An investigation of the Peutz-Jeghers gene (LKB1) in sporadic breast and colon cancers

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Abstract

Aims—To explore the role of the Peutz-Jeghers gene (LKB1) in sporadic breast and colon cancers.

Methods—Thirty consecutive sporadic carcinomas of the breast and 23 of the colon were selected. DNA was extracted from paraffin wax embedded tissue and analysed for loss of heterozygosity (LOH) at microsatellite markers D19S886 and D19S565 close to the LKB1 gene. Tumours showing LOH were screened for LKB1 mutations by single strand conformational polymorphism (SSCP).

Results—Five breast carcinomas showed LOH (21% and 7% of those informative for D19S886 and D19S565, respectively). Five of the colorectal carcinomas showed LOH (15% and 36% of those informative for D19S886 and D19S565, respectively), with one sample showing allele loss with both markers. Screening of these 10 carcinomas by SSCP identified one migrational shift but sequencing revealed an intronic polymorphism only. Therefore, no coding mutations were found in these carcinomas.

Conclusions—These findings suggest that although allele loss at the LKB1 locus occurs relatively frequently in sporadic breast and colon cancers, mutations do not seem to be a feature.

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First reported in the literature in 1921 by Peutz¹ and further characterised by Jeghers *et al* in 1949,² Peutz-Jeghers syndrome is a rare, autosomal dominant condition with near complete penetrance. It is manifest clinically by the development of mucocutaneous pigmentation and morphologically characteristic gastrointestinal hamartomatous polyps.

Initially believed to run a benign course, it has become increasingly apparent since the 1960s that the syndrome is associated with an increased incidence of malignancy, including cancers of the breast, gut, pancreas, ovary, and endometrium. In addition, the malignancies appear to develop at a younger age in those with Peutz-Jeghers syndrome than in the general population.³ Furthermore, two rare tumours, sex cord tumour with annular tubules and adenoma malignum of cervix, have been associated with the syndrome, with up to 10% of all cases of adenoma malignum occurring in patients with Peutz-Jeghers syndrome.⁴ This increased risk was quantified in 1987 by Giardiello and co-workers, who reported an 18-fold increase in the risk of cancer development in

Peutz-Jeghers syndrome compared with the general population.³ This finding has been refined to give relative risks of 18.5 in women and 6.2 in men, with the relatively greater risk in women being attributable to the development of breast and gynaecological malignancy.⁵

Using comparative genomic hybridisation and linkage analysis techniques, the Peutz-Jeghers gene (LKB1) has been mapped to the short arm of chromosome 19 at 19p13.3.^{6,7} It has been suggested that a second locus on the long arm of the same chromosome is responsible for the syndrome in a minority of patients.⁸ The LKB1 gene encodes a serine threonine kinase (STK11).⁹ Several mutations of LKB1 have been reported that, in the main, lead to truncations of the protein and resultant loss of enzyme activity. It is this loss of enzyme activity that is believed to be responsible for the Peutz-Jeghers syndrome phenotype. Loss of heterozygosity studies of the hamartomas and adenocarcinomas occurring in patients with Peutz-Jeghers syndrome^{6,10} have identified loss of the wild-type allele, thus conforming with Knudson's two hit hypothesis of tumorigenesis, and suggesting that the Peutz-Jeghers gene is a tumour suppressor gene.¹¹ Interestingly, Peutz-Jeghers syndrome is the first cancer susceptibility syndrome that occurs as a result of inactivation of a protein kinase.

Tumour suppressor genes identified in other cancer syndromes (such as familial adenomatous polyposis) are frequently implicated in sporadic cancers. In this context, the aim of our study was to explore the role of LKB1 in sporadically occurring breast and colon cancers. Breast and colon cancers were chosen because they occur with relative frequency in both Peutz-Jeghers syndrome and in the general population. The tumours were screened for loss of heterozygosity (LOH) at 19p13.3 using microsatellite markers and those showing LOH were analysed for mutations of the remaining allele.

Methods

Thirty consecutive, sporadic carcinomas of the breast and 23 of the colon treated by surgical excision in Dundee, Scotland in 1997 were included in our study. In each case, tumour tissue was matched with normal tissue from the same specimen, usually an uninvolved lymph node. DNA was extracted from paraffin wax embedded tissue using a commercial kit (Hybaid, Ashford, Middlesex, UK) and amplified by the polymerase chain reaction (PCR) using the microsatellite markers D19S886 and D19S565. These markers have been shown previously to map closely to the Peutz-Jeghers syndrome locus.⁶ The reverse primers were

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Figure 1 Loss of heterozygosity in a breast carcinoma. (A) Normal tissue: two tall peaks representing the two different normal alleles. (B) Breast carcinoma tissue: owing to loss of an allele (loss of heterozygosity), the second tall peak (far right) is missing.

labelled fluorescently—D19S886 with Fam and D19S565 with Hex. Amplification of D19S886 was initiated by hot start for five minutes at 94°C, followed by 35 cycles of one minute at 94°C, 30 seconds at 50°C, and 30 seconds at 72°C. The conditions for D19S565 were similar apart from an annealing temperature of 55°C. The amplified products were resolved by electrophoresis on an agarose gel (1.5% wt/vol in 0.5× Tris borate EDTA buffer) containing ethidium bromide (0.5 mg/ml) and viewed under UV light. LOH was detected using the ABI 310 fragment analyser, comparing normal and tumour samples in each case.

