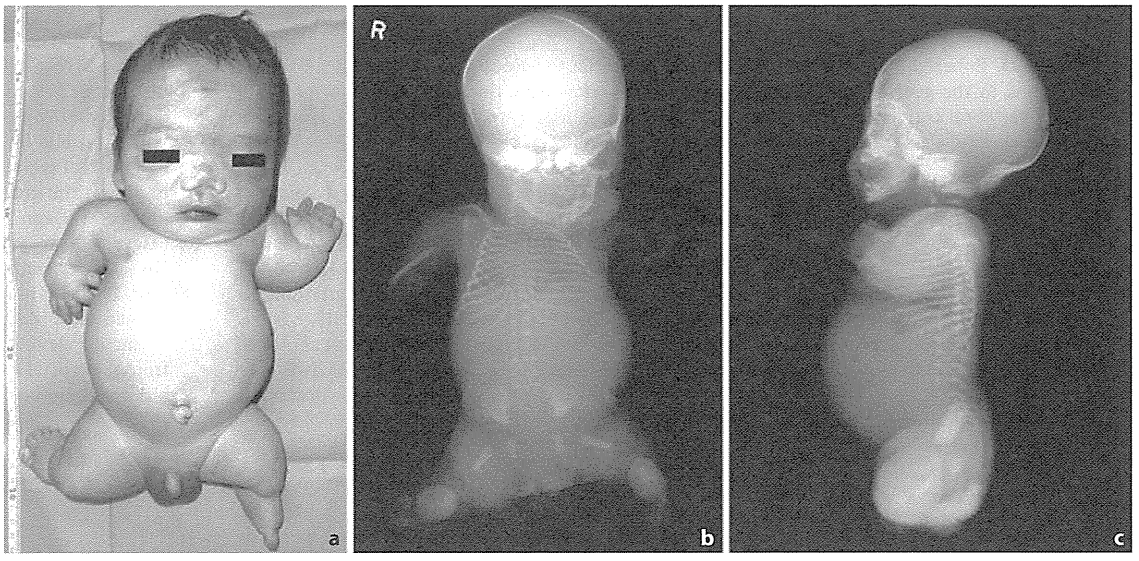

Fig. 3. The postmortem images of the patient. The nasal bridge was flattened, and the extremities were short and flexed: macroscopic front view (**a**), X-ray front view (**b**) and X-ray rear side view (**c**).

The key findings that facilitated the diagnosis of boomerang dysplasia in this instance included the absence of two of the three long bones in each limb, underossification of some components of the vertebrae and disordered ossification of the metacarpals. These findings can also be observed in atelosteogenesis type I, an observation that reflects the close phenotypic relatedness of these two conditions. Importantly, however, the milder potentially survivable condition, atelosteogenesis III, does not feature nonossification of the long bones of the limbs [4, 7]. In this instance demonstration of the characteristic bent bone morphology in the limbs by 3D-CT added diagnostic certainty and facilitated prognostication and genetic counseling for the parents. This was possible because the images obtained by 3D-CT enabled the visualization of some additional details of the fetal skeleton which were not clearly recognized in the ultrasonographic evaluation. Furthermore, the reconstructed 3D-CT enabled visualization of the whole fetal skeleton without contamination from maternal anatomy [8, 9].

The mutation observed in this patient, c.605T>C is the third causative mutation described in this disorder, and like the other two known mutations (p.Leu171Arg, p.Ser235Pro) leads to substitution of an amino acid residue in the actin-binding domain of FLNB [5]. Reflecting their close relatedness, a previously reported mutation, c.604A>G, occurring at the same codon predicts the substitution p.Met202Val and results in an atelosteogenesis I phenotype. The parents of our patient did not give permission to perform their own genetic analysis to check whether the change is de novo. However, this substitution (p.Met202Thr) changes polarity and hydrophilic property of the amino acid residue. Therefore, it might be pathogenic due to the potential of protein structural and functional change. Similar mutations leading to atelosteogenesis I and Larsen syndrome leads to an increased avidity of FLNB for cytoskeletal actin [10], but the mechanism by which this impacts on skeletogenesis and ossification of bone is not understood.

