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Japanese and North American/European patients with Beckwith–Wiedemann syndrome have different frequencies of some epigenetic and genetic alterations

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Beckwith–Wiedemann syndrome (BWS) is an imprinting-related human disease. The frequencies of causative alterations such as loss of methylation (LOM) of *KvDMR1*, hypermethylation of *H19-DMR*, paternal uniparental disomy, *CDKN1C* gene mutation, and chromosome abnormality have been described for North American and European patients, but the corresponding frequencies in Japanese patients have not been measured to date. Analysis of 47 Japanese cases of BWS revealed a significantly lower frequency of *H19-DMR* hypermethylation and a higher frequency of chromosome abnormality than in North American and European patients. These results suggest that susceptibility to epigenetic and genetic alterations differs between the two groups.
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Introduction

Beckwith–Wiedemann syndrome (BWS), a well-known imprinting-related human disease, is characterized by macrosomia, macroglossia, and abdominal wall defects (OMIM #130650). Genomic imprinting is an epigenetic phenomenon that is responsible for parent-of-origin-specific expression of genes. The relevant imprinted chromosomal region in BWS, 11p15.5, consists of two independent imprinted domains, *IGF2*/*H19* and *CDKN1C*/*LIT1*. Imprinted genes within each domain are regulated by the imprinting control region (ICR), which in this case is either *H19-DMR* or *KvDM1* (Figure 1a).[1] In North American and European BWS patients, several causative alterations have been identified: *KvDMR1* loss of methylation (LOM) (~50%), *H19-DMR* hypermethylation (2–7%), paternal uniparental disomy (patUPD; ~20%), *CDKN1C* mutations (~10%), duplications of 11p15 (<1%), and inversions or translocations involving 11p15 (<1%).[1] The cause is unknown for ~15% of patients.[1] Comprehensive analyses of Japanese patients with BWS, however, have not been carried out to date. To add to our understanding of frequencies of epigenetic mutations in the Japanese population, we analyzed 47 cases of BWS. Compared with North American and European groups, the frequency of *H19-DMR* hypermethylation was significantly lower (0%) and that of chromosome abnormality (13%) was higher in the Japanese patients. These results suggest that suscept-

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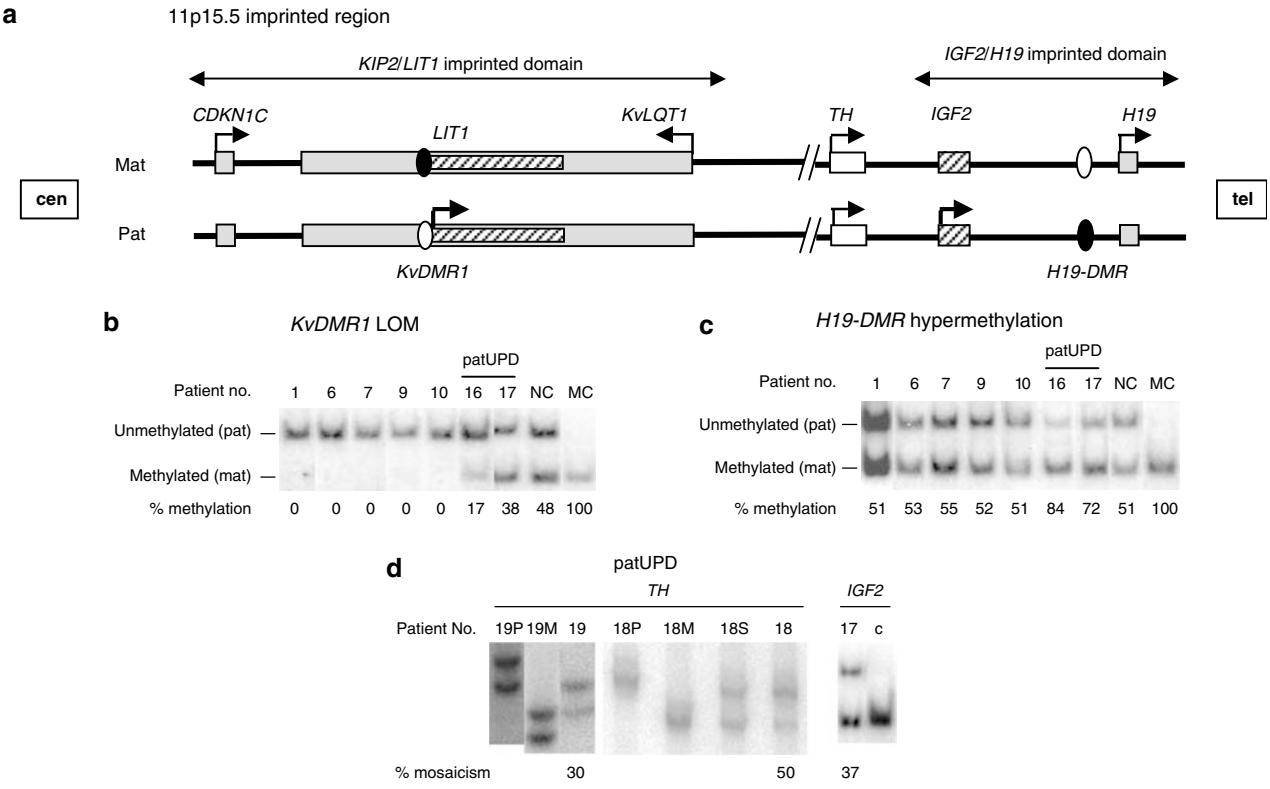

