Differences in GH-related parameters and GH responses between patients with Prader-Willi syndrome due to deletion and maternal uniparental disomy 15.

Keiko Matsubara[1,2], Yuki Kozu[2], Kazuo Obata[2], Nobuyuki Murakami[2], Tsutomu Ogata[1], Toshiro Nagai[2]

[1] National Research Institute for Child Health and Development, Department of Molecular Endocrinology, Tokyo, Japan
[2] Dokkyo Medical University of Koshigaya Hospital, Department of Pediatrics, Koshigaya, Japan

INTRODUCTION

Individuals with PWS have several endocrine abnormality, including GH deficiency. They have short stature, a decreased spontaneous and provoked GH secretion, low IGF-I levels, and a positive response to GH treatment. Some data had demonstrated that the birth length were longer among the patients due to uniparental disomy (UPD) than deletion.

In adult patients, GH response to provocative test by GHRH – arginine is significantly lower in the patients due to UPD than deletion. (Grugni et al. 2006)

These data indicated that individuals with PWS due to UPD tended to be shorter and might be good responders to GH replacement therapy.

We hypothesized that there could be differences of the response to GH treatment between genetic subtypes among children with PWS. To examine this possibility, we compared peak level of GH secretion provoked by arginine or insulin and GH-related parameters between patients due to deletion and UPD.

PATIENTS

76 patients were included in this study.
(55 with deletion and 21 with UPD)

All participants :

(i) were genetically confirmed PWS by methylation studies, fluorescent in situ hybridization (FISH) and analysis of microsatellite makers.
(ii) were prepubertal at start of GH. (defined as Tanner breast stage ≦2 for girls and testicular volume <4ml for boys and with age below 12 or 14 years in girls or boys, respectively.)
(iii) had undergone a GH provocative test by arginine (0.5g/kg) and/ or insulin (0.1U/kg) before the start of GH treatment.
(iv) were treated with recombinant hGH (Genotropin, Pfizer, New York, NY) 0.245 mg/ kg/ week.

RESULTS

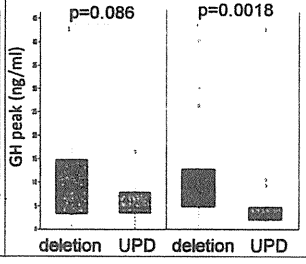
Characteristics of participants

Deletion 55 (m/f 35/20), UPD 21 (m/f 12/9)

	Deletion				UPD			
	mean	± SE	median	range	mean	median	range	p-value
birth length (cm)	47.7 ±	2.3	47.8	41.5 - 51.5	45.3 ± 1.6	45.1	32 - 51	0.12
birth weight (g)	2530 ±	376	2565	1714 - 3316	2243 ± 178	2260	622 - 3180	0.12
age (y) *			3.5	8m - 13.5y		3.6	10m - 11y	0.97
$Ht SDS_{PWS}$*	-0.52 ±	0.14	-0.54	-3.1 - 1.7	-0.72 ± 0.15	-0.6	-2.6 - 0.32	0.46
BMI (kg/m^2) *	17.2 ±	0.72	15.3	12.3 - 30.2	12.3 ± 1.2	16.8	13 - 22.3	0.35
IGF-I (ng/ml) *	85.1 ±	13.8	69	10 - 383	62.2 ± 15.0	63.7	4 - 160	0.41
IGF-I SDS *	-1.4 ±	0.18	-1.7	-2.6 - 2.5	-1.64 ± 0.29	-1.9	-3.0 - 2.8	0.38
IGFBP3 (μg/ml) *	1.34 ±	0.09	1.3	0.51 - 2.33	1.24 ± 0.16	1.1	0.33 - 2.9	0.45
IGFBP3 SDS *	-2.4 ±	0.2	-2.1	-4.7 - -0.43	-2.4 ± 0.33	-2.6	-3.9 - 1.6	0.48

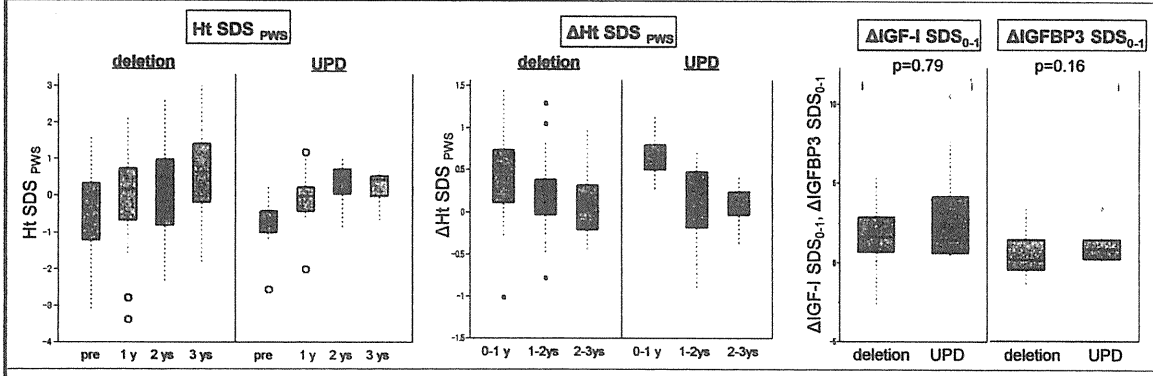
Height data are expressed in SDS for age- and sex- references for individuals with PWS of Japanese
IGF-I and IGFBP3 SDS are expressed for age- and sex- references for Japanese.
Differences were calculated using Mann-Whitney test.
* Data at GH start

Provocative test



GH peak (ng/ml)	Arginine	Insulin
Deletion Median (icq)	8.7 3.4 – 14.7	9.5 5.0 – 14.4
UPD Median (icq)	4.6 3.9 – 7.5	3.2 2.2 – 4.6
P-value	0.086	0.0018

Box-and-whisker plot showing median, interquartile range (icq), and values within ±1.5 icq.
Open circles are outliers.



	deletion				UPD			
	pre	1 year	2 years	3 years	pre	1year	2 years	3 years
Ht SDS PWS Median (icq)	-0.54 -1.3 – 0.01	-0.1 [a] -1.0 – 0.66	-0.03 -1.0 – 0.55	0.24 [a] -0.63 – 0.81	-0.6 -0.97 – 0.42	-0.006 [c] -0.33 – 0.19	0.39 [c] 0.05 – 0.68	0.26 [b] -0.007 – 0.48
ΔHt SDS PWS Median (icq)		0.49 [d] 0.16 – 0.77	0.17 [d] -0.08 – 0.34	0.24 [d] -0.63 – 0.81		0.61 [d] 0.57 – 0.79	0.26 [d] -0.14 – 0.47	0.13 [d] 0.02 – 0.24
ΔIGF-I SDS Median (icq)		1.78 [d] 0.81 – 2.88				1.46 [d] 0.75 – 3.1		
ΔIGFBP3 SDS Median (icq)		0.95 [d] 0.22 – 2.4				1.68 [d] 1.0 – 2.3		

[a] P < 0.05, [b] P < 0.005, [c] P < 0.001 compared to baseline levels
[d] No significant differences between deletion and UPD

Conclusion

GH peak induced by insulin in patients with deletion was significantly higher than those with UPD.

Height SDS improved by GH- treatment among the patients with PWS.

There was no significant difference of GH - induced changes in growth and plasma levels of IGF-I / IGFBP3 between genetic subtypes.

Journal of Human Genetics (2011) 56, 566–571

www.nature.com/jhg

ORIGINAL ARTICLE

Maternal age effect on the development of Prader–Willi syndrome resulting from upd(15)mat through meiosis 1 errors

Keiko Matsubara[1,2,3], Nobuyuki Murakami[2,3], Toshiro Nagai[2] and Tsutomu Ogata[1]

Prader–Willi syndrome (PWS) is primarily caused by deletions involving the paternally derived imprinted region at chromosome 15q11.2–q13 and maternal uniparental disomy 15 (upd(15)mat). The underlying mechanisms for upd(15)mat include trisomy rescue (TR), gamete complementation (GC), monosomy rescue and post-fertilization mitotic error, and TR/GC is mediated by non-disjunction at maternal meiosis 1 (M1) or meiosis 2 (M2). Of these factors involved in the development of upd(15)mat, M1 non-disjunction is a maternal age-dependent phenomenon. We studied 117 Japanese patients with PWS and identified deletions in 84 patients (Deletion group) and TR/GC type upd(15)mat through M1 non-disjunction in 15 patients (TR/GC (M1) group), together with other types of abnormalities. Maternal age was significantly higher in TR/GC (M1) group than in Deletion group (median (range), 37 (35–45) versus 30 (19–42); $P=1.0\times10^{-7}$). Furthermore, delayed childbearing age became obvious since the year 2003 in Japan, and relative frequency of TR/GC (M1) group was significantly larger in patients born since the year 2003 than in those born until the year 2002. The results imply that the advanced maternal age at childbirth is a predisposing factor for the development of upd(15)mat because of increased M1 errors.
Journal of Human Genetics (2011) **56**, 566–571; doi:10.1038/jhg.2011.59; published online 2 June 2011

Keywords: maternal age effect; meiosis 1; non-disjunction; Prader-Willi syndrome; upd(15)mat

INTRODUCTION

Prader–Willi syndrome (PWS) is a developmental disorder associated with various dysmorphic, neurologic, cognitive, endocrine and behavioral/psychiatric features.[1] It is caused by absent expression of paternally derived genes on the imprinted region at chromosome 15q11.2–q13, and previous studies have indicated that deletions of the paternally derived imprinted region and maternal uniparental disomy 15 (upd(15)mat) account for ~70 and ~25% of PWS patients, respectively.[1] The remaining PWS patients have rare abnormalities such as epimutations (hypermethylation) of the PWS imprinting center (IC), at the differentially methylated region encompassing exon 1 of *SNRPN* and microdeletions involving the PWS-IC or HBII-85 small nucleolar RNAs distal to the PWS-IC.[2–4]

