LETTER TO JMG

Is the locus for Costello syndrome on 11p?

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Costello syndrome (CS) is characterised by postnatal growth retardation, relative macrocephaly, distinctive facies, loose, hyperpigmented skin with deep palmar and plantar creases, and developmental delay.^{1–4} Cutaneous papillomata, which when present are the hallmark of this syndrome, develop in childhood. Cardiac anomalies, either structural defects, hypertrophic cardiomyopathy, or tachyarrhythmia, are present in 60% of cases.⁵

The cause of CS is unknown. Segregation analysis and recognition of advanced paternal age support autosomal dominant inheritance, with most cases being the result of de novo mutations.⁶ For uncommon conditions such as CS, which are generally sporadic, gene identification often relies upon clinical observation or rare cases with chromosomal rearrangements. A single translocation has been described in CS, t(1;22)(q25;q11).⁷ Recently, malignant tumours have been reported in a significant number of patients with CS, suggesting that a predisposition to malignancy is part of the condition. Bladder carcinoma has been reported twice.^{8–9} Three patients developed ganglioneuroblastomas.^{10–11} There have been single reports of epithelioma¹² and vestibular schwannoma.¹³ However, rhabdomyosarcoma (RMS) has been reported in 10 patients.^{14–18} In seven of these cases, the tumour histology was embryonal; one was pleomorphic, one of unknown subtype,¹⁸ and the other alveolar.¹⁵ Based on the published cases of CS, with and without tumours, a tumour frequency as high as 17% has been suggested.¹⁸

We hypothesise that the increased malignancy risk in CS is the result of involvement of a tumour suppressor gene in the causation of CS, either by deletion or mutation. We suggest that the predisposition to malignancy occurs when a second mutation in the tumour suppressor gene occurs. Demonstration of loss of heterozygosity (LOH) is a proven technique for localisation of tumour suppressor genes.

We report here the outcome of LOH and candidate gene studies in five embryonal rhabdomyosarcomas from patients with CS. Four patients have previously been published^{14–16–18} and the fifth was diagnosed by one of us (PS).

MATERIALS AND METHODS

Rhabdomyosarcoma tissue and genomic DNA was obtained from all five patients. Primers for microsatellite markers

Key points

- It has been recently shown that patients with Costello syndrome (CS), a rare multiple congenital anomalies/mental retardation syndrome, are predisposed to develop malignant tumours, most commonly rhabdomyosarcomas.
- We propose that the increasing numbers of reports of childhood malignancy suggest the involvement of a tumour suppressor gene in the molecular aetiology of CS. We have shown loss of heterozygosity at the 11p15.5 locus in embryonal rhabdomyosarcoma (E-RMS) tumour samples from five patients with CS.
- We hypothesise that LOH at 11p15.5 is causally related to the genetic basis of both E-RMS and CS. A constitutional deletion at this locus has been excluded in a series of patients, and two candidate genes from this region have been screened for mutations.
- Further studies of this region in tumours from CS patients may provide an opportunity to understand the mechanisms, hitherto poorly defined, underlying the role of this region in a number of malignant tumours.

obtained from the Genome Data Base were screened for loss of heterozygosity.

RESULTS

At 11p15.5 all five tumours tested showed LOH. For two tumours (tumours 4 and 5, table 1), for all informative markers tested, we compared the genotype of tumour tissue with that of patient's lymphocytes and showed LOH across a region spanning 3–20 cM on the Genethon genetic map flanked by markers D11S1984–D11S1349. For the three remaining tumours, for which limited DNA was available, evidence of LOH was sought, and found, for the microsatellite marker D11S860 that lies within a region in which LOH has previously been reported in embryonal rhabdomyosarcomas not associated with Costello syndrome. In 2/5 cases the paternal origin

Table 1 Outcome of LOH studies in rhabdomyosarcoma samples in five patients with Costello syndrome

	Position on genetic map (cM)	Tumour 1	Tumour 2	Tumour 3	Tumour 4	Tumour 5
D11S1984	3	–	–	–	LOH	LOH
D11S1758	8	NI	LOH	NI	–	–
D11S4088	8	–	–	–	LOH	LOH
D11S860	8	LOH	LOH	LOH	–	–
D11S1349	20	–	–	–	LOH	LOH

Figure 1 Schematic representation of transferable 2.5 Mb fragment implicated in tumorigenesis on 11p15.

of the remaining allele was shown, as has been shown for other tumours showing allelic loss of this region.

We looked for LOH at other genomic regions for which there is circumstantial evidence for involvement either in RMS or CS. Those tested included (1) 16q23, the second most frequent region of LOH in embryonal RMS¹⁹; (2) chromosome 22q, implicated in the cause of Costello syndrome because of the reported translocation as well as a single patient with CS and a unilateral vestibular schwannoma¹³; (3) the region on chromosome 1q25 associated with the other translocation breakpoint. However, no consistent pattern of LOH was shown for any of these regions. Furthermore, sequencing of the *hCHK2* gene, a candidate tumour suppressor gene which lies at 22q11.2 close to the NF2 locus, did not show any unusual variants in two patients.