Figure 1 11p15.5 imprinted region and methylation and patUPD analysis. (**a**) 11p15.5 imprinted region. Representative genes are shown. Gray boxes and shaded boxes indicate maternal and paternal expressed genes, respectively. *KvDMR1* and *H19-DMR* are the ICRs for each domain. Black and white ovals indicate methylated and unmethylated ICRs, respectively. Broken arrows indicate the transcriptional direction. (**b**, **c**) Representative results for methylation analyses of *KvDMR1* and *H19-DMR* using hot-stop COBRA. patUPD patients had partial *KvDMR1* LOM and partial *H19-DMR* hypermethylation. (**d**) Representative results for patUPD analysis. Bands transmitted from the father were stronger than those from the mother, indicating patUPD. 18P and 19P, DNA from patient's father; 18M and 19M, DNA from patient's mother; 18S, unaffected sister; c, control for complete digestion. MC, methylated DNA control; NC, normal control.

ibility to epigenetic and genetic alterations differs according to ethnicity.

Materials and methods

BWS patients

Forty-seven Japanese patients who had been diagnosed with BWS by clinical geneticists were analyzed. Diagnoses were made according to the criteria of DeBaun and Tucker[2] with all patients having at least two of the five most common features (macroglossia, birth weight >90th percentile, hypoglycemia in the first month of life, ear creases or ear pits, and abdominal wall defects). DNA was extracted from lymphoblastoid cell lines, peripheral blood lymphocytes, or tissues. One sample from each patient was used. This study was approved by the Ethical Committee for Human Genome and Gene Analyses of the Faculty of Medicine, Saga University.

Chromosomal analysis

Metaphase chromosomes were analyzed using the standard G-banding method.

Methylation analyses

We investigated methylation status at the *Acc*II site 16 bp downstream of the *Not*I site in *KvDMR1*, and the *Mlu*I site approximately 80 bp downstream of the CTCF binding site 6 (CTS6) in *H19-DMR*. Combined bisulfite restriction analyses (COBRA) using the hot-stop method were employed as described previously.[3,4] For *H19-DMR*, bisulfite sequencing was performed using the same primers as used for COBRA. PCR primers and conditions are shown in Supplementary Table 1. The percentage methylation was calculated as: intensity of methylated band/(intensity of methylated band + intensity of unmethylated band) × 100.