Upd(15)mat are primarily caused by four mechanisms; that is, trisomy rescue (TR), gamete complementation (GC), monosomy rescue (MR) and post-fertilization mitotic error (PE).[5] TR refers to a condition in which chromosome 15 of paternal origin is lost from a zygote with trisomy 15, formed by fertilization between a disomic oocyte and a normal sperm. GC results from fertilization of a disomic oocyte with a nullisomic sperm. MR refers to a condition in which chromosome 15 of maternal origin is replicated in a zygote with monosomy 15, formed by fertilization between a normal oocyte and a nullisomic sperm. PE is an event after formation of a normal zygote. In this regard, a disomic oocyte specific to TR and GC is produced by non-disjunction at meiosis 1 (M1) or meiosis 2 (M2), and non-disjunction at M1 is known to increase with maternal age, probably because of a long-term (10–50 years) meiotic arrest at prophase 1.[6]

It is predicted, therefore, that the relative frequency of TR/GC-type upd(15)mat through M1 non-disjunction is high in PWS patients born to aged mothers and is increasing in countries where childbearing age is rising. In this context, previous studies have revealed a significantly higher maternal age in PWS patients with upd(15)mat than in those with deletions,[7,8] a significantly higher relative frequency of upd(15)mat in patients born to mothers aged ⩾35 years than in those born to mothers aged <35 years[9] and a significantly increased relative frequency of upd(15)mat in PWS patients <5 years of age in United Kingdom where childbearing age is increasing.[10] In these

[1]Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan and [2]Department of Pediatrics, Dokkyo Medical University Koshigaya Hospital, Saitama, Japan
[3]These authors contributed equally to this work.
Correspondence: Dr T Ogata, Department of Molecular Endocrinology, National Research Institute for Child Health and Development, 2-10-1 Ohkura, Setagaya, Tokyo 157-8535, Japan.
E-mail: tomogata@nch.go.jp
Received 3 March 2011; revised 29 April 2011; accepted 8 May 2011; published online 2 June 2011

studies, however, as underlying mechanisms for upd(15)mat have not been examined, it remains to be clarified whether such maternal age effect on the occurrence of upd(15)mat is primarily mediated by M1 non-disjunction. Furthermore, after studying underlying mechanisms for upd(15)mat by microsatellite analysis, Robinson *et al.*[11] have mentioned that maternal age effect is similar between M1 and M2 errors. Thus, it remains to be clarified whether advanced maternal age is relevant to the occurrence of TR/GC type upd(15)mat through M1 errors.

Here, we report that the advanced maternal age at childbirth constitutes a risk factor for TR/GC type upd(15)mat through M1 non-disjunction.

MATERIALS AND METHODS

This study was approved by the Institute Review Board Committees at the National Center for Child Health and Development and Dokkyo University Koshigaya Hospital, and performed after obtaining informed consent.

PWS patients

This study consisted of 117 Japanese PWS patients (72 male patients and 45 female patients) who satisfied the following selection criteria: (1) normal karyotype in all the 50 lymphocytes examined, (2) hypermethylated PWS-IC that was confirmed by methylation analysis for bisulfite-treated leukocyte genomic DNA, using methylated and unmethylated allele-specific PCR primers (Supplementary Figure 1),[12] and (3) positive data on the maternal age at childbirth (parental age was not found in two aged patients who had left our follow-up and whose hospital records had been discarded and in one patient who was born after artificial insemination by donor).

Molecular studies

We performed fluorescence *in situ* hybridization analysis, microsatellite analysis and multiplex ligation-dependent probe amplification (MLPA) analysis. For fluorescence *in situ* hybridization analysis, an ~125-kb probe identifying a region encompassing *SNRPN* was hybridized to lymphocyte metaphase spreads, together with a CEP 15 probe for *D15Z1* and a probe for *PML* on 15q22 utilized as internal controls. The probe for the *SNRPN* region was labeled with digoxigenin and detected by rhodamine anti-digoxigenin, and the control probes were detected according to the manufacturer's protocol (Abbott, Chicago, IL, USA). For microsatellite genotyping, PCR amplification was performed for 13 microsatellite loci on chromosome 15, using fluorescently labeled forward primers and unlabeled reverse primers. Subsequently, the PCR products were determined for size on a CEQ8000 autosequencer (Beckman Coulter, Fullerton, CA, USA). For MLPA analysis, we utilized a commercially available MLPA probe mix (ME028-B1) for multiple segments on the chromosome 15 imprinted region, including the PWS-IC and three portions within the HBII-85 small nucleolar RNAs (MRC-Holland, Amsterdam, The Netherlands). The procedure was as described in the manufacturer's instructions. The primers utilized in this study are summarized in Supplementary Table 1.

Classification of PWS patients

The PWS patients were classified into several groups, according to the underlying (epi) genetic causes (Figure 1). In particular, upd(15)mat was divided into three groups by the previously reported methods[13] (Supplementary Figure 2): (1) heterodisomy for at least one of the three adjacent pericentromeric (<4 Mb from the centromere) microsatellite loci (*D15S541*, *D15S542* and *D15S1035*) was regarded as indicative of TR/GC type upd(15)mat through M1 non-disjunction (TR/GC (M1) group), (2) the combination of isodisomy for the pericentromeric microsatellite loci and heterodisomy for at least one middle to distal microsatellite loci was interpreted as indicative of TR/GC type upd(15)mat through M2 non-disjunction (TR/GC (M2) group) and (3) isodisomy for all the informative microsatellite loci was regarded as indicative of MR/PE type upd(15)mat (MR/PE group). However, it is usually impossible to distinguish between TR and GC, and between MR and PE on the basis of microsatellite data, although identification of segmental isodisomy or mosaicism with a normal cell lineage is unique to PE.[14,15]

Analysis of parental ages

We compared parental ages between different groups and between two different time periods (until the year 2002 and since the year 2003), and relative frequency of each group between the two time periods. The setting of the two time periods was based on the Annual Vital Statistics Data from the Japanese Ministry of Health, Labor and Welfare (http://www.mhlw.go.jp/toukei/list/81-1.html). The maternal age producing the largest number of live births changed from 25–29 years to 30–34 years, and that producing the third largest number of live births changed from 20–24 years to 35–39 years, between the two time periods (Supplementary Figure 3).

Statistical significance of the median age was examined by the Mann–Whitneys *U*-test, that of the correlation between parental ages by Spearman's rank correlation test, and that of relative frequency by the Fisher's exact probability test. $P<0.05$ was considered significant.

RESULTS

Classification of PWS patients

The results are shown in Figure 1. Fluorescence *in situ* hybridization analysis revealed heterozygous deletions in 84 of the 117 patients (Supplementary Figure 4;Deletion group). Then, microsatellite genotyping was carried out in 27 of the 33 patients without deletions, classifying 15 patients as TR/GC (M1) group, seven patients as TR/GC (M2) group and three patients as MR/PE group (Figure 2;in the remaining six patients, further studies were refused by the parents). There was no finding indicative of segmental isodisomy or mosaicism. Finally, MLPA was performed in the remaining two non-upd(15)mat patients, identifying no microdeletion affecting the PWS-IC. Thus, the two patients were classified as Epimutation group.

Analysis of parental ages

Distribution of parental ages in each group is shown in Figure 3a, and parental age data are summarized in Table 1. Maternal ages were invariably ⩾35 in TR/GC (M1) group. Furthermore, comparison of maternal ages in Deletion, TR/GC (M1) and TR/GC (M2) groups with >5 patients revealed significant difference between Deletion and TR/GC (M1) groups ($P=1.0\times10^{-7}$), but not between Deletion and TR/GC (M2) groups ($P=0.19$), and between TR/GC (M1) and TR/GC (M2) groups ($P=0.085$). Paternal ages showed similar tendency, with

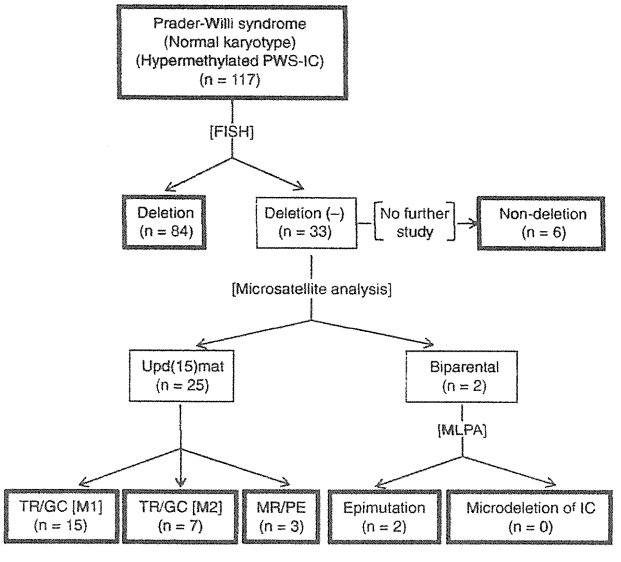


Figure 1 Classification of 117 Japanese patients with Prader–Willi syndrome phenotype.

