Genetic Mapping of a Third Li-Fraumeni Syndrome Predisposition Locus to Human Chromosome 1q23

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Abstract
Li-Fraumeni syndrome (LFS) is a clinically and genetically heterogeneous inherited cancer syndrome. Most cases (~70%) identified and characterized to date are associated with dominantly inherited germ line mutations in the tumor suppressor gene TP53 (p53) in chromosome 17p13.1. In a subset of non-p53 patients with LFS, CHEK2 in chromosome 22q11 has been identified as another predisposing locus. Using a genome-wide scan for linkage with complementing parametric and nonparametric analysis methods, we identified linkage to a region of approximately 4 cM in chromosome 1q23, a genomic region not previously implicated in this disease. Identification of a third predisposing gene and its underlying mutation(s) should provide insight into other genetic events that predispose to the genesis of the diverse tumor types associated with LFS and its variants. (Cancer Res 2005; 65(2): 427-31)

Introduction
Li and Fraumeni (1, 2) reported a rare, familial cancer syndrome characterized by autosomal dominant inheritance and early onset of tumors, multiple tumors within an individual, and multiple affected family members (3, 4). In contrast to other inherited cancer syndromes, which are predominantly characterized by site-specific cancers, Li-Fraumeni syndrome (LFS; MIM 151623) families present with a variety of tumor types. The most common types are soft tissue sarcomas (STS) and osteosarcomas (OST), breast cancer, brain tumors, leukemia, and adrenocortical carcinoma. Other less frequent tumors include melanoma and carcinomas of the lung, pancreas, cervix, and prostate (2). Classic LFS is defined as a proband with a sarcoma before the age of 45 years and a first-degree relative age <45 years with any cancer and one additional first- or second-degree relative in the same lineage with any cancer age <45 years or a sarcoma at any age (1, 3).

The inheritance of a single mutant p53 allele results in predisposition to tumor development with high penetrance, and p53 germ line mutation carriers have a dramatically elevated cancer risk relative to non–mutation carriers (5–9). In most tumors, both alleles are altered—one by inherited mutation, the other by somatic loss of the wild-type allele (10, 11). To date, germ line p53 mutations have been identified in approximately 70% to 75% of LFS families fulfilling the classic criteria (12–14). Based on updated information of the types of tumors and the ages of onset in affected families, Birch et al. (12, 15) defined Li-Fraumeni–like syndrome (LFL) as a proband with any childhood cancer, or a sarcoma, brain tumor, or adrenocortical tumor age <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 age <60 years. Brugieres et al. (16) defined incomplete LFS (LFI) as any kindred with at least two cases consistent with LFS at any age instead of the minimum of three cases required for classic LFS or LFL. Germ line mutations in p53 have been identified in 40% of LFL (12, 17–23) and 6% of LFI (24). Based on segregating germ line mutations, CHEK2 in 22q12.2 was recently implicated as a second predisposing LFS locus and a tumor suppressor gene (25). The role of CHEK2 mutations has been somewhat controversial due to the presence of related genes on various other chromosomes in the human genome. However, the 1100delC sequence variant clearly seems to be a disease-causing mutation in LFS (17, 18, 25–29).

Materials and Methods
Subjects. We previously ascertained a large cohort of LFS and LFL kindred through systematic surveys of childhood and adolescent patients with sarcoma (9, 30, 31). In several of these LFS kindred direct sequencing of all coding exons and splice junctions and Southern blot analysis revealed no mutations or deletions in p53 (32). Together these data provide significant evidence for high-risk LFS families with a clinical phenotype similar to that of classic LFS with no detectable p53 or CHEK2 germ line mutations, hereafter collectively referred to as non-p53 LFS. Based on the phenotypic similarities between p53 and non-p53 LFS kindred, we hypothesize that the inherited susceptibility in these kindreds is the result of a highly penetrant, dominantly acting tumor suppressor gene.

