Brief Communication

Normal Dosage of the Insulin and Insulin-Like Growth Factor II Genes in Patients with the Beckwith-Wiedemann Syndrome

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SUMMARY

Several patients in whom the Beckwith-Wiedemann syndrome (BWS) is associated with duplication of chromosomal region 11p15 have recently been observed. The genes encoding insulin and insulin-like growth factor II (IGF-II), proteins that affect cellular growth and pancreatic function, have been mapped to 11p15, and their increased expression might, thus, account for the physical features of BWS. To determine whether BWS is frequently associated with small duplications of 11p15, we performed dosage analyses of the insulin and IGF-II genes in somatic DNAs of seven patients with BWS. In each case, we observed apparent diploid representation of these genes. These data suggest that BWS is not frequently associated with small duplications of 11p15 material that embed the insulin and IGF-II genes.

INTRODUCTION

The Beckwith-Wiedemann syndrome (BWS) [1, 2] is a generalized somatic overgrowth disorder with variable features that include large birth size, exomphalos, macroglossia, visceromegaly, adenocortical cytomegaly, renal medullary dysplasia, and, frequently, neonatal hypoglycemia associated with islet cell hyperplasia and transient hyperinsulinemia. Patients with BWS also have an increased incidence of embryonal tumors, including Wilms tumor, gonadoblastoma, hepatoblastoma, rhabdomyosarcoma, and adrenal carcinoma [3, 4]. A number of these features have been previously noted to be similar to those

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| Patient no. | Karyotype* | Birth wt (gm) [gestation age (wks); percentile] | Omphalocele | Macroglossia | Lobular ear creases | Hypoglycemia | Other facial features | Other features |
|------------|------------|-----------------------------------------------|-------------|-------------|---------------------|-------------|-----------------------|---------------|
| 1 .... 46,XY (M) | 3,300 [40, 40%] | + | - | + | - | Submucous cleft palate, severe underbite | Aniridia, bilat. inguinal hernias |
| 2 .... 46,XY (P) | 4,353 [38, >97%] | + | + | + | - | Bilateral zygomatic hypoplasia, micrognathia | Bilateral inguinal hernias, pectus carinatum, bilateral 5th finger clinodactyly [7, 9, 11] |
| 3 .... 46,XY (P) | 2,322 [33½, 50%] | + | + | + | + | ... | Bilateral cryptorchidism, bilateral inguinal hernias, VSD, “borderline intelligence,” brother of patient 3 [7, 9, 11] |
| 4 .... 46,XX (M) | 3,020 [34, 90%] | + | + | - | - | Wide nasal base, nuchal skin redundancy | Enlarged right kidney, dislocated knees, wide space between 1st and 2nd toes bilateral |
| Case | Karyotype | Chromosome | Features                                                                 | Clinical Features                                                                 |
|------|-----------|-------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| 5    | 46,XY (P) | 3,775 [40, 75%] | + + + - | Wide nasal base | First-degree hypospadias, double left ureter, paroxysmal atrial tachycardia |
| 6    | N.D.      | 3,620 [40, 50%] | + + + - | Hypoplastic malar eminences, prognathism, wide nasal base, synophris, prominent lips | Bilateral cryptorchidism, intestinal malrotation, seizure disorder, I.Q. 72, 2nd cousin of patients 2 and 3, brother also affected with BWS [7, 9, 11] |
| 7    | 46,XY (P) | 2,803 [37, 25%] | + + + - | Dolichocephaly, “barrel-shaped” nose, mandibular prognathism, high arched palate | Hydramnios, pre-eclampsia, birth length 53 cm (> 90%), bilateral cryptorchidism, right inguinal hernia, renal medullary dysplasia, agenesis of left testis, absent xiphisternum, hypoplastic lumbar vertebrae, brainstem astrocytoma [11] |

* M, metaphase karyotype; P, prometaphase karyotype; N.D., not determined.
resulting from prenatal hyperinsulinemia seen in some infants born to diabetic mothers [5]. Most cases of BWS are sporadic; however, many instances of familial recurrence have been reported [2, 6–17]. Although cytogenetic abnormalities have not been detected in the great majority of cases, four unrelated cases of BWS were recently reported with the common feature of trisomy 11p15 [18, 19], and another has been reported with a balanced 11p:22q translocation [20]. Two genes encoding proteins that affect cellular growth and pancreatic function have been mapped to the region 11p15: insulin [21, 22] and insulin-like growth factor II (IGF-II) [23, 24]. We have performed dosage analyses of these genes to determine whether BWS could be associated with small duplications of genetic material within chromosomal region 11p15 and thus account for the physical characteristics of the disorder. We present evidence here for diploid representation of these genes in somatic tissues from seven patients with BWS, including three sporadic cases, three familial cases, and one sporadic case with both BWS and aniridia.

MATERIALS AND METHODS

All patients were examined by the University of Wisconsin Clinical Genetics Center staff and found to have BWS based on typical clinical criteria. Patient data are presented in table 1 and [7, 9, 11].

Human DNAs from the seven BWS patients and four normal individuals were prepared from peripheral blood leukocytes or cultured skin fibroblasts by a modification of the method of Blin and Stafford [25], digested to completion with EcoRI, electrophoresed through a 0.8% agarose slab gel, and transferred [26] to a nylon membrane (Gene Screen Plus; New England Nuclear). Probes used were a 310 basepair (bp) PstI fragment from the human genomic insulin plasmid pHIGx310 [27], a 1.5-kilobase (kb) EcoRI fragment from the human IGF-II cDNA plasmid p8–1 [28], and an approximately 440-bp HindIII or EcoRI-PstI fragment from plasmid p9D11 that contains a human genomic DNA fragment from chromosome region 13q22 [29] as a referent. 32P nick-translated 11p15 (insulin or IGF-II) plus 13q22 (9D11) probes were hybridized simultaneously to the membrane as described [30] and autoradiographed, and the gene dosages determined by scanning densitometry analysis [31]. The same membrane was used for both the insulin and IGF-II gene-dosage analyses after removing the previous probes by denaturation as recommended by the manufacturer.