Paternal uniparental disomy

To analyze patUPD, we used DNA polymorphic markers for 11p15.5, including tetranucleotide repeats in the tyrosine hydroxylase gene (*TH*), an *Ava*II (*Apa*I) polymorphism in *IGF2*, an *Rsa*I polymorphism in *H19*, and an *Nsi*I polymorphism in *KCNQ1OT1*. Hot-stop PCR was employed for PCR–RFLP to eliminate heteroduplex formation. The percentage mosaicism of patUPD was calculated as:[5] % mosaicism = $(k-1)/(k+1) \times 100$, where k is the ratio of the

Table 1 Results of genetic and epigenetic analyses in Japanese BWS patients

Pt no.	*Pt ID*	*Tissue*	*KvDMR1 LOM*	*H19-DMR hypermethylation*	*PatUPD (% mosaicism)*	*CDKN1C mutation*	*Chromosome abnormality*	*Alteration type*	*Tumor*
1	YN	LCL	+	−	−	−	−	*KvDMR1* LOM	
2	NA	PBL	+	−	−	−	−	*KvDMR1* LOM	
3	ST	PBL	+	−	−	−	−	*KvDMR1* LOM	
4	SR	PBL	+	−	−	−	−	*KvDMR1* LOM	
5	KT	PBL	+	−	−	−	−	*KvDMR1* LOM	
6	OZ	LCL	+	−	−	−	−	*KvDMR1* LOM	
7	MTT	LCL	+	−	−	−	−	*KvDMR1* LOM	
8	TH	PBL	+	−	−	−	−	*KvDMR1* LOM	
9	TY	LCL	+	−	−	−	ND	*KvDMR1* LOM	
10	OY	LCL	+	−	−	−	ND	*KvDMR1* LOM	
11	IM	LCL	+	−	−	−	ND	*KvDMR1* LOM	
12	SK	LCL	+	−	−	−	ND	*KvDMR1* LOM	
13	MH	PBL	+	−	−	−	ND	*KvDMR1* LOM	Rhabdomyosarcoma
14	MR	PBL	+	−	−	−	ND	*KvDMR1* LOM	
15	Kbt-To	Tongue	+	ND	−	ND	ND	*KvDMR1* LOM	
16	TK	LCL	Partial	Partial	+ (66%)	−	−	PatUPD	Hepatoblastoma
17	KY	PBL	Partial	Partial	+ (37%)	−	−	PatUPD	
18	A-MS	PBL	Partial	Partial	+ (50%)	−	−	PatUPD	
19	MI	PBL	−	Partial	+ (30%)	−	ND	PatUPD	Hepatoblastoma
20	A87-Lu	Lung	Partial	ND	+ (45%)	ND	ND	PatUPD	
21	S99-Li	Liver	Partial	ND	+ (77%)	ND	ND	PatUPD	
22	S94-Pa	Pancreas	Partial	ND	+ (72%)	−	−	PatUPD	
23	MT	LCL	−	−	−	399C>T (mat)	−	*CDKN1C* mutation	Cardiac atrial tumor
24	SA	PBL	ND	ND	ND	570delCTinsG (mat)	−	*CDKN1C* mutation	
25	SA-S	PBL	ND	ND	ND	570delCTinsG (mat)	−	*CDKN1C* mutation	
26	Fjm-To	Tongue	−	ND	−	1086delTinsAG	−	*CDKN1C* mutation	
27	SzM	LCL	−	Partial	−	−	Trisomy 11 due to t(11;14) (pat)	Chromosome abnormality	
28	SY	LCL	Partial	Partial	ND	ND	Trisomy 11 due to t(11;X) (pat)	Chromosome abnormality	
29	YT	LCL	−	Partial	−	−	Trisomy 11 (pat)	Chromosome abnormality	
30	MS	LCL	ND	ND	ND	ND	Trisomy 11	Chromosome abnormality	
31	MK	LCL	−	−	−	−	t(11;12)	Chromosome abnormality	
32	SdM	LCL	−	−	−	−	46,XX,−17,+der(17),t(14;17) (q32;p13)	Chromosome abnormality	
33	OM	LCL	−	−	−	−	−	Unknown	
34	TM	LCL	−	−	−	−	−	Unknown	
35	IY	LCL	−	−	−	−	−	Unknown	
36	AK	LCL	−	−	−	−	−	Unknown	
37	HD	LCL	−	−	−	−	−	Unknown	
38	HY	LCL	−	−	−	−	−	Unknown	
39	KK	LCL	−	−	−	−	−	Unknown	
40	KH	LCL	−	−	−	−	−	Unknown	
41	MtS	LCL	−	−	−	−	−	Unknown	