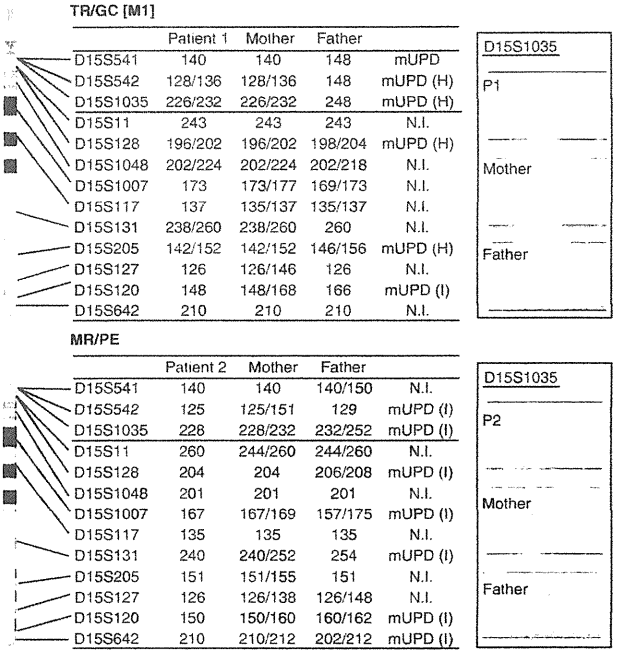


Figure 2 Chromosomal locations of the examined microsatellite loci and representative results. MUPD, maternal uniparental disomy (unknown for heterodisomy or isodisomy); mUPD (H), maternal uniparental heterodisomy; mUPD (I), maternal uniparental isodisomy; N.I., not informative. Pericentromeric loci are present in a heterodisomic status in patient 1, and this is consistent with trisomy rescue/gamete complementation (meiosis 1) (TR/GC (M1)) type maternal uniparental disomy 15 (upd(15)mat). For D15S1035, for example, both of the heterozygous maternal alleles are inherited by patient 1, whereas the homozygous paternal alleles are not transmitted to patient 1; this demonstrates mUPD (H) for this locus. In patient 2, all informative loci are present in an isodisomic condition, and this is compatible with monosomy rescue/post-fertilization mitotic error (MR/PE) type upd(15)mat. For D15S1035, for example, one of the two heterozygous maternal alleles is transmitted to patient 2, whereas both of the heterozygous paternal alleles are not inherited by patient 2; this demonstrates mUPD (I) for this locus.

significant difference between Deletion and TR/GC (M1) groups ($P=8.8\times10^{-5}$), but not between Deletion and TR/GC (M2) groups ($P=0.39$), and between TR/GC (M1) and TR/GC (M2) groups ($P=0.39$). However, whereas a significant correlation was observed between maternal and paternal ages in Deletion and TR/GC (M2) groups, there was no significant correlation between maternal and paternal ages in TR/GC (M1) group because of relatively advanced maternal ages in this group (Figure 3b). In addition, whereas maternal ages at childbirth were grossly similar between Deletion and TR/GC (M2) groups and the Japanese general population (the mean parental ages at childbirth in Japan were based on the data registered in the Ministry of Health, Labor and Welfare; http://www.mhlw.go.jp/toukei/list/81-1.html), they were obviously higher in TR/GC (M1) group than in the Japanese general population. Paternal ages at childbirth were grossly similar between Deletion group and the Japanese general population and tended to be higher in TR/GC (M1) and TR/GC (M2) groups than in the Japanese general population.

Relative frequency of each group markedly differed between 75 patients born until 2002 and 42 patients born since 2003 (Figure 3c). Here, TR/GC (M1) was indicated in three of the 75 patients born until the year 2002, and six non-deletion type patients were invariably born until the year 2002. Thus, TR/GC (M1) group accounted for at least three and up to nine of the 75 patients born until the year 2002, and 12 of the 42 patients born since the year 2003. Thus, the relative frequency of TR/GC (M1) was assessed to be significantly different, with the *P*-values being 1.8×10^{-7} for 3/75 versus 12/42, and 0.025 for 9/75 versus 12/42. In addition, there was no significant change in the parental ages of each group between the two time periods, although the maternal ages at birth of all the patients significantly differed between the two time periods.

DISCUSSION

The present study revealed deletions in 84 patients, upd(15)mat in 25 patients and epimutations in 2 patients. In addition, whereas microsatellite and MLPA analyses were not performed in six patients with non-deletion, the present and the previous studies argue that most of them have upd(15)mat, especially TR/GC (M1) type upd(15)mat.[1,13] Thus, the relative frequency of deletions, upd(15)mat and other rare causes appears to be similar between Japanese patient and previously reported Caucasian patients.[1]

Notably, the present study implies that advanced maternal age at childbirth constitutes a risk factor for the development of TR/GC (M1) type upd(15)mat. Indeed, maternal ages were significantly higher in TR/GC (M1) group than in Deletion group, which is free from maternal age effect. Although a significant difference was not found between maternal age-dependent TR/GC (M1) group and maternal age-independent TR/GC (M2) group, this would primarily be due to the small number of TR/GC (M2) group. Furthermore, the relative frequency of TR/GC (M1) group significantly increased since the year 2003 when delayed childbearing age became obvious, and the advanced maternal ages at birth since the year 2003 were primarily associated with the high frequency of TR/GC (M1) group rather than the advanced maternal ages in each group. Although it was impossible to distinguish between TR and GC, and between MR and PE,[16] this would not pose a major problem. The patients with M1 non-disjunction are included only in TR/GC (M1) group.

Paternal and environmental factors should also be considered for the present results. For a paternal factor, the frequencies of micro-deletions and nullisomic sperms might increase with age.[17] However, paternal ages at childbirth in each group were similar between the two time periods, and the relative frequency of Deletion group actually decreased since the year 2003. Furthermore, whereas nullisomic sperms can be a background of the development of GC, concomitant occurrence of a nullisomic sperm and a disomic oocyte must be extremely rare. Rather, nullisomic sperms would primarily constitute an underlying factor for the development of maternal age-independent MR. For an environmental factor, it is predicted that chemical materials are increasing with time and that aged parents are exposed to such materials for a long time. In this regard, it has been reported that exposure to environmental chemicals may exaggerate the occurrence of aneuploidies in females.[18] Thus, the environmental factor might be relevant to the recent increase of TR/GC (M1) group, although it is unlikely that this factor constitutes the major cause of the increased TR/GC (M1) type upd(15)mat. In males, whereas it has been reported that exposure to chemical materials might facilitate the occurrence of PWS, the relative frequency of genetic causes remained unchanged in PWS patients born to such males.[19–21] Collectively, the effects of such non-maternal age factors would remain small, if any, although further careful examinations are required for the precise evaluation of the maternal age effect on the occurrence of TR/GC (M1).

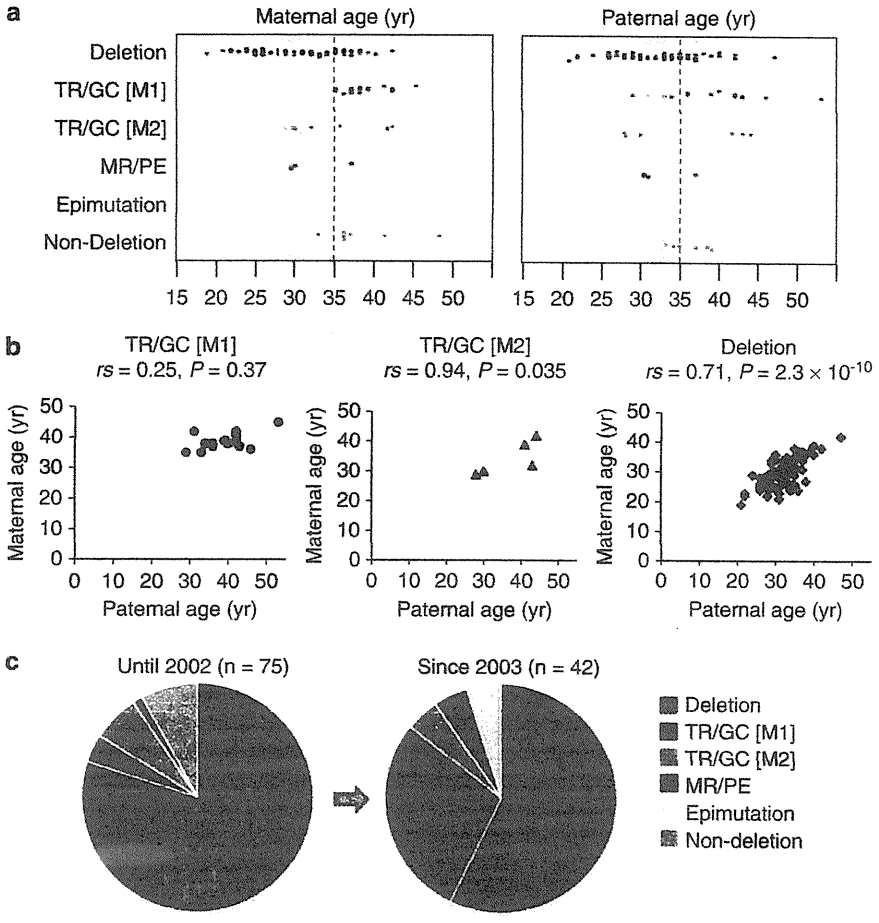


Figure 3 Analysis of parental ages at childbirth. (a) The distribution of parental ages in each group. The light pink and blue vertical bars represent the mean maternal and paternal ages at childbirth from the year 1970 to the year 2008. (b) Correlation between maternal and paternal ages at childbirth. Significant correlation is observed in trisomy rescue/gamete complementation (meiosis 2) (TR/GC (M2)) and Deletion groups, but not in trisomy rescue/gamete complementation (meiosis 1) (TR/GC (M1)) group because of relatively advanced maternal age. (c) Relative frequency of each group in 75 patients born until the year 2002 (n=60, 3, 5, 1, 0 and 6 for Deletion, TR/GC (M1), TR/GC (M2), monosomy rescue/post-fertilization mitotic error (MR/PE), epimutation and non-deletions groups, respectively) and in 42 patients born since the year 2003 (n=24, 12, 2, 2, 2 and 0 for Deletion, TR/GC (M1), TR/GC (M2), MR/PE, Epimutation and Non-deletions groups, respectively).

