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Advances in Brief

A Germ Line Mutation in Exon 5 of the *p53* Gene in an Extended Cancer Family[1]

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Abstract

Germ line p53 point mutations have been reported for some families with Li-Fraumeni syndrome, a syndrome characterized by a dominantly inherited increased susceptibility for the development of early age of onset neoplasms of diverse origin in multiple family members. All of the initially reported *p53* germ line mutations have been found exclusively within a single conserved, nonpolymorphic region of the gene between condons 245 and 258. The restricted distribution of these inherited mutations has led to speculation that germ line *p53* mutations have unique properties [B. Vogelstein, Nature (Lond.), *348:* 681–682, 1990]. We report here on the identification of a *p53* germ line mutation at codon 133 (ATG → ACG) in nine members of an extended Li-Fraumeni syndrome family. This mutation leads to an amino acid substitution in the protein and is shown to completely cosegregate with Li-Fraumeni syndrome associated cancer in this family. Its location extends the region of the *p53* gene where inherited mutations predisposing to cancer are observed and suggests that their distribution may be diverse.

Introduction

Point mutations within the *p53* tumor suppressor gene are a common finding in a variety of sporadic tumors in man (see Ref. 1 for review). Additionally, *p53* germ line mutations have been identified in a familial form of cancer known as Li-Fraumeni syndrome. Most tumor associated mutations and all reported germ line mutations occur within well defined nonpolymorphic regions of the gene (1–3). The *p53* gene has been conserved during evolution. Comparison of the amino acid sequence in various species reveals significant nonrandom homology concentrated in five discrete blocks (conserved blocks I–V). These regions are believed to be important protein functional domains (4). Conserved blocks II–V, which are scattered throughout exons 4–8, have been implicated as the most common sites for tumor associated *p53* mutations (1). LFS[3] is a familial form of cancer in which affected family members display diverse tumor types including soft tissue sarcomas, breast carcinoma, brain tumors, osteosarcoma, leukemias, and adrenocortical carcinoma. Cancer commonly develops at an early age and additional diverse primary tumors commonly develop in survivors of the initial cancer. Segregation of tumors within these families is consistent with an autosomal dominant mode of inheritance of cancer susceptibility (5–7). The *p53* germ line mutations which have been previously identified in LFS families (2, 3) all cluster within a span of 42 nucleotides of conserved block IV and have been limited to one of four codons (Fig. 1). These point mutations result in amino acid substitutions in the protein and are believed to represent loss of function mutations leading to an increased susceptibility for cancer in carriers, as would be predicted by the two-hit model of Knudson (8) and DeMars (9). The significance of the restricted distribution of these initially reported germ line mutations is uncertain but has led to speculation that germ line *p53* mutations may have unique properties (10). The overall purpose of this study was to test for germ line mutations in the *p53* gene in families exhibiting a genetic predisposition to cancer. One such mutation in an extended cancer prone family is described here.

Materials and Methods

Our study population consists of an extended LFS family in which 12 members, with various cancers, were identified over 4 generations descending from a woman who died of breast cancer at the age of 52. This family was initially ascertained through a systematic study of families of 3-year survivors of childhood soft tissue sarcomas and provided strong statistical evidence for an autosomal dominant mode of inheritance for cancer susceptibility in Li-Fraumeni syndrome (7).

Genomic DNA was extracted by standard techniques (11) from lymphocytes obtained from peripheral blood samples. The conserved block regions of the gene were amplified by PCR. A 1.1-kilobase fragment containing conserved blocks II and III spanning an area of genomic DNA from codon 51 (exon 4) to codon 199 (exon 6) was amplified with primers (PA3) 5′-ACA ATG GTT CAC TGA AGA CCC AGG-3′ and (PA8) 5′-CTT CCA CTC GGA TAA GAT GC-3′. A second 638-base pair fragment containing conserved blocks IV and V comprised all of exon 7 and 8 and was amplified with primers (PA5) 5′-TCT CCT AGG TTG GCT CTG AC-3′ and (PA6) 5′-CTT ACC TCG CTT AGT GCT CC-3′. Multiple internal sequencing primers were designed in order to obtain sequencing data on both strands for all four conserved blocks (Table 1). Nucleotide sequencing was performed by use of the dideoxynucleotide chain terminating method (12) and was carried out directly on the double stranded PCR amplification products (13). Since sequencing was performed directly on PCR amplified products, the resulting sequencing ladder would represent the consensus sequence of the entire population of DNA molecules. Random PCR related misincorporation errors should not be detectable unless they occur during the initial PCR cycles (14). To rule out this possibility each sample was amplified in triplicate and the products were pooled prior to sequencing. The entire amplification and sequencing process was done in duplicate on those individuals found to carry the codon 133 mutation. Both DNA strands were sequenced in order to confirm the mutation.