Table 1 (*Continued*)

Pt no.	*Pt ID*	*Tissue*	*KvDMR1 LOM*	*H19-DMR hypermethylation*	*PatUPD (% mosaicism)*	*CDKN1C mutation*	*Chromosome abnormality*	*Alteration type*	*Tumor*
42	AT	LCL	–	–	–	–	–	Unknown	
43	KcH	LCL	–	–	–	–	–	Unknown	
44	SmM	PBL	–	–	–	–	–	Unknown	
45	TR	PBL	–	–	–	–	–	Unknown	
46	IH	PBL	–	–	–	–	–	Unknown	
47	YS	PBL	–	–	–	–	–	Unknown	Hepatoblastoma

LCL, lymphoblastoid cell line; LOM, loss of methylation; mat, maternal transmission; ND, not done; patUPD, paternal uniparental disomy; PBL, peripheral blood lymphocyte.
#24 and #25 were siblings.

intensity of the paternal to maternal alleles of the sample. We defined patUPD as more than 30% mosaicism.

CDKN1C mutations

Mutations of CDKN1C were investigated by sequencing as described previously.[6]

Statistical analysis

Differences between Japanese and North American and European patients with BWS were calculated using the χ^2-test or Fisher's direct probability method. Probability levels of <0.05 were considered statistically significant.

Results and discussion

We used hot-stop COBRA for quantitative analysis of methylation, the results of which were confirmed in several patients by methylation-sensitive Southern blot analysis. We found that 15 patients (#1–15) had complete LOM of *KvDMR1* and 7 patients (#16–18, #20–22, #28) had partial LOM (Table 1 and Figure 1b). We also found seven patients (#16–19, #27–29) with partial hypermethylation of *H19-DMR*, but found no patient with complete hypermethylation (Figure 1c). patUPD manifested in a mosaic pattern, and trisomy 11 was associated with an extra 11p, which may have affected the results of methylation analyses at *KvDMR1* and *H19-DMR*. Indeed, all our patients with partial *KvDMR1* LOM and/or partial *H19-DMR* hypermethylation had mosaic patUPD (#16–22) or trisomy 11 (#27–29; Figure 1b and c). We thus concluded that 15 (#1–15) patients (34%) had isolated *KvDMR1* LOM. For *H19-DMR*, since the *Mlu*I site used for COBRA was approximately 80 bp downstream of CTS6, we examined whether the methylation status of the *Mlu*I site correctly reflected that within the core sequence of CTS6 by bisulfite sequencing. A total of 258 clones generated from 13 patients (11–20 clones per patient; 5 patients with *KvDMR1* LOM, 3 with patUPD, 1 with the *KIP2* mutation, and 4 with cause unknown) and 5 normal individuals (11–12 clones per individual) were sequenced. We found that 235 clones (91%) showed the same methylation status at the *Mlu*I site and CTS6 (data not shown). Thus, we concluded that COBRA based on the *Mlu*I site accurately reflected the methylation status of CTS6. We also concluded that no patient in this study had isolated *H19-DMR* hypermethylation.

When PCR products generated from the paternal allele were more intense than those from the maternal allele, an allelic imbalance was indicated (Figure 1d). We found seven patients with patUPD (15%), as mentioned above, and six patients (#27–32) with chromosome abnormalities (13%); via karyotyping, four of these patients were found to have translocations and two were found to have trisomy 11 (Table 1). Two (#27 and #28) of the four translocation patients had partial trisomy for 11p due to a translocation.

