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Parental Origin of De Novo Constitutional Deletions of Chromosomal Band 11p13

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Summary

One-half of all cases of Wilms tumor (WT), a childhood kidney tumor, show loss of heterozygosity at chromosomal band 11p13 loci, suggesting that mutation of one allele and subsequent mutation or loss of the homologous allele are important events in the development of these tumors. The previously reported nonrandom loss of maternal alleles in these tumors implied that the primary mutation occurred on the paternally derived chromosome and that it was "unmasked" by loss of the normal maternal allele. This, in turn, suggests that the paternally derived allele is more mutable than the maternal one. To investigate whether germinal mutations are seen with equal frequency in maternally versus paternally inherited chromosomes, we determined the parental origin of the de novo germinal 11p13 deletions in eight children by typing lymphocyte DNA from these children and from their parents for 11p13 RFLPs. In seven of the eight cases, the de novo deletion was of paternal origin. The one case of maternal origin was unremarkable in terms of the size or extent of the 11p13 deletion, and the child did develop WT. Transmission of 11p13 deletions by both maternal and paternal carriers of balanced translocations has been reported, although maternal inheritance predominates. These data, in addition to the general preponderance of paternally derived, de novo mutations at other loci, suggest that the increased frequency of paternal deletions we observed is due to an increased germinal mutation rate in males.

Introduction

Wilms tumor (WT) is a childhood kidney tumor that can occur either unilaterally or bilaterally. In 8% of all WT cases, congenital abnormalities such as aniridia, genitourinary anomalies, and mental retardation are also observed (Matsunaga 1981; Breslow and Beckwith 1982; Breslow et al. 1988). Approximately 1% of patients present with aniridia and a cytogenetically detectable constitutional deletion of chromosomal band 11p13; conversely, 50%–60% of patients with an 11p13 deletion and aniridia develop WT (Narahara et al. 1984). The observation of these germ-line deletions, the majority of which are de novo mutations, led to the hypothesis that a gene at 11p13 was important in the etiology of WT. The presence of tumor-specific alterations, detected cytogenetically or molecularly as loss of heterozygosity at polymorphic 11p loci in one-half of all tumors, further implicated an 11p13 gene in tumorigenesis (Kaneko et al. 1981; Fearon et al. 1984; Koufos et al. 1984; Orkin et al. 1984; Reeve et al. 1984; Dao et al. 1987). These data, in addition to epidemiological observations on the age at onset and laterality of tumors, have led to the model that mutation of one 11p13 allele and subsequent mutation or loss of the remaining normal allele are critical steps in the development of tumors (Knudson and Strong 1972). Implicit in this model is the assumption that both normal alleles are equivalent and that either can sustain the first mutation. This has been challenged, however, by the

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observation that maternal alleles are preferentially lost in tumors—both unilateral and bilateral—that show loss of heterozygosity for 11p markers (Reeve et al. 1984; Schroeder et al. 1987; Grundy et al. 1988; Mannens et al. 1988; Williams et al. 1989; Huff et al., submitted).

This nonrandom loss of maternal alleles implies that in these tumors the primary mutation occurred on the paternally derived chromosome and that this mutation was unmasked by the loss of the normal maternal allele. This, in turn, suggests that the paternally derived allele is more mutable than the maternally derived allele. To investigate whether germinal 11p13 mutations are observed with equal frequency in maternally versus paternally inherited chromosomes, we ascertained the parental origin of the chromosome deletion in children with de novo constitutional 11p13 deletions.

Methods

DNA was isolated from peripheral lymphocytes or lymphoblastoid cell lines from eight children with constitutional 11p13 deletions and also from their parents. Seven children had cytogenetically detectable deletions that were confirmed by molecular dosage studies (fig. 1), and one had a smaller 11p13 deletion that was only detectable by gene dosage studies (Compton et al. 1988). In all cases, the child's deletion was a new mutation; no 11p13 deletions were detected in parental DNA samples. In all cases, the deletions encompassed the region of the 11p13 WT and aniridia genes. All of the children had aniridia; most also developed WT, and three had genitourinary anomalies. Clinical and genetic data on four of the children have been reported elsewhere (for LCS134, see Riccardi et al. 1980; for LCS036, see Riccardi et al. 1982; Compton et al. 1988; for LCS144, see Francke et al. 1979; and for LCS120, see Riccardi et al. 1978).