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RESEARCH LETTER

Low prevalence of genetic prenatal diagnosis in Japan

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KEY WORDS: prenatal diagnosis; maternal serum marker screening; amniocentesis; chorionic villus sampling

Advances in molecular genetics, cytogenetics, and ultrasound diagnosis have revealed many abnormalities of fetuses *in utero*. In addition to technical advances and economic aspects, ethical considerations influence how prenatal diagnosis is used in communities. The outcome of prenatal diagnoses may be associated with trends in babies born with congenital disorders. We surveyed the number of genetic prenatal diagnoses to examine the trends in prenatal diagnosis in Japan.

In 2002, we conducted a survey on genetic prenatal diagnoses performed in Japan during the previous 5-year period (Sago *et al.*, 2005). This report was the first to explore the trends in prenatal diagnosis in Japan. The study surveyed clinical laboratories nationwide and was assumed to cover most cases of genetic prenatal tests performed in Japan. Adding the results of a recent 6-year survey, we have now completed an 11-year survey for the period between 1998 and 2008. Based on the methods in the previous survey, we sent questionnaires to both commercial and academic clinical laboratories thought to be performing prenatal diagnostic tests and obtained answers regarding the numbers of genetic tests of maternal serum markers, amniocentesis, and chorionic villus sampling (CVS) conducted between January 2003 and December 2008. We combined the data from this new 6-year survey with that of the previous survey conducted between 1998 and 2002 and examined the trends over this 11-year period. Of the 59 responding laboratories, 25 laboratories had actually conducted some sort of prenatal genetic test. Yearly changes in the numbers of maternal serum marker screenings, amniocentesis procedures, and CVS procedures are shown in Table 1. The annual number of maternal serum marker screenings reached a maximum in 1998 and then decreased markedly until 2001, after which time it increased. The annual number of amniocentesis procedures was about 10 000 between 1998 and 2002, increasing by approximately 30% in 2008. On the other hand, the number of CVS procedures was very low.

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According to a report by the Ministry of Health, Labor, and Welfare, the Japanese population in 2008 was 127.7 million and the total number of births in 2008 was 1.09 million. The total number of prenatal genetic tests was about 32 000 in 2008. Consequently, only 3% of all pregnant women received prenatal diagnoses based on maternal serum marker screening or chromosome analysis; however, some pregnant women received both maternal serum marker screening and chromosome analysis (i.e. some overlap existed). The rate of prenatal genetic diagnosis is extremely low in Japan, compared with the rates in other advanced countries where the maternal ages are also high, similar to the situation in Japan.

Japanese law permits abortions for maternal economic or health problems but not for fetal abnormalities. Actually, abortions because of fetal abnormalities are, in many cases, performed with maternal health problems given as the reason. Pregnant women may be deterred from receiving a prenatal diagnosis because abortions for fetal abnormalities are not permitted legally and many people believe that abortions are unethical even if a fetus has serious abnormalities.

Maternal serum marker screening was introduced to Japan in 1994 and soon began to be used clinically; however, insufficient genetic counseling for this test facilitated anxiety in pregnant women, creating a social problem. Therefore, in 1999, the Expert Committee on Prenatal Diagnosis of the Sciences Council for Evaluating Advanced Medical Techniques of Japan published the 'View on Prenatal Serum Marker Screening'. This view stated that physicians were not required to give information on this test to pregnant women vigorously and that physicians should not recommend this test. Thereafter, the number of maternal serum screening tests decreased for a few years and remained at about 15 000 annually. However, the number began to increase in 2003, albeit slightly, and reached 18 000 in 2008. A nuchal translucency scan at 11–13 weeks of gestation was also rarely performed, although ultrasonography is performed during the first trimester to check the fetal heartbeat and growth in almost all pregnant women in Japan. The lack of information on prenatal diagnosis provided by doctors

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Table 1—The number of prenatal genetic tests and liveborns in Japan 1998–2008

Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Liveborn infant[a]	1 203 147	1 177 669	1 190 560	1 170 662	1 153 855	1 123 610	1 110 835	1 062 530	1 092 674	1 089 818	1 091 150
Pregnant women of ≥35 years[b]	127 442	130 497	141 653	142 784	147 636	157 369	169 516	173 754	192 905	211 711	228 444
Maternal serum screening	21 708	18 312	15 927	15 308	15 627	16 591	16 613	16 279	17 558	17 333	18 209
Amniocentesis	10 419	10 516	10 627	10 070	9 926	11 284	11 261	11 557	11 703	12 458	13 402
Chorionic villus sampling	76	58	96	120	129	65	80	43	36	36	46

[a] The number of liveborn infants has been significantly decreasing (number of infants = 1 200 079.13312 × (year-1998), ($R^2 = 0.85$).
[b] Advanced age pregnancies has been significantly increasing (proportion of over 35 years = 0.10 + 0.01 × (year-1998), $R^2 = 0.96$).

Table 2—Prenatal screening policies and screening rates in industrialized countries

Countries	Screening policies	Total fertility rates[a] (2000/2008)	Screening rates
Australia	W	1.76/1.97	98% (2007)[b]
Denmark	W	1.77/1.89	84.4% (2006)[c]
England and Wales	W	$1.64/1.90_{(2007)}$	88% (2009)[d]
France	W	1.88/2.00	—
Germany	W	1.38/1.38	—
Italy	W	1.26/1.41	—
Switzerland	W	1.50/1.48	—
USA	W	$2.06/2.12_{(2007)}$	—
Netherlands	S	1.72/1.78	—
Spain	S	1.23/1.46	—
Japan	S	1.36/1.37	3%(2008)

W, prenatal screening offered in whole country; S, no national policy but some form of screening in some area.
[a] From United Nations Statistics Division, 2008.
[b] From Genetics Education in Medicine Consortium, 2007.
[c] From Ekelund *et al.* (2008).
[d] From National Down Syndrome Cytogenetic Register, 2009.

is thought to be one of the reasons why relatively few pregnant women receive genetic prenatal diagnosis.

A comparison of the status of prenatal diagnosis between Japan and other advanced countries is difficult because nationwide data are scarce (Boyd *et al.*, 2008). Available data of screening policies and screening rates in industrialized countries are shown in Table 2. Many industrialized countries have a national policy to offer first or second trimester screening for all pregnant women. More than half of pregnant women are assumed to undergo genetic prenatal screening tests in advanced countries (Genetic Education in Medicine Consortium, 2007; Ekelund *et al.*, 2008; National Down Syndrome Cytogenetic Registry, 2009). On the other hand, only 3% of all pregnant women receive genetic prenatal screening or diagnostic tests in Japan and most chromosomal analyses were performed using amniocentesis in the 11-year survey.

Recently, the number of births in Japan has been decreasing yearly and the number of advanced-age pregnancies has been increasing markedly (Table 1). This situation raises the concern that the number of babies born with congenital disorders related to maternal age, such as Down syndrome, may increase. Kajii reported that if the current situation continues, the number of babies born with Down syndrome would reach 3000 per year in 2011, representing a 50% increase over the previous 5-year period (Kajii, 2008).

The present study clarified the genetic prenatal diagnosis trends in Japan over the past 11 years. Although the number of prenatal tests being performed has increased slightly, the rate of prenatal testing in pregnant women remains extremely low at about 3%, and amniocentesis was the main invasive procedure used to confirm a diagnosis. We should carefully observe how these results affect society in terms of the number of

babies born with congenital disorders. The data presented herein provides valuable information for assessing the effect of medical care on society.

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SHORT COMMUNICATION

Prevalence of c.1559delT in *ALPL*, a common mutation resulting in the perinatal (lethal) form of hypophosphatasia in Japanese and effects of the mutation on heterozygous carriers

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Hypophosphatasia (HPP) is an inherited disorder caused by mutations in *ALPL* that encodes an isozyme of alkaline phosphatase (ALP), TNSALP. One of the most frequent *ALPL* mutations is c.1559delT, which causes the most severe HPP, the perinatal (lethal) form (pl-HPP). c.1559delT has been found only in Japanese and its prevalence is suspected to be high; however, the allele frequency of c.1559delT in Japanese remains unknown. We designed a screening system for the mutation based on high-resolution melting curve analysis, and examined the frequency of c.1559delT. We found that the c.1559delT carrier frequency is 1/480 (95% confidence interval, 1/1562–1/284). This indicates that ~1 in 900 000 individuals to have pl-HPP caused by a homozygous c.1559delT mutation. In our analysis, the majority of c.1559delT carriers had normal values of HPP biochemical markers, such as serum ALP and urine phosphoethanolamine. Our results indicate that the only way to reliably detect whether individuals are pl-HPP carriers is to perform the *ALPL* mutation analysis.
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Keywords: *ALPL*; c.1559delT; perinatal form of hypophosphatasia; serum alkaline phosphatase; skeletal dysplasia; urine phosphoethanolamine