Table 2 Frequencies of alterations in North American and European and Japanese patients with BWS

Alteration type	*North American and European*[a]	*Japanese*	
KvDMR1 LOM	44% (164/375)	32% (15/47)	$P=0.1222$ (χ^2)
H19-DMR hypermethylation	9% (40/428)	0% (0/47)	$P=0.0235$ (F)
patUPD	16% (69/428)	15% (7/47)	$P=0.8275$ (χ^2)
CDKN1C mutation	5% (24/531)	9% (4/47)	$P=0.2729$ (F)
Chromosome abnormality	2% (2/97)	13% (6/47)	$P=0.0150$ (F)
H19-DMR microdeletion	11 patients from 6 families[9–11]	0% (0/17)	
Unknown	25% (71/278)	32% (15/47)	$P=0.3595$ (χ^2)

LOM, loss of methylation; patUPD, paternal uniparental disomy.
χ^2, χ^2-test; F, Fisher's direct probability method.
[a]Data on North American and European patients were obtained from the previously published literature.[12, 16–18]

Partial *KvDMR1* LOM and/or partial *H19-DMR* hypermethylation in patients #27–29 indicated that they had two copies of paternal 11p. Patient #27 was previously described as having paternal origin duplication of 11p.[7] Whether the chromosomal abnormalities in the remaining two cases (#30 and #31) were of paternal origin was unknown. Although patient #32, who suffered from umbilical hernia, macroglossia, ear creases, and ear pits, had a translocation, it did not involve 11p or any molecular abnormalities. Thus, an unknown causative factor, which may or may not be due to the translocation, is involved in this patient. We found three mutations in *CDKN1C*, that is, 399C>T, 570delCTinsG, and 1086delTinsAG, in four patients (9%; #23–#26; #24 and #25 were siblings), as reported previously.[8]

Of the 47 patients, 15 (32%; #33–47) did not have any identifiable alterations, and so their BWS was designated as cause unknown. The frequencies of macrosomia and abdominal wall defects were lower in these patients than in patients with known causes (data not shown). Although microdeletion of *H19-DMR* has been reported,[9–11] we did not find it in any patient examined, including the 15 patients with cause unknown and two patients (#18 and #28) who had patUPD and trisomy 11 due to t(11;X), respectively (data not shown).

The frequency of isolated *H19-DMR* hypermethylation was significantly lower in Japanese patients with BWS than in the North American and European patients (Table 2). The Japanese patients had more frequent chromosomal abnormality. Although our sample number was not large, our data suggest that susceptibility to epigenetic and genetic alterations leading to BWS varies according to ethnicity. BWS predisposes patients to embryonal tumors, especially Wilms' tumor, in which loss of imprinting (LOI) of *IGF2* accompanied by *H19-DMR* hypermethylation is involved. Furthermore, a strong association between *H19-DMR* hypermethylation and Wilms' tumor development in BWS has been reported.[12,13] In Japanese BWS patients, however, the frequency of Wilms' tumor tends to be lower than expected,[14] and low frequencies of *H19-DMR* hypermethylation and *IGF2* LOI in Wilms' tumors in Japanese children have been reported.[15] Of the 47 patients in the present study, 5 patients developed tumors (3 hepatoblastomas, 1 rhabdomyosarcoma, and 1 cardiac atrial tumor). Although overall tumor incidence (11%) was consistent with that reported for North American and European patients, there were no patients with Wilms' tumor in our sample. The low frequency of *H19-DMR* hypermethylation may account for this. Further investigations will be necessary to understand whether the different frequencies of epigenetic and genetic alterations are due to DNA polymorphisms, such as SNPs and/or low copy repeats, at 11p.

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Competing interests

None declared.

References

1 Weksberg R, Shuman C, Smith AC: Beckwith–Wiedemann syndrome. *Am J Med Genet C Semin Med Genet* 2005; **137**: 12–23.
2 DeBaun MR, Tucker MA: Risk of cancer during the first four years of life in children from the Beckwith–Wiedemann Syndrome Registry. *J Pediatr* 1998; **132**: 398–400.