RESULTS

Insulin

Figure 1 shows hybridization of the human insulin and 9D11 chromosome 13 probes to EcoRI-cleaved DNAs from the four normal individuals and seven patients with BWS studied. As previously noted, the human insulin probe hybridizes to a single EcoRI fragment of approximately 13.6 kb [32]. The precise size of this fragment varies in the human population because of size variation within a tandem repeat array 5' to the insulin structural gene [32–34]. Three principal types of alleles occur: I (13.6 kb), II (14.3 kb), and III (15.0 kb), in order of ascending number of repeat units. The approximate allele frequencies in American whites are, respectively, .67, .01, and .32 [35]. Control numbers 1 and 2 are homozygous for type III and I alleles, respectively, and control numbers 3 and 4 are I/III heterozygotes. Patients 4 and 7 are homozygous for
type I alleles, and patients 1–3, 5, and 6 are I/III heterozygotes. No type II alleles were detected.

The 9D11 chromosome 13 probe hybridizes to a single 8.2-kb EcoRI fragment. No polymorphism of this fragment was observed.

As shown in table 2, none of the BWS patients had insulin/9D11 hybridization ratios that were significantly different from the control mean. Furthermore, in all individuals who were heterozygotes for type I and type III insulin alleles, the amount of hybridization of each allele was similar. Although the hybridization of the type III alleles tended to be somewhat less than that of the type I alleles, none of these differences was statistically significant, and in no case was the difference greater than 1.4-fold. These data demonstrate that the insulin gene is not duplicated on either copy of chromosome 11 in the BWS patients that we studied.

**IGF-II**

Figure 2 shows hybridization of the human IGF-II and 9D11 chromosome 13 probes to the same filter illustrated in figure 1. The IGF-II probe hybridized to a single EcoRI fragment of approximately 16 kb.

As shown in table 2, none of the BWS patients had IGF-II/9D11 hybridization ratios that were significantly different from the control mean. These data demonstrate that the IGF-II gene is not duplicated on either copy of chromosome 11 in the BWS patients that we studied.

**DISCUSSION**

Considerable circumstantial evidence suggests an association between BWS and abnormalities of the short arm of chromosome 11. Anomalous prenatal somatic growth and pancreatic function are characteristic of BWS, and some genes involved in the control of cellular growth and pancreatic function, includ-
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TABLE 2
RATIO OF 11p15 TO 13q22 PROBE HYBRIDIZATION

| Controls           | INS [SD = .09] | IGF-II [SD = .11] |
|--------------------|----------------|-------------------|
| 1                   | 1.1 (0, 1.0)   | .9                |
| 2                   | 1.0 (.6, .4)   | .9                |
| 3                   | 1.1 (.5, .5)   | 1.2               |
| 4                   | 1.0 (.5, .5)   | 1.0               |

| Beckwith-Wiedemann patients | INS | IGF-II |
|-----------------------------|-----|--------|
| 1                           | 1.1 (.5/5) | .9 |
| 2                           | 1.0 (.6/4) | .9 |
| 3                           | 1.0 (.6/4) | 1.0 |
| 4                           | 1.0 (1.0/0) | .9 |
| 5                           | 1.1 (.6/4) | 1.0 |
| 6                           | .9 (.5/5) | 1.1 |
| 7                           | 1.0 (1.0/0) | 1.1 |

**NOTE:** Ratios of 11p15 to 13q22 probe hybridization were determined, and the mean of the control values was defined as 1.0; nos. in brackets are the standard deviations of the control means. All individual ratios are presented as multiples of the control means. Nos. in parentheses are the fraction of the total insulin hybridization attributable to type I and type III alleles, respectively (see text).

...ing insulin and IGF-II, have been mapped to 11p15. Chromosomal duplication of 11p15 has been observed in at least four cases of BWS, and BWS shares many clinical features with the effects of fetal hyperinsulinism seen in infants born to diabetic mothers. Deletion of the distal half of chromosome region 11p13 is associated with the syndrome of aniridia, Wilms tumor, and gonado-

![Fig. 2.—Southern blot of EcoRI-cleaved DNAs hybridized to human IGF-II and 9D11 probes. IGF-II fragment size is 16 kb and 9D11 fragment size is 8.2 kb. Lanes as in figure 1.](image-url)
blastoma [36, 37]. The frequencies of Wilms tumor and gonadoblastoma are elevated among patients with BWS, and one of the patients studied here (patient 1) had both BWS and aniridia. Expression of the IGF-II gene is elevated in Wilms tumor tissues [38, 39], and apparent homo- or hemizygosity for some 11p markers has recently been observed in Wilms tumor [40–43], hepatoblastoma, and rhabdomyosarcoma [44] tissues.

We have shown here that the insulin and IGF-II genes are not duplicated in seven patients with BWS. Three of these patients had sporadic BWS, one had BWS plus aniridia, and three were from a family with multiple individuals affected with BWS [7, 9, 11]. All six patients whose chromosomes were analyzed had normal prometaphase or metaphase-banded karyotypes. These data, together with the recent observation of normal prometaphase-banded karyotypes in 19 other BWS patients [45], suggest that BWS is not commonly associated with duplication of the insulin or IGF-II genes. Instead, it is likely that some patients with 11p15 duplications share some clinical features with BWS [45].

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