Genotyping and Analysis. To map this predisposition locus, we carried out a genome-wide scan for linkage initially focused on four extended, non-p53 LFS pedigrees (STS1, STS2, STS3, and OST1) shown in Fig. 1, with a total of 62 constitutive DNA samples. In power simulations (33), these kindreds were found most likely to provide the highest LOD scores in parametric linkage analysis. We did a genome-wide scan for linkage with 400 highly polymorphic microsatellite markers [Linkage Mapping Set Version 2 (LMSv2), ABI, Foster City, CA] at an average resolution of ~10 cM (62 samples × 400 markers = ~25,000 genotypes). For linkage analysis, we used a combination of parametric (two-point and multipoint) and nonparametric linkage analyses, done using

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LINKAGE/FASTLINK (34), GENEHUNTER (35, 36), SIMPLE (37), VITESSE (38), and SIMWALK2 (39, 40). Linkage analysis was conducted assuming the genetic model defined by Lustbader et al. (31) in which individuals from 159 families ascertained through early-onset soft tissue sarcomas were classified into 10 age-, sex-, and cohort-specific groups according to similarity in population-based risks. Segregation analysis of these families provided estimates of the cumulative risks for all cancers. We applied the approach first suggested by Margaritte et al. (41) in which interval- and genotype-specific probabilities were assigned to individuals who developed cancers, and genotype-specific disease free probabilities were used for noncarriers (Table 1). This approach to analysis gave highest weight to early-onset cancers and very little weight to late-onset cancers.

Results

Consistent with the previous exclusion of \(p53\) (32), the 17p13.1 region containing \(p53\) showed no evidence of linkage. Moreover, no linkage to the 22q12.1 region containing \(CHEK2\) was observed. No evidence of suggestive linkage to any of the other previously considered and excluded LFS candidate genes was seen, namely, \(MDM2/HDM2\) in 12q15, \(PTEN\) in 10q23.3, \(CDKN2A\) in 9p21.3, \(BCL2\) in 18q21.3, \(TP73L\) (\(p63\)) in 3q28, \(CHEK1\) in 11q24.2, and \(TP73\) in 1p36.32.

Initially, the highest genome-wide LOD scores providing evidence for suggestive linkage were obtained by two-point linkage analysis for marker \(D5S96\) at 169.40 cM (LOD = 1.81 at \(\theta = 0.02, \) HomogLOD = 2.04). Examination of kindred-specific LOD scores indicated that both STS1 and STS3 contributed positively (with STS3 providing the strongest evidence for linkage), whereas STS2 contributed little, and OST1 contributed negatively to the overall LOD score. Multipoint linkage analysis provided further evidence for suggestive linkage with the highest LOD scores approaching 3.00 in the region between \(DIS484/\)

![Figure 1. Pedigrees of four non-\(p53\) Li-Fraumeni syndrome kindreds included in the genome-wide scan for linkage. Males are represented by squares and females by circles. Filled symbols are considered affected. A slash through a symbol indicates the person is deceased.](image)

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Figure 2. Graph showing separate and combined results of SIMWALK2 multipoint analysis for all four non-p53 LFS kindred on whom the genome-wide scan for linkage was done. Chromosome 1 gave the highest results; shown are the results for 54 markers in the analysis. The LOD score for kindred STS3 shows a significant peak value of 3.455 at 155 cM from pter (1q23). This strong positive signal is also seen in the total and heterogeneity LOD (H-LOD) scores. Kindred OST1 seems to exclude chromosome 1 over most of its length. Most of the remainder of the chromosome is excluded.

Figure 3. SIMWALK2 LOD scores for all 400 microsatellite markers across the genome. X axis, cM on the deCODE map; Y axis, LOD scores. Each family is represented by a separate graph.
157.51 cM and D1S413/194.98 cM in 1q23.3-q32.1 with STS1 and STS3 again making the largest contributions. Using additional markers in the 1q candidate region, we confirmed the initial linkage results and obtained definitive evidence for linkage with multipoint LOD scores >3.00 between D1S2635/154.28 cM and D1S1677/161.50 cM (Homolog.LOD = 2.42; SIMWALK2, H-LOD = 3.57). The same region seemed to be excluded in STS1 with LOD scores ≤−2.00 throughout, indicating possible additional genetic heterogeneity. The results of the SIMWALK2 analysis for chromosome 1 are shown in Fig. 2. SIMWALK2 LOD scores across the entire genome are shown for each family separately in Fig. 3.