INTRODUCTION

Hypophosphatasia (HPP) is an inherited disorder characterized by defective mineralization of the bone and low activity of alkaline phosphatase (ALP; EC 3.1.3.1).[1,2] HPP is a clinically heterogeneous disease and classified into five forms according to severity and age of onset: perinatal (lethal), infantile (OMIM 241500), childhood (OMIM 241510), adult (OMIM 146300) and odontohypophosphatasia.[1] All forms of HPP display reduced activity of unfractionated serum ALP and the presence of either one or two pathologic mutations in *ALPL*, the gene encoding an ALP isozyme (TNSALP).

The perinatal (lethal) form of HPP (pl-HPP) is the most severe HPP with an autosomal recessive mode of inheritance. pl-HPP is more common in Japan than in other countries.[3] Parents of pl-HPP are heterozygous carriers of *ALPL* mutations. They show no clinical symptoms, but have reduced serum ALP activity and increased urinary phosphoethanolamine (PEA).[4–8]

ALPL is the only gene known to be associated with HPP.[1] More than 200 *ALPL* mutations have been described, accounting for most phenotype variabilities.[9] HPP is frequently caused by p.E191K and p.D378V in Caucasians,[1] whereas p.F327L[10] and c.1559delT[10,11] are more common in Japanese.[1] To date, c.1559delT has only been found in Japanese.[11] Some patients with pl-HPP are homozygous for c.1559delT, with parents who are heterozygous carriers for the mutation but with no evidence of consanguinity.[12,13]

To identify c.1559delT genotype and to examine its frequency in Japanese, we designed a screening system based on a high-resolution melting curve analysis.[14] In addition, we examined serum ALP activity and urine PEA in heterozygous c.1559delT carriers to determine whether these markers can identify the HPP carriers.

MATERIALS AND METHODS

This study was approved by the Institutional Genetic Research Ethics Committee at Nippon Medical School and RIKEN, Center for Genomic Medicine. Blood samples were collected under written informed consents from 3844 healthy Japanese without HPP and its related findings confirmed by orthopedic surgeons. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. The c.1559delT genotype screening was performed by the small amplicon genotyping method based on high-resolution melting curve

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analysis.[14] PCR primers for c.1559delT were designed to flank the mutation leaving only single base, including the mutation between the primers: 5′-TT TAAATTCTCGCGCTGGCCCTCTACCCC-3′ (forward) and 5′-TTTAAATTCC CTCAGAACAGGACGCTC-3′ (reverse). PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 45 cycles at 94°C for 30 s and annealing at 67°C for 30 s. After PCR, high-resolution melting was performed in a 96-well plate LightScanner (Idaho Technology, Salt Lake City, UT, USA), which collected data from 55°C to 97°C at a ramp rate of 0.10°C sec^{-1}. The observed number of c.1559delT carriers was divided by the total number of individuals tested to determine the carrier frequency. Serum ALP activity and urine PEA were measured in c.1559delT-heterozygous parents of pl-HPP patients.

RESULTS

Three *ALPL* c.1559delT genotypes (wt/wt, wt/c.1559delT and c.1559delT/c.1559delT) were distinguished by the modified small amplicon genotyping method (Figure 1). A heterogeneous c.1559delT mutation (wt/c.1559delT) was detected in 8 of 3844 healthy Japanese subjects, indicating a carrier frequency of 1/480 in the Japanese population (95% confidence interval, 1/1562–1/284).

The numerical value of ALP activity and urinary PEA varied in heterozygous c.1559delT carriers in parents of perinatal HPP patients. The majority of heterozygous c.1559delT carriers had normal levels of both ALP activity (five out of six males and three out of four females) and urinary PEA (three out of six males and four out of five females) (Figure 2).

