Constitutional 1p36 Deletion in a Child with Neuroblastoma

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Summary

We describe a child with dysmorphic features, as well as developmental and growth delay, who developed neuroblastoma at 5 mo of age. Cytogenetic analysis of blood lymphocytes revealed an interstitial deletion of 1p36.1→1p36.2, which was apparent only with high-resolution banding. Molecular analysis with a collection of polymorphic DNA probes for 1p confirmed an interstitial deletion involving subbands of 1p36. Deletions of this region are a common finding in neuroblastoma cells from patients with advanced stages of disease. Therefore, these results (a) suggest that constitutional deletion of this region predisposed the patient to the development of neuroblastoma and (b) support the localization of a neuroblastoma tumor-suppressor locus to 1p36.

Introduction

Neuroblastoma is the most common extracranial solid tumor of childhood and accounts for 8%–10% of all childhood cancers (Young et al. 1986). Although the majority of cases are presumed to be sporadic, a genetic predisposition to neuroblastoma has been hypothesized on the basis of reports of familial cases. In multigeneration cases or in families where more than one sibling is affected, multiple primary tumors and a younger age at diagnosis (<12 mo of age) are more common than in the apparently sporadic cases (Knudson and Strong 1972; Kushner et al. 1986). Knudson and Strong (1982) proposed that 20%–25% of neuroblastomas are heritable and that the gene may act in a tumor-suppressor-like function.

No specific constellation of congenital abnormalities has been associated with neuroblastoma, and no constitutional chromosomal abnormalities have been identified that predispose to the development of this disease (Brodeur 1991). Rather, most of the genetic data on neuroblastoma have been derived from studies

of the neuroblastoma cells in these patients. Such studies have implicated the presence of a tumor-suppressor gene at the distal short arm of chromosome 1 (Brodeur et al. 1977, 1981; Gilbert et al. 1984; Fong et al. 1989, 1992; Weith et al. 1989; Brodeur 1990).

We now report a case of neuroblastoma in a child with a constitutional deletion of 1p36. This case and a recent case reported by Laureys et al. (1990) of a constitutional translocation involving 1p36 in a neuroblastoma patient support the localization of a neuroblastoma tumor-suppressor locus to this region of chromosome 1.

Subject, Material, and Methods

Patient History

The patient was initially evaluated because of dysmorphic features and developmental delay. She was the 7-lb 14-oz product of a full-term pregnancy and was born to a 21-year-old G_1P_1 mother and 23-year-old father. The infant was noted to be hypotonic at birth and to feed poorly. At 8 d of age, the patient had a respiratory arrest and was intubated. A cardiac workup revealed a small ventricular septal defect that subsequently closed.

Physical examination at 10 wk of age revealed all growth parameters to be delayed, including head cir-

cumference (50% for 6 wk), weight (50% for 2 wk), and length (50% for 4 wk). Examination of the head revealed a hirsute forehead and prominent occipital shelf. There was evident hypertelorism, a depressed nasal bridge, high arched palate, protuberant tongue, short frenulum, and a flat, long philtrum. The chest exam was notable for widely spaced nipples. The neurologic exam was abnormal, with head lag, poor tone, and no fixing or following. Dermatoglyphics were normal.

At 5 mo of age, the child was readmitted to the hospital because of increased abdominal girth and poor feeding. Two bluish subcutaneous nodules were present on the abdomen and groin, and the liver was enlarged and palpable 5 cm below the right costal margin. Computed tomography (CT) scans revealed a left adrenal mass, an enlarged liver studded with tumor nodules, large bilateral paraaortic nodes extending upward beyond the diaphragm, and a large anterior and posterior mediastinal mass surrounding the trachea and extending inferiorly with extensive subcarinal lymphadenopathy. Her 24-h urinary vanillylmandelic acid and homovanillic acid were markedly elevated. Serum ferritin was normal. A bone scan was normal, but a bone marrow biopsy was 50% replaced by neuroblastoma cells. She was treated according to Children's Cancer Group protocol 3881 and received 10 mo of chemotherapy with cyclophosphamide, doxorubicin, cisplatin, and etoposide. The liver returned to normal size and CT appearance, and the abnormal mediastinal mass, paratracheal and paraaortic nodes, bone marrow disease, and skin nodules disappeared. A small suprarenal mass was noted. Biopsy of this mass and of the liver revealed only mature ganglioneuroma. She has no evidence of disease 4 mo after chemotherapy was discontinued. Genetic evaluation at 19 mo of age again demonstrated significant growth delay (head circumference, 50% for 8 mo; length, 50% for 12 mo) and developmental delay.

