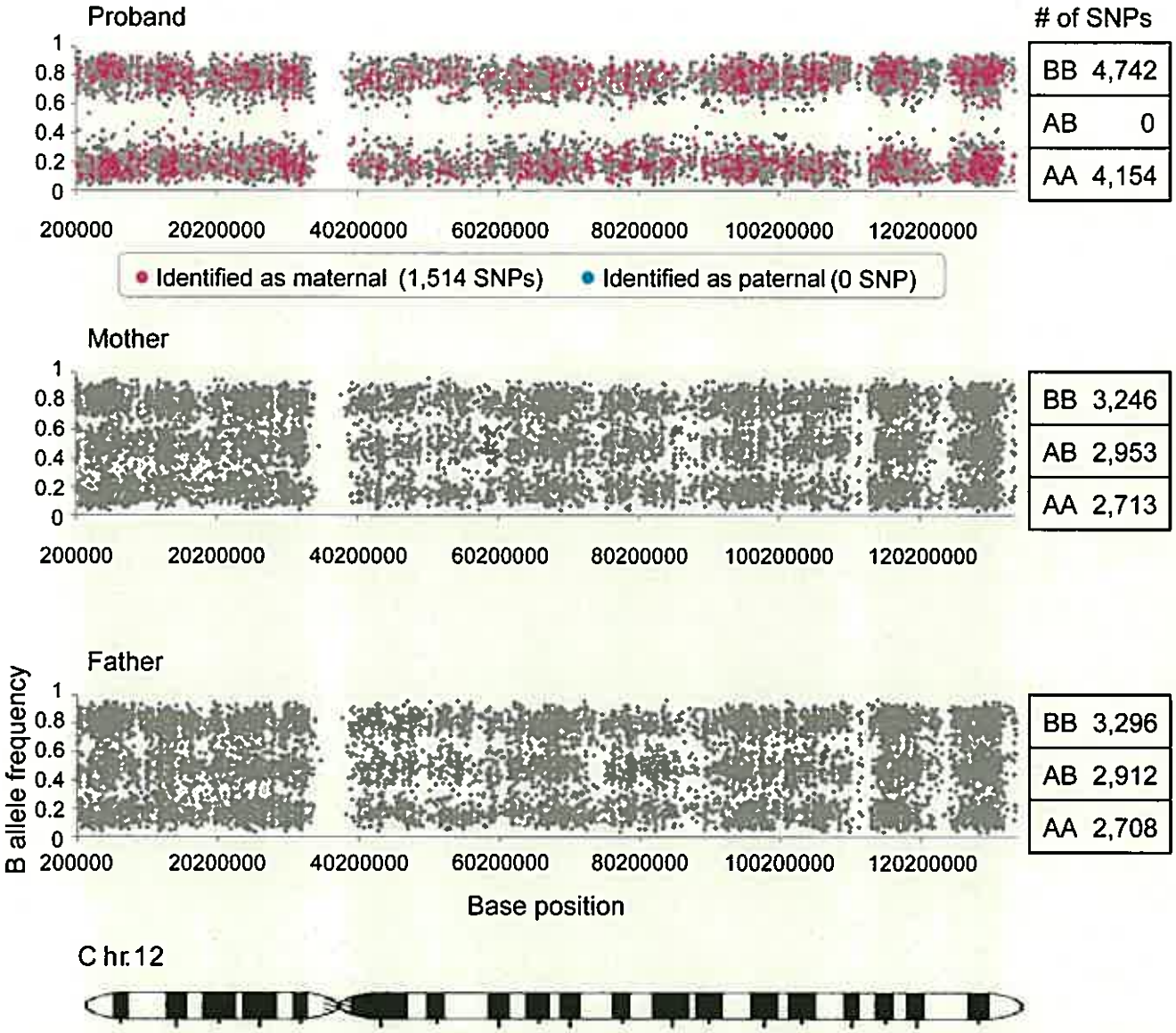


**Fig 3. Genome-wide SNP Array Indicating Complete Maternal Isodisomy of Chromosome 12.** B allele frequencies of chromosome 12 in the proband, the mother, and the father are shown. Allele segregation in the proband comprised AA (4,154 SNPs) and BB (4,742 SNPs) homozygotes only, and loss of heterozygosity (AB; 0 SNP). The parental allele segregation of chromosome 12 showed a heterozygous pattern. All other chromosomes had normal homo/heterozygous patterns. In the diagram for the proband, the pink spots (1,514 SNPs) represent SNPs identified as maternal, with no evidence of paternal SNPs (which would be blue).

doi:10.1371/journal.pone.0131157.g003

## Clinical course

Initial treatment with increasing amounts of alfacalcidol up to 5 μgkg/day with oral calcium 60 mg/kg/day failed to improve her symptoms. After detecting the *VDR* mutation, she was admitted to another hospital for further treatment. After increasing her oral elemental calcium (calcium lactate) dose to 300 mg/kg/day (divided 3 times), her serum calcium and PTH levels improved (Fig 4A). The oral calcium was subsequently reduced to 240 mg/kg/day when high

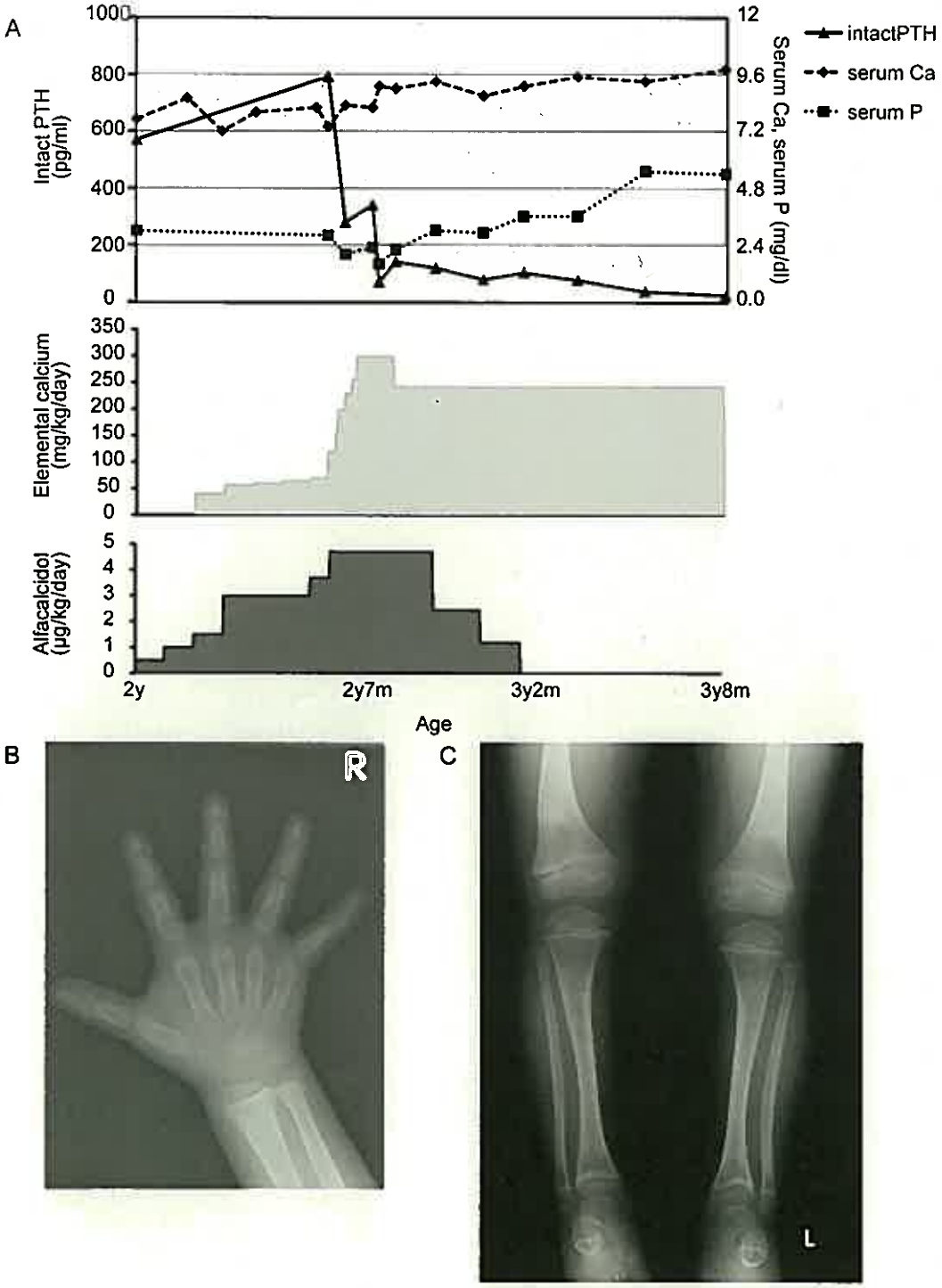


**Fig 4. After Treatment with High-dose Oral Calcium Supplementation.** (A) Treatment course and laboratory data are shown. (B) Bone roentgenogram at 3 years and 8 months of age, showing markedly improved signs of rickets.

doi:10.1371/journal.pone.0131157.g004

urinary calcium excretion started; the alfacalcidol dose was stopped because it was considered ineffective based on the genetic analysis. After 12 months of therapy, her laboratory data, including alkaline phosphatase levels, had normalized, her height gain improved, she started to run, and a repeat bone roentgenogram showed an improvement in the features of rickets (Fig 4B and 4C). Her most recent $1,25(OH)_2D$ level was 20 pg/mL, whereas her FGF23 was 12 pg/mL, and her urine calcium/creatinine ratio was 0.5. Although her rickets improved, alopecia has remained. Her amblyopia was noticed at age three.

## Discussion

This is the first report of an HVDRR caused by UPD of the mutant allele. We also found that genome-wide SNP array was useful for the detection of the complete isodisomy. We first noted an unusual homozygous state in this non-consanguineous family, and found that only the mother was heterozygous for the mutation. Such non-Mendelian inheritance implied the possibility of gross deletion of the father's allele, non-paternity, UPD, or some *de novo* mutation. However, common SNP sequencing and STR analysis suggested a homozygous chromosome 12 and confirmed paternity, whereas subsequent G-band karyotyping excluded gross deletion of chromosome 12. Finally, the genome-wide SNP array confirmed that complete maternal isodisomy of chromosome 12 was present. This molecular diagnosis is fundamental to ensure accurate genetic counseling of the risk of recurrence in the next child, which would be far less than 25%, as would typically be the case for a recessive disorder.

