified as being components of Li-Fraumeni syndrome were found to be strongly associated with a germline TP53 mutation, with the exception of leukemia, which was not found to be a major component. However, Wilms’ and malignant phylloides tumors of the breast were found at significantly higher frequency. The increased cancer risk was most marked at younger ages, and decreased with age. The only common adult epithelial tumor apart from breast to be found at an increased frequency was pancreas, and there was no increased risk of lung, ovary, bladder, bowel, or head and neck tumors. The latter tumors are particularly interesting because somatic TP53 mutations are found in around 60% of sporadic tumors at these sites [Soussi et al., 2000]. Clearly then, the tumors that are seen at elevated frequency in Li-Fraumeni syndrome are not simply those in which somatic mutations are common. The tissue/cell specificity associated with inheritance of a germline TP53 mutation is still not understood, but is very striking.

**GENOTYPE–PHENOTYPE STUDIES IN LI-FRAUMENI FAMILIES**

We have also carried out genotype–phenotype analysis on a cohort of families with or without germline TP53 mutations. Families with mutations within the central core domain of the gene have generally more cancers, and at younger ages than families with null mutations (nonsense, splice, deletions, and insertions) or with no germline TP53 mutation [Birch et al., 1998]. In addition, loss of the wild-type allele is less common in tumors from patients with the former type of mutation. In general, mutations within the core DNA-binding domain of p53 have a gain-of-function or are dominant, and therefore it might be expected that there is no selective pressure in tumors for loss of the wild-type allele. In contrast, in tumors with a germline null allele there will be selection for the remaining wild-type allele to be inactivated or deleted in order for a tumor to develop.

**TP53 GERMLINE MUTATIONS IN PATIENTS WITH TUMORS TYPICAL OF LFS**

Many studies have been carried out analyzing the frequency of germline TP53 mutations in cohorts of patients with tumors typical of Li-Fraumeni syndrome, or with multiple primary tumors. The greater the level of selection in cohorts of patients, the higher the frequency of germline mutations. This is exemplified by studies of osteosarcoma, where only 3% of children with osteosarcoma were found to have germline mutations [McIntyre et al., 1994], whereas when patients with either a family history or multiple primary tumors were studied, the figure rose [Toguchida et al., 1992]. Other studies have looked at cohorts of patients with a variety of sarcomas [Iavarone et al., 1992; Porter et al., 1992; Toguchida et al., 1992; McIntyre et al., 1994; Diller et al., 1995], breast cancer [Børresen et al., 1992; Prosser et al., 1992; Sørdal et al., 1992], brain tumors [Chung et al., 1991; Kyritsis et al., 1994; Chen et al., 1995;
Felix et al., 1995; Li et al., 1995; Zhou et al., 1999], and childhood adrenocortical carcinoma [Sameshima et al., 1992; Wagner et al., 1994; Varley et al., 1999; Ribeiro et al., 2001].

Germline TP53 mutations have been detected infrequently in breast cancer, although the figure does appear to be higher in cases of premenopausal breast cancer [Børresen et al., 1992; Sidransky et al., 1992]. We have recently completed a study looking at the frequency of germline mutations in TP53, BRCA1, and BRCA2 in 99 women presenting with breast cancer aged 30 years or younger. Detailed family histories were taken, and 31 of the women had a family history of breast cancer aged under 65 years and/or ovarian cancer, five had a history conforming to LFS/LFL, and the remainder had no significant family history. Overall, 4% of the women in the cohort had germline TP53 mutations, two were in LFS/LFL families, and the other two had no family history [Lalloo et al., 2002]. We have also examined a series of 21 families that do not conform to LFS, but that were ascertained as a proband with a sarcoma where either that patient or a first-degree relative has developed breast cancer aged under 65 years and/or ovarian cancer, five had a history conforming to LFS/LFL, and the remainder had no significant family history. Overall, 4% of the women in the cohort had germline TP53 mutations, two were in LFS/LFL families, and the other two had no family history [Lalloo et al., 2002].

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In our study of children with ACC unselected for family history, the majority of mutations occurred at codons 152 or 158 [Varley et al., 1999]. There was no evidence of a founder mutation, and as yet we have no explanation for this clustering of mutations. It is worth noting that in a previous study Wagner et al. [1994] also identified a germline codon 152 mutation in a case of childhood ACC. In a recent study of Brazilian children with ACC, Ribeiro et al. [2001] found an identical codon 337 mutation in 35 of 36 children, again with no founder effect. Both we and Ribeiro et al. [2001] have proposed that there may be low penetrance TP53 alleles which predispose to childhood ACC at high frequency. The markedly different mutation spectra in the two studies may be due to exposure of the Brazilian children to carcinogenic insults, particularly pesticides [Sandrini et al., 1997]. While the codon 152 mutation has been shown to be defective in a number of functions including transactivation in the yeast functional assay [Boyle et al., 1999], the codon 337 mutation identified by Ribeiro et al. [2001] has not until recently been shown to have any functional consequences [Lomax et al., 1997; Davison et al., 1998]. However an explanation for the tumor specificity of the codon R337H mutation has been proposed recently with the identification of pH-dependent instability of the mutant p53 tetramer [DiGiammarino et al., 2002]. These authors propose that the vastly increased risk of children with this mutation of developing ACC is a consequence of the increased pH levels in apoptotic adrenal cortex cells during the pre- and post-natal development of the adrenal gland.