Cytogenetic Studies and Cell Lines

Chromosomes were prepared from 72-h cultures of PHA-stimulated peripheral blood lymphocytes, according to routine methods. High-resolution banding was accomplished by exposure of cells to actinomycin D for 30 min before harvesting. At least 20 metaphase cells were examined from the patient and each parent. Permanent lymphoblastoid cell lines were established by Epstein-Barr–virus transformation of fresh samples of peripheral blood from the patient, mother, and father. Cell lines were maintained in

RPMI 1640 supplemented with 15% FCS, glutamine, and antibiotics.

Molecular Studies

The procedures for DNA preparation and quantitation have been described elsewhere (Brodeur et al. 1984). For Southern analysis, 5 µg of DNA were digested with the appropriate restriction enzyme (Promega) according to the manufacturer's protocols. Samples were electrophoresed on 0.8% agarose gels, transferred to nylon membranes (Sure Blot; Oncor), and hybridized to the appropriate probe, according to the method described by Brodeur et al. (1984). Nine DNA markers that map to distal 1p by genetic linkage were used for Southern analysis (Dracopoli et al. 1991). These include the telomeric hypervariable marker D1Z2 (Buroker et al. 1987), D1S47 (Donis-Keller et al. 1987), pronatriodilatin (PND) (Frossard and Coleman 1986), D1S56 (Donis-Keller et al. 1987), liver/bone/kidney alkaline phosphatase (ALPL) (Weiss et al. 1987), α-L-fucosidase (FUCA1) (Darby et al. 1986), the FGR proto-oncogene at 1p36 (Dracopoli et al. 1988), the VNTR marker D1S79 at 1p35 (Nakamura et al. 1988), and the VNTR marker D1S57 at 1p34 (Nakamura et al. 1984). The order of the markers on chromosome 1, from telomere to centromere, is as listed above. The Na^+/H^+ antiporter (APNH) gene has been mapped to 1p35-36.1 by genetic linkage analysis and in situ hybridization (Mattei et al. 1988; Lifton et al. 1990). Probes were labeled with ^{32}P by the random-primer technique (Feinberg and Vogelstein 1983). Filters were washed and exposed to X-OMAT AR autoradiography film (Kodak) for 1–5 d. Single-strand conformation polymorphism (SSCP) analysis of the APNH locus will be described elsewhere (authors' unpublished data).