Several points should be made with regard to the present study. First, we classified upd(15)mat primarily on the basis of the results of three pericentromeric microsatellite loci, with the assumption of no recombination between the centromere and the three loci, as have been employed in the previous study.[13] The methods would be basically acceptable, because the three loci reside within a 4 Mb region from the centromere and a recombination is relatively rare in the centromeric regions.[22] However, it remains possible that a cryptic recombination(s) might have occurred in the pericentromeric region.

Second, upd(15)mat may also be caused by maternal age-dependent meiotic sister chromatid pre-division that can lead to aneuploid oocytes, including disomic oocytes specific to TR/GC.[23] In this regard, as such disomic oocytes can have various patterns of isodisomic and heterodisomic regions, it is impossible to discriminate between upd(15)mat through sister chromatid pre-division and that through conventional meiotic non-disjunction by microsatellite analysis. Thus, the patients classified as TR/GC (M1) group may have upd(15)mat due to maternal age-dependent conventional non-disjunction at M1 and maternal age-dependent sister chromatid pre-division, whereas those classified as TR/GC (M2) group may have upd(15)mat due to maternal age-independent conventional non-disjunction at M2 and maternal age-dependent sister chromatid pre-division. However, even if not all the patients classified as TR/GC (M1) group have upd(15)mat due to conventional non-disjunction at M1, it can be concluded that maternal age-dependent factors still have a critical role in the occurrence of upd(15)mat in patients classified as TR/GC (M1) group. In addition, possible mixture of maternal age-dependent and -independent factors in patients classified as TR/GC (M2) group may be relevant to the lack of significant difference in the maternal age between TR/GC (M2) and Deletion groups, and between TR/GC (M2) and TR/GC (M1) groups.

Lastly, whereas fluorescence *in situ* hybridization analysis has been routinely performed at commercial laboratories since the year 1993 in Japan, detailed molecular studies including microsatellite analysis are usually available only in institutional laboratories. Thus, a substantial fraction of patients without deletions may have remained undiagnosed or misdiagnosed, without receiving further studies including micro-

Table 1 Parental ages (year) at childbirth

	Deletion	*TR/GC (M1)*	*TR/GC (M2)*	*MR/PE*	*Epimutation*	*Non-deletion*	*All patients*	*General population*
Maternal age								
Total								
Median	30	37	31	30	38.5	36	32	27.5–30.9
Range	19–42	35–45	29–42	29–37	38–39	30–48	19–48	
Number	84	15	7	3	2	6	117	
Until 2002								
Median	29	37	32	29	—	36	30[a]	
Range	19–42	35–37	29–42	—	—	30–48	19–48	
Number	60	3	5	1	0	6	75	
Since 2003								
Median	32.5	38.5	35.5	33.5	38.5	—	35[a]	
Range	23–39	35–45	30–41	30–37	38–39	—	23–45	
Number	24	15	2	2	2	0	42	
Paternal age								
Total								
Median	32.5	40	35.5	31	41.5	36	33	30.6–33.0
Range	21–47	29–53	28–44	28–37	38–45	33–39	21–53	
Number	82[b]	15	6[c]	3	2	6	114[b,c]	
Until 2002								
Median	32.5	43	35.5	28	—	36	33	
Range	21–47	33–43	28–44	—	—	33–39	21–47	
Number	58[b]	3	4[c]	1	0	6	72[b,c]	
Since 2003								
Median	32.5	39.5	35.5	34	41.5	—	34.5	
Range	22–40	29–53	30–41	31–37	38–45	—	22–53	
Number	24	12	2	2	2	0	42	

Abbreviations: GC, gamete complementation; M1, meiosis 1; M2, meiosis 2; MR,monosomy rescue; PE, post-fertilization mitotic error; TR, trisomy rescue.
The data of the general population indicate the range of the mean parental ages at childbirth from the year 1970 to 2008.
[a]*P*-value=0.00017.
[b]Paternal age was not found in two old patients who had left our follow-up and whose hospital records had been discarded.
[c]Paternal age was not identified in one patient who was born after artificial insemination by donor.

satellite analysis at appropriate institutions. In this regard, considering the opportunity to receive detailed molecular studies, it is possible that upd(15)mat is overlooked more frequently in aged patients than in young patients. If so, this may be relevant to the significant difference in the relative frequency of TR/GC (M1) group between the two time periods ('since the year 2003' versus 'until the year 2002').

In summary, the results imply that the advanced maternal age at childbirth is a predisposing factor for the development of upd(15)mat because of increased M1 errors. This notion is applicable to maternal upd in general, as well as to trisomies. However, there are several caveats as discussed in the above, and the number of patients, especially those classified as TR/GC (M2) group, is small. Thus, further careful studies using a large number of patients are necessary in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

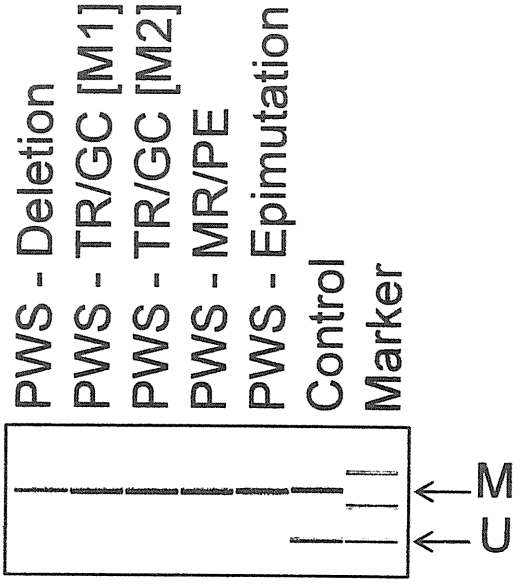
ACKNOWLEDGEMENTS

This work was supported by Grants for Research on Intractable Diseases (H22-165) and for Health Research on Children, Youth and Families (H21-005) from the Ministry of Health, Labor and Welfare, and by Grants-in-Aid for Scientific Research (A) (22249010) and Grant-in-Aid for Young Scientists (B) (22791022) from the Japan Society for the Promotion of Science (JSPS).

1 Cassidy, S. B. & Driscoll, D. J. Prader-Willi syndrome. *Eur. J. Hum. Genet.* **17,** 3–13 (2009).
2 Buiting, K., Grob, S., Lich, C., Gillessen-Kaesbach, G., El-Maarri, O. & Horsthemke, B. Epimutation in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *Am. J. Hum. Genet.* **72,** 571–577 (2003).
3 Sahoo, T., del Gaudio, D., German, J. R., Shinawi, M., Peter, S. U., Person, R. E. *et al.* Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nat. Genet.* **40,** 719–721 (2008).
4 de Smith, A. J., Purmann, C., Walters, R. G., Ellis, R. J., Holder, S. E., VanHaelst, M. *et al.* A deletion of the HBII-85 class of small nucleolar RNAs (snoRNAs) is associated with hyperphagia, obesity and hypogonadism. *Hum. Mol. Genet.* **18,** 3257–3265 (2009).
5 Shaffer, L. G., Agan, N., Goldberg, J. D., Ledbetter, D. H., Longshore, J. W. & Cassidy, S. B. American College of Medical Genetics statement on diagnostic testing for uniparental disomy. *Genet. Med.* **3,** 206–211 (2001).
6 Jones, K. T. Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age. *Hum. Reprod. Update.* **14,** 143–158 (2008).
7 Mitchell, J., Schinzel, A., Langlois, S., Gillessen-Kaesbach, G., Schuffenhauer, S., Michaelis, R. *et al.* Comparison of phenotype in uniparental disomy and deletion Prader-Willi syndrome: sex specific differences. *Am. J. Med. Genet.* **65,** 133–136 (1996).
8 Cassidy, S. B., Forsythe, M., Heeger, S., Nicholls, R. D., Schork, N., Benn, P. *et al.* Comparison of phenotype between patients with Prader-Willi syndrome due to deletion 15q and uniparental disomy 15. *Am. J. Med. Genet.* **68,** 433–440 (1997).
9 Ginsburg, C., Fokstuen, S. & Schinzel, A. The contribution of uniparental disomy to congenital development defects in children born to mothers at advanced childbearing age. *Am. J. Med. Genet.* **95,** 454–460 (2000).
10 Whittington, J. E., Butler, J. V. & Holland, A. J. Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. *Eur. J. Hum. Genet.* **15,** 127–130 (2007).
11 Robinson, W. P., Langlois, S., Schuffenhauer, S., Horsthemke, B., Michaelis, R. C., Christian, S. *et al.* Cytogenetic and age-dependent risk factors associated with uniparental disomy 15. *Prenat. Diagn.* **16,** 837–844 (1996).