Patient and parental DNA was restricted and analyzed by Southern blots according to a method described elsewhere (Huff et al. 1990). The DNA probes used were those that detected RFLPs at 11p13 and were located within the deleted region on each child's chromosome (fig. 1). They included the catalase intron probe, pINT800, and the anonymous probes, p8B1.25, JA8-1, p60H1.4, p5BE1.2, p32–1, and p56H2.4 (Feder et al. 1985; Quan et al. 1985, 1986; Kidd et al. 1987; Huff et al. 1987, 1990; Compton et al. 1988; Ton et al. 1990). In this way we could determine which parent had not transmitted alleles to the child and, therefore, on which parental chromosome the de novo deletion had occurred.

Results

Autoradiograms of patient/parent DNA samples for two families are shown in figure 2. In both of these cases, paternally derived alleles were not present in the child's constitutional DNA, indicating that the germinal 11p13

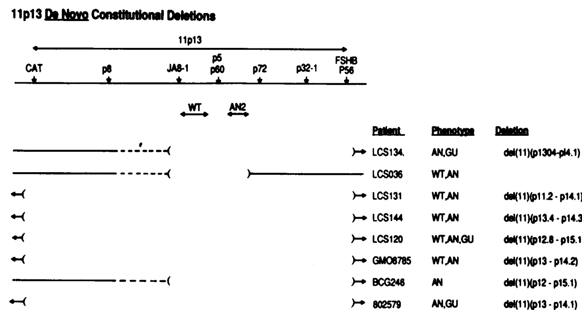

Figure 1 Schematic of 11p13 de novo constitutional deletions in eight children, along with karyotypic and phenotypic data. Diagram at top shows position of DNA probes and the WT and AN2 loci within chromosomal band 11p13. Brackets enclose deleted region; brackets with arrows indicate deletion breakpoints outside of 11p13; dashed lines indicate region within which a breakpoint has occurred. AN2 = aniridia; GU = genitourinary anomalies.

Paternal Origin of De Novo Constitutional 11p13 Deletions

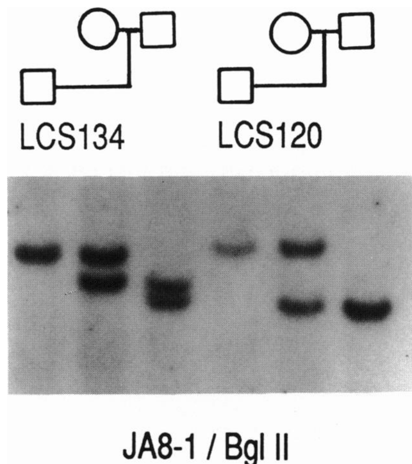

Figure 2 Paternal origin of the de novo germinal 11p13 deletion in two children. Lymphocyte DNA from the children and their parents was restricted with *Bgl*II and hybridized to the 11p13 probe, JA8–1. The absence of paternally inherited alleles in the children's DNA is shown in the autoradiograms. □ = Male; ○ = females.

deletion in the child had occurred on the paternal chromosome. RFLP data at informative loci for all eight patient/parent trios are shown in table 1. The de novo deletion was of paternal origin in seven of eight cases. This is statistically significant ($P = .03$) by exact binomial probability calculations (Sokal and Rohlf 1981). In only one child (LCS131) did the deletion occur on the maternal chromosome. This case was unremarkable in terms of size or extent of the deletion, and it is notable that this child did develop WT.

Discussion

We have determined by RFLP analysis that the de novo 11p13 deletion in seven of eight children occurred on the paternally derived chromosome. Furthermore, the paternal origin for one additional de novo 11p13 deletion has recently been reported (Glaser et al. 1989). This significant excess of paternal mutations is similar to that reported for retinoblastoma and von Recklinghausen neurofibromatosis (Dryja et al. 1989; Zhu et al. 1989; Jadayel et al. 1990) and for chromosome deletions, including 13q14 deletions in retinoblastoma (Kondo et al. 1985; Ejima et al. 1988) and 15q deletions in Prader-Willi syndrome (Niikawa and Ishikiriyama 1985; Butler et al. 1986). A general preponderance of paternally derived, de novo structural chromosome abnormalities has been noted elsewhere and has been attributed to a mutation rate that is higher during spermatogenesis than during oogenesis (Chamberlin and Magenis 1980; Emanuel 1988; Olson and Magenis 1988). Our data would suggest that, as in much of the genome, deletions at 11p13 occur more frequently during male gametogenesis.

