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Invited Review

Genomic imprinting and Beckwith-Wiedemann syndrome

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Summary. Genomic imprinting is the parental-allele-specific expression of genes. Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth syndrome with increased risk of childhood tumors, is one of the well-known diseases caused by imprinted genes. The imprinted genes causing BWS are discussed in this review.

Key words: Genomic imprinting, Imprinted, Beckwith-Wiedemann syndrome, *p57KIP2*, IGF2, 11p15

Introduction

Imprinted genes in mammals are exclusively expressed from one of the parental chromosomes (Solter, 1988; Barlow, 1994; Efstratiadis, 1994; Nicholls, 1994; Ohlsson et al., 1994; Razin and Cedar, 1994). For example, *Igf2* is exclusively expressed from the paternal allele and the maternal allele is repressed. On the contrary, *Igf2r* is exclusively expressed from the maternal allele and the paternal allele is repressed. The importance of imprinted genes in development is implicated in a number of genetic diseases and developmental failure of embryos produced by parthenogenesis, gynogenesis or androgenesis.

Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth syndrome with an estimated incidence of 1/13700, is one of the well-known diseases caused by imprinted genes. This syndrome is characterized by associations of macroglossia, gigantism, abdominal wall defects including omphalocele and umbilical hernia, visceromegaly, earlobe creases or pits, renal dysplasia, facial flame nevus, hypoglycemia, adrenal cytomegaly, skeletal anomalies and increased risk of childhood tumors including Wilms' tumor, adrenocortical carcinoma, rhabdomyosarcoma and hepatocellular carcinoma. Although most cases of BWS are sporadic, familial cases constitute 15%. Familial BWS is inherited as an autosomal dominant trait with incomplete penetrance and the BWS gene(s) was mapped to 11p15 (Ping et al., 1989). Penetrance is stronger when the mother is the transmitting parent and cases of transmitting males with affected children are very rare (Lubinsky et al., 1974; Best and Hoekstra, 1981; Moutou et al., 1992). Another 25% of cases are paternal disomy, which indicates replacement of the maternal locus with duplication of the paternal locus (Henry et al., 1991). The region most commonly involved in paternal disomy includes 11p15. In BWS patients with balanced translocations and inversion of chromosome 11, the chromosomal aberration is of maternal origin (Mannens et al., 1994). These findings indicate that the BWS gene(s) is imprinted.

Loss of heterozygosity at 11p15 has been observed in a number of human cancers including breast cancer, bladder, lung, ovarian, kidney and testicular carcinoma. Several types of childhood tumors including Wilms' tumor, adrenocortical carcinoma, rhabdomyosarcoma and hepatocellular carcinoma, display specific loss of maternal 11p15 alleles, suggesting that genomic imprinting plays an important role (Schroeder et al., 1987; Scrable et al., 1989; Pal et al., 1990). Loss of imprinting of IGF2 on 11p15 has been frequently observed in Wilms' tumor (reveiewed by Yun, 1998).

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Mutations of the *p57KIP2* gene

The *p57KIP2* gene is localized to the 11p15 (Matsuoka et al., 1995). This gene is related to *p21CIP1* and *p27KIP2*, and is a potent tight-binding inhibitor of several G1 cyclin/CDK complexes which control the cell cycle (Matsuoka et al., 1995; Mong-Hong et al., 1995). Overexpression of *p57KIP2* arrests cells in G1, and its expression during development and in adults is correlated with terminal cellular differentiation. The *p57KIP2* gene is imprinted and expressed predominantly from the maternal allele (Hatada and Mukai, 1995; Hatada et al., 1996a). Mutation analysis of *p57KIP2* in BWS patients revealed mutations that alter the structure of the protein in 17% of cases (n=24, Hatada et al., 1996b, 1997a). All patients were heterozygous for the mutant allele. In all cases where the parents were examined, the mothers showed mutant alleles whereas the father did not. Each mother was heterozygous for the mutant allele and the phenotype was normal. In each

mother, the mutant allele was speculated to have been inherited from the father (grandfather of the patient). In the mother, the expression was repressed and the normal allele expressed. In contrast, in each patient, the expressed maternal allele was a mutant and the repressed paternal allele was normal (Fig. 1). Consequently, little or no active $p57^{KIP2}$ protein existed resulting in overgrowth in the BWS patient. These findings are correlated with predominant transmission of BWS through the mother.

Human $p57^{KIP2}$ consists of three domains (Matsuoka et al., 1995; Mong-Hong et al., 1995): an amino-terminal Cdk-inhibitory domain which is markedly similar to $p21^{CIP1}$ and $p27^{KIP2}$ (residues 28-91); a region containing proline-alanine repeats (PAPA repeats, residues 142-219); and a carboxyl-terminal domain conserved with $p27^{KIP2}$ (QT domain, residues 238-316). The functions of the PAPA repeat and QT domain are unknown. One of the common features of the mutations is the absence of the complete QT domain (Fig. 2). The human $p57^{KIP2}$ protein contains a proliferating cell nuclear antigen (PCNA) binding domain in this region. Truncated $p57^{KIP2}$ protein that lost 41 C-terminal residues as a result of a T to AG transversion/addition at codon 276 found in BWS exhibited no detectable PCNA binding activity (Watanabe et al., 1998). The QT domain can prevent PCNA-dependent DNA synthesis in vitro and S phase entry *in vivo*. Disruption of the PCNA binding domain partially reduces the ability to suppress myc/RAS-mediated transformation in primary cells (Watanabe et al., 1998).