DISCUSSION

Based on our results, we estimated the frequency of c.1559delT-homozygous individuals (for example, those with pl-HPP) to be 1/900 000. Previous studies showed that all Japanese pl-HPP patients carried the c.1559delT mutation in at least one allele; half (10/20) were homozygous for c.1559delT and half (10/20) were compound heterozygous for c.1559delT,[9–13,15] which gives a pl-HPP prevalence of 1/450 000 for patients that are homozygous or compound heterozygous for c.1559delT mutation. The other common mutation on *ALPL* in Japan, p.F327L, is a mild allele whose product retained ~70% of its enzymatic activity. Patients compound heterozygous for c.1559delT and p.F327L are not associated with pl-HPP.[10]

Biochemical markers, serum ALP activity and urinary PEA levels fell within their normal ranges in the majority of the c.1559delT carriers examined in this paper, whereas heterozygous carriers of the severe forms in other *ALPL* mutations were reported to have reduced serum ALP activity and increased urinary PEA.[4–8] Some possible reasons why c.1559delT carriers display normal marker levels are as follows: the first is the protein properties caused by the different mutation positions. The c.1559delT mutation causes a frameshift downstream of codon L503, resulting in the elimination of the termination codon at 508 and the addition of 80 amino acids at the C-terminus. The mutant protein forms an aggregate that is polyubiquitinated and then degraded in the proteasome. However, the aggregates possess enzyme activity, and may, therefore, influence physiological processes before their destruction.[16] Second, serum ALP activity is affected by some other factors. The genetic modifier of ALP is reported to have a potential influence on serum ALP activity.[17] Total ALP value is also elevated by some environmental factors, in vitamin D deficiency[2] or in the third trimester of gestation by the increasing placental ALP, which is not affected by TNSALP.[18] Recently, it was shown that patients who are homozygous for the c.1559delT mutation differed in the severity of HPP, including both their symptoms and serum ALP activity.[15]