3 Soejima H, Nakagawachi T, Zhao W *et al*: Silencing of imprinted CDKN1C gene expression is associated with loss of CpG and histone H3 lysine 9 methylation at DMR-LIT1 in esophageal cancer. *Oncogene* 2004; **23**: 4380–4388.
4 Satoh Y, Nakadate H, Nakagawachi T *et al*: Genetic and epigenetic alterations on the short arm of chromosome 11 are involved in a majority of sporadic Wilms' tumours. *Br J Cancer* 2006; **95**: 541–547.
5 Itoh N, Becroft DM, Reeve AE, Morison IM: Proportion of cells with paternal 11p15 uniparental disomy correlates with organ enlargement in Wiedemann–Beckwith syndrome. *Am J Med Genet* 2000; **92**: 111–116.
6 Hatada I, Ohashi H, Fukushima Y *et al*: An imprinted gene p57KIP2 is mutated in Beckwith–Wiedemann syndrome. *Nat Genet* 1996; **14**: 171–173.
7 Mannens M, Hoovers JM, Redeker E *et al*: Parental imprinting of human chromosome region 11p15.3-pter involved in the Beckwith–Wiedemann syndrome and various human neoplasia. *Eur J Hum Genet* 1994; **2**: 3–23.
8 Hatada I, Nabetani A, Morisaki H *et al*: New p57KIP2 mutations in Beckwith–Wiedemann syndrome. *Hum Genet* 1997; **100**: 681–683.
9 Sparago A, Cerrato F, Vernucci M, Ferrero GB, Silengo MC, Riccio A: Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and Beckwith–Wiedemann syndrome. *Nat Genet* 2004; **36**: 958–960.
10 Prawitt D, Enklaar T, Gartner-Rupprecht B *et al*: Microdeletion of target sites for insulator protein CTCF in a chromosome 11p15 imprinting center in Beckwith–Wiedemann syndrome and Wilms' tumor. *Proc Natl Acad Sci USA* 2005; **102**: 4085–4090.
11 Sparago A, Russo S, Cerrato F *et al*: Mechanisms causing imprinting defects in familial Beckwith–Wiedemann syndrome with Wilms' tumour. *Hum Mol Genet* 2007; **16**: 254–264.
12 Weksberg R, Nishikawa J, Caluseriu O *et al*: Tumor development in the Beckwith–Wiedemann syndrome is associated with a variety of constitutional molecular 11p15 alterations including imprinting defects of KCNQ1OT1. *Hum Mol Genet* 2001; **10**: 2989–3000.
13 Rump P, Zeegers MP, van Essen AJ: Tumor risk in Beckwith–Wiedemann syndrome: a review and meta-analysis. *Am J Med Genet A* 2005; **136**: 95–104.
14 Kobayashi Y: Malformation syndromes and childhood tumors; in Shimizu K, Misugi K (eds): *Shouni Geka Byourigaku (Pediatric Surgical Pathology)*. Tokyo: Bunkodo, 1995, pp 12–18.
15 Fukuzawa R, Breslow NE, Morison IM *et al*: Epigenetic differences between Wilms' tumours in white and east-Asian children. *Lancet* 2004; **363**: 446–451.
16 Gaston V, Le Bouc Y, Soupre V *et al*: Analysis of the methylation status of the KCNQ1OT and H19 genes in leukocyte DNA for the diagnosis and prognosis of Beckwith–Wiedemann syndrome. *Eur J Hum Genet* 2001; **9**: 409–418.
17 DeBaun MR, Niemitz EL, McNeil DE, Brandenburg SA, Lee MP, Feinberg AP: Epigenetic alterations of H19 and LIT1 distinguish patients with Beckwith–Wiedemann syndrome with cancer and birth defects. *Am J Hum Genet* 2002; **70**: 604–611.
18 Bliek J, Gicquel C, Maas S, Gaston V, Le Bouc Y, Mannens M: Epigenotyping as a tool for the prediction of tumor risk and tumor type in patients with Beckwith–Wiedemann syndrome (BWS). *J Pediatr* 2004; **145**: 796–799.

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