Uniparental isodisomy refers the inheritance of two identical copies of the same chromosome, and different from uniparental heterodisomy, which is the inheritance of the two different homologous chromosomes from one parent. Both of isodisomy and heterodisomy can cause genome imprinting disorders, but only uniparental isodisomy can cause recessive disorders if the chromosome harbors a disease-causing mutation. As a result, the mutated allele of her mother became homozygous in the proband to present non-Mendelian inheritance of the autosomal recessive disease, HVDRR. Although high maternal age is suggested as a risk factor for maternal UPD in imprinting disorders, it is mostly meiotic nondisjunction causing heterodisomy and differs for this case of isodisomy [22].

For the molecular detection of UPD, microsatellite marker analysis has been used conventionally, which needs parents' samples to demonstrate UPD [23]. Recently, the new technology of genome-wide SNP array had facilitated the detection of UPD [9,24–26]. SNP array can detect isodisomy by loss of heterozygosity of the segment or whole chromosome without parents' samples. It is sufficient to demonstrate UPD if there are no chromosomal aberration by cytogenetic analysis. More recently, using a signal intensity analysis of the SNP array, it is possible to evaluate the copy number changes, so that even chromosome banding is unnecessary. Furthermore, we have shown it possible to detect the origin of the homozygous chromosome by analyzing together with the parents' allele. For example, if the proband's allele is AA, mother's AA or AB, and father's allele BB, the proband's allele reveals to be derived only from the mother. In this point, our study is distinguished from others.

There are only two reported cases of chromosome 12 UPD leading to recessive disorders, one maternal and one paternal [27,28], making this the third case. The occurrence of UPD in each chromosome does not appear to be equivalent, and is rare in chromosome 12. UPD of chromosomes 6, 7, 11, 14, 15, and 20 has been reported to cause imprinting disorders; however, none is known for chromosome 12. Although we show the limited period of one case, the fact that our patient shows no other than typical symptoms of HVDRR by the age of 3, suggests that there are no genomic imprinting diseases caused by UPD of maternal chromosome 12. Moreover, it is also suggested that the UPD allele in the proband contained no other disease-

causing mutations that would present by the age of 3. In theory, there may be at least 3 other recessive disease-causing mutations in this homozygous chromosome (average mutations > 200 per genome, maternal chromosome 12 constitution approximately 2%) [26,29]. The discovery of UPD will require careful observation for late manifestations of other chromosome 12 related genetic disorders. Furthermore, we consider from this study that, although there are relatively few reports of recessive diseases caused by UPD, this phenomenon may be more frequent and only have not analyzed.

Our patient with deleterious *VDR* mutation was successfully treated with high-dose oral calcium. She had no trouble taking large doses of calcium lactate orally after every meal. Most of the reported cases with severe HVDRR have required intravenous calcium infusions for initial treatment, which often lead to prolonged hospitalizations and increased risks of catheter-related complications [6,30]. Although oral calcium with vitamin D therapy is reportedly effective in some cases, it is mainly reserved for use as a maintenance therapy [18,31]. Although this is one unusual case with UPD, the clinical course of our case suggested that oral calcium therapy is effective in the initial treatment in some cases of severe HVDRR.

Among the intestinal calcium absorption, active transport of calcium through calcium transporters is induced by VDR and $1,25(OH)_2D$. On the other hand, high dietary calcium with lactose can induce passive transport, which is considered to be VDR-independent [32,33]. It has been shown in the VDR-null mice that bone abnormalities can be rescued by high calcium diet [34,35]. We consider that although the mutant VDR in this case is inactive, high calcium diet induced passive calcium transport at the intestine and improved her rickets. However, alopecia is considered as VDR-mediated but not calcium-mediated phenotype, and was unresponsive to high calcium diet, which was also similar to the observations on VDR-null mice [36].

## Conclusions

HVDRR in this case was caused by a rare and complete UPD of maternal chromosome 12 with a *VDR* mutation. Genome-wide SNP array helped to detect the isodisomy and parental origin of the allele. Such comprehensive examination of the homozygous state is essential for accurate genetic counseling of recurrence risk and appropriate monitoring for other chromosome 12 related disorders. The treatment course suggested that oral calcium therapy is effective as an initial treatment for rickets in some cases with severe HVDRR.

## Supporting Information

**S1 Table. Primers and PCR conditions used to amplify the coding region of *VDR*.**
(DOCX)

## Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (to T.I. and S.K., grant number 23591489), and for Research on Intractable Diseases from the Ministry of Health, Labor and welfare (to K.O.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Author Contributions

Conceived and designed the experiments: SK. Performed the experiments: TI MK KT SK. Analyzed the data: MT TI MK KT SK. Contributed reagents/materials/analysis tools: MT TI MK HY KY TK NN KO KT SK. Wrote the paper: MT TI MK KT SK. Study supervision: AO KO KT SK.

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PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

*Pediatrics International* (2015) **57**, 864–869 doi: 10.1111/ped.12657

Original Article

# Mineral status of premature infants in early life and linear growth at age 3

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**Abstract** ***Background:*** Preterm infants are at significant risk of reduced bone mineral content and subsequent bone disease (metabolic bone disease of prematurity, MBDP). MBDP is frequently found in very low-birthweight (VLBW) infants, but long-term height prognosis is not well known.

***Methods:*** VLBW infants from two major neonatal intensive care units were studied. Medical records were reviewed. A total of 143 subjects were analyzed after excluding subjects who died, or who had severe complications that could affect linear growth, Silver–Russell syndrome, severe cholestasis, and/or chromosomal abnormality. The relationship between MBDP and height at age 3 was investigated.

***Results:*** Height standard deviation score (SDS) at age 3 negatively correlated with peak serum alkaline phosphatase (ALP) activity in early life ($r=-0.30$, $P=0.0003$) and positively correlated with serum phosphorus (P) at peak ALP ($r=0.33$, $P=0.0002$). In addition, serum P independently affected height SDS at 3 years of age ($\beta=0.19$, $P=0.018$), and was significantly different between infants with and without catch-up growth in height (difference: 0.23 mmol/L, 95%CI: 0.09–0.36, $P=0.0010$).

***Conclusions:*** MBDP, particularly hypophosphatemia in the early period of life, is associated with linear growth until 3 years of age in VLBW infants.

**Key words** metabolic bone disease of prematurity, mineral supplementation, short stature, very low-birthweight infant.

Continuous advances in the intensive care of preterm newborns have led to a progressive decrease in mortality. Infant mortality dropped among all races between 1980 and 2000,[1] but this success is not always followed by subsequent normal growth and development of the newborn. Adult height of very low-birthweight (VLBW) infants was reported to be shorter in both men (3–5 cm) and women (1–8 cm ).[2–5] Considering that there has been a steady increase in survival rate for children born extremely preterm (gestational age at birth <28 completed weeks) during the past two decades,[4] adult height of recent VLBW infants would be lower than that reported for VLBW infants who were born approximately two decades earlier.

Mineral homeostasis and skeletal mineralization are multifaceted mechanisms requiring adequate mineral supplies.[6] The fetal accretion of calcium and phosphorus during the last trimester is approximately 20 g and 10 g, respectively, representing accretion rates of 100–120 mg/kg/day for calcium and 50–65 mg/kg/day for phosphorus.[6–8] Preterm infants are born during the most rapid phase of fetal mineral accretion, but, in clinical practice, it is difficult to ensure that they receive adequate mineral intake during the neonatal period, especially in sick infants. Therefore, preterm infants are at significant risk of reduced bone mineral content (BMC) and subsequent bone disease, varyingly termed "metabolic bone disease of prematurity" (MBDP), "osteopenia of prematurity", or "neonatal rickets". Radiological changes attributable to rickets have been found in 55% of infants with birthweight <1000 g,[9] and in 23% of infants with birthweight <1500 g.[10] Although MBDP occurs with a high frequency, there are few reports on the long-term prognosis of suboptimal early bone mineralization because MBDP is a self-resolving disease.[6]

Disturbances of intrauterine and postnatal growth during critical periods of human development may have long-term implications for adult health. Adaptations that occur to the fetus or young infant when undernourished induce alterations in metabolism, hormonal output, and distribution of cardiac output, resulting in central obesity, diabetes, and cardiovascular disease in middle age.[11] Regarding disturbance of mineral homeostasis in early life, Fewtrell *et al.* reported that former preterm infants were shorter, lighter, and had lower BMC than controls at 8–12 years of age.[12] Similarly, Chan *et al.* reported that children who were born prematurely with birthweight <1500 g tended to be significantly smaller for age and have lower lumbar spinal bone mineral content and density at 5–9 years of age compared with children born at term gestation.[13] Although these reports suggest that reduced mineralization in the neonatal period would affect later bone health and linear growth, there are no reports on more severe patients hospitalized in neonatal intensive care units (NICU) with current practice vitamin and mineral supplementation. In this study, we investigated the association

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Received 18 October 2014; revised 1 February 2015; accepted 19 February 2015.

between early demineralization and linear growth under the hypothesis that early demineralization would affect linear growth at 3 years of age.

## Methods

Infants from two major NICU in Tokyo (i.e. University of Tokyo Hospital and Tokyo Metropolitan Bokutoh Hospital) with birthweight <1500 g (VLBW infants) were studied. Medical records were reviewed, and data were collected on factors and laboratory findings that would influence bone mineralization and linear growth. The factors included the following: perinatal events, parity and gestation of the mothers, sex, birthweight, birth height, singleton or multiple birth, nutrition, neonatal complications, medications, serum calcium (Ca), serum phosphorus (P), serum alkaline phosphatase (ALP) activity, and auxological data at follow up. This study was performed with approval from the Ethics Committees of both hospitals.