Comparison of BWS patients' clinical features (Table 1) and their mutant $p57^{KIP2}$ protein structure (Fig. 2) revealed that only the mutant $p57^{KIP2}$ protein of patients with tumors lost both the complete Cdk-inhibitory and QT domains. All other mutant $p57^{KIP2}$ products of patients without tumors retained the complete Cdk-inhibitory domain. Disruption of either the Cdk inhibitory or QT domain partially reduced

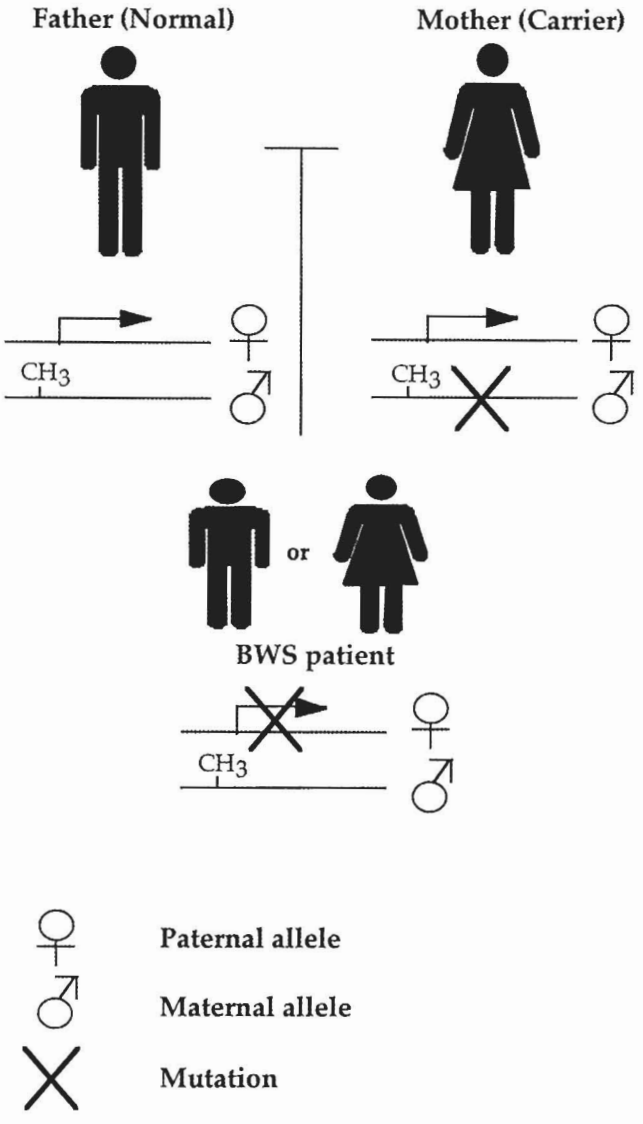

Fig. 1. Transmission of the $p57^{KIP2}$ mutation in a BWS family. Mutated allele is transmitted from the mother.

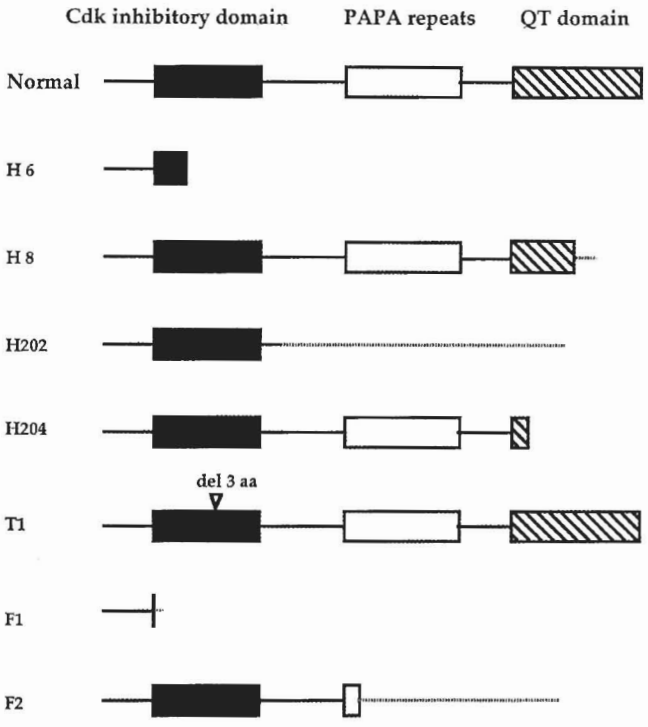

Fig. 2. Structure of normal and mutant $p57^{KIP2}$. H6 and H8 are patient 6 and patient 8 reported by Hatada et al. (1996b), respectively. H202 and H204 are patient 202 and patient 204 reported by Hatada I et al. (1997a), respectively. T1 is patient 1 reported by O'Keefe et al. (1997). F1 and F2 are patient 1 and patient 2 reported by Lee et al. (1997), respectively. Broken lines indicate unusual amino acid sequences caused by the frameshift mutation.