Results and Discussion

Twenty-eight members of this family (Fig. 2) were examined for the possibility of *p53* germ line mutations in conserved blocks II–V. Eight of these family members were affected with cancer, seven with various LFS associated neoplasms and one with Hodgkin's disease. A *p53* germ line point mutation was identified at codon 133 of conserved block II (ATG → ACG) and shown to be present in the heterozygous condition in each of nine family members (Fig. 3). All affected members with LFS associated tumors were heterozygous for this *p53* mutation; they include the proband (IV-12), his sister (IV-13), four

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[3] The abbreviations used are: LFS, Li-Fraumeni syndrome, PCR, polymerase chain reaction.

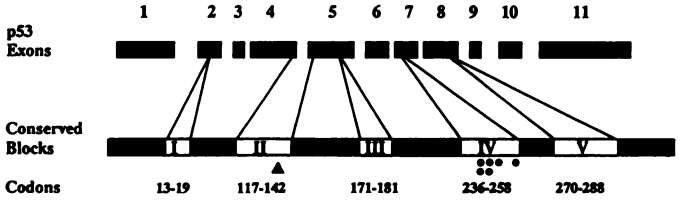

Fig. 1. Evolutionary conserved blocks of the *p53* gene. Conserved blocks II–V have been shown to be hot spots of mutation in sporadic tumors. Location of hereditary mutations in LFS are indicated by *circles* (previously identified: codons 245, 248, 252, 258) and *triangle* (this report: codon 133).

Table 1 *DNA sequencing oligonucleotide primers*

Primer sequence	Strand sequenced	Conserved blocks included
5′-CTG TCA TCT TCT GTC CCT TC-3	Coding	IIA[a]
5′-TG AAG TCT CAT GGA AGC CAG-3′	Noncoding	IIA
5′-AC TCT GTC TCC TTC CTC TTC-3′	Coding	IIB
5′-CGT CAT GTG CTG TGA CTG C-3′	Noncoding	IIB
5′-TC CGC GCC ATG GCC ATC TAC-3′	Coding	III
5′-GCC AGA CCT AAG AGC AAT CAG-3′	Noncoding	III
5′-TCT CCT AGG TTG GCT CTG AC-3′	Coding	IV
5′-AG GCC AGT GTG CAG GGT GGC-3′	Noncoding	IV
5′-CCT ATC CTG AGT AGT GGT AA-3′	Coding	V
5′-CTC CCC TTT CTT GCG GAG A-3′	Noncoding	V

[a] Conserved block II is split by a 600-base pair intron and was divided into IIA and IIB for sequencing purposes.

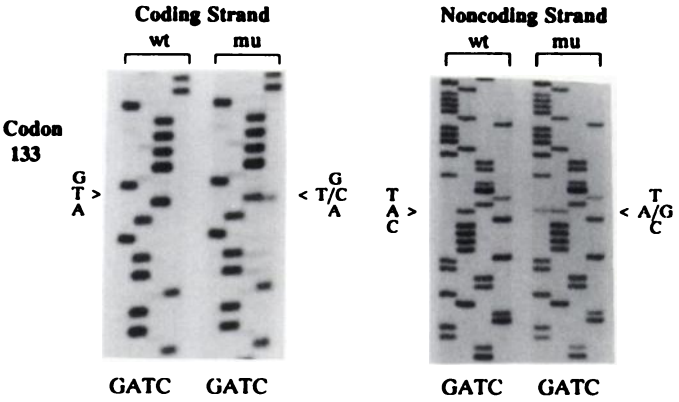

Fig. 3. Location of *p53* germ line mutation in a LFS family. Nucleotide sequence autoradiogram of coding and noncoding strands for conserved block II (exon 5) from homozygous wild type (*wt*) and heterozygous mutant (*mu*) samples confirms a single base substitution at codon 133. The base substitution mutation is depicted by a second sequencing band migrating at the same position as the normal band from the nonmutated allele (ATG → AT/CG) coding strand, CAT → CA/GT noncoding strand).

Table 2 *Germ line p53 mutation at codon 133 and cancer in members of a Li-Fraumeni syndrome family*

Family member	Sex	Neoplasm	Age at diagnosis (yr)	Codon 133 mutation
III.1	Male	Colon	39	+[a]
		Soft tissue sarcoma	39	
III.3	Female	Breast carcinoma	32	+
III.5	Female	Acute myelogenous leukemia	30	+
III.7	Female	Breast carcinoma	31	+
III.9	Female	Breast carcinoma	41	+
IV.10	Female	Hodgkin's disease	21	−
IV.12	Male	Soft tissue sarcoma (fibrosarcoma)	1	+
IV.13	Female	Soft tissue sarcoma (fibrosarcoma)	1	+

[a] +, codon 133 mutation present in one *p53* allele; −, codon 133 mutation not present in either *p53* allele.

paternal aunts (III-3, 5, 7, 9), and a paternal male cousin (III-1). The mutation was not present in family member IV-10 who was affected with Hodgkin's disease. Two family members who were not affected with cancer were heterozygous for the codon 133 mutation. The two unaffected carriers, proband's father (III-13) and paternal female cousin (IV-6), do demonstrate mendelian segregation of the mutation in their immediate families and may be at increased risk for cancer.