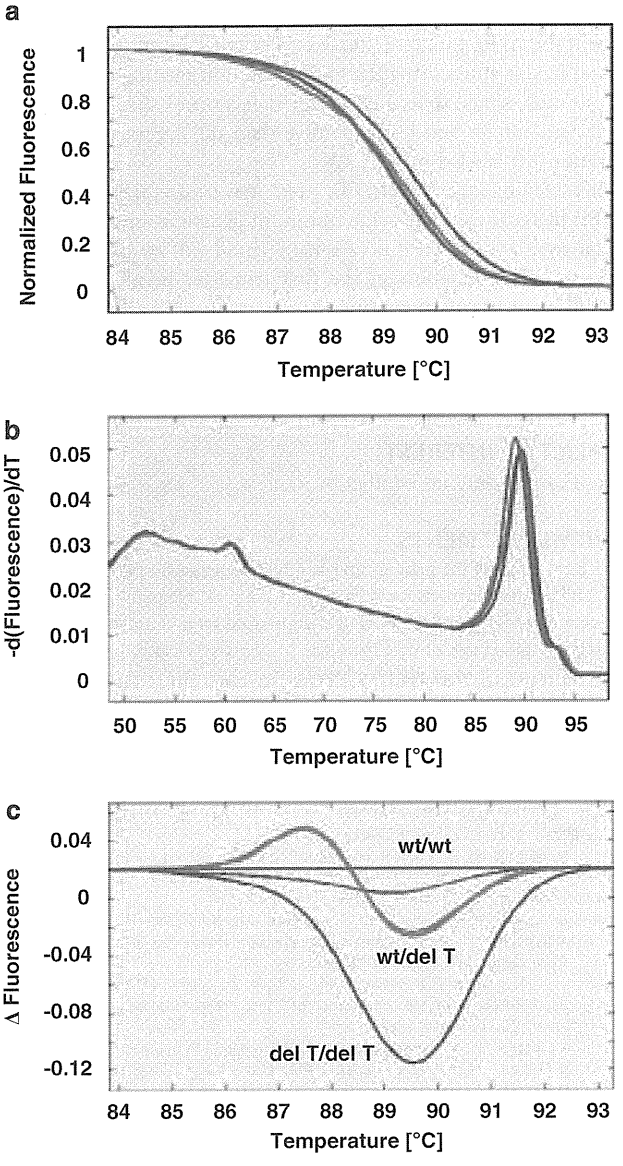

Figure 1 Identification of c.1559delT mutation in *ALPL* by small amplicon genotyping (SAG) method. (**a**) Normalized fluorescence plots. (**b**) d(fluorescence)/dT plot. (**c**) The corresponding fluorescence difference plots. Wild-type (wt/wt) samples are in gray; samples heterozygous for c.1559delT (wt/c.1559delT) are in red; and samples homozygous for c.1559delT (c.1559delT/c.1559delT) are in blue. The three genotypes were clearly distinguishable in the SAG method.

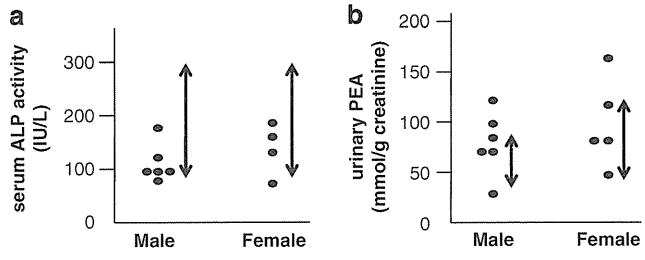

Figure 2 Biochemical marker levels in heterozygous carriers of the *ALPL* c.1559delT mutation. The serum ALP activity (**a**) and urinary PEA (**b**) levels in the majority of heterozygous carriers (wt/c.1559delT) fell within normal ranges (indicated by arrows).

Thus, the only way to reliably detect the pl-HPP carriers is to perform the *ALPL* mutation analysis. The small amplicon genotyping method in this study using the high-resolution melting curve analysis is a one-step, single-tube method for detection of specific mutations and faster, simpler and less expensive than the approaches requiring separations or labeled probes.[19]

The screening for c.1559delT in *ALPL* may be useful for diagnosis of pl-HPP in Japanese to provide optimum genetic counseling for fetal skeletal dysplasia. pl-HPP occasionally could not be diagnosed with sonographic examination in the first trimester because incomplete ossification is an usual finding at this stage of development.[20] To diagnose pl-HPP, collaborations between obstetricians and clinical geneticists are important and could provide support for parents of prenatal patients suspected of having skeletal dysplasia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CASE REPORT

Prenatal diagnosis of Kniest dysplasia with three-dimensional helical computed tomography

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Abstract

Objective. Fetal three-dimensional helical computed tomography (3D-CT) has attracted attention in the diagnosis of fetal skeletal dysplasias because of limited diagnostic capabilities of standard ultrasonography to delineate the skeleton. Here we report the first instance of diagnosing Kniest dysplasia with 3D-CT.

Methods. Fetal 3D-CT was performed for a fetus at 28 weeks' gestation after ultrasonography at 24 weeks had shown moderate shortening of the limbs, mild narrow thorax, and polyhydramnios. The imaging parameters were set so as to reduce estimated fetal irradiation dose to 12.39 mGy of the CT dose index volume and 442 of the dose length product.

Results. Fetal 3D-CT revealed dumbbell-shaped femora and platyspondyly with coronal cleft of the lumbar vertebral body. This warranted a diagnosis of Kniest dysplasia and corresponded well with postnatal radiographic findings. In retrospect, however, spinal deformation was somewhat underestimated due to image smoothing associated with image processing in 3D-CT. Genetic testing for *COL2A1* confirmed Kniest dysplasia; i.e., a *de novo* mutation of A–C transversion at the splice acceptor site of the 3′ end of intron 16.

Conclusions. The combined use of 3D-CT with ultrasonography is a power tool for the prenatal diagnosis of congenital skeletal dysplasias.

Keywords: *Kniest dysplasia, skeletal dysplasia, helical CT, three-dimensional, prenatal diagnosis*

Introduction

Kniest dysplasia (OMIM 156550) is an autosomal dominant (AD) skeletal dysplasia characterized by characteristic mid-face hypoplasia and distinctive skeletal changes, including dumbbell deformity of the long bones and platyspondyly with coronal clefts [1]. The exact incidence remains to be determined. The disorder is caused by a heterozygous mutation of the type II collagen gene (*COL2A1*), however, most cases are attributed to sporadic mutations. Reports on the prenatal diagnosis of Kniest dysplasia have been very limited.