Whether paternal age or exposure to hazardous compounds influences the rate of 11p13 deletions is not

Table 1

11p13 Alleles Present in Lymphocyte DNA from Deletion Patients and Their Parents

DNA Sample	pINT800/ *Taq*I	p8B1.25/ *Bgl*II	JA8-1/ *Bgl*II or *Pst*I	p60H1.4/ *Taq*I	p5BE1.2/ *Hae*III	p32-1/ *Msp*I[a]	p56H2.4/ *Pst*I
LCS134 ...			4.3/ –				
Mother			4.3/3.9				
Father			3.9/3.78				
LCS036 ...					.66/ –		
Mother					.66/.66		
Father					.88/.88		
LCS131 ...		16/ –		7.5/ –			
Mother		11,5/11,5		4.9/4.9			
Father		16/16		7.5/7.5			
LCS144 ...	2.5,1.0/ –			7.5/ –			
Mother	3.5/2.5,1.0			7.5/7.5			
Father	3.5/3.5			4.9/4.9			
LCS120 ...			4.3/ –				
Mother			4.3/3.78				
Father			3.78/3.78				
GM08785 ..		11,5/ –		7.5/ –		B/ –	
Mother		16/11,5		7.5/4.9		A/B	
Father		16/16		4.9/4.9		A/A	
BCG246 ...			2.00/ –				9.4/ –
Mother			2.00/2.00				9.4/5.8
Father			2.28/2.21				5.8/5.8
802579			2.49/ –				
Mother			2.49/1.79				
Father			2.28/2.21				

NOTE.—Data are given for informative loci only; sizes of alleles are in kilobase pairs.

[a] Alleles consist of multiple fragments and are designated A, B, or C.

known. Data of Olson and Magenis (1988) provide no evidence for an effect of paternal age on the occurrence of de novo structural chromosome abnormalities throughout the genome. However, a paternal age effect has been reported for several dominant disorders (Vogel and Rathenberg 1975). Differences in the mechanisms by which mutations occur (e.g., point mutations vs. chromosome deletions and translocations) may be an important factor in whether paternal age is influential. In our study, the mean age of the fathers when the children with the 11p13 deletions were born was 31.5 years (range 23–39 years). Thus, we have no strong data to suggest a paternal age effect for 11p13 deletions. An association between WT and paternal occupational exposures to a variety of hazardous compounds has been noted and would offer an explanation for the paternal bias we have observed (Kantor et al. 1979; Kwa and Fine 1980; Hicks et al. 1984; Bunin et al. 1989). Unfortunately, data on paternal occupational exposures are unavailable for the fathers in our sample.

An alternative explanation for our data is that deletion of the maternally derived 11p13 genes is selected against during early development. Such a selective process could not be completely stringent, however, since we did observe one case in which the maternal chromosome carried the 11p13 deletion. A further argument against a selective mechanism comes from studies of five families in which children have inherited 11p13 deletions from parents who are carriers of balanced translocations involving chromosome 11. In four of the five, the mothers were the carrier parents, and thus the children inherited 11p13 deletions from their mothers (Hittner et al. 1979; Strobel et al. 1980; Yunis and Ramsey 1980; Kousseff and Agatucci 1981; Nakagome and Nagahara 1985; Lavedan et al. 1989).

In conclusion, we find that de novo 11p13 deletions are more frequently paternal in origin, although we did observe one maternal deletion (in a sample of eight deletions). This suggests that, as has been reported for many chromosomal abnormalities, there is an increased rate of paternal deletion on the short arm of chromosome 11. This increased mutation rate would result in the preferential, germinal inactivation of the paternal allele and subsequent somatic loss of the maternal allele, as is observed in bilateral WT. This is analogous to the model proposed for retinoblastoma (Dryja et al. 1989; Zhu et al. 1989). However, whereas loss of either parental allele has been reported for sporadic, unilateral retinoblastoma (Dryja et al. 1989; Zhu et al. 1989), only maternal allele loss is observed in unilateral as well as bilateral WT (Reeve et al. 1984; Schroeder et al. 1987; Grundy et al. 1988; Mannens et al. 1988; Williams et al. 1989; Huff et al., submitted). An increased mutation rate during spermatogenesis cannot account for preferential maternal allele loss in unilateral tumors in which both mutations are thought to be somatic events. For these cases, a selective mechanism or imprinting of a linked gene on chromosome 11 may indeed be important.

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