A total of 201 VLBW infants were admitted: 83 to University of Tokyo Hospital between January 2001 and December 2006, and 118 to Tokyo Metropolitan Bokutoh Hospital between January 2006 and December 2007. Infants were excluded from the study if they died or had complications during the neonatal period that would obviously affect their linear growth. We also excluded those diagnosed with Silver–Russell syndrome, cholestasis (defined as conjugated bilirubin >34 μmol/L), or chromosomal abnormality. In total, 26 subjects were excluded due to death (n = 10), chromosomal abnormality (n = 3), congenital heart disease accompanied by impaired growth (n = 5), surgical disease accompanied by impaired growth (n = 4), hydrocephaly after intracranial hemorrhage (n = 2), Silver–Russell syndrome (n = 1), or cholestasis (n = 1). Surviving infants underwent follow up at the outpatient clinics of the two hospitals until at least 3 years of age, but 32 infants could not be followed up. The remaining 143 subjects were enrolled in this study.

In the two hospitals, serum Ca, P, and ALP were routinely measured in all VLBW infants every 1 or 2 weeks in the morning before feeding until the infants had shown a clear pattern that ALP was no longer rising. Recommended practice in the two hospitals regarding mineral supplementation was i.v. Ca and P until enteral feeding was established (100 mL/kg/day), then, fortified powder (HMS-1, Morinaga, Tokyo, Japan) was added to breast milk. The fortified breast milk contained 95 mg/100 mL Ca and 54 mg/100 mL P, equivalent to that of the intrauterine gain of a normal fetus. Moreover, if serum P was <1.6 mmol/L, additional P was administered orally or through a feeding tube. And if serum ALP was rising in spite of proper mineral supplementation, vitamin D supplementation was added. The Ca and P amounts, and vitamin D supplementation were determined by the primary care physicians.

Although there is insufficient evidence that serum Ca, serum P, and serum ALP are valid biochemical markers of MBDP,[14] osteopenia has usually been diagnosed on serum biochemistry, and it has been reported that osteopenia is characterized by low serum P and/or an increase in serum ALP fivefold higher than the upper reference range used for adults.[15] Serum ALP rose in newborns in the first 2–3 weeks of life and increased further if there was insufficient mineral supply.[16] In addition, high peak ALP in low-birthweight infants was strongly associated with impaired linear growth.[17] Among the laboratory findings, we considered that peak ALP after 1 month of age, and serum Ca and P at peak ALP could be potential indicators of early bone mineralization in the neonatal period, and we adopted these factors to predict height standard deviation score (SDS) at 3 years of age.

Under the current practices in NICU, steroid is often used mainly for chronic lung disease (CLD) and late-onset circulatory collapse due to adrenal insufficiency.[18] Therefore we included these factors because steroid administration might affect linear growth. CLD is defined as requirement of supplemental oxygen at 36 weeks corrected gestational age, which means moderate or severe bronchopulmonary dysplasia.[19] Late-onset circulatory collapse due to adrenal insufficiency was defined as presence of more than two of the five signs of adrenal insufficiency (hypotension, oliguria, hyponatremia, lung edema, and increased demand for oxygen treatment) at >1 week after birth.[18]

To investigate whether height SDS at 3 years of age is affected by factors and laboratory findings related to bone mineralization and linear growth, both univariate and multivariate analyses were performed. We also studied whether height catch-up growth (defined as height SDS at 3 years of age > −2) is influenced by mineral status in the neonatal period and neonatal factors. Neonatal factors were compared between two groups according to presence of height catch-up.

### *Biochemistry*

Serum ALP, Ca, and P were measured using an autoanalyzer with commercially available reagents (Shino-test, Tokyo, Japan; Sekisui Medical, Tokyo, Japan; and Serotec, Sapporo, Japan, respectively). The within-subject biological variations of ALP, Ca, and P in adults were 1.4%, 0.71%, and 0.54%, respectively. Other measurements were determined using standardized and certified procedures.

### *Statistical analysis*

The results are expressed as mean ± SD, or as n (%). Weight and length SDS at birth were calculated using Japanese standards for premature infants according to sex and gestational age,[20] and weight and height SDS at 3 years of age were calculated using Japanese standards for normal children by sex and age.[21] The factors predicting height SDS at 3 years of age were investigated. First, univariate analyses were performed to extract factors correlating with height SDS at 3 years of age. Univariate comparison was done using Student's *t*-test for continuous variables and the chi-squared test or Fisher's exact test for discrete variables. Correlations were performed with Pearson's test. Then, factors predicting height SDS at 3 years of age were investigated on multiple linear regression analysis with height SDS at 3 years of age as the dependent variable and potential predictors as independent variables (multivariate analysis 1). Among the potential predictors, however, there existed multi-colinearities. Therefore, a second multiple linear regression analysis was performed considering multi-colinearities among factors (multivariate analysis 2).

All analysis was performed using JMP 8 (SAS Institute, Cary, NC, USA), and $P < 0.05$ was considered statistically significant.

## Results

Table 1 lists the subject characteristics. Mean gestational age was 27.7 ± 3.3 weeks and mean birthweight was 958 ± 287 g. Mean age at peak serum ALP was 97 ± 42 days, and all infants had peak serum ALP after 1 month of age. Forty-two infants (42%) suffered from CLD. Twenty-five infants (17.5%) suffered from late-onset circulatory collapse due to adrenal insufficiency. The subjects had lower gestational age and were smaller than those of the reported cohort for longitudinal outcome of MBDP.[12,13]

There was a strong negative correlation between peak serum ALP in early life after 1 month and height SDS at 3 years of age ($r=-0.30$, $P=0.0003$; Fig. 1a) and a strong positive correlation between serum P at peak serum ALP and height SDS at 3 years of age ($r=0.33$, $P=0.0002$; Fig. 1b). There was no correlation between serum Ca at peak serum ALP and height SDS at 3 years of age ($r=0.026$, $P=0.76$; Fig. 1c).

Table 2 summarizes the regression analysis. On univariate analysis, gestational age, body length SDS at birth, bodyweight SDS at birth, peak serum ALP, serum P at peak serum ALP, CLD and vitamin D supplementation were extracted as predictors of height SDS at 3 years of age (Table 2). Then, multiple linear regression analysis was performed using these predictors (multivariate analysis 1, Table 2). CLD, however, strongly correlated with gestational age ($r=0.69$, $P<0.0001$), and height SDS at birth strongly correlated with weight SDS at birth ($r=0.83$, $P<0.0001$) and with gestational age ($r=-0.55$, $P<0.0001$) on univariate analysis. Therefore, a second multiple linear regression analysis was performed after excluding CLD and height SDS at birth (multivariate analysis 2). Gestational age, weight SDS at birth, and serum P at peak serum ALP were identified as predictors for height SDS at 3 years of age independently. According to the relationship between height catch-up growth and markers of mineral status during the neonatal period, serum ALP and P of infants with height catch-up growth were statistically different from those of infants without height catch-up growth (difference, −468 U/L, 95% CI: −886 to −49.3, $P=0.029$; difference, 0.23 mmol/L, 95%CI:

**Table 1** Subject characteristics (n = 143)

| Neonatal factors | Mean ± SD or n (%) |
|---|---|
| Gestational age (weeks) | 27.7 ± 3.3 |
| Male | 83 (58.0) |
| Birth length (cm) | 34.6 ± 3.9 |
| Birth length SDS | −0.37 ± 1.4 |
| Birthweight (g) | 958 ± 287 |
| Birthweight SDS | −0.69 ± 1.3 |
| Multiple birth | 32 (22.4) |
| Chronic lung disease | 42 (29.4) |
| Late-onset circulatory collapse | 25 (17.5) |
| Small for gestational age | 37 (25.9) |
| Peak serum ALP (U/L) | 1,970 ± 939) |
| Age at peak serum ALP (days) | 97 ± 42 |
| Serum Ca (mmol/L; at peak serum ALP) | 2.4 ± 0.19 |
| Serum P (mmol/L; at peak serum ALP) | 1.8 ± 0.30 |
| Height SDS at 3 years of age | −1.0 ± 1.0 |

ALP, alkaline phosphatase; Ca, calcium; P, phosphorus; SDS, standard deviation score.

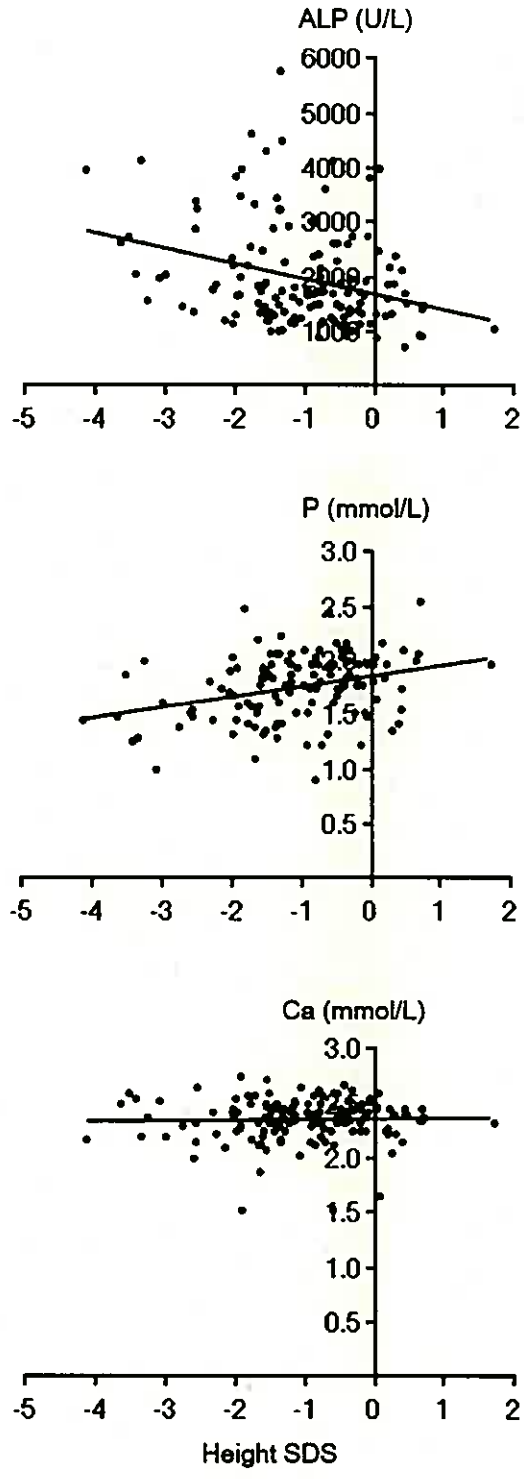


**Fig. 1** Correlations between height SD score (SDS) at 3 years of age and serum (a) alkaline phosphatase (ALP), (b) phosphorus (P) and (c) calcium (Ca). (a) There was a strong negative correlation between peak serum ALP during the neonatal period after 1 month and height SDS at 3 years of age ($r=-0.30$, $P=0.0003$). (b) There was a strong positive correlation between serum P at peak serum ALP and height SDS at 3 years of age ($r=0.33$, $P=0.0002$). (c) No correlation was seen between serum Ca at peak serum ALP and height SDS at 3 years of age ($r=0.026$, $P=0.76$).

