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RAPID COMMUNICATION

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New p57^{KIP2} mutations in Beckwith-Wiedemann syndrome

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Abstract Beckwith-Wiedemann syndrome (BWS) is characterized by numerous growth abnormalities and an increased risk of childhood tumors. The gene for BWS is localized in the 11p15.5 region, as determined by linkage analysis of autosomal dominant pedigrees. The increased maternal transmission pattern seen in the autosomal dominant-type pedigrees and the findings of paternal uniparental disomy reported for a subgroup of patients indicate that the gene for BWS is imprinted. Previously, we found p57^{KIP2}, which is a Cdk-kinase inhibitor located at 11p15, is mutated in two BWS patients. Here, we screened for the mutation of the gene in 15 BWS patients.

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Introduction

Beckwith-Wiedemann syndrome (BWS) is characterized by numerous growth abnormalities, including macroglossia, gigantism, exomphalos, viceromegaly, and an increased risk of childhood tumors, including Wilms' tumor, adrenocortical carcinoma, rhabdomyosarcoma, and hepatocellular carcinoma (Wiedemann 1983). Although most cases of BWS are sporadic and karyotypically normal, there are patients with chromosome 11 duplications (Okano 1986; Waziri et al. 1986) or translocations (Pueschel and Padre-Mendoza 1984) and a few families with autosomal dominant transmission (Moutou et al. 1992; Niikawa et al. 1986). The increased maternal transmission pattern seen in the autosomal dominant-type pedigrees (Lubinsky et al. 1974) and the findings of paternal uniparental disomy reported for a subgroup of patients (Henry et al. 1991) indicate that the gene for BWS is imprinted.

The gene for BWS is localized in the 11p15.5 region, as determined by linkage analysis of autosomal dominant pedigrees (Koufos et al. 1989; Ping et al. 1989). p57^{KIP2}, which is an imprinted gene (Hatada and Mukai 1995; Hatada et al. 1996a) located at 11p15 (Matsuoka et al. 1995), is a potent tight-binding inhibitor of several G_1 cyclin/Cdk complexes and is a negative regulator of cell proliferation (Lee et al. 1995; Matsuoka et al. 1995). Mutations in p57^{KIP2} have been found in two BWS patients (Hatada et al. 1996b). Here, we screened for the mutation of the gene in 15 BWS patients.

Materials and methods

Patients

We used samples from 14 Japanese and one Austrian BWS patient. Three of them had childhood tumors. Patient 202 (patient III-3 of family 3; Niikawa et al. 1986) was born at 39 weeks of gestation. He had the following abnormalities: gigantism, peculiar faces with capillary hemangiomas on the forehead, prominent eyes, large protruding tongue, bilateral skin grooves along the infraorbital ridge, linear earlobe indentations, accessory nipples, omphalocele, left

inguinal hernia, bilateral cryptorchidism, enlargement of liver, spleen, and kidneys, and bilateral postaxial polydactyly. The sister of patient 202 was born after 40 weeks of gestation. She had the following abnormalities: gigantism, peculiar faces, exomphalos, macroglossia, macrostomia, ear lobe grooves, mental retardation, and ventricular septal defect. The father did not have any signs of BWS. The mother had gigantism during infancy.

Patient 204 was born at 35 weeks of gestation. He had the following abnormalities: macroglossia, omphalocele, hepatomegaly, bilateral cryptorchidism, and ear lobe grooves.

PCR amplification and direct DNA sequencing

The p57^{KIP2} gene was examined for mutations by direct sequencing of five PCR-amplified fragments as previously described (Hatada et al. 1996b). The primers used were: fragment 1, 5′-CGTTCCACAGGCCAAGTGCG-3′ and 5′-GCTGGTGCGCACTAGTACTG-3′; fragment 2, 5′-CGTCCCTCCGCAGCACATCC-3′ and 5′-CCTGCACCGTCTCGCGGTAG-3′; fragment 3, 5′-TGGACCGAAGTGGACAGCGA-3′ and 5′-GGGGCCAGGACCGCGACC-3′; fragment 4, 5′-CGGAATTCCGGAGCAGCTGCCTAGTGTC-3′ and 5′-CTTTAATGCCACGGGAGGAGG-3′; and fragment 5, 5′-CGGCGACGTAAACAAAGCTG-3′ and 5′-GGTTGCTGCTACATGAACGG-3′. The amplified bands were excised from gels and purified using a Qiaex II gel extraction kit (Qiagen). Sequencing was performed using an ABI Prism dye terminator cycle sequencing kit (ABI).