Results

Cytogenetic Studies

Cytogenetic studies were initially requested at 2 mo of age to rule out a chromosomal basis for the patient's phenotypic abnormalities and growth delay. At that time, the karyotype (at 450-band resolution) was felt to be normal, 46,XX. These studies were repeated with high-resolution banding and with particular reference to chromosome 1, at the time the diagnosis of neuroblastoma was made. Examination of multiple karyotypes suggested that there was a deletion involving 1p36. Idiograms of the normal and deleted chro-
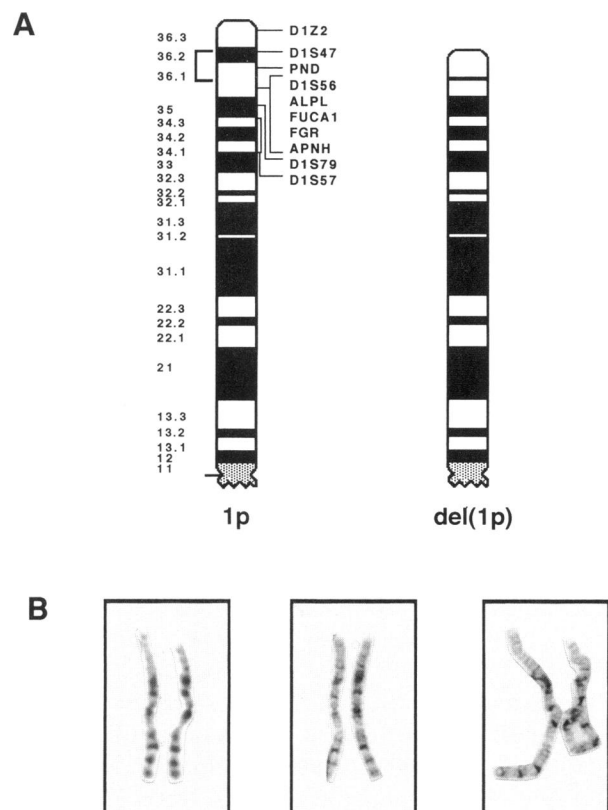
Figure 1   *A,* Idiogram of the normal chromosome 1p, shown on the left, with the location of the polymorphic loci examined. The bracket on the left indicates the deleted region, which results in the deleted chromosome, shown on the right. *B,* Three partial karyotypes of the chromosomes 1 from the patient. In each, the normal homologue is on the left, and the deleted homologue is on the right.

mosome 1p are shown in figure 1*A.* Partial karyotypes of the normal and deleted chromosomes 1 from the patient are shown in figure 1*B.* Constitutional karyotypes of both parents were normal.

Molecular Studies

In order to confirm the region of deletion indicated by cytogenetic analysis, DNA was examined from the patient for allelic loss on distal 1p. Heterozygosity studies were performed on lymphoblastoid cell-line DNAs established from the patient and both parents by using a panel of 10 polymorphic DNA markers that map to the region 1p34→1p36. DNA probes from nine loci, each of which identified a conventional RFLP, were employed. An additional polymorphism was detected by the SSCP technique (Orita et al. 1989) at the APNH locus (Mattei et al. 1988; Lifton et al.

1990). Representative autoradiograms of the RFLPs and SSCP are shown in figure 2, and the chromosomal locations of the markers used are shown in figure 1*A.*

At the hypervariable locus D1Z2, which has been localized to 1p36.3 (Buroker et al. 1987; Lamb et al. 1988), the patient inherited several unique bands from each parent (fig. 2*A*), indicating that she had inherited alleles from both parents at this locus. This indicates that the constitutional deletion was proximal to D1Z2. However, allelic loss was apparent at D1S47, a locus that appears to map more proximally at 1p36.2 (Donis-Keller et al. 1987). At D1S47, the patient had retained the 7.6-kb allele inherited from her father
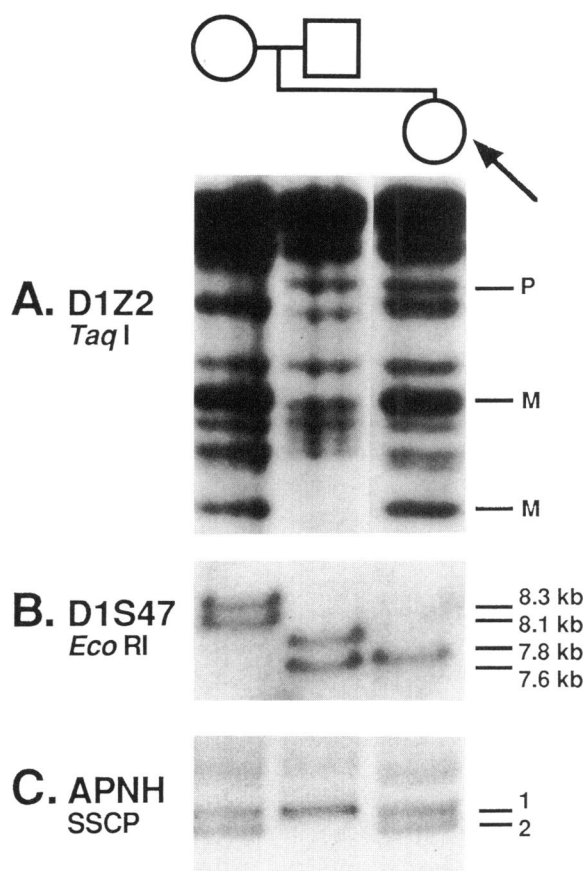