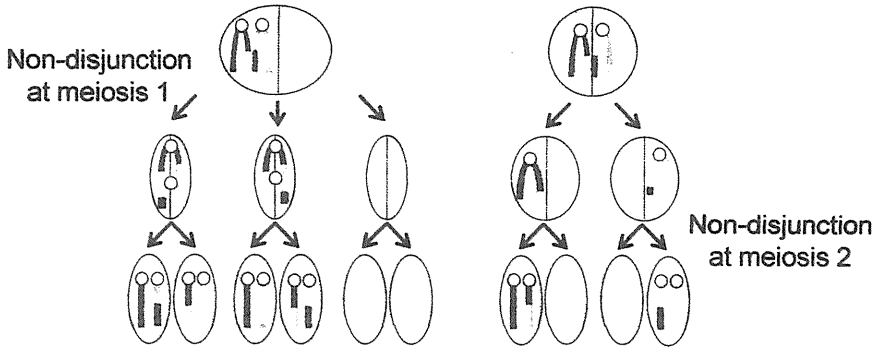
12 Kubota, T., Das, S., Cristian, S. L., Baylin, S. B., Herman, J. G. & Ledbetter, D. H. Methylation-specific PCR simplifies imprinting analysis. *Nat. Genet.* **16,** 16–17 (1997).
13 Robinson, W. P., Kuchinka, B. D., Bernasconi, F., Peterson, M. B., Schulze, A., Brondum-Nielsen, K. *et al.* Maternal meiosis I non-disjunction of chromosome 15: dependence of the maternal age effect on level of recombination. *Hum. Mol. Genet.* **7,** 1011–1019 (1998).
14 Robinson, W. P. Mechanisms leading to uniparental disomy and their clinical consequences. *Bioessays* **22,** 452–459 (2000).
15 Kotzot, D. Advanced parental age in maternal uniparental disomy (UPD): implications for the mechanism of formation. *Eur. J. Hum. Genet.* **12,** 343–346 (2004).
16 Oliver, T. R., Feingold, E., Yu, K., Cheung, V., Tinker, S., Yadav-Shah, M. *et al.* New insights into human nondisjunction of chromosome 21 in oocytes. *PLoS Genet.* **4,** e1000033 (2008).
17 Sloter, E., Nath, J., Eskenazi, B. & Wyrobek, A. J. Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. *Fertil. Steril.* **81,** 925–943 (2004).
18 Pacchierotti, F., Adler, I. D., Eichenlaub-Ritter, U. & Mailhes, J. B. Gender effects on the incidence of aneuploidy in mammalian germ cells. *Environ. Res.* **104,** 46–69 (2007).
19 Strakowski, S. M. & Butler, M. G. Paternal hydrocarbon exposure in Prader-Willi syndrome. *Lancet* **330,** 1458 (1987).
20 Cassidy, S. B., Gainey, A. J. & Butler, M. G. Occupational hydrocarbon exposure among fathers of Prader-Willi syndrome patients with and without deletions of 15q. *Am. J. Hum. Genet.* **44,** 806–810 (1989).
21 Akefeldt, A., Anvret, M., Grandell, U., Nordlinder, R. & Gillberg, C. Parental exposure to hydrocarbons in Prader-Willi syndrome. *Dev. Med. Chil. Neurol.* **37,** 1101–1109 (1995).
22 Robinson, W. P., Bernasconi, F., Mutirangura, A., Ledbetter, D. H., Langlois, S., Malcom, S. *et al.* Nondisjunction of chromosome 15: origin and recombination. *Am. J. Hum. Genet.* **53,** 740–751 (1993).
23 Pellestor, F., Andreo, B., Anahory, T. & Hamamah, S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. *Eur. J. Med. Genet.* **49,** 103–116 (2006).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

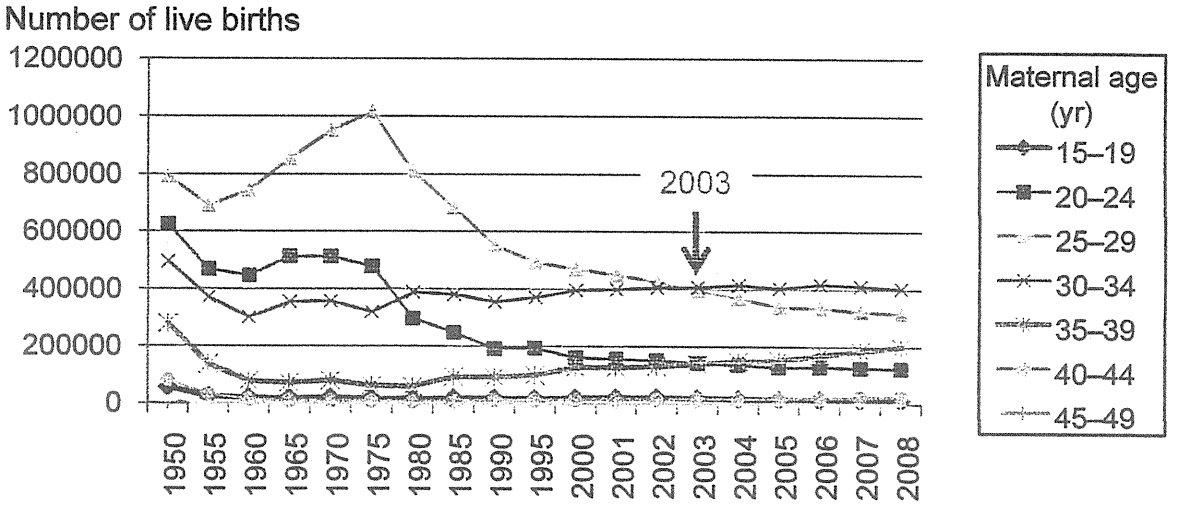


Supplementary Figure 1 Representative results of methylation analysis for the PWS-IC. Bisulfite-treated leukocyte genomic DNA was PCR-amplified for the PWS-IC, using methylated (M) and unmethylated (U) allele-specific primers (Kubota *et al.*, 1997). After PCR amplification, the PCR products were loaded onto LabChip with Gel-Dye Mix (Agilent, Santa Clara, CA). While both M and U alleles are delineated in a control subject, only M allele is identified in Prader-Willi syndrome (PWS) patients irrespective of the underlying causes. The PCR fragment size is 174 bp for the M allele and 100 bp for the U allele. The marker sizes are 100 bp, 150 bp, and 200 bp.

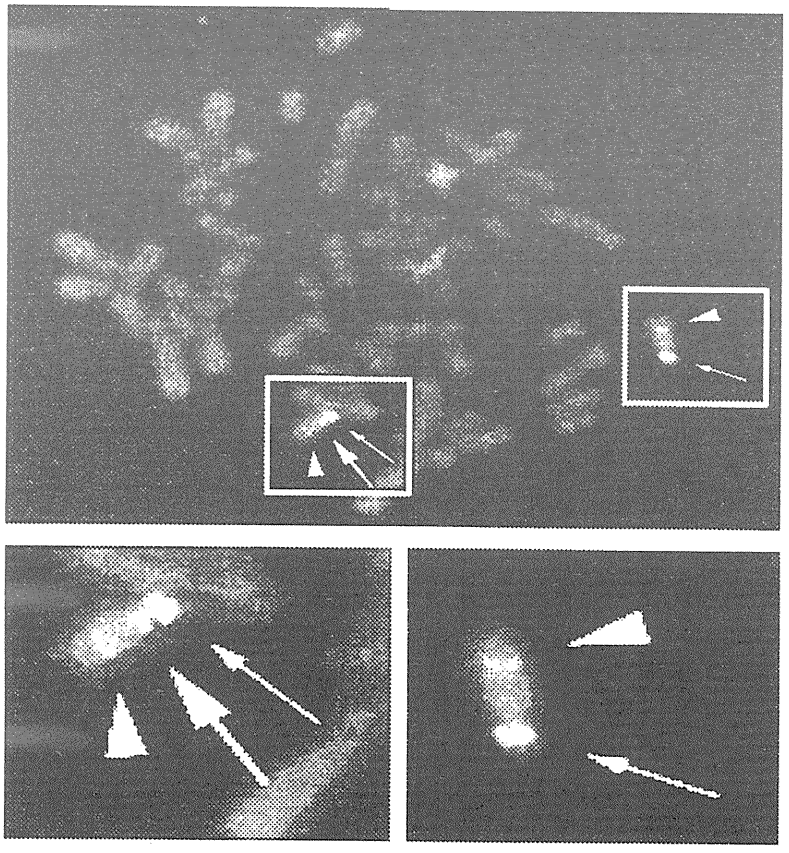
Locus	Position	Results		
D15S541	q11.2	Heterodisomy	Isodisomy	Isodisomy
D15S542	q11.2			
D15S1035	q11.2			
D15S11	q11.2	Heterodisomy or Isodisomy	Heterodisomy for at least one locus	
D15S128	q11.2			
D15S1048	q13.1			
D15S1007	q14			
D15S117	q21.1			
D15S131	q23			
D15S205	q25.2			
D15S127	q26.1			
D15S120	q26.3	UPD by TR/GC (Meiosis 1)	UPD by TR/GC (Meiosis 2)	UPD by MR/PE
D15S642	q26.3			



Supplementary Figure 2 Classification of upd(15)mat.
Upper part: Schematic representation of the methods utilized in this study. When at least one of the three pericentromeric loci is present in a heterodisomic condition, this indicates TR/GC [M1] type upd(15)mat. When the combination of isodisomy for the pericentromeric loci and heterodisomy for at least one locus in the middle to distal region is present, this indicates TR/GC [M2] type upd(15)mat. When all the loci are present in an isodisomic situation, this indicates MR/PE type upd(15)mat.
Lower part: Schematic representation showing that heterodisomy for centomeric loci is compatible with TR/GC [M1] type upd(15)mat, and that the combination of isodisomy for centromeric loci and heterodisomy for at least one middle to distal locus is consistent with TR/GC [M2] type upd(15)mat. Here, one recombination is assumed for 15q. When two recombinations take place, this can influence the status (isodisomy or heterodisomy) of middle to distal loci, but not that of centromeric loci. Thus, the status (isodisomy or heterodisomy) of centromeric loci is informative for the classification of upd(15)mat.



