A Germ Line Mutation in Exon 5 of the p53 Gene in an Extended Cancer Family

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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 codons 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 non-polymorphic 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 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 DeMaris (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 GTC 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 TGG CCT 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 dideoxyribonucleotide 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
paternal aunts (III-5, 7, 9), and a paternal male cousin (III-13). 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 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.

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. 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 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
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 ascertainment 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.

References


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