The routine use of ultrasonography in obstetric management has apparently increased occasions of encountering fetal skeletal dysplasias. The technical development of ultrasonography has continuously improved the diagnostic accuracy for these disorders *in utero* [2]. However, a precise diagnosis is not necessarily warranted by ultrasonography alone; thus, guidelines for the prenatal diagnosis of fetal skeletal dysplasias have emphasized combined use of multi-modalities, including *in-utero* radiography, fetal magnetic resonance imaging (MRI), three-dimensional ultrasonography, and genetic testing [3]. Recently, fetal three-dimensional helical computed tomography (3D-CT) has been introduced as a powerful tool for the prenatal diagnosis of skeletal dysplasias [4–6]. Accurate diagnosis accomplished with fetal 3D-CT has been shown to help perinatal management and genetic counseling.

We report here on the first case in which fetal 3D-CT warranted a prenatal diagnosis of Kniset dysplasia caused by a *de novo COL2A1* mutation. Fetal ultrasonography at 24 weeks' gestation did not provide optimal images for the definitive diagnosis, while fetal 3D-CT at 28 weeks' gestation did. The diagnosis was postnatally confirmed on radiological and molecular grounds.

Case report

A 23-year-old Japanese primigravida was referred at 24 weeks' gestation because of fetal limb shortening found with ultrasonography. Fetal parameters measured by ultrasonography were biparietal diameter (BPD) 64.4 mm (−0.2 SD), femoral length (FL) 32.6 mm (−3.8 SD), humeral length

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(HL) 30.5 mm (−4.5 SD), fetal trunk area (FTA) 35.9 cm^2, and estimated fetal body weight (EFBW) 756 g (−1.2 SD). The fetal thorax was deemed to be slightly hypoplastic. An amniotic fluid index (AFI) exceeding 27 cm constituted the diagnosis of polyhydramnios at 24 weeks' gestation. Other findings, including abnormal facial and head profiles with a short and flat nose, did not help make the diagnosis definite. The limb shortening was not so severe as to consider a lethal skeletal dysplasia [7], and the overall ultrasonographic findings strongly indicated a diagnosis of achondroplasia. However, polyhydramnios at 24 weeks' gestation was considered uncommon in achondroplasia. Thus, fetal 3D-CT was planned to obtain further information. We also offered chromosomal analysis by amniocentesis to the couple, but they did not agree with genetic examination.

At 28 weeks' gestation, fetal 3D-CT was performed after informed consent. Fetal 3D-CT images were obtained using a 16 slice scanner (SOMATOM Sensation 16-Slice Cardiac CT Scanner; Siemens) with 12.39 mGy of the CT dose index volume (CTDIvol). CT images were obtained using volume rendering with three-dimensional reconstruction (3D-VR) and maximum intensity projection (MIP). The 3D-VR images showed platyspondyly, limb shortening, and a dumbbell appearance of the tubular bones (Figure 1a). MIP images revealed a coronal cleft of the 4th lumber vertebral body and facilitated identification of the dumbbell deformity (Figure 1b, c). Given these findings, a diagnosis of Kniest dysplasia was the most plausible diagnosis. Since Kniest dysplasia is not associated with bone fragility or macrocephaly, we concluded that the vaginal delivery would be possible and safe for the fetus, and the couple also agreed this decision. We then repeated amniocentesis with withdrawal of amniotic fluid to reduce polyhydramnios.