Table 1. Phenotypes of BWS patients, *p57KIP2* disruption, and *IGF2* overexpression.

PHENOTYPE	BWS PATIENTS WITH *P57KIP2* MUTATION						MICE	
	H6	H8	H202	H204	T1	F1	*p57KIP2* KO	*Igf2* Tg
Gigantism	+	+	+	+			-	+
Macroglasia	+	+	+	+	+	+	-	+
Abdominal wall defects	+	+	+	+	+	+	+	-
Visceromegaly				+			-	+
Renal dysplasia							+	
Adrenal cytomegaly							+	
Cleft palate	+						+	
Lens defect							+	
Hemihypertrophy							-	-
Tumor	+					+	-	-
Hypoglycemia	+	+				+		-

p57KIP2 ability to suppress myc/RAS-mediated transformation in primary cells, while loss of both domains completely eliminated *p57KIP2*'s suppressive activity (Watanabe et al., 1998). These findings suggest that disruption of both Cdk inhibitory and QT domains is required for the tumor formation in BWS patients and disruption of the QT domain alone does not lead to the development of tumors.

The molecular pathogenesis of a paternal disomy case can be explained by the loss of expression of the *p57KIP2* gene because this gene is predominantly expressed from the maternal allele.

Targeted disruption of *p57KIP2*

Yan et al. (1997) and Zhang et al. (1997) reported targeted disruption of the mouse *p57KIP2* gene. These mice have a number of similar phenotypes but there are certain significant differences, which may be due to differences in strain background. The observed defects which correspond to BWS clinical features include: abdominal wall defect, visceromegaly, renal dysplasia, adrenal cytomegaly, skeletal anomalies, cleft palate and lens defect (Table 1). Cleft palate was observed in both sets of mice at high frequency. However, this clinical feature is not frequently associated with BWS. Only one patient with the *p57KIP2* mutation had a cleft palate (Table 1). In this patient, a truncated mutant product showed loss of most of the protein (Fig. 2). The products of both knockout mice also showed loss of most of the *p57KIP2* protein. Therefore, cleft palate may be a typical phenotype of null *p57KIP2* alleles.

Gigantism, macroglossia, visceromegaly, hemihypertrophy, and hypoglycemia were not observed in these mice. However, all features except for hemihypertrophy were observed in the BWS patient with the *p57KIP2* mutation. This can be explained by species differences because strain differences can cause significant phenotype differences.

Abnormal expression of IGF2

The presence of trisomy cases including two paternal 11p15 and one maternal 11p15 case is difficult to explained by *p57KIP2* because unlike paternal disomy cases, *p57KIP2* should be expressed from the one maternal 11p15. Duplicating the imprinted paternal *p57KIP2* allele may affect the maternal allele's expression. Similar effects were reported for some imprinted genes (Hatada et al., 1997b; Lee and Jaenisch, 1997; Sun et al., 1997). An alternative explanation is the presence of the paternally expressed gene mapped to 11p15 which has the opposite function of *p57KIP2*. In the trisomy case, increased expression of the paternally expressed gene cancels the *p57KIP2* effect resulting BWS. The *IGF2* is probably the involved gene and three indirect findings support this theory. The first finding is that *IGF2* is the only known paternally expressed gene mapped to 11p15 although there could be another unknown paternally expressed gene in this locus. The second finding is that *IGF2* has the opposite function of *p57KIP2*. The third finding is the biallelic expression of *IGF2* observed in 60% of BWS patients (Reik and Maher, 1997). In these patients, *IGF2* is expressed from the maternal allele in addition to the paternal allele. Biallelic expression may increase the *IGF2* expression level. Similar effects are known as the imprinting mutation in Prader-Willi syndrome (PWS), which was mapped to 15q11-13. At this site, many imprinted genes cluster as seen at 11p15 (Buiting et al., 1995; O'Keefe et al., 1997). In an imprinting mutation case, a microdeletion affected the expression of at least three imprinted genes in the cluster (Sutclife et al., 1994). This finding suggests that abnormal expression of another imprinted gene mapped to 11p15 is responsible for a part or all of the BWS phenotype in *IGF2* biallelic expression cases. Therefore, it is important to determine whether or not *IGF2* alone causes the BWS phenotype. Identifying an *IGF2* mutation case of BWS would support this theory. However, no mutation of the *IGF2* gene has yet been reported. Creating mice that overexpress *igf2* by introducing transgenes may be useful to clarify the role of the *IGF2* gene. Increased expression of *igf2* in mice showed some defects associated with BWS (Sun et al., 1997). These defects included gigantism, macroglossia, visceromegaly, and

skeletal anomalies. Although other BWS clinical features including abdominal wall defects, hemihypertrophy, tumor, and hypoglycemia were not observed, the findings suggest that *IGF2* is responsible for at least part of the BWS phenotype.

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