Consistent with linkage, affected patients from the linked kindreds (STS1 and STS3) showed excess marker and haplotype sharing for the candidate region. Whereas kindred STS1 showed a LOD score >2.0, this was much less than the maximum expected by simulation (ELODmax = 4.967). Therefore, for purposes of defining the critical region, we elected to make the conservative assumption that this peak might represent a false-positive result in this family. Based on a region shared identical-by-descent in affected individuals of kindred STS3, which had a significant positive LOD score, the critical region was initially found to be located between D1S2635/154.28cM and D1S1677/161.50 cM, an interval of ~8.6 cM contained within a 4.6-Mb region. Further fine mapping of the critical region was significantly aided by the recent completion of the human genome draft sequence (42) and subsequent completion of the finished sequence. By using additional microsatellite markers from the region, both known and novel, we identified informative cross-overs in affected members of pedigree STS3, which provided the strongest evidence for linkage, and further narrowed the critical region to less than 1.88 Mb. On the centromeric side, individual III:4 has a cross-over between intron 1 of KCNJ10 and marker D1S2771. On the telomeric side, individual IV:1 has a cross-over between a (GT)n repeat located in NRR13 and a (GT)n, repeat located in intron 1 of ATF6. Alleles seen for microsatellite markers on the affected chromosomes in the critical region are shown in Fig. 4. For reasons of space and readability, it is impractical to display the entire pedigree with all alleles and haplotypes. However, a total of 73 microsatellite markers were included in the haplotype analysis and 54 were used for the linkage analysis. Primer sequences for novel markers are provided in Table 2.

### Discussion

The existence of familial predisposition to cancer has been instrumental in the mapping and subsequent cloning of several tumor suppressor genes, such as BRCA1 (43) and BRCA2 (44). The identification of high-penetrance inherited cancer genes in, for example, breast cancer or colorectal cancer, has provided considerable fundamental and unexpected insights into many aspects of cancer biology (45–47). LFS, as a childhood cancer, is a unique model system to study the underlying genetic events associated with a complex cancer syndrome, presumably because fewer somatic alterations are needed to give rise to the associated cancers. Successful mapping of a novel LFS predisposition locus in 1q23 and, subsequently, the identification and characterization of the gene and its mutation(s) will be significant in several respects. First, it will immediately benefit affected patients and their relatives.

### Table 2. Primer sequences for custom markers

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<th>Marker</th>
<th>Forward primer sequence*</th>
<th>Reverse primer sequence†</th>
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<td>CAGATGGAGGTTAACCTTGGCGAATGGG</td>
<td>CTAATTTCCTTTCACTTGATGAGGAC</td>
</tr>
<tr>
<td>KCNJ9-i1_GT</td>
<td>CCTCCAAGACGAGATGTTGAGGAAG</td>
<td>CTGGTGGGAGAAGAGGATGTC</td>
</tr>
<tr>
<td>KCNJ10_i1_GT</td>
<td>GMACACTTTAAGAGGCGTCTACAC</td>
<td>GAATGGGTGATTTGCTGTATCTCTG</td>
</tr>
<tr>
<td>RhoGap_i5_TC</td>
<td>GCAACAATTTAAGAGGGAGGAGGAGG</td>
<td>CTACGCCAAGAAATCTCCAC</td>
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<tr>
<td>ADAMTS4_CA</td>
<td>GGCCTCTCCCTACCCTCACTCT</td>
<td>CCTGGTCAGGGTGTAGCACATAC</td>
</tr>
<tr>
<td>NRR13_GT</td>
<td>GACAGGACAGAGACTTCTCATCT</td>
<td>GAAAACCCCGAGATAGGGAGG</td>
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<tr>
<td>ATF6_i1_GT</td>
<td>GGTATAGCGATCCTCCTTACCT</td>
<td>CATGAGATGCTTTCCTTGT</td>
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</table>

NOTE: PCR was carried out using 3 primers; a forward primer containing a 5’ universal tail, a reverse primer containing the 5’ pigtail sequence, and a Universal primer with the sequence GGGGACACCGCTGATGCTTTGA. Reaction conditions are described in Bachinski et al. (48).  
*Each forward primer was designed with a Universal tail at its 5’ end, having the sequence GACGGGACACCGCTGATGCTTTGA.  
†Each reverse primer contains a pigtail sequence (GTTTCTT) at its 5’ end to facilitate nontemplated polyadenylation of the PCR product.
families, if so desired, by providing the means for DNA-based genetic testing either for linkage or for specific mutations (since the actual gene has been identified). Second, identification of the mutated gene and determination of its mutational spectrum will provide the basis to determine phenotype/genotype correlations to explain the highly variable clinical phenotype seen in the affected LFS kindred. Given the importance of p53 in p53 and the Li-Fraumeni syndrome. Methods Mol Biol 2003:221:117–29.


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