Table 2 Independent predictors of height SD score at 3 years of age

| Predictors | Univariate analysis | | Multivariate analysis 1 | | Multivariate analysis 2 | |
|---|---|---|---|---|---|---|
| | R | *P* value | β | *P* value | β | *P* value |
| Gestational age | 0.27 | 0.0014 | 0.55 | <0.0001 | 0.66 | <0.0001 |
| Sex | 0.025 | 0.77 | | | | |
| Body length SDS at birth | 0.17 | 0.052 | −0.041 | 0.75 | | |
| Bodyweight SDS at birth | 0.22 | 0.0095 | 0.66 | <0.0001 | 0.68 | <0.0001 |
| Multiple birth | 0.029 | 0.73 | | | | |
| Parity | 0.093 | 0.27 | | | | |
| Peak serum ALP | −0.30 | 0.0003 | −0.067 | 0.44 | −0.059 | 0.49 |
| Serum Ca (at peak serum ALP) | 0.026 | 0.76 | −0.039 | 0.63 | −0.043 | 0.58 |
| Serum P (at peak serum ALP) | 0.33 | 0.0002 | 0.16 | 0.043 | 0.19 | 0.018 |
| Chronic lung disease | 0.39 | <0.0001 | 0.20 | 0.045 | | |
| Steroid use | 0.095 | 0.26 | 0.0071 | 0.93 | | |
| Vitamin D supplementation | 0.17 | 0.051 | −0.11 | 0.18 | | |

ALP, alkaline phosphatase; Ca, calcium; P, phosphorus; SDS, standard deviation score.

0.09–0.36, $P=0.0011$, respectively; Fig. 2a,b), but serum Ca was not different between the two groups (Fig. 2c). When we performed these analyses in only small-for-gestational-age (SGA) infants, serum P in infants with height catch-up growth was higher than that in infants without height catch-up growth (difference, 0.19 mmol/L, 95%CI: 0.0016–0.37, $P=0.048$), but serum ALP was not different between the two groups ($P=0.11$). Neither gestational age nor weight SDS at birth, which were identified as predictors for height SDS at 3 years of age, were different between the two groups ($P=0.054$ and $P=0.13$, respectively).

Chronic lung disease is reported to be a risk factor of poor linear growth,[22] although some studies found no relationship between CLD prevalence and linear growth.[23] Thus, we also investigated this after excluding subjects with CLD, and found that serum P at peak serum ALP after 1 month was an independent predictor of height SDS at 3 years of age ($\beta=0.19$, $P=0.049$).

## Discussion

We found that there was a strong negative correlation between peak serum ALP in early life after 1 month and height SDS at 3 years of age ($r=-0.30$, $P=0.0003$), in a group of severe VLBW infants recently hospitalized in NICU (Table 1) with current practice vitamin and mineral supplementations (Fig. 1a). This is in accordance with a previous study done 30 years ago, in which high peak neonatal plasma ALP was found to be associated with a long-term stunting effect persisting up to 12 years later.[12] We also found that there was a strong positive correlation between serum P at peak serum ALP and height SDS at 3 years of age ($r=0.33$, $P=0.0002$; Fig. 1b). In addition, we showed that serum P at peak serum ALP after 1 month of age is an independent predictor of height SDS at 3 years of age. Because growth is a complex process, and the reasons for the poor growth seen in premature infants are multifactorial, it is difficult to conclude that MBDP with hypophosphatemia in accordance with highly elevated ALP would affect linear growth in later life. Serum P, however, was also identified as an independent predictor of height SDS at 3 years of age after excluding subjects with CLD, which was reported to be a risk factor of poor linear growth.[22] Taken together, the present study provides evidence that MBDP with hypophosphatemia in early life with highly elevated ALP would affect linear growth at 3 years of age in VLBW infants with current practice vitamin and mineral supplementation.

We also found that there was a statistically significant difference in serum P between infants with/without height catch-up growth at 3 years of age (Fig. 2b). When the analysis was done in only SGA infants, this difference was still seen. This suggests that some VLBW infants with MBDP and hypophosphatemia could not achieve catch-up growth in height at 3 years of age. Catch-up growth in height before the age of 3 years is reported to occur in 81% of extremely low-birthweight infants,[24] and it is still not clear why the remaining infants are incapable of catch-up growth. We suggest that MBDP with hypophosphatemia in accordance with highly elevated ALP might be a reason for poor growth during infancy. This proposal is consistent with *in vitro* experiments in which increased extracellular phosphorus facilitated the proliferation of chondrocytes by upregulation of cyclin D1 expression.[25] Such MBDP may be preventable with proper phosphorus supplementation.

Metabolic bone disease of prematurity is often asymptomatic and is generally detected on biochemistry or incidentally on X-ray done for other purposes. Whether this suboptimal early bone development results in later consequences has been a topic of research and is relevant to the programming of bone health by early nutrition.[26] There are few studies, however, that focus on this matter, especially on the later consequences of linear growth. To the best of our knowledge, this is the first report showing a correlation between MBDP and later linear growth in a group of recently treated VLBW infants. The incidence of radiological evidence of MBDP in this population is reported to be approximately 30% and is similar to that previously reported.[27] This suggests that MBDP remains a significant comorbidity in a group of recently treated preterm infants. Further research concerning the later effect of MBDP is vital for best clinical practice in neonatology.

There are some limitations of this study. First, in this retrospective study, we could not obtain serum vitamin D for the subjects or the mothers. The primary care physicians determined the dosage of vitamin D, and serum vitamin D status was not always measured. Low vitamin D intake during pregnancy is associated with reduced intrauterine long bone growth.[28] Thus, serum vitamin D status

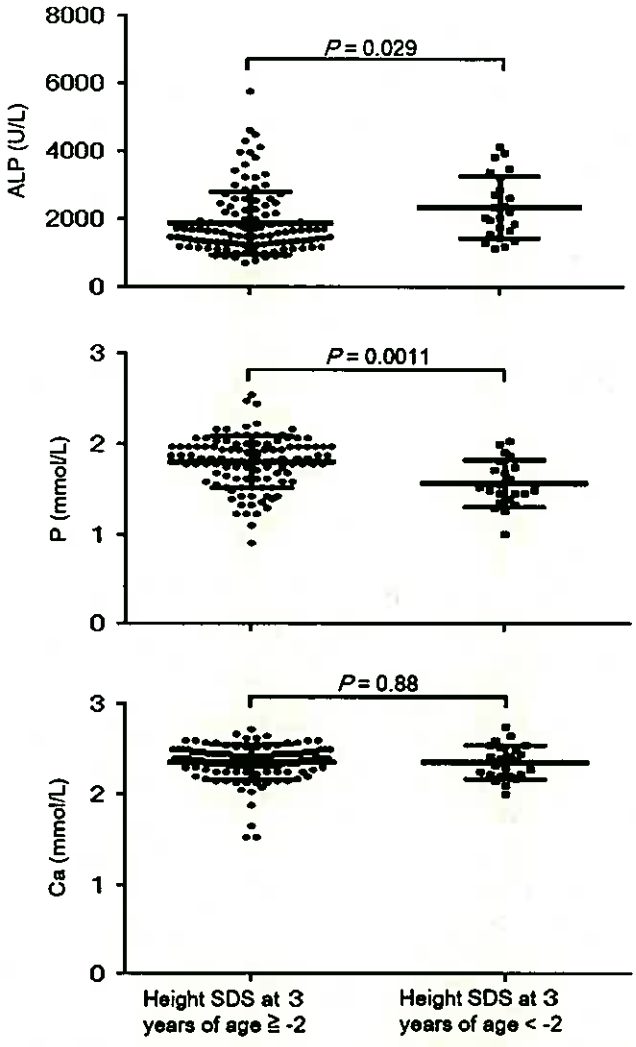


**Fig. 2** Serum (a) alkaline phosphatase (ALP), (b) phosphorus (P) and (c) calcium (Ca) according to presence of height catch-up growth. (a) Serum ALP in infants with height catch-up growth was lower than that in infants without height catch-up growth (difference, −468 mmol/L, 95%CI: −886 to −49.3, $P = 0.029$). (b) Serum P in infants with height catch-up growth was higher than that in infants without height catch-up growth (difference, 0.23 mmol/L, 95%CI: 0.09–0.36, $P = 0.0011$). (c) Serum Ca was not different between the two groups.