Supplementary Figure 3 Secular trend in the number of live-births according to maternal ages in Japan. Constructed by the authors on the basis of the Annual Nationwide Survey Data from the Ministry of Health, Labor and Welfare.



Supplementary Figure 4 FISH finding in a Prader-Willi syndrome patient with a microdeletion. Only a single red signal has been detected by a FISH probe for a region encompassing *SNRPN* (a thick arrow), whereas two green signals and two red signals have been identified by a CEP 15 probe for *D15Z1* (thin arrows) and by a probe for *PML* on 15q22 (arrowheads), respectively.

Supplementary Table 1 Primers utilized in this study.

	Forward primer	Reverse primer	PS	AT
<Methylation analysis of the *SNRPN*-DMR>[a]				
Methylated	TAAATAAGTACGTTTGCGCGGTC	AACCTTACCCGCTCCATCGCG	174	62
Unmethylated	GTAGGTTGGTGTGTATGTTTAGGT	ACATCAAACATCTCCAACAACCA	100	62
<Microsatellite analysis>[b]				
D15S541	GCATTTTTGGTTACCTGTATG	GTCTTCCAGGTTTATGGTTGTC	~150	60
D15S542	AGCAGACTCCGGAACCTCATC	CCTGCCTTCTTGCTGGGGCTG	~140	59
D15S1035	CACCCCCATGCAGAGT	AAGGCCAAGACCTGCC	172–262	60
D15S11	GACATGAACAGAGGTAAATTGGTGG	GCTCTCTAAGATCACTGGATAGG	~243	57
D15S128	GCTGTGTGTAAGTGTGTTTTATATC	GCAAGCCAGTGGAGAG	193–209	60
D15S1048	AGCCGTCTTTGTGCCA	TGCAGCCACTGTGGAA	197–233	57
D15S1007	GGGGAACCTACACTTCCG	CCAGGAATCTCAAATGGCTT	165–189	57
D15S117	GCACCAACAACTTATCCCAA	CCCTAAGGGGTCTCTGAAGA	132–150	60
D15S131	GAAAGGCACCTCATCTCG	TTAAAAACTCTGGAGCAGCG	238–274	60
D15S205	CTTAATGGTTTGGCAGGATA	AGCTTAAAANCAAAATCTCCC	128–170	60
D15S127	CCAACCACACTGGGAA	AACAGTTGCCCACGGT	~137	57
D15S120	TTTGTGATGGTCTTTTATAGGCATA	GGCTCAAAGTGTTTGCACTG	150–174	57
D15S642	CAGTTACCCAGGAAGCTGAA	AGATGCCGCCTGTACTAATG	195–218	60

PS: product size (bp), and AT: annealing temperature (°C).

[a] Kubota et al. (1997) Methylation-specific PCR simplifies imprinting analysis. *Nat. Genet.*, **16**, 16–17.

[b] NCBI Database (http://www.ncbi.nlm.nih.gov/unists).

Prader-Willi Syndrome : From Basic Research to Clinical Practice

Prader-Willi 症候群の基礎と臨床

総編集

永井敏郎　福島医科大学越谷病院小児科教授

編集

大野耕策　高知大学医学部脳神経小児科教授

緒方　勤　浜松医科大学医学部小児科教授

横谷　進　国立成育医療研究センター生体防御系内科部部長

診断と治療社

I 基本概念

獨協医科大学越谷病院小児科 永井敏郎

Prader-Willi 症候群（PWS）は 1956 年，スイスのチューリッヒ子ども病院の Prader, Labhart と Willi の 3 名が雑誌 Schweiz Med Wochenschr（Swiss Medical Weekly）に，ドイツ語により“Ein Syndrom von Adipositas, Kleiwuch, Kryptorchidismus und Ologophrenie nach mytoniertigem Zustand im Neugehorenalter”のタイトルではじめて発表した症候群である[1]．英文訳では，“A Syndrome Characterized by Obesity, Small Stature, Cryptorchidism, and Oligophrenia Following a Myotonia-like Status in Infancy”となる．男児 5 名，女児 4 名の短い報告で，患者年齢は，最高齢が男性 23 歳，女性 15 歳で，他は 5～10 歳の年齢であった．症状をそろえるため，あえて低年齢の患者は対象から除外してある．肥満，低身長，停留精巣，精神発達遅滞，筋力低下が主症状で，頻度は決してまれではないだろうと報告された．

その後，多くの患者の集積から，本症の多彩な症状，徴候が報告されてきている．そのため，患者のケアには遺伝，内分泌，神経，精神・心理，栄養，理学療法などの専門家による包括医療の必要性が求められている．本症の病因は，従来の Mendel 遺伝学では説明がつかず，ゲノム刷り込み現象，片親性ダイソミー（uniparental disomy：UPD）などの新しい遺伝学の発見により，ようやく疾患の原因が解明されてきている．

本項では，病因と診断法，症状と治療法につき過去，現在を簡単にレビューし，後の章への一助にしたい．

1 病因と診断法

本症は，1956 年に報告されて以来，患者の集積は順調に行われたが，その病因は長く不明であった．1980 年代になり染色体分析に高度分染法が導入され，本症に染色体 15q11-13 の欠失症例が散見されるようになった[2]．次いで 1990 年頃には，この染色体 15q11-13 欠失はすべて父親由来の染色体であることが報告された[3]．母親由来の染色体 15q11-13 欠失は，Angelman 症候群という全く異なる疾患となる．このように，同じ遺伝子でもその由来が父親か母親かにより遺伝子の働きが異なる現象，すなわち，ゲノムの上に情報の刷り込みが起こっている現象（ゲノム刷り込み現象：genomic imprinting）の概念がはじめて臨床の場に登場した．次いで，染色体 15q11-13 の欠失がない患者で本症を呈する患者の病因が，染色体 15 番が父親から伝播せず，2 本ともに母親由来である現象（maternal uniparental disomy：母性 UPD）に起因することが解明された．欠失例も母性 UPD 例もともに，父親由来の染色体 15q11-13 の部分が伝播されていないときに本症を発症する．欠失例が約 75%，UPD 例が約 25%の頻度といわれている．さらに本症の約 2%の患者で，ゲノム刷り込みをコントロールするセンター（imprinting center：IC）異常（欠失やエピ変異）に由来するまれな例が報告されている．この IC 欠失による異常は最高 50%の頻度で再発リスクを有するため，発生頻度はまれであるが，遺伝相談上，重要となる．

診断法は，欠失例は FISH（fluorescence *in situ*

表1 年齢に伴い変化する症状

胎生期	胎動微弱
出生時	頭位で帝王切開 出生時体重減少
新生児～乳児期	色素低下 筋緊張低下 哺乳障害 外性器低形成 （陰嚢，陰唇低形成，停留精巣）
幼小児期	3歳頃からはじまる過食 小さな手足 アーモンド様の目 前頭部狭小
学童期	発達遅滞 しつこく頑固な性格 過食に伴う体幹部中心の肥満 低身長
思春期	二次性徴発来不全 頑固で爆発的性格，異常行動 2型糖尿病 強度肥満 低身長
成人期	性格障害・異常行動 肥満・糖尿病とその合併症 重度肥満に伴う呼吸障害

（永井敏郎：Prader-Willi 症候群の自然歴．日児誌 103：2-5, 1999）

表2 Holm らの診断基準

大症状

1．新生児期から乳児期の筋緊張低下と哺乳障害．年齢とともに軽快
2．哺乳障害のための体重増加不良
3．1～6歳までにはじまる急激な体重増加と体幹部中心の肥満
4．特徴的顔貌（短頭，前頭部狭小，アーモンド様の目，薄い上口唇と小さな口，口角の低下）など三つ以上の症状
5．外性器低形成
6．発達遅滞
7．6歳までにはじまる過食症・食べ物あさり・食べ物に対する執着
8．染色体 15q11-13 欠失あるいは片親性ダイソミーの証明

小症状

1．胎動微弱と弱々しい泣き声．年齢とともに改善
2．頑固，過激，盗み，うそつきなどの性格障害
3．睡眠障害，睡眠時無呼吸など
4．低身長
5．色素低下
6．小さな手
7．狭小な手幅とまっすぐな尺骨側の手
8．斜視や近視などの眼科的異常
9．粘稠な唾液
10．構音障害
11．皮膚の引っ掻き

補助的症状

1．痛覚鈍麻
2．少ない嘔吐
3．乳児期の体温不安定．成人では温度感覚異常
4．側彎と前彎
5．早発陰毛
6．骨密度低下
7．ジグソーパズルが得意
8．神経・筋疾患なし

大症状は1点，小症状 0.5 点．補助的症状は点数加算はないが臨床的に参考となる所見．3歳以下では5点以上必要，うち4点は大症状から．3歳より上では8点以上が必要，うち5点以上は大症状から

（Holm VA, *et al.*：Prader-Willi syndrome consensus diagnostic criteria. *Pediatrics* 91：398-402, 1993 より引用，一部改変）

hybridization）法で検索可能であるが，UPD 型は DNA メチル化試験が必要である．しかし，わが国では DNA メチル化試験は保険適用がないことが大きな問題である．2008 年に snoRNA（小さな RNA で他の RNA の働きを修飾する RNA）が本症の病因として大きく関与し，HBII-85 の微細欠失は PWS の身体所見に，HBII-52 の微細欠乏は性格障害に大きく関与していることが報告された[4]．しかし，いまだ臨床症状と遺伝子の関連は不明な点が多い．