Management of patients with germline TP53 mutations is still a difficult issue. The range of different sites and types of cancer associated with LFS, and the variable age of onset complicate screening. There are no national UK guidelines for either genetic testing for a germline TP53 mutation, or for presymptomatic screening, although such guidelines have been published in France [Frebourg et al., 2001]. We still follow the procedures and recommendations that we have described previously [Varley et al., 1997a]. The definitions of LFS and LFL are reliable predictors for a germline mutation as around 80% and 40% of such families respectively carry germline TP53 mutations (see above). In addition, children with ACC have an extremely high frequency of germline mutations, and so germline testing may be indicated. Defining other groups who may be predicted to have a significant proportion of germline mutations is more difficult. From our own

GERMLINE TP53 MUTATIONS IN CHILDHOOD ADRENOCORTICAL CARCINOMA

The most striking association between germline TP53 mutations and cancer is found in cases of childhood adrenocortical carcinoma (ACC). ACC was identified as a component tumor of Li-Fraumeni syndrome from the earliest studies [Li and Fraumeni, 1969a,b; Lynch et al., 1978; Li and Fraumeni, 1982], and this has been borne out by all subsequent analyses [e.g., Kleihues et al., 1997; Birch et al., 1998; Nichols et al., 2001; Chompret, 2002]. Two small studies have been reported that analyzed germline TP53 mutations in children with ACC [Sameshima et al., 1992; Wagner et al., 1994], and we subsequently investigated the frequency of germline mutations in a cohort of children with ACC unselected for family history [Varley et al., 1999]. Of the patients studied, over 80% had a germline TP53 mutation, and in addition all 12 LFS/LFL families that we have studied in which there is a case of ACC have a germline TP53 mutation. In total, of all families in which there is a case of ACC, plus children with ACC unselected for family history, 22 out of 25 have a germline TP53 mutation (88%).
studies looking at families with a case of breast cancer and sarcoma, the frequency of germline mutations was low [Evans et al., 2002], and these two tumor types are known to be associated with Li-Fraumeni syndrome. It must be stressed at this point that germline testing should be offered only in the context of appropriate clinical support and consideration of the wealth of clinical, social, psychological, and ethical issues associated with such testing, even if the chances of finding a causative mutation are high.

Screening patients in Li-Fraumeni families or those with verified germline TP53 mutations is also not simple, and there are no widely accepted procedures. Our group recommends annual clinical review with access to informed clinicians. MRI can be offered to women at risk of developing breast cancer, and abdominal ultrasound for children. We no longer carry out a blood count in children as the leukemia risk is not sufficiently high (DGR Evans, personal communication). A number of studies have now been published detailing the occurrence of radiation-induced second malignancies in mutation carriers [Hisada et al., 1998; Varley et al., 1999; Limacher et al., 2001; Nutting et al., 2001]. Together with experimental data in systems looking at the radiation sensitivity of cells carrying a mutant TP53 allele, there is a strong indication that radiotherapy should be avoided if possible.

Finally, it needs to be noted that germline TP53 mutations are not responsible for all cases of Li-Fraumeni syndrome. A number of studies have excluded various candidate genes including MDM2 [Birch et al., 1994b], PTEN, p16 [Burt et al., 1999; Brown et al., 2000; Portwine et al., 2000], Bcl10 [Stone et al., 1999], p63 [Bougeard et al., 2001], and hChk1 [Bell et al., 1999; Vahteristo et al., 2001]. However mutations in the cell-cycle checkpoint kinase hChk2 have been reported in Li-Fraumeni families [Bell et al., 1999; Lee et al., 2001; Vahteristo et al., 2001], and there is evidence that these mutations are functionally compromised [Lee et al., 2001; see Varley, 2003]. The importance of germline hChk2 mutations in Li-Fraumeni syndrome and in familial breast cancer is still somewhat unclear, with recent reports that the same germline mutation which was found in an LFS family [Bell et al., 1999] is a low-penetration susceptibility gene in breast cancer families [Meijers-Heijboer et al., 2002; Vahteristo et al., 2002].

Certainly Li-Fraumeni syndrome and the germline mutations associated with it remain fascinating and informative, but there are still many unanswered questions and unresolved issues.

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REFERENCES