during the intrauterine and neonatal periods might influence linear growth. Height SDS at 3 years of age, however, was not statistically different among subjects with/without vitamin D supplementation (Table 2), and similar correlations between height SDS at 3 years of age and peak ALP or serum P at peak ALP were detected (data not shown). Second, we could not obtain urinary Ca and P for all subjects because they were not routinely measured. Urinary data would provide more information on Ca and P status, especially on phosphorus overload. Phosphorus overload results in relative calcium deficiency, and relative calcium deficiency could induce hyperparathyroidism. Judging from available urinary data, however, no subject was given more phosphorus than was necessary. Therefore, it was assumed that hypophosphatemia was mainly due to P insufficiency. In addition, without urinary data, serum ALP >900 U/L associated with serum P <1.8 mmol/L has a diagnostic sensitivity of 100% and specificity of 70% for MBDP.[29] From the present data, serum ALP >2200 U/L and serum P <1.7 mmol/L might cause failure of catch-up growth. Considering that serum ALP, which was measured using the Japan Society of Clinical Chemistry standardized reagent, was 2–3-fold higher than that reported in other countries,[30] the present data (ALP > 2200 U/L) is consistent with the reported level (ALP > 900 U/L). Third, this study did not include any factors relating to early parenteral nutrition, especially in the first weeks of life. Improvement of early nutritional support from the first weeks of life may improve growth, but it is frequently associated with a higher incidence of hypophosphatemia and an increase in serum ALP.[31,32] But when we conducted the same analysis including serum ALP in the first weeks of life and serum P at that time, serum P at peak serum ALP after 1 month of age was still identified as an independent predictor of height SDS at 3 years of age (data not shown). This suggests that MBDP might affect height at 3 years of age independently of early nutritional support, although the data did not include any factors of early parenteral nutrition. Fourth, parental height was not available for this study. Given that height is usually influenced by parental height, this limitation could affect the results, but the present study included other critical factors, and serum P at peak serum ALP after 1 month was confirmed as an independent predictor. Fifth, as many as 32 infants could not be followed up. Because this study was a retrospective study, infant presentation to hospital was determined by parental decision, therefore milder infants tended to be missing. The mean gestational age, mean birthweight, and mean peak ALP of the 32 infants were 31.4 ± 2.8 weeks, 1249 ± 212 g, and 1118 ± 384 U/L, respectively. In clinical practice, such mild infants usually do not have many problems. Therefore, we believe that this limitation would not affect the results very much. Finally, the long-term prognosis was not able to be determined. In a cohort from a prospective study of 30 years ago, the long-term stunting effect had not continued into adulthood.[33] Because we do not know whether early demineralization would affect linear growth into adulthood in a group of recently treated VLBW infants, further follow up of these infants is vital. Regardless of these limitations, the present findings provide additional evidence of the relationship between suboptimal early bone development and later consequences.

We found that height SDS at 3 years of age strongly correlated with peak serum ALP in early life and serum P. In addition, we found that serum P at peak serum ALP was a critical factor for height SDS at 3 years of age and catch-up growth. MBDP would affect height and catch-up growth at 3 years of age, and maintenance of normal serum phosphorus is strongly recommended.

## Acknowledgments

We thank all the patients and physicians for their time spent and commitment to this retrospective cohort. The authors declare no conflicts of interest.

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# Growth references for Japanese individuals with Noonan syndrome

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**BACKGROUND:** Noonan syndrome (NS) is a clinically and genetically heterogeneous syndrome characterized by distinctive facial features, short stature, congenital heart diseases, and other comorbidities. NS-specific growth charts are essential for NS care, but currently no such charts are available for Asian populations.

**METHODS:** We conducted a nationwide survey by collaborating with three academic societies in Japan. We obtained the data of 356 clinically diagnosed NS subjects from 20 hospitals. The Lambda-Mu-Sigma method was used for establishing growth charts.

**RESULTS:** A total of 308 subjects (males: 159 and females: 149) were analyzed after excluding 48 subjects because of missing auxological data (26 subjects), presence of complications affecting growth (5 subjects), and extreme longitudinal growth aberrations which lay more than three standard deviation scores from the mean in this population (17 subjects). Genetic analyses were performed in 150 patients (48.7%); 103 (68.7%) were reported to have some abnormalities in the known causative genes. Cardiovascular diseases were found in 256 patients (83.1%). The NS-specific height, weight, and BMI charts were constructed with 3,249 mixed longitudinal and cross-sectional measurements.

**CONCLUSION:** Growth standards for Japanese individuals with NS were established. These charts are expected to be used in various clinical settings.

Noonan syndrome (NS) is a clinically and genetically heterogeneous syndrome characterized by short stature, chest deformity, congenital heart disease, distinctive facial features that change with age, and other comorbidities (1,2). The clinical features in NS can vary within the same family but become more subtle in adult life (3). NS is relatively common, with an estimated prevalence of between 1 in 1,000 to 1 in 2,500 live births (2,4,5). Based on the cardinal features, the follow-up and comprehensive care for NS patients require a multidisciplinary team of specialists. As NS is characterized by marked variable dysmorphic appearances (6), it is often diagnosed by pediatric geneticists. Pediatric cardiologists also have an important role in NS care as it is the second most common syndromic cause of congenital heart disease, exceeded in prevalence only by trisomy 21 (7). In addition, children with NS often present to pediatric endocrinologists because of their short stature, delayed puberty, or undescended testes in males (3).

The *protein-tyrosine phosphatase, nonreceptor type 11* (*PTPN11*) gene was the first causative gene identified in NS patients. It encodes a tyrosine phosphatase protein involved in the Ras/mitogen-activated protein kinase signaling pathway (8). The protein is expressed throughout the body and plays a crucial role in cellular response to growth factors, hormones, cytokines, and cell adhesion molecules. There is increasing evidence that germline mutations in the genes involved in the Ras/mitogen-activated protein kinase signaling pathway cause NS and Noonan-related syndrome (9). While mutations in such genes were identified in ~60–70% of patients with NS phenotype (10,11), the diagnosis of NS remains clinical (2,12). Several scoring systems are currently available to aid in the diagnostic process, with the one developed by van der Burgt *et al.* (13) in 1994 being the most widely used.

At present, there are disease-specific growth charts for several syndromes, such as Prader–Willi syndrome, Down syndrome, Williams syndrome, and Turner syndrome (14–18). These charts are generally used in the clinical setting for monitoring the growth and development of patients with these syndromes and allow the identification of those with severe growth deficit who may need additional medications. To date, three NS-specific growth curves have been established in Brazil, North America, and Europe. Two of them were developed with data mainly collected by pediatric geneticists (19,20), while the other was constructed with data gathered by pediatric endocrinologists and cardiologists (21). Evidently, NS-specific charts should be created with data collected by a multidisciplinary team of specialists who is involved in the follow-up of NS patients. To that end, we conducted a nationwide survey of pediatric geneticists, pediatric cardiologists, and pediatric

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Received 1 June 2015; accepted 17 September 2015; advance online publication 6 January 2016. doi:10.1038/pr.2015.254

endocrinologists who are responsible for Japanese NS patients to establish growth standards for Japanese patients with NS.

## RESULTS

Twenty of the 31 hospitals (64.5%) responded to our questionnaire, and the data of 356 subjects clinically diagnosed with NS were obtained. From these data, a total of 48 subjects were excluded because of missing auxological data (26 subjects), presence of complications that affected growth (5 subjects), and extreme longitudinal growth aberrations which lay more than three standard deviation scores (SDS) from the mean in this population (17 subjects). The remaining 308 subjects (159 males and 149 females) were analyzed. Their birth years ranged from 1970 to 2012 (median: 1998). Gestational age was 38.4 ± 2.4 wk ($n$ = 247), birth length 48.2 ± 3.0 cm (male, $n$ = 94) and 47.8 ± 3.4 cm (female, $n$ = 104), birth weight 3.04 ± 0.55 kg (male, $n$ = 137) and 3.03 ± 0.57 kg (female, $n$ = 141), target height 170.9 ± 5.3 cm (male, $n$ = 58) and 157.5 ± 4.8 cm (female, $n$ = 66). Target height was calculated by the formula adjusted for Japanese before the secular trend reached a plateau (22). Table 1 shows the cumulative cardiac findings of the 308 patients. Cardiovascular disease was seen in 256 patients (83.1%). Genetic analyses were performed in 150 patients (48.7%), among them 103 individuals (68.7%) were found to have abnormalities in the causative genes (i.e., *PTPN11*: 67, *KRAS*: 3, *SOS1*: 7, *RAF1*: 14, *BRAF*: 7, *SHOC2*: 3, and *RIT1*: 2).

To establish the growth standards, 3,249 height measurements (males: 1,674 and females: 1,575) and 3,111 weight measurements (males: 1,564 and females: 1,547) were eventually included after the exclusion of 23 height measurements and 19 weight measurements derived from three outliers. Scatter plots of height and weight measurements were plotted on growth charts for normal population in **Supplementary Figures S1 and S2** online, respectively. The majority of subjects (males: $n$ = 126 (79.2%) and females: $n$ = 127 (85.2%)) had repeated measurements (male: median 10, range 2–63; female: median 11, range 2–46). Table 2 lists the number of measurements by sex and age. There was no statistically significant difference in height SDS between those with and without genetic analysis (difference: 0.076, 95% confidence interval (CI): −0.020 to 0.17, $P$ = 0.12). In contrast, height SDS in patients with *PTPN11* mutation was significantly smaller than that with other gene mutation (difference: 0.39, 95% CI: 0.26–0.53, $P$ < 0.0001).

Centile curves were fitted to the data using the Lambda-Mu-Sigma (LMS) method. For height, the distribution was generally assumed normal, while for weight and BMI, there was appreciable skewness, and the age-varying power transformations were adjusted. Equivalent degrees of freedom for (L, M, S) of height, weight, and BMI for males are (0, 12, 10) with age rescaled, (4, 9, 5) with age rescaled, and (3, 7, 4) with age transformed, respectively. Equivalent degrees of freedom for (L, M, S) of height, weight, and BMI for females are (0, 10, 7) with age rescaled, (4, 11, 5) with age rescaled, and (3, 6, 4) with age transformed, respectively. **Supplementary Tables S1–S3** online provide values for L, M, and S of height, weight, and BMI by sex and age,

**Table 1.** Cardiac findings in our series of 308 patients with NS

| Cardiac defect | Number[a] | Rate (%) |
|---|---|---|
| Pulmonary stenosis | 162 | 52.6 |
| Hypertrophic cardiomyopathy | 84 | 27.3 |
| Atrial septal defect (ostium secundum type) | 66 | 21.4 |
| Ventricular septal defect | 35 | 11.4 |
| Mitral valve anomalies | 19 | 6.2 |
| Patent ductus arteriosus | 10 | 3.2 |
| Aortic valve stenosis | 9 | 2.9 |
| Atrioventricular septal defect | 3 | 0.97 |
| Aortic coarctation | 2 | 0.65 |
| Pulmonary arterial hypertension | 2 | 0.65 |
| Tetralogy of Fallot | 2 | 0.65 |
| Bicupsid aortic valve | 1 | 0.32 |
| Coronary artery abnormality | 1 | 0.32 |
| Double outlet right ventricle | 1 | 0.32 |
| Aortic valve regurgitation | 1 | 0.32 |
| Truncus arteriosus communis | 1 | 0.32 |
| No finding | 52 | 16.9 |

NS, Noonan syndrome.
[a]Number indicates the cumulative total number of patients.