Results and discussion

We analyzed DNA samples from 14 Japanese and one Austrian BWS patient. Three of them had childhood tumors. Human p57^{KIP2} encodes a 316-amino acid protein consisting of three structurally distinct domains (Lee et al. 1995; Matsuoka et al. 1995): an amino-terminal Cdk-inhibitory domain with marked similarity to p21^{CIP1} (Harper et al. 1993; Toyoshima and Hunter 1994) and p27^{KIP1} (Polyak et al. 1994); a region containing proline–alanine repeats (PAPA repeats); and a carboxyl-terminal domain conserved with p27^{KIP1} (QT domain). We analyzed the entire coding region of the gene including intron/exon boundaries by direct sequencing of five PCR-amplified fragments.

Mutations were detected in two patients (patients 202 and 204; Fig. 1). Patient 202 was one of two familial cases we examined. In this patient, direct sequencing analysis and sequencing of each cloned allele revealed a heterozygous CT to G transversion/deletion at nucleotide 570, leading to a frameshift at codon 104 resulting in the loss of the QT domain and the PAPA repeats. His father was normal, but his mother had gigantism during infancy. His sister was diagnosed as having BWS. Sequencing analysis of his affected sister revealed the same mutation. Familial cases of BWS are inherited exclusively through the mother (Lubinsky et al. 1974). To examine the transmission of the mutation in this familial case, sequencing analysis of his parents was performed. The mutation was not found in his father and the same mutation was found in his mother, indicating maternal transmission of the mutation, as in a previously studied case (Hatada et al. 1996b).

Direct sequencing analysis of patient 204 revealed a heterozygous C to A transversion at nucleotide 1000, changing a serine (TCG) to a termination (TAG) codon at codon 247. This resulted in a truncated polypeptide of 246 residues with a disruption of the QT domain. This mutation indicates an important role of the QT domain in growth regulation. Overexpression of the Cdk domain arrests cells in the G_1 phase (Matsuoka et al. 1995) However, of the four cases including the previous report (Hatada et al. 1996b), three of them have the complete Cdk inhibitory domain. Therefore, the QT domain seems to have a regulatory function for the p57^{KIP2} protein.

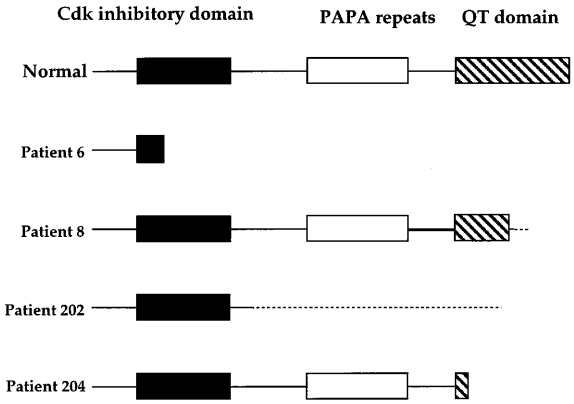

Fig. 1 Schematic representation of the normal and mutant structure of p57^{KIP2}. The mutations in patient 6 and patient 8 have been previously reported (Hatada et al. 1996b). *Broken lines* indicate unusual amino acid sequences caused by the frameshift mutation

Another possibility is involvement of other loci. There are three othre known BWS balanced translocations that mapped to several megabases away from this region (Hoovers et al. 1995). IGF2 could also be involved because we could not explain the etiology of paternal duplications by p57^{KIP2} since, in this class, a maternal allele is present and presumably functional.

The total mutation rate, including data from the previous study (Hatada et al. 1996b), was 17% (n = 24). However, we only analyzed the entire coding region of the gene including intron/exon boundaries. BWS with no mutations could be due to the reduced expression of p57^{KIP2} by paternal uniparental disomy, loss of imprinting or maternal translocation.

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