2 臨床症状と治療法

本症の臨床症状，自然歴はすでに解明されており，その大きな特徴は年齢ごとにその症状が変化することである（表1）[5]．新生児～乳児期は，筋緊張低下，哺乳障害，色素低下，外性器低形成などの症状が本症診断の契機となる．幼小児期は，3歳頃からはじまる過食と肥満，しつこい性格，学童期は過食，肥満，発達遅滞，性格障害など，思春期頃は二次性徴発来不全，肥満，頑固で爆発的な性格障害，異常行動など，成人期には肥満に伴う糖尿病，呼吸障害，性格障害などがみられるようになり，本人と家族への負担は大きい．多彩な症状を呈するが，その本体は間脳視床下部に存在する諸々の中枢の障害で説明可能である．満腹中枢不全による過食・肥満，体温中枢不全による冬

DNA 検査適応の診断基準

評価時年齢	DNA 検査適応のための症状
出生時～2 歳	1．哺乳障害を伴う筋緊張低下
2～6 歳	1．筋緊張低下に加え哺乳障害の既往 2．全般的発達遅滞
6～12 歳	1．筋緊張低下と哺乳障害の既往 2．全般的発達遅滞 3．過度の食欲，体幹部中心の肥満
12 歳～成人	1．軽度発達遅滞 2．過度の食欲，体幹部中心の肥満 3．視床下部性性腺機能不全と特徴的行動異常

(Gunay-Aygun M, *et al.*：The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. *Pediatrics* 108：92, 2001 より引用，一部改変)

の低体温，夏の高体温，呼吸中枢不全による中枢性呼吸障害に起因した突然死，性の中枢不全による二次性徴発来不全，情緒中枢不全による性格障害などを呈する．

1993 年に Holm らが，診断基準を報告した(表)[6]．また 2001 年に Cassidy らのグループが，DNA 検査を実施すべきか否かを決める簡単な臨床症状の診断基準を発表している(表)[7]．Holm らの診断基準は臨床症状中心であったが，Cassidy らのものは臨床症状は可能な限り簡略化されて，検査重視の方向になっている．このような診断基準の変化は，診断技術の進歩が背景にあると思われる．

治療法の根幹は，①食事療法，②運動療法，③成長ホルモン補充療法，④性ホルモン補充療法，⑤性格障害，異常行動への取り組み，の 5 本柱である．食事療法と運動療法は，生涯にわたり不可欠である．摂取カロリーは身長 1 cm あたり 10 kcal/日が低年齢から成人まで使用可能な指標である．運動は，患者が筋力低下，肥満を伴うため実践には困難を伴うが，水泳で成功している例は少なくない．

2002 年からわが国でも成長ホルモン補充療法が開始され，劇的に患者の QOL 改善をもたらしている．成長ホルモンは，本症では低身長改善目的で認可されているが，本症で成長ホルモンを使用する最大の目的は，体組成改善による将来の心臓・脳血管の血管障害のリスクを下げること，筋力向上に伴う活動性増加（粗大運動能力の改善）である．しかし，現在の成長ホルモン適応認可が低身長のみのため，患者の約半数は適応基準から外れること，また，成人での使用が認められないため成長ホルモン中止後の体組成悪化などの問題がある．今後，成長ホルモン使用の適応拡大が不可欠となる．

PWS 患者では性腺機能不全は必発であり，それに起因した臨床症状も多い．二次性徴発来不全，骨密度低下，筋力低下，精神的劣等感などである．そのため，性ホルモン補充は理にかなった治療法である．女性への性ホルモン補充は反対意見がないが，男性への男性ホルモン補充は患者の攻撃性を増悪させ，性格障害を悪化させることが危惧され，実際はあまり行われていないのが実情である．男性ホルモン補充に伴う性格障害悪化には学問的根拠はなく，全く寓話的な話であり，性ホルモン補充は実践すべき治療法である．

性格障害への取り組み，とりわけ向精神薬の選択に関しては今後の課題である．現在まで，前述の snoRNA 中，HBII-52 がセロトニン受容体作用に関連する遺伝子であることが証明されており，以前から臨床現場で選択的セロトニン再取込み阻害薬(selective serotonin reuptake inhibitor：SSRI)の効果が認められていたことなどから，今後，薬物治療法の整備が望まれる．

本疾患患者で生涯にわたり問題となるのは，性格障害，異常行動である．これらに対する対応の仕方には，家族，医師，看護師，教師，心理士などのチーム医療なくして成功は望めない．患者の異常行動は，時として反社会的傾向の行動（放火，盗み，虚言，火災警報器を押す，ストーカー的行動など）を呈するため，社会から孤立することが少なくない．この疾患の難しさは，病因の複雑さのみならず，周囲に理解されることが困難な症状にもある．今後，ますますの疾患への理解と多職種の人たちによるきめ細かい連携が必要である．

文　献

1) Prader A, *et al.*：Ein syndrom von adipositas, kleiwuchs, kryptorchidismus und ologophrenie nach mytoniertigem zustand im neugehorenalter. *Schweiz Med Wschr* 86：1260-1261, 1956
2) Ledbetter D, *et al.*：Deletions of chromosome 15 as a cause

of Prader-Willi syndrome. *N Engl J Med* 304：325-329, 1981
3) Nicholls RD, *et al.*：Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. *Nature* 342：281-285, 1989
4) Sahoo T, *et al.*：Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nat Genet* 40：719-721, 2008
5) 永井敏郎：Prader-Willi 症候群の自然歴．日児誌 103：2-5, 1999
6) Holm VA, *et al.*：Prader-Willi syndrome consensus diagnostic criteria. *Pediatrics* 91：398-402, 1993
7) Gunay-Aygun M, *et al.*：The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. *Pediatrics* 108：92, 2001

2 成長パターン：成長曲線

獨協医科大学越谷病院小児科　永井敏郎

Prader-Willi 症候群（PWS）は，低身長，肥満，糖尿病，性腺機能不全など多くの内分泌学的異常を呈する疾患である．特に低身長に対して，わが国では 2002 年から健常者の－2 SD 以下の低身長に対する成長ホルモン（growth hormone：GH）使用が小児慢性特定疾患の適応になっている．小児慢性特定疾患に指定されることで本症の社会的知名度は飛躍的に向上し，医療関係者のみならず，行政，学校，マスコミなどの関係者らが PWS について少なからず知識をもつようになってきた．この小児慢性特定疾患適応認可を厚生省（現在の厚生労働省）から得るにあたり，筆者らの作成した日本人 PWS 疾患特異的成長曲線が大きな力を発揮した．疾患特異的成長曲線を作成することは，将来の患者最終身長予測が可能になるばかりでなく，たとえば GH 治療による治療効果の客観的判定も可能となる．事実，現在まで小児慢性特定疾患で GH 使用が認可されている疾患（Turner 症候群，軟骨無形成症，PWS）では，日本人独自の疾患特異的成長曲線が完成している．

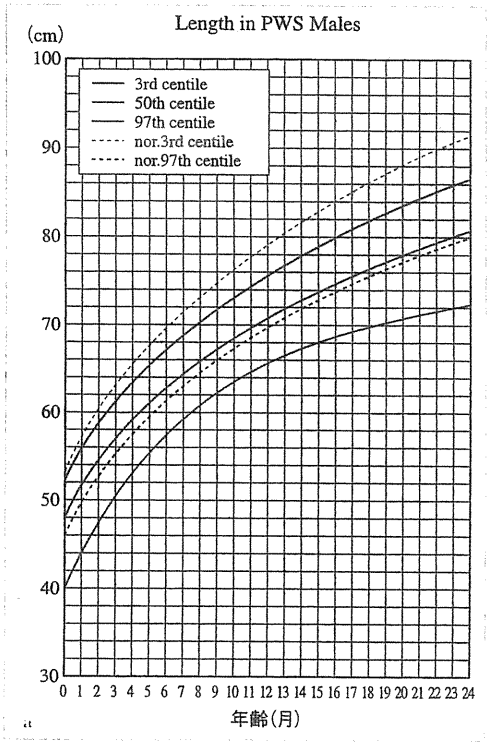
1 日本人 PWS 患者の身長（図 1，図 2）と体重（図 3，図 4）の成長曲線作成

筆者らの作成した成長曲線[1]は，臨床的に Holm らの診断基準[2]（*p.4* の表 1 を参照のこと）で PWS と診断された 252 人（男 153 人，女 99 人，年齢 0～24 歳）をもとに作成した．身長のデータか，または，可能なかぎり年余にわたる縦断的データ集積を目指したが，一部，横断的データも含まれており，成長曲線としては，セミ縦断的成長曲線となったが，臨床的には十分対応可能である．対象患者中 198 人が染色体 15q11-13 欠失，26 人が片親性ダイソミー（uniparental disomy：UPD），28 人が検査結果不明であった．平均観察期間は，男性で 7 年 3 ヶ月，女性で 6 年 1 ヶ月であった．このデータをもとに，重み付け移動平均法で 1 ヶ月ごとのデータを予測算出した．このデータを用い，0～2 歳は length（長さ；仰臥位で計測），2～17 歳は height（身長；立位で計測）の成長曲線を，最終的には eye-fitting 法にて決定した．

体重成長曲線は，食事制限や盗食による過食などによる体重の人為的関与が大きすぎるため，重み付け移動平均法による 1 ヶ月ごとの予測値の算出は不可能であった．そのため，体重の成長曲線は生データをそのままプロットし，PWS 患者の体重の範囲を体重の成長曲線としたが，今後，改善の余地がある．