**Table 2.** Age distribution of numbers of measurements used for analysis

| Age (years) | Height | | Weight | | Body mass index | |
|---|---|---|---|---|---|---|
| | Boy | Girl | Boy | Girl | Boy | Girl |
| 0 | 300 | 279 | 322 | 311 | 300 | 279 |
| 1 | 175 | 174 | 164 | 177 | 164 | 174 |
| 2 | 128 | 121 | 125 | 121 | 125 | 121 |
| 3 | 155 | 125 | 144 | 127 | 144 | 125 |
| 4 | 132 | 94 | 126 | 91 | 126 | 91 |
| 5 | 128 | 75 | 121 | 77 | 121 | 75 |
| 6 | 105 | 90 | 99 | 86 | 99 | 86 |
| 7 | 79 | 78 | 72 | 78 | 72 | 78 |
| 8 | 70 | 87 | 69 | 87 | 69 | 87 |
| 9 | 52 | 63 | 51 | 61 | 51 | 61 |
| 10 | 46 | 60 | 46 | 57 | 46 | 57 |
| 11 | 58 | 48 | 51 | 44 | 51 | 44 |
| 12 | 47 | 47 | 41 | 45 | 41 | 45 |
| 13 | 43 | 47 | 33 | 45 | 33 | 45 |
| 14 | 43 | 44 | 33 | 42 | 33 | 42 |
| 15 | 35 | 30 | 25 | 27 | 25 | 27 |
| 16 | 21 | 30 | 14 | 27 | 14 | 27 |
| 17 | 16 | 24 | 10 | 22 | 10 | 22 |
| 18 | 9 | 13 | 4 | 9 | 4 | 9 |
| 19 | 10 | 13 | 5 | 5 | 5 | 5 |
| 20+ | 22 | 33 | 9 | 8 | 9 | 8 |
| Total | 1,674 | 1,575 | 1,564 | 1,547 | 1,542 | 1,508 |

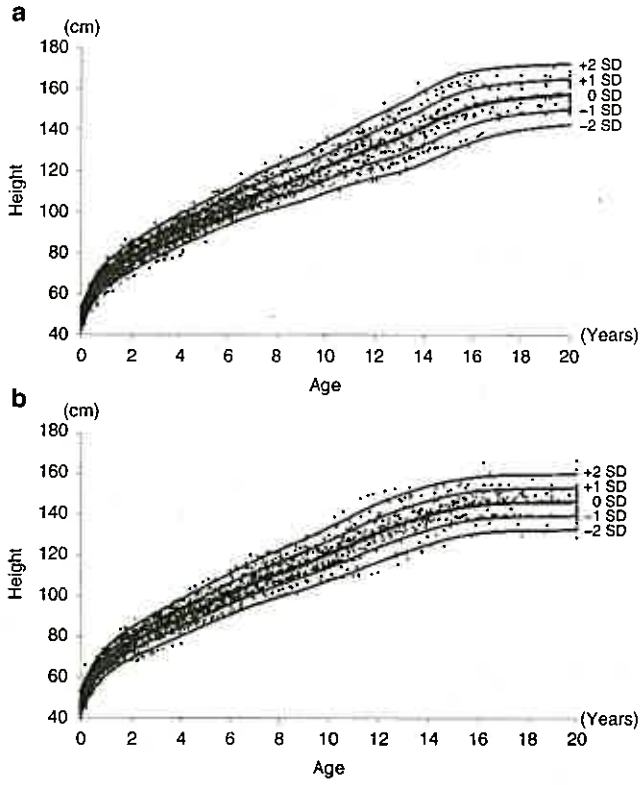


**Figure 1.** Scatter plots and smoothing curves of +2 SD, +1 SD, median, −1 SD, and −2 SD of height in Japanese (**a**) male and (**b**) female subjects with NS.

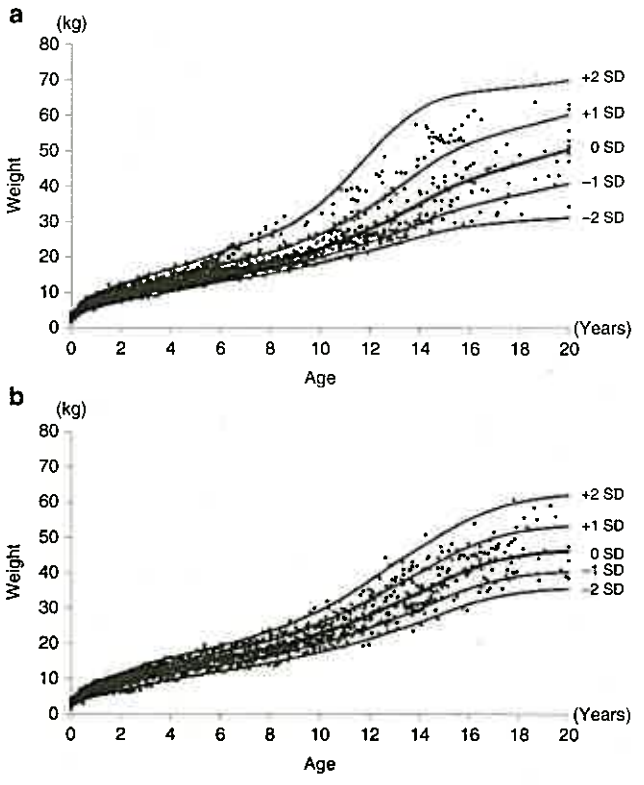


**Figure 2.** Scatter plots and smoothing curves of +2 SD, +1 SD, median, −1 SD, and −2 SD of weight in Japanese (**a**) male and (**b**) female subjects with NS. NS, Noonan syndrome.

respectively. Clinical growth charts with scatter plots of subjects for height, weight, and BMI are shown in Figures 1–3, respectively. A comparison with previously published mean height data for NS (19–21) was given in Figure 4. Growth curves for height, weight, and BMI are superimposed on those of normal population as shown in **Supplementary Figures S3–S5** online, respectively. Male weight and BMI charts are superimposed on those of female as shown in **Supplementary Figure S6** online.

## DISCUSSION

From the nationwide survey data of NS patients followed up by specialists from different fields, we have established growth references for Japanese NS patients. To our knowledge, these are the first NS-specific growth references in an Asian population. They were constructed by the LMS method, which we believe to be one of the most widely applied approaches (23). The LMS method is applicable to not only cross-sectional data but also longitudinal data, if all subjects were measured broadly and the number of measurements per individual did not reflect the growth charts (24). In this study, we collected the data in the following way so that certain individuals would not have much influence on the established charts. Our questionnaire required the minimal interval between two anthropometric values to be 1 mo when the subjects were measured before 1 y of age or 3 mo after 1 y of age. In fact, after 10 y of age, the intervals between two anthropometric values were more than half a year.

The number of subjects analyzed in this study ($n$ = 308) was sufficient compared with the numbers analyzed in the construction of the other three existing NS-specific charts ($n$ = 112, 137, and 144, respectively) (19–21). The diagnosis of NS in the subjects in this study was very reliable because it was performed by specialists certified for NS care in Japan by one of the three collaborating societies. The diagnosis was further validated using the scoring system developed by van der Burgt *et al.* (13). All auxological data were properly selected by excluding data with previous growth-promoting treatment, reported complications that may affect growth, and extreme longitudinal growth aberration. In addition, the percentage of detected abnormalities in the causative genes (68.7%) was reasonable compared to the reported ratios (10,11). Notably, the frequency of cardiovascular disease in this cohort (83.1%) is very similar to that of a recent report of 293 NS patients (81%) (25). Taken together, the newly established charts can be considered as representative of the growth in individuals with NS who are ordinarily seen in clinical practice.

The growth pattern of height was in line with previous reports (19–21,26). The average birth length is slightly shorter but within normal limit; however, there is a rapid decline in length/height SDS for normal population during the first year of life. Thereafter, mean height lies below the −2 SDS line for normal

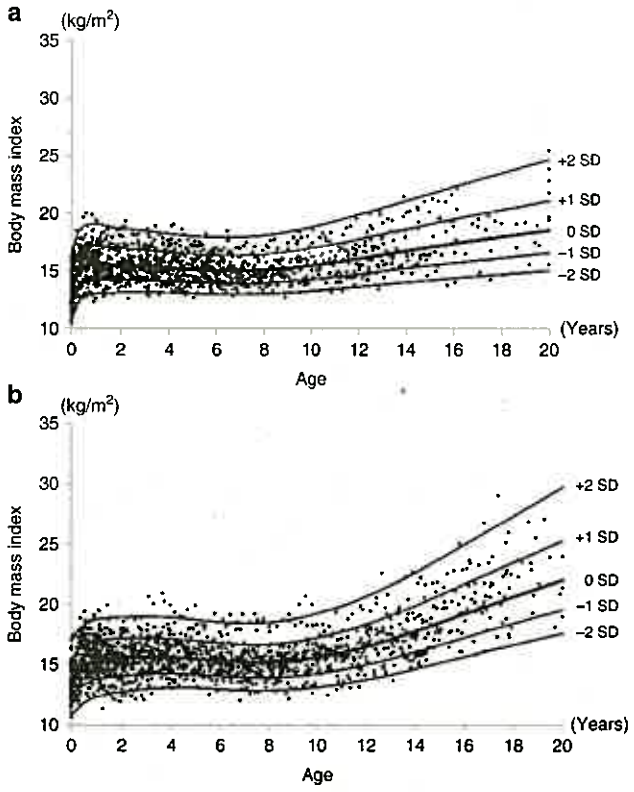


**Figure 3.** Scatter plots and smoothing curves of +2 SD, +1 SD, median, −1 SD, and −2 SD of body mass index in Japanese (**a**) male and (**b**) female subjects with NS. NS, Noonan syndrome.

population until 12 y in boys and 10 y in girls. Afterwards, there is a further decline due to delayed pubertal spurt, and then, the height SDS for normal population tends to recover. The adult height from the newly established growth charts is 157.3 ± 7.4 cm for boys (−2.3 ± 1.3 SDS) and 146.8 ± 6.9 cm for girls (−2.1 ± 1.3 SDS), respectively, when it is defined as the mean height at the age of 20 y. These adult height SDS for normal population are considered to be valid when compared to adult height SDS reported in other countries (male: −2.5 to −1.4, female: −2.2 to −1.8) (19–21,26,27), although small differences likely due to the different definitions of adult height were noted. In view of these, our NS-specific growth standards give adequate information about the natural growth of NS and are expected to be useful for evaluating height in various clinical settings such as potential growth-promoting treatments in NS.