Those samples showing LOH were selected for mutation analysis. DNA extracted from paraffin wax embedded tissue using standard techniques was amplified by PCR (initiated by hot start for 15 minutes at 94°C, followed by 35 cycles of one minute at 94°C, 45 seconds at 64°C, and 30 seconds at 72°C). Primers were selected to cover the nine exons of LKB1.^{12, 13} The amplified products from each exon were screened by single strand conformational polymorphism (SSCP) analysis at room temperature on 10% polyacrylamide gels (ratio, 49 : 1) containing 10% glycerol. Any sample showing a migration shift compared with the normal control was sequenced.

Results

Nineteen of the 30 breast and 11 of the 23 colon carcinomas were informative for D19S886. Of these, four breast and four colon carcinomas showed LOH (fig 1). With the marker D19S565, one of 15 informative breast carcinomas and two of 13 informative colon carcinomas showed LOH. One colon carcinoma showed LOH with both markers. Thus, we identified 10 carcinomas showing allele loss at the Peutz-Jeghers syndrome locus. Of these,

only one sample (a colon carcinoma) showed a band shift on SSCP analysis. Sequencing revealed a T to C transition but this occurred in an intronic region not crucial for splicing and is therefore likely to be non-coding and of no pathogenetic importance.

Discussion

The existence of cancer associated syndromes provides direction for the study of sporadic cancers. Accumulating epidemiological evidence suggests that Peutz-Jeghers syndrome predisposes to cancer development, at sites both within and outside the gastrointestinal tract. There is increasing interest in a “hamartoma–adenoma–carcinoma” sequence to explain the development of cancer in patients with Peutz-Jeghers syndrome.¹⁴ However, many of the malignancies occur at sites outside the gastrointestinal tract and those occurring within do not necessarily arise from hamartomas.

The recent literature contains several studies that explore the role of the Peutz-Jeghers syndrome gene in sporadic cancers; in particular, looking for allele loss and evidence of mutations at this locus. The results show some inconsistencies. Most studies show a notable frequency of allele loss but mutational rates are variable between populations (table 1). No mutations were found in a UK study of 62 sporadic breast cancers,¹² none in a study from Norway of 75 colon cancers,¹⁵ and only one in an Italian series of 71 colorectal cancers.¹⁶ However, a study from Korea gives contradicting results, identifying seven mutations in 23 colorectal carcinomas.¹³ Interestingly, these workers separated their series into right and left sided colorectal carcinomas and found that the mutations were present in the left sided carcinomas only (seven of 13). This said, their incidence of mutations is still higher than that reported elsewhere without taking anatomical segregation into account. Why this study should be different from the others remains unexplained, but it could be related to the different incidence of colorectal carcinoma in Korea and to population differences, with all the other studies having been performed on Western populations. Other studies of testicular and gastric cancers have found similar results to the Western studies.^{15, 17} Indeed, a recent large study of 129 cancers from a variety of sites identified only three mutations.¹⁸

Our findings are in keeping with other work on Western populations and with the only previous UK study on breast cancers. To date, similar work has not been reported on colon cancers in a UK population. No mutations were detected in our study. However, we did identify an intronic variation, confirming that our methods were capable of detecting sequence changes. It could be that mutations are occurring at the LKB1 locus that cannot be detected by SSCP analysis—for example, large genomic rearrangements. Alternatively, it is possible that other mechanisms such as promoter methylation are occurring as the “second hit” phenomenon.

Thus, although it seems that allele loss at LKB1 in sporadic breast and colon cancers is a

Table 1 Allele loss and mutation rates for different populations

Population	Carcinoma	Number	LOH	Marker	Mutations	Ref
UK	Breast	62	3/40	D19S565	Nil	12
Norwegian	Colorectal	75	13/50	D19S886	Nil	15
Italian	Colorectal	71	10/52	D19S886	1	16
Korean	Colorectal	23	9/17	D19S886	5	13
			6/15	D19S883	4	
			6/22	D19S565	3	
UK	Breast	30	1/15	D19S565	Nil	Current
	Colorectal	23	4/19	D19S886		
			2/13	D19S565	Nil	
			4/11	D19S886		

LOH, loss of heterozygosity, given as allele loss/number informative.

relatively frequent event, mutations at this locus appear not to be a feature. Hemizygosity for LKB1, even in the absence of a mutation, may in itself be deleterious. Alternatively, another as yet unspecified gene might be involved.

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Your time and help are appreciated. We especially thank all those ACP members who returned questionnaires circulated in the September 1999 journal issue for their valuable feedback.

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