Eighteen family members, including all spouses who married into the cancer prone family, did not have cancer and did not carry the *p53* germ line mutation. There is complete association of this mutation with LFS associated neoplasms in this family (Table 2). Two cancers not commonly associated with LFS were identified in this family. Hodgkin's disease was diagnosed in the proband's paternal female cousin (IV-10) but neither she or her parents carry the codon 133 mutation. Additionally, a paternal male first cousin of the proband's father (III-1) was diagnosed with colon cancer and soft tissue sarcoma at 39 years of age and was found to carry the codon 133 mutation. These data would seem to indicate that Hodgkin's disease is not a component cancer of LFS in this family but colon cancer may be. No other *p53* mutations were identified in conserved blocks II–V. Additionally, 11 unrelated individuals who were not affected with cancer and had no family history of excess cancer were examined and found to have wild type *p53* sequences in conserved blocks II–V. These data are consistent with the nonpolymorphic nature of these conserved regions (2).

Current evidence suggests that the normal *p53* protein is essential for normal cell growth, perhaps as a negative regulator of the cell cycle, and that its alteration or inactivation is necessary for the development of malignancy (reviewed in Ref. 15). The codon 133 mutation is located in a conserved domain and would likely lead to altered protein function. The base transition of T to C leads to the amino acid substitution of a hydrophilic threonine for a hydrophobic methionine. Tumor

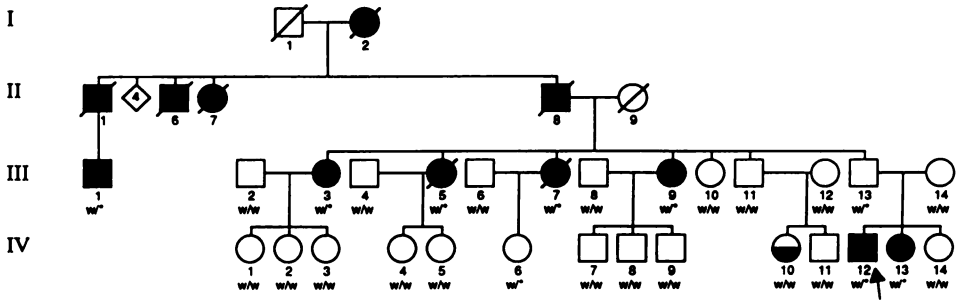

Fig. 2. Partial pedigree of an extended LFS family. Pedigree key: male (□); female (○); LFS associated cancer (■, ●); Hodgkin's disease (⊖); deceased individual (/); proband (↖); four individuals, sex not specified (◈). Individuals found to be homozygous wild type for the *p53* nucleotide sequence in conserved block II–V are indicated by (*w*/*w*); those individuals found to carry the codon 133 mutation (ATG → ACG) are indicated by (*w*/*m*).

specific mutations within this region have been shown to lead to altered protein function with loss of tumor suppressor activity (16–18). The cosegregation of this *p53* germ line mutation with all LFS associated neoplasms in this family strongly suggests that the mutation is functionally significant and that carriers are at an increased risk for developing cancer. Interestingly, at the time of the proband's diagnosis, none of the ascertained family members depicted in Fig. 2 (III and IV) had yet developed cancer. This observation underscores the difficulty in attempting to identify Li-Fraumeni syndrome patients.

The location of a *p53* mutation in conserved block II is unique for a germ line mutation but is not uncommon for sporadic tumors. A *p53* mutation in human tumor tissue from sporadic cancer of the colon was previously reported for codon 133 (19). It differs from the germ line mutation reported here in that it involved an A to T transversion of the first base of codon 133 (ATG → TTG) and results in a different amino acid substitution (methionine → leucine). Similarly, two of the previously reported *p53* germ line mutations occur in codons (245, 248) which have also been implicated in sporadic tumors (20). This study indicates that LFS associated germ line *p53* mutations are not restricted to a specific conserved region of the gene, as might be concluded from the clustering of initially reported germ line mutations in conserved block IV. Additionally, the fact that codons 133, 245, and 248 have been shown to be mutated in both hereditary and sporadic cancer would suggest that germ line mutations may not be unique. It is reasonable to suspect the *p53* germ line mutations in LFS are as diverse in nature and location as those that occur in a variety of sporadic tumors.

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