With regard to the growth pattern of weight, birth weight is within normal limit; however, there is also a rapid decline in weight SDS to approximately −2 SDS for normal population during the first year of life, which may reflect the feeding problem in NS. Thereafter, growth retardation of weight is not apparently progressive. There seems to be a sexual difference of growth pattern of weight in older ages. Distributions of weight in male NS individuals are wider than those in female NS individuals, although it is difficult to interpret the implication with only auxological measurements in this study. On the other hand, BMI is similar to that of normal populations until ~5 y of age, but gradual increase after BMI rebound age is milder than that of normal population. These results are in line with the Brazilian BMI charts (20) and the reportedly low prevalence of overweight in adult NS patients (28). In addition, it is noteworthy that there is also a sexual difference in the growth pattern of BMI; gradual increase after BMI rebound age in male is milder than that in female. The interpretation of this difference remains unclear in the present study.

A difference in height SDS was detected throughout the whole period between those with *PTPN11* mutation and with other gene mutation (difference: 0.39, 95% CI: 0.26–0.53, $P < 0.0001$). Although it may be due to the fact that patients with *PTPN11* mutation often have low concentrations of insulin-like growth factor 1 (7), there are conflicting data on the severity of short stature between *PTPN11* mutation-positive and mutation-negative groups. In some studies, there were no differences in growth parameters between 51 *PTPN11* mutation-positive and 64 mutation-negative subjects (29), as was the case in an analysis of adult height in 56 patients (30). In another study, 82% of 34 *PTPN11* mutation-positive cases and 57% of the 23 *PTPN11* mutation-negative subjects had a height of less than −2 SDS (mean −3.1 SDS and −2.4 SDS, respectively) (31). There are similar reports that *PTPN11* mutation-positive subjects are shorter than *PTPN11* mutation-negative subjects (32,33). Although further investigation is needed, our study provides additional clinical data that NS individuals with *PTPN11* mutation might be 0.39 SDS shorter than those with other gene mutations.

There are several limitations in this study. First, the number of measurements of near-adult ages, especially in weight and BMI (Table 2), is low. Therefore, our charts may not be sufficiently reliable at these ages. Notably, this limitation is shared by all the other NS-specific charts (19–21). Nonetheless, growth patterns shown by our charts are in line with other studies of longitudinal natural history of NS (27,30). The adult heights from our charts were also valid when compared to other reports discussed above. The second limitation is a selection bias. For establishing disease-specific charts, recruiting unbiased subjects is challenging but nevertheless crucial. As NS is a clinically and genetically heterogeneous syndrome, it is critical to determine which subjects should be analyzed. For example, if we collected only the data of NS patients being confirmed genetically, there would have been an inherent selection bias because individuals with clinically confirmed NS may not necessarily undergo genetic analyses nor receive genetically confirmed diagnosis. However, the difference in height SDS between those with and without genetic analyses was not statistically significant, which suggested that these two groups had similar characteristics in height. Another type of selection bias regarding unreliable clinical diagnosis may derive from clinicians' limited specialty. However, this was largely overcome because this study was a nationwide survey of clinically diagnosed NS patients cared for by specialists for NS care.

In conclusion, we constructed the growth standards for Japanese individuals with NS using 3,249 measurements

from 308 NS patients nationwide. These are the first Asian NS-specific growth charts and are expected to be widely used in various clinical settings.

## METHODS

### Subjects

The nationwide survey of NS was conducted in collaboration with three academic societies in Japan (i.e., The Japan Society of Pediatric Genetics; The Japanese Society of Pediatric Cardiology and Cardiac Surgery; and The Japanese Society for Pediatric Endocrinology). We collected the data of patients in 31 hospitals nationwide who had been clinically diagnosed with NS by specialists certified by one of the three societies, and whose birth year was not earlier than 1970 considering the secular trend in height in Japan (17). We sent a questionnaire that included auxological measurements together with a minimal set of clinical and genetic data. Data on height and weight were collected in a mixed longitudinal and cross-sectional fashion, and the minimal interval between two anthropometric values was 1 mo when the subjects were measured before 1 y of age or 3 mo after 1 y of age. BMI was calculated as weight in kilograms divided by the square of height in meters. Clinical and genetic data included the following: clinical features according to the scoring system developed by van der Burgt *et al.* (13), classification of congenital heart disease, sex, birth year, birth weight, birth length, gestational periods, complications, comorbidities such as growth hormone deficiency, age at start of puberty, parents' height, family history, initial clues to the diagnosis, results of genetic analysis if available, and identified mutation(s) in the causative genes. We excluded subjects with missing description of auxological data, reported complications that significantly affected their growth, and extreme longitudinal growth aberrations which lay more than three SDS from the mean in this population. Subjects with growth-promoting treatment were included only if they had pretreatment auxological data, but measurements after the commencement of the growth-promoting therapy were excluded from our analysis. This study was performed with approval from National Center for Child Health and Development Ethics Committee and all the other Ethics Committees in institutes which participated in this study. We did not collect any information which may identify individuals, such as name and address, and so did not obtain informed consent from participants. The Japanese guidelines permit the use of data from epidemiological survey without consent if the data are anonymous. The information on the present survey has been disclosed to the public on the website of National Center for Child Health and Development Ethics Committee at http://www.ncchd.go.jp/center/information/epidemiology/index.html.

### Methods

Data cleaning was performed at several stages. Bivariate plots of height and weight were used to identify gross disproportion. Data points were scrutinized, going back to the source data if necessary, and descriptive errors were corrected. If a value was deemed highly unlikely (more than 3 SDS from the mean in the population), even though there was no evidence of a descriptive error, such a point was deleted.

To establish the growth charts, the LMS method (34) was used, with the assumption that the data can be transformed to normality by a suitable power transformation (L), and the distribution summarized by the median (M) and coefficient of variation (S). Using penalized likelihood, three curves (L, M, and S) can be fitted as cubic splines by nonlinear regression, and the extent of smoothing was controlled by equivalent degrees of freedom. The values of L, M, and S were constrained to change smoothly with age, and fitted values can be used to construct any required centile curves. Fitting and smoothing were done with lmsChartMaker Pro ver.2.3 (Medical Research Council, London, UK). The SD score (Z-score) of each measurement (y value) could be calculated from the L, M, and S curves, using values appropriate for the age and gender, by the following equation: $Z = [(y/M)^{L-1}]/(L \times S)$, and if $L = 0$, $Z = \ln(y/M)/S$.

### Statistical Analysis

The results are expressed as the mean ± SD or by frequency and percentage. To investigate the height difference between those with and without genetic analyses, height SDS, which were calculated by Japanese standards for normal children by sex and age (35), were compared with ANOVA considering the adjustment for the number of measurements in each subjects. Similar analyses were performed with other factors. These analyses were performed by JMP 8 (SAS Institute, Cary, NC); $P$ values <0.05 were considered statistically significant.

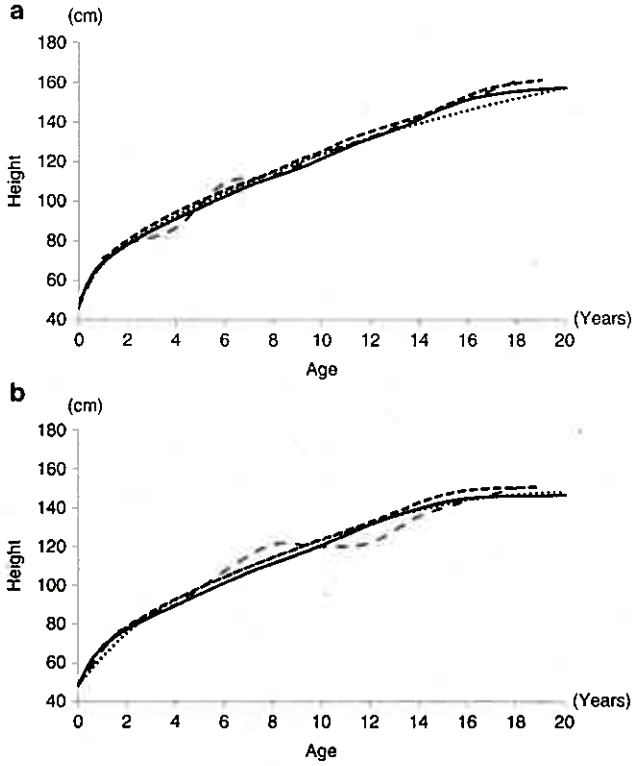


**Figure 4.** Comparison of Japanese NS mean height curves with previously published foreign curves, (**a**) male and (**b**) female, respectively. Black, black dotted, black dashed, and gray broken lines indicate Japanese, Brazilian, European, and North American NS mean height curves, respectively. NS, Noonan syndrome.

