

Exclusion of the genes *CDKN2* and *PTEN* as causative gene defects in Li–Fraumeni syndrome

EC Burt¹, G McGown¹, M Thorncroft¹, LA James¹, JM Birch² and JM Varley¹

¹CRC Section of Molecular Genetics, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, UK; ²CRC Paediatric and Familial Cancer Research Group, Royal Manchester Children's Hospital, Manchester M27 1HA, UK

Summary We have analysed Li–Fraumeni syndrome families, previously shown to be negative for mutations in *TP53*, for mutations to the tumour suppressor genes *PTEN* and *CDKN2*. These genes function in cell cycle progression or are mutated in a variety of tumours. We have detected no mutations in the family members tested.

Keywords: Li–Fraumeni syndrome; *PTEN*; *CDKN2*; p16; p19^{ARF}

Li–Fraumeni syndrome (LFS) is an autosomal, dominant, inherited cancer disorder, characterized by a broad but specific spectrum of tumours observed at an early age, in particular a predominance of bone and soft tissue sarcomas, brain and breast cancer (Li et al, 1988). LFS is defined by a strict clinical definition, described previously (Li et al, 1988). This definition has been relaxed to include Li–Fraumeni-like (LFL) cases (Birch et al, 1994).

In 1990, the tumour suppressor gene *TP53* was identified as the candidate for mutation in a number of LFS families (Malkin et al, 1990). To date more than 50 families have been identified with germline *TP53* mutations (Varley et al, 1997a). Previously we have detected mutations in approximately 70% of LFS and 20% of LFL families, when all exons, including non-coding regions, have been sequenced (Varley et al, 1997b). Therefore, 30% of LFS and the majority of LFL families do not have a detectable germline mutation within the coding region of *TP53*. The p53 protein functions in checkpoint control at G1/S and G2 phase of the cell cycle. It is feasible that characterization of related genes, for example other cell cycle inhibitors, may identify germline mutations within these families. We have selected genes for analysis that are involved in p53-related pathways or are known to be mutated in Li–Fraumeni-associated tumours, in particular the tumour suppressor genes *CDKN2*, which encodes p16 and p19^{ARF}, and *PTEN*. Like p53, both p16 and p19^{ARF} function as negative regulators of the cell cycle, with the p16 gene shown to be mutated in many tumour types (Kamb, 1995; Quelle et al, 1995). *PTEN* mutations have also been observed in a number of tumours (Steck et al, 1997). We have analysed 16 families, previously shown to be negative for mutations to *TP53* (Varley et al, 1997b), for mutations to these sequences.

MATERIALS AND METHODS

Families were ascertained as previously described (Varley et al, 1997b). Blood samples were obtained from either the proband or a first-degree relative in each family (Table 1). DNA was isolated from whole blood using the Dynabeads DNA direct System (Dyna), according to manufacturers instructions.

Genomic DNAs were amplified by nested polymerase chain reaction (PCR) to generate products corresponding to each exon of *PTEN* and *CDKN2*. Primers and PCR conditions are available from authors. PCR products were sequenced directly using either an ABI377 Sequencer with Dye Primer chemistry (Perkin-Elmer Applied Biosystems), or a Sequenase kit (USB).

Table 1 Mutation analysis of *PTEN* and *CDKN2* in LFS and LFL families^a

Family ^b	Person ^b	Mutation found ^c	
		Nucleotide change ^d	Amino acid change
LFS Families			
22	III-5	None	
81	III-5	None	
82	IV-5	None	
88	II-2	None	
119	III-1	None	
1779	III-7	None	
LFL Families			
80	V-4	None	
253	IV-2	<i>CDKN2</i> : 442 G>A	p16: Ala148Thr ^e
328	II-3	None	
338	III-2	None	
348	III-2	None	
352	IV-1	None	
353	III-3	None	
2093	IV-3	None	
2613	III-1	None	
2634	III-3	None	

^aFor details of tumour types and relationship to proband in each family, refer to Varley et al (1997b). ^bFamily and Person numbers are as previously (Varley et al, 1997b). ^cIncludes mutations found in *PTEN* and *CDKN2*.

^dNucleotide positions are derived from the amended p16 gene sequence (Okamoto et al, 1994). ^eAla148Thr is a common polymorphism, previously reported as Ala140Thr (Hussussian et al, 1994).

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Correspondence to: JM Varley

RESULTS AND DISCUSSION

We report the results of sequencing six LFS and ten LFL samples for mutations in tumour suppressor genes *PTEN* and *CDKN2*. The *CDKN2* gene consists of four exons (1 α , 1 β , 2 and 3), and encodes two proteins: p16 and p19^{ARF}. The p16 protein is encoded by exons 1 α , 2 and 3, and functions as a negative regulator in the G1 phase of the cell cycle. The *p16* gene is a major target in carcinogenesis, and has been shown to be inactivated in a number of tumour cell lines and primary tumours including adenocarcinomas and glioblastoma (Kamb, 1995). Germline mutations in the gene can result in hereditary predisposition to the development of melanoma and pancreatic cancer (Hussussian et al, 1994; Foulkes et al, 1997). In addition, an alternatively spliced transcript, encoding p19^{ARF}, is derived from the same locus as p16 and contains a novel first exon (1 β), located upstream of exon 1 α . Both transcripts share exons 2 and 3 but are translated in a different reading frame, and hence p16 and p19^{ARF} have no amino acid similarity. The p19^{ARF} protein also functions to block cell cycle progression at G1 and G2, although in a p53-dependent manner (Quelle et al, 1995). We have been unable to identify any germline mutations in either the *p16* or *p19^{ARF}* genes in the Li–Fraumeni families tested, although we have detected a single heterozygous polymorphism at codon 148 of p16 in IV-2, family 253 (Table 1). This polymorphism is prevalent in the population (Hussussian et al, 1994), and has no effect on p19^{ARF}.

The tumour suppressor gene *PTEN* (*MMAC1*) was isolated by mapping of homozygous deletions commonly found in glioblastomas (Li et al, 1997). Subsequently somatic mutations in *PTEN* have been observed in a number of tumours including breast and brain tumours, and malignant melanoma (Steck et al, 1997). Germline mutations in the gene have been identified in Cowden syndrome, which is associated with early onset breast cancer (Tsou et al, 1997). However, a recent study of 136 breast cancer families has shown that *PTEN* is not linked to familial breast cancer (Chen et al, 1998). The gene encodes a dual specificity phosphatase, with enzymatic activity required for tumour suppressor function (Myers et al, 1997). We have not detected any *PTEN* mutations in the families analysed.

We have therefore excluded these genes as candidates for mutation in Li–Fraumeni syndrome. However, this does not preclude other cell cycle control genes as targets for mutation, for example the Rb and p21 genes.

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