2 PWS 患者の成長パターン

PWS 患者の平均生下時身長は，男子 48.0 cm（n＝117），女子 48.0 cm（n＝85）で，健常児と比較して有意差はなかった[1]．身長の成長パターンは男女とも類似のパターンを示し，思春期まで PWS の平均身長は健常者の－2 SD に沿って経過し，思春期あたりで（男性は 13 歳頃，女性は 11 歳頃），健常者の 3 rd%を大きく下回った．最終身長は，男性で 147.7±7.7 cm，女性で 141.2±4.8 cm であった．最終身長は健常者に比較して，男性で 21.9 cm，女性では 15.8 cm 下回り，男性の低身長が目立った．欧米のデータをみても，健常者の平



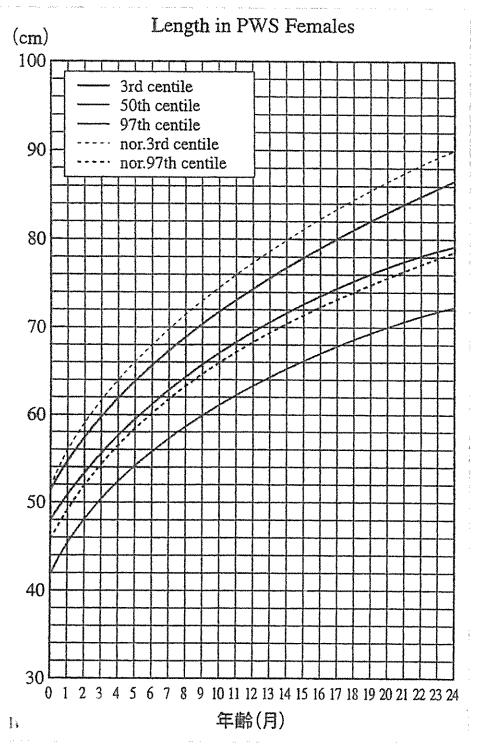
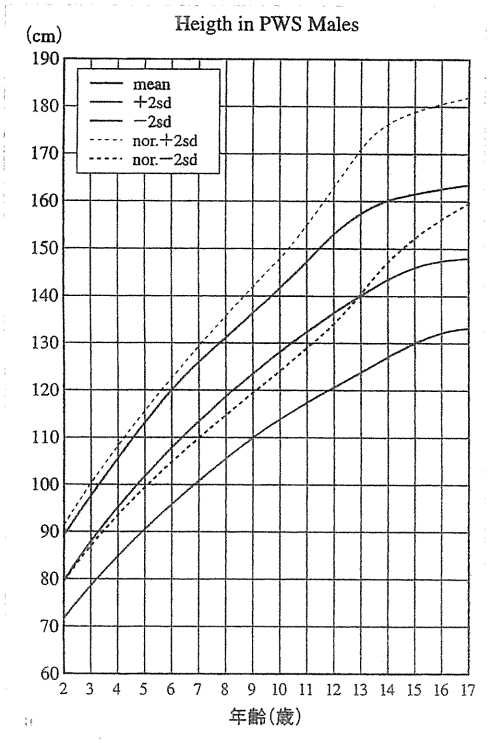


図1　2歳までの身長の成長曲線

a：男子，b：女子〔製作者　永井敏郎　獨協医科大学越谷病院小児科(禁無断転載，複製)〕



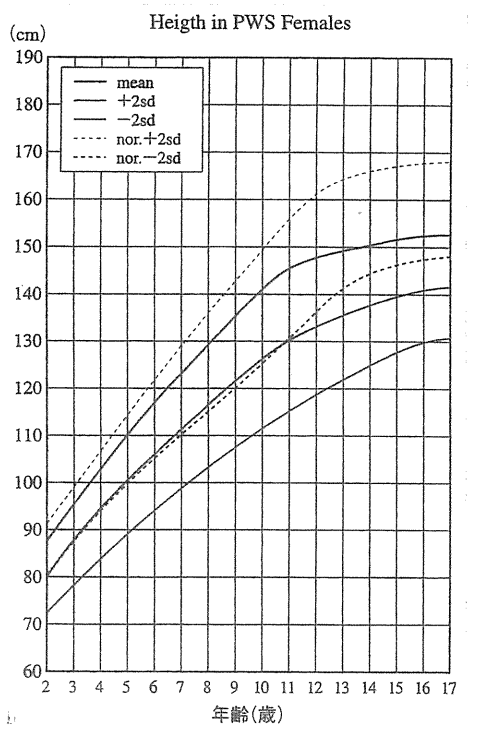
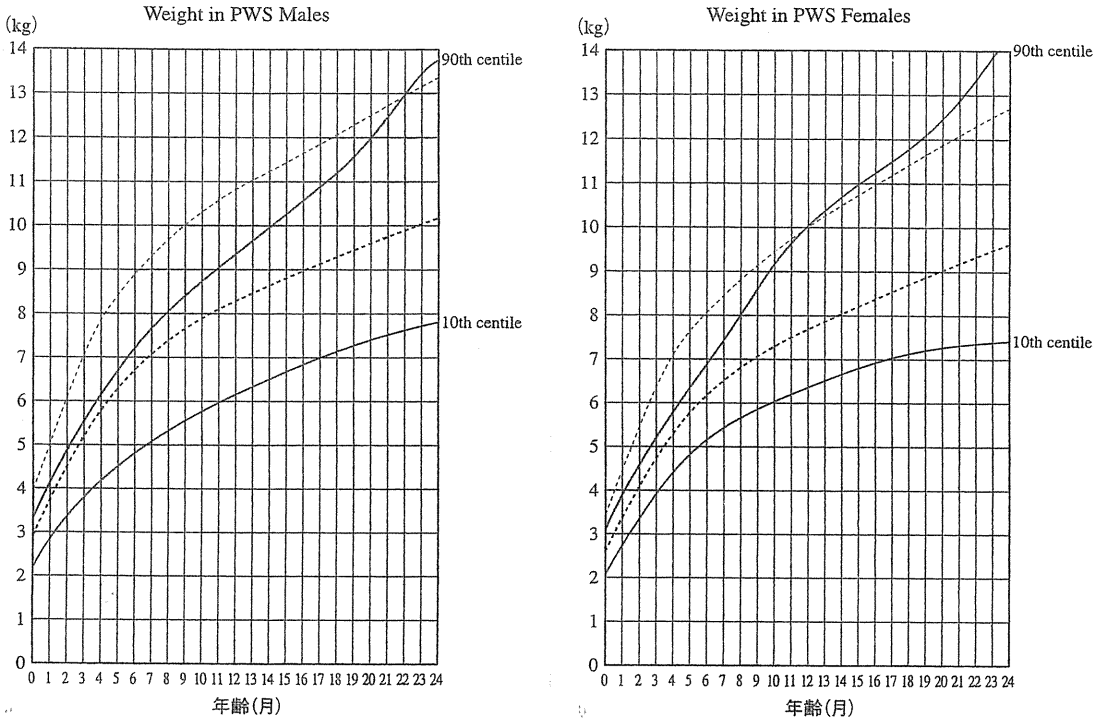
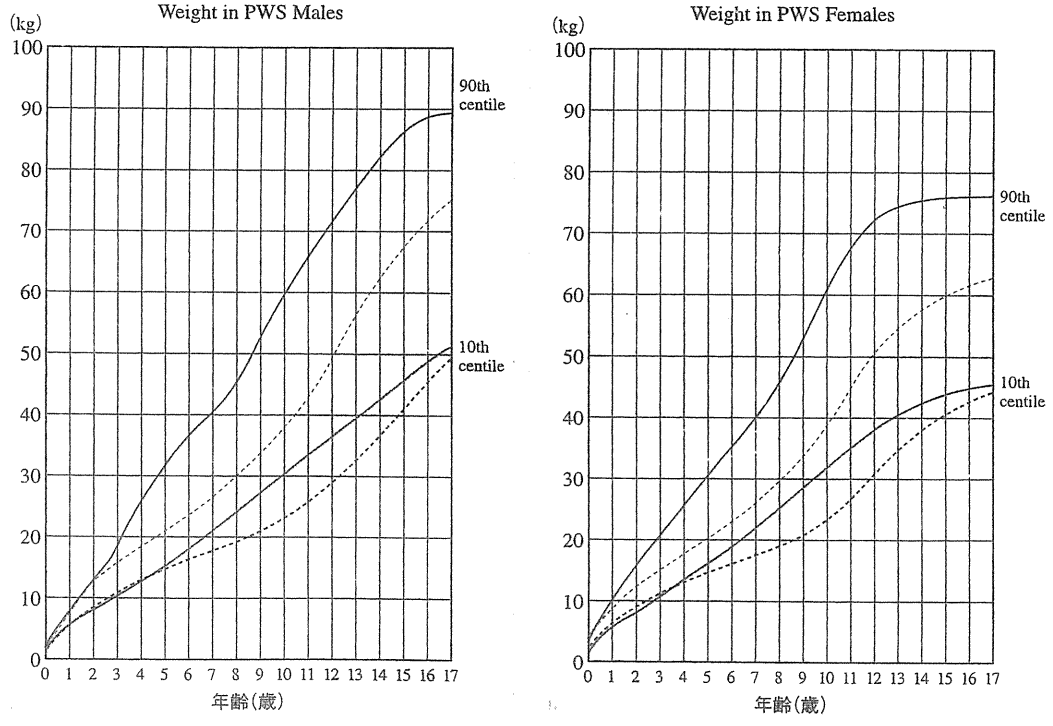


図2　17歳までの身長の成長曲線

a：男子，b：女子〔製作者　永井敏郎　獨協医科大学越谷病院小児科(禁無断転載，複製)〕



2 歳までの体重の成長曲線
：男子，　：女子〔製作者　永井敏郎　獨協医科大学越谷病院小児科(禁無断転載，複製)〕



17 歳までの体重の成長曲線
：男子，　：女子〔製作者　永井敏郎　獨協医科大学越谷病院小児科(禁無断転載，複製)〕