DISCUSSION

Allelic loss at 11p15.5 is the most frequent genetic alteration in embryonal rhabdomyosarcoma,^{19–22} whereas it is rarely found in the alveolar form of the tumour. LOH at the 11p15.5 region is also observed in other embryonal tumours of childhood as well as adult cancers, including bladder carcinoma. Our study confirms that CS associated E-RMS display molecular abnormalities that are shared with other, sporadic E-RMS cases.

CS predisposes to E-RMS. Furthermore, E-RMS whether sporadic or associated with CS is frequently associated with loss of the 11p15.5 region. This evidence strongly implicated the distal region of 11p as a candidate region harbouring a gene(s) important for the development of CS, either by deletion or mutation. A constitutional deletion at 11p15.5 was excluded in 11 CS patients; biparental inheritance was seen for all informative markers, excluding a constitutional deletion or uniparental disomy at this locus.

Previously RMS tumour cell growth arrest has been shown as a result of subchromosomal transfer of a 2.5 Mb region of chromosome 11p15.5^{23–24} that contains both imprinted and non-imprinted genes (fig 1). It has been hypothesised that

this region contains at least one gene which is critical in the pathogenesis of RMS as well as other tumour types that show similar patterns of loss at 11p15.5. It has been suggested that an imprinted gene may be involved in RMS, the “first hit” that predisposes to tumorigenesis being functional (monoallelic expression resulting from imprinting) rather than mutational. However, should a two hit suppressor-suppressor gene underlie the development of CS (first hit) and RMS (second hit) then this would implicate a non-imprinted, developmentally expressed gene. Imprinted genes are likely to be important in determining which allele (paternal/maternal) is lost. In those tumour types where there is loss of 11p15.5, the retained chromosome is usually of paternal origin, and we have confirmed this in 2/5 tumours associated with CS. Since we have shown that loss of the second allele is associated with large scale loss of 11p, this suggests that retention of the paternal allele, and in particular a subset of paternally expressed genes, may be critical to cell survival.

Within the 2.5 Mb subchromosomal fragment, genes that escape the imprinting process²⁵ include *TSSC4* and *TSSC6*.²⁶ Such genes therefore represent attractive candidates to exclude in CS. The *SLC22A1L* gene (also previously termed *TSSC5* and *BWR1A*), which is maternally imprinted and encodes an organic cation transporter-like molecule, also lies within this region. The gene has previously been examined in tumours, including rhabdomyosarcoma, that show loss of 11p15.5 and sequence variants on the retained allele.²⁷ We undertook analysis of the gene by SSCP/heteroduplex analysis in three E-RMS (tumours 1–3) associated with CS. We identified intragenic polymorphisms that were found in the normal population that confirmed that LOH occurred in the tumours at this location. Using this methodology, we therefore found no evidence to support the hypothesis that alteration of the remaining copy of this gene underlies CS. *STIM1* (previously known as *GOK*), a gene that is highly expressed in the skeletal muscle, is also located at 11p15. *STIM1* has also been proposed as a candidate tumour suppressor gene in RMS because there

is no detectable *STIM1* mRNA in cell lines derived from rhabdomyosarcoma or rhabdoid tumours.²⁸ Moreover, overexpression of *STIM* leads to suppression of growth and survival in cells from these tumours, whereas it has no effect on renal carcinoma cells and breast cancer cell lines. Sequencing of the entire coding region of this gene in three CS patients showed no abnormalities.

It is notable that the differential diagnosis of CS in the newborn includes Beckwith-Wiedeman syndrome (BWS). BWS is also characterised by an increased risk of embryonal tumours, including Wilms tumour and, at a lower frequency, rhabdomyosarcoma. While the genetic mechanisms underlying BWS are complex, they are known to involve alteration and differential methylation of several genes on 11p15. These include *IGF2*, a gene known to be overexpressed in RMS. The highest risk of tumours in BWS appears to be associated with particular epigenetic mechanisms, paternal uniparental disomy or aberrant methylation of H19.²⁹ Involvement of *IGF2* in CS has also been suggested because of the resemblance of the early phenotype to leprechaunism.³⁰

Multiple loci are involved in the genetic basis of single tumours.³¹ While LOH at 11p15.5 is likely to be causally related to the genetic basis of E-RMS it is not certain that this is a primary event rather than one of a number of secondary processes in the cellular events leading to malignant transformation. It remains possible that such events relate solely to the tumour types involved and that they are distinct from those earlier events in RMS tumorigenesis which also predispose to CS. However, we believe that further studies of 11p15.5 in tumours from patients affected with CS will be of value and may provide an opportunity to understand the mechanisms, hitherto poorly defined, underlying the role of this region in a number of malignant tumours.

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