Figure 2   *Top,* Diagram of the patient's pedigree. The arrow indicates the patient. Symbols overlie the respective autoradiograms for selected genetic loci. *A,* Partial autoradiogram for the D1Z2 probe. Unique bands from each parent (P = paternal; and M = maternal) can be seen in the patient's pattern. *B,* Autoradiogram of the D1S47 RFLP. The patient inherited the 7.6-kb band from her father, but neither of the mother's bands is evident in the patient's DNA. *C,* Autoradiogram of the SSCP for the APNH locus. Band 1 from the father and band 2 from the mother are evident in the patient's DNA, indicating retention of heterozygosity at this locus.

but showed no evidence of either the 8.3- or 8.1-kb maternal alleles (fig. 2B). Finally, heterozygosity was retained at the APNH locus, which has been mapped to 1p35→1p36.1 (Mattei et al. 1988). The patient's mother is heterozygous, demonstrating both allele 1 and allele 2, while her father is homozygous for allele 1 (fig. 2C). The patient is heterozygous, indicating obligate inheritance of allele 1 from her father and allele 2 from her mother. Consequently, the constitutional deletion breakpoint must be distal to the APNH locus. Allelotyping was performed with seven additional distal 1p markers, but the patient was not informative at any of these loci.

The results of the allelotyping studies establish that the patient carries a de novo germ-line deletion involving subbands of 1p36 and that this deletion includes the D1S47 locus from the mother. The largest possible region of deletion is bounded distally by D1Z2 and proximally by the APNH gene, the two markers for which retention of heterozygosity was observed. Thus, the allelotype analysis confirms that the region of deletion lies within subbands of 1p36, as suggested by the cytogenetic data, and that the deletion is interstitial in nature.

Discussion

This study describes the cytogenetic and molecular analysis of a constitutional deletion involving the distal short arm of chromosome 1 in a child with neuroblastoma, dysmorphic features, and developmental and growth delay. By cytogenetic analysis, the deletion appeared to involve 1p36.1→1p36.2. The molecular analysis provided confirmation that the patient had constitutional loss of a portion of the maternal homologue in this same region. We were unable to define the region of loss more precisely, because many of the probes available in this region were uninformative in this family. However, the cytogenetic and molecular data were consistent with constitutional deletion in this region and presumably account for the patient's phenotypic findings and developmental and growth delay.

There are 13 reported cases of constitutional deletions of the short arm of chromosome 1p in the literature (Howard and Porteus 1990). Although no specific phenotype is associated with deletion of 1p36, several of the features that our patient has, including mental retardation, microcephaly, hypertelorism, high arched palate, and a heart defect, have been seen in other cases of 1p deletion.

The constitutional loss of DNA from the short arm of chromosome 1 in a child who developed neuroblastoma, however, is particularly interesting because there is frequent loss of DNA from this region in the tumor cells of many neuroblastomas (Brodeur et al. 1977, 1981; Gilbert et al. 1984; Fong et al. 1989, 1992; Weith et al. 1989; Brodeur 1990). Thus, it is likely that loss of one copy of a gene or genes in this region resulted in predisposition to the development of neuroblastoma. The one other reported case of a neuroblastoma patient with a constitutional abnormality involving 1p36 was a balanced translocation t(1;17)(p36;q12-21) (Laureys et al. 1990). Our case thus adds substantial supportive evidence to the hypothesis that there is a predisposition locus for neuroblastoma, on the short arm of chromosome 1.