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

### ACKNOWLEDGMENTS

We thank the following members of this nationwide survey study: Hironori Tanahashi, Department of Pediatrics, Asahikawa Medical College School of Medicine; Masato Yokozawa, Department of Pediatrics, Hokkaido Medical Center for Child Health and Rehabilitation; Yoko Aoki, Department of Medical Genetics, Tohoku University School of Medicine; Ikuma Fujiwara, Department of Pediatrics, Tohoku University School of Medicine; Hitoshi Horigome, Department of Child Health, Graduate School of Comprehensive Human Science, University of Tsukuba; Hirofumi Ohashi, Division of Medical Genetics, Saitama Children's Medical Center; Hiroshi Mochizuki, Division of Endocrinology and Metabolism, Saitama Children's Medical Center; Chie Takahashi, Yasuhiro Naiki, Reiko Horikawa, Division of Endocrinology and Metabolism, National Center for Child Health and Development; Reina Ishizaki, Hiroyuki Yamagishi, Department of Pediatrics, Keio University School of Medicine; Kenjiro Kosaki, Center for Medical Genetics, Keio University School of Medicine; Hiroshi Yoshihashi, Division of Medical Genetics, Tokyo Metropolitan Children's Medical Center; Yukihiro Hasegawa, Division of Endocrinology and Metabolism, Tokyo Metropolitan Children's Medical Center; In-Sam Park, Department of Pediatrics, Sakakihara Heart Institute; Sachiko Kitanaka, Department of Pediatrics, Graduate School of Medicine, The University of Tokyo; Kenji Kurosawa, Division of

Medical Genetics, Kanagawa Children's Medical Center, Masanori Adachi, Department of Endocrinology and Metabolism, Kanagawa Children's Medical Center; Ayumi Uematsu, Department of Endocrinology and Metabolism, Shizuoka Children's Hospital; Yasuo Ono, Department of Cardiology, Shizuoka Children's Hospital; Haruo Mizuno, Shinji Saito, Department of Pediatrics and Neonatology, Nagoya City University Graduate School of Medical Sciences; Masako Izawa, Takashi Hamajima, Division of Endocrinology and Metabolism, Aichi Children's Health and Medical Center; Yasuko Shoji, Shinobu Ida, Department of Pediatric Gastroenterology, Nutrition, and Endocrinology, Osaka Medical Center and Research Institute for Maternal and Child Health; Nobuhiko Okamoto, Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health; Yoko Miyoshi, Noriyuki Namba, Keiichi Ozono, Department of Pediatrics, Osaka University Graduate School of Medicine; Yoshikazu Nishi, Department of Pediatrics, Hiroshima Red Cross Hospital. We also thank Julian Tang, Department of Education for Clinical Research, National Center for Child Health and Development, for reviewing and editing this manuscript.

STATEMENT OF FINANCIAL SUPPORT

All authors have no financial relationships relevant to this article to disclose.

Disclosure: All authors have no conflicts of interest to disclose.

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# ビタミンD欠乏性くる病
Vitamin D Deficient Rickets

北中幸子* KITANAKA Sachiko

## 1 基本病因，発症機序

くる病とは，カルシウムやリンの低下により，骨基質の石灰化不全が起こる病態の総称である。成長期にみられるもので，骨変形と成長障害を主徴とする小児期の代表的骨疾患である。骨端線閉鎖後の成人においては，同様の病態は骨軟化症となる。くる病は大きく分けて，ビタミンD作用の欠乏により低カルシウム血症を主体とする場合と，主にリン排泄増加により低リン血症を主体とする場合がある（表1）。ビタミンD作用の欠乏によるものには，ビタミンD欠乏性くる病と，先天性にビタミンDの合成や作用機構に異常がある遺伝性のビタミンD依存性くる病がある。

ビタミンD欠乏性くる病は，栄養状態が良くなかった昔に多くみられたが，近年再び世界的に問題となっている[1]。わが国でも同様で，くる病の原因のなかで近年もっとも発症数が多い。ビタミンD欠乏による症状は，乳児期にはビタミンD欠乏性低カルシウム血症として発症することが多く，幼児期以降にはビタミンD欠乏性くる病として発症することが多い。いずれも病因は同じであるため，ビタミンD欠乏性低カルシウム血症とビタミンD欠乏性くる病をあわせて，ビタミンD欠乏症という。ビタミンD欠乏症が近年増加してきた要因は，以下のとおり大きく三つある[1]。

一つ目の要因は，母乳栄養が推進されたことである。母乳は，免疫など赤ちゃんにとって好ましいことが多いが，人工乳に比較して格段にビタミンD含有量が少ない。これまでビタミンD欠乏症を発症した児は，ほとんどすべて母乳栄養児である。症状がなくても，多くの母乳栄養児はビタミンD不足状態にある。

二つ目の要因は，日光浴の不足である。昔，くる病が多くみられたころは，赤ちゃんに日光浴がすすめられ，一時期ビタミンD欠乏性くる病はほとんどみられなくなった。ところが，白色人種で紫外線と皮膚癌の関連が明らかになってから，紫

表1 くる病をきたす疾患

| |
|---|
| 1. ビタミンDの異常，カルシウムの欠乏 |
| 1）ビタミンD欠乏（摂取不足，日光不足） |
| 2）ビタミンD依存性くる病1型（ビタミンD1α水酸化酵素欠損）（*CYP27B1* 変異） |
| 3）ビタミンD依存性くる病2型（ビタミンD受容体異常）（*VDR* 変異） |
| 4）ビタミンD吸収障害（消化管疾患，胆汁分泌不全） |
| 5）慢性腎不全 |
| 6）抗けいれん薬によるくる病，肝性くる病 |
| 7）カルシウム欠乏・吸収障害 |
| 2. リンの異常・欠乏 |
| 1）X連鎖性低リン血症性くる病（HYP）（*PHEX* 変異） |
| 2）常染色体優性低リン血症性くる病（ADHR）（*FGF23* 変異） |
| 3）常染色体劣性低リン血症性くる病（ARHR）（*DMP1*，*ENPP1* 変異） |
| 4）高カルシウム尿症を伴う遺伝性低リン血症性くる病（HHRH）（*SLC34A3* 変異） |
| 5）腫瘍性低リン血症性くる病/骨軟化症（TIO） |
| 6）Fanconi 症候群 |
| 7）Dent 病 |
| 8）尿細管性アシドーシス |
| 9）McCune-Albright 症候群 |
| 10）リン欠乏・吸収障害 |
| 3. その他 |
| 1）未熟児くる病 |
| 2）低フォスファターゼン症（*TNSALP* 変異） |

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外線対策が盛んにとられるようになった。白色人種では，小児期の紫外線曝露も皮膚癌に関与するというデータがあることから，赤ちゃんにまで紫外線対策の活動が広まっている。さらに若年女性は，皮膚老化，いわゆるしみやしわ予防のためにも紫外線対策がとられ，妊婦のビタミンD不足も多い。ところが，日本人では，紫外線の関連する悪性の皮膚癌は少ないといわれている。ビタミンDが合成されるためには，ある程度の日光が必要であり，紫外線曝露を過度に避けるあまりに日光をまったく浴びないと，ビタミンD欠乏となる。ビタミンD欠乏症は，北海道など緯度の高い地域，外出を制限している乳幼児，日焼け止め使用や紫外線対策が過剰な児に多くみられている。

三つ目の要因は，食事制限や食事の偏りである。そのなかでももっとも多いのは，食事アレルギーやアトピー性皮膚炎がある幼児で，卵や動物性タンパク質を制限している場合である。ビタミンDは魚，卵，きのこなどに多く含まれている。アレルギーを心配して親が勝手に食事制限をしていたり，制限を行う際に適切な代替食品の指導が行われていなかったりする場合に，ビタミンD欠乏症が起こる。そのほかにも菜食主義や偏食によって起こる場合がある。

## 2 基本病態

ビタミンDは，食事から摂取したり，皮膚において紫外線（UV-B）の存在下に7-dehydrocholesterolから合成される。ビタミンDには主に植物由来のビタミン$D_2$および動物由来のビタミン$D_3$がある。食事から吸収されるか皮膚で合成されたビタミンDは，まず肝臓で25水酸化ビタミンD［25（OH）D］となり，さらに腎臓近位尿細管で1α水酸化酵素により活性型ビタミンD［1,25$(OH)_2D$］となる。

活性型ビタミンDは，標的組織（腸管，腎，骨，副甲状腺など）において，ビタミンD受容体（VDR）に結合して作用する。その作用は，腸管からのカルシウム吸収や骨への石灰化促進などによる生体内カルシウム濃度維持をはじめ，免疫や細胞の分化増殖など多くの生理作用を発揮する。このいずれかの段階での異常によりビタミンDの作用が欠乏すると，血中カルシウム濃度を維持することができず低カルシウム血症となる。

さらに，低カルシウムによる二次性の副甲状腺機能亢進が加わることにより，血中リン濃度も低下しくる病となる。

## 3 病態生理からみた臨床症状

症状は，低カルシウム血症で発症する場合とくる病で発症する場合がある。1歳未満の乳児では，低カルシウム血症で発症することが多く，低カルシウム血症による全身性のけいれんが初発症状となる。低カルシウム血症は，感染症などの発熱時に悪化しやすいので，熱性けいれんとして発症することもある。熱性けいれんと思われる児でも，必ず血中カルシウム値のチェックは必要である。

1歳すぎの幼児では，くる病として発症することが多い。症状としては，O脚（内反膝）がもっとも多く，そのほかに成長障害，歩行開始の遅れ，歩行異常，骨変形，X脚（外反膝）などがある。乳幼児は生理的にもO脚となるが，程度が強い場合はX線検査が必要である。O脚あるいはX脚は，骨幹部に生じた骨軟化症による骨強度の低下により生じる。骨強度の低下は，横隔膜付着部における肋骨の陥凹（Harrison溝），頭蓋骨が柔らかくへこむ頭蓋癆（craniotabes）になる。成長障害は，骨気質の石灰化障害により，骨の縦方向への成長が障害されるために起こる。骨変形は，関節部において，石灰化されない類骨が横方向へ拡大するため，手首など長幹骨骨端部の関節の膨隆が起こる。また，肋軟骨部に生じた同様の変化は，肋骨骨移行部の腫大となって肋骨念珠（rachitic rosary）となる。ほかの症状として運動発達の遅れや筋力低下は，低カルシウム血症によると考えられている。歯のエナメル質形成不全がみられることもある。より年長児になると，低カルシウム血症によるテタニーで発症することが多い。成人では骨軟化症となり，骨痛などをきたす。