Cytogenetic analysis of tumor tissue has identified deletions of the short arm of chromosome 1, as well as cytogenetic evidence of gene amplification in selected cases (Brodeur et al. 1977, 1981; Gilbert et al. 1984; Brodeur and Fong 1989). Molecular analysis has confirmed the deletions of this chromosome arm and has localized the region of deletion to subbands of 1p36, which is similar to the region constitutionally deleted in our case (Fong et al. 1989; Weith et al. 1989). Molecular analysis of neuroblastoma cells has also identified another region of allelic loss on the long arm of chromosome 14 (Suzuki et al. 1989), and this finding has been confirmed in another study (Fong et al. 1992). There has also been a study of chromosome transfer in neuroblastoma cells, which has confirmed that there is an important locus on 1p (Bader et al. 1991) but which also suggests a locus that maps to chromosome 17 that suppresses tumorigenicity. Thus, there may be other genes, in addition to the gene at 1p36, that are involved in the development or progression of neuroblastoma.

The findings seen in this patient are reminiscent of two other pediatric tumors in which there are constitutional chromosome deletions or rearrangements that predispose to tumor development. For retinoblastoma, there is a predisposition locus at 13q14, which contains the RB-1 gene (Friend et al. 1986; Fung et al. 1987; Lee et al. 1987). New germinal deletions or rearrangements involving this locus predispose to the development of retinoblastoma. Familial retinoblastoma, in the absence of gross chromosomal deletions, also maps to this region (Sparkes et al. 1983; Cavenee et al. 1986). Similarly, patients with Wilms tumor may have gross deletions or rearrangements of two regions on the short arm of chromosome 11, at 11p13 (WT-1

locus) (Gessler et al. 1990; Rose et al. 1990) and 11p15 (WT-2 locus) (Henry et al. 1989; Koufos et al. 1989; Reeve et al. 1989). In contrast to familial retinoblastoma, however, some familial Wilms tumors do not map to the short arm of chromosome 11 (Grundy et al. 1988; Huff et al. 1988), suggesting the existence of a third locus.

It is not clear whether constitutional abnormalities of the 1p36 region are representative of the single-locus model provided by retinoblastoma or the multilocus model provided by Wilms tumor. Previous cytogenetic analyses of constitutional karyotypes in patients with neuroblastoma have not revealed any consistent rearrangement. However, the recent case described by Laureys et al. (1990), considered together with this case, strongly suggests that constitutional deletions or abnormalities involving a locus at 1p36 may predispose to the development of neuroblastoma. Given the difficulty in detecting deletions in this region of chromosome 1, it is possible that submicroscopic deletions are present in other patients but are not detectable by routine cytogenetic analysis, even with high-resolution banding.

Familial neuroblastoma is not common, but there are a sufficient number of families to document an autosomal dominant mode of inheritance (Kushner et al. 1986; Brodeur 1991). Transmission of a submicroscopic 1p36 deletion would also mimic an autosomal dominant mode of inheritance. Because of the generally poor outcome of patients with neuroblastoma, there are few extended pedigrees in which genetic linkage can be tested. However, as the locus is further defined, it will be important to determine whether familial neuroblastoma is linked to 1p36 as well.

Finally, genomic imprinting has been shown to play a role in determining which allele is lost at suppressor loci in retinoblastoma and certain other tumors (Dryja et al. 1989; Scrable et al. 1989; Toguchida et al. 1989; Zhu et al. 1989; Leach et al. 1990). It is not known yet whether imprinting plays a role in determining which allele is preferentially lost from 1p36 in neuroblastomas, but it is clear that the maternal allele was lost in our patient. Future studies should confirm whether genomic imprinting at the 1p36 locus is an important mechanism in the development of this disease.

Acknowledgments

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