A female neonate was vaginally delivered following spontaneous onset of labor pain at 37 weeks' gestation. Birth weight was 2863 g and length 40.8 cm. Apgar score at 5 min was 6, but the newborn progressed quickly to respiratory distress requiring intubation, and then artificial ventilation was carried out for 10 days. Physical findings included a flat mid-face, depressed nasal bridge, micrognathia with cleft palate, and micromelia. Hearing impairment was noted later on brainstem auditory evoked response. The radiological findings were identical with those on the previous fetal 3D-CT; i.e., broad thorax, platyspondyly, dumbbell appearance of the long bones, coronal cleft of the 4th lumber vertebral body and broad ilia with hypoplasia of the basilar portion (trefoil-shaped pelvis) (Figure 2a, b). However, flat and deformed vertebral bodies with anterior wedging were severer than expected based on the fetal 3D-CT imaging. A diagnosis of Kniest dysplasia was made, but dyssegmental dysplasia Rolland–Desbuquois type was not completely excluded. Genetic testing for *COL2A1* revealed a *de novo* mutation of A–C transversion in the splicing acceptor site of intron 16 (Figure 3) and confirmed the diagnosis of Kniest dysplasia.

Discussion

Ultrasonography has played a central role in the prenatal diagnosis of skeletal dysplasias in the second or third trimesters of pregnancy. However, conventional two-dimensional ultrasonography has provided limited diagnostic success, with only 50–68% of cases being accurately diagnosed [7,8]. The accuracy may decline when we have to address a very rare disorder with no family history [9]. A systemic approach to the fetal skeleton with conventional ultrasonography enables one to identify shortening and/or curvature (bowing) of the limbs, bone fractures, thoracic

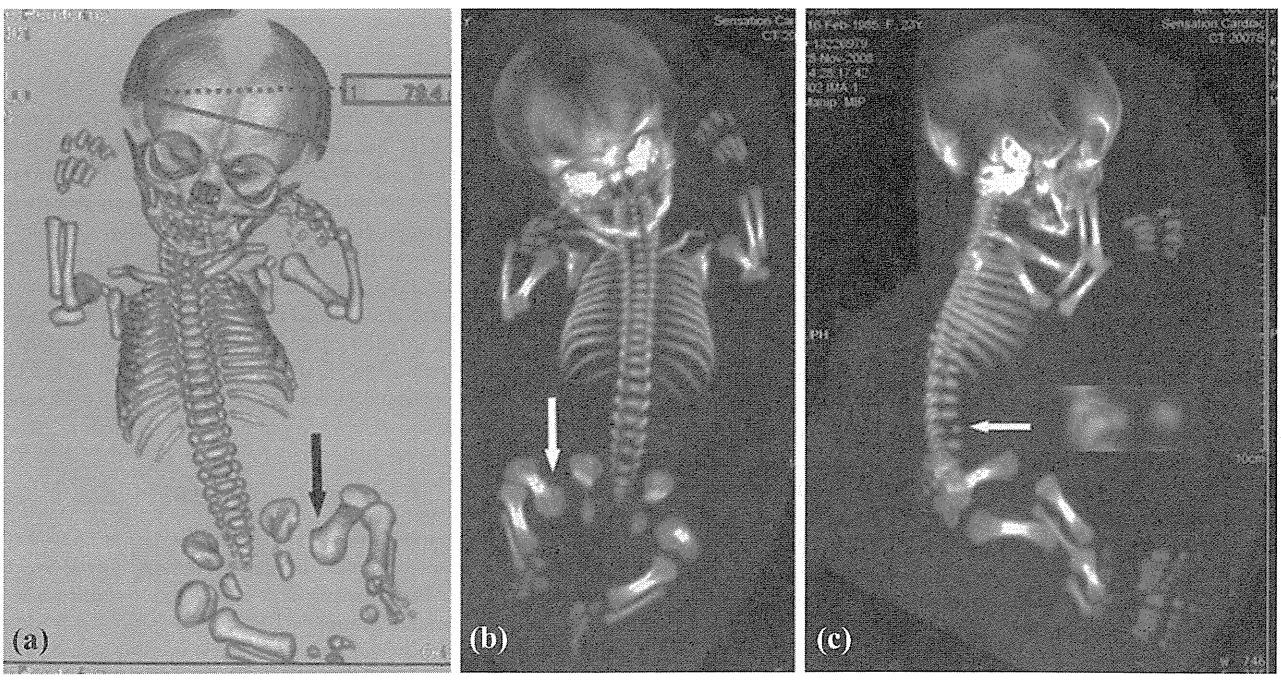

Figure 1. Images of helical CT of the fetus. (a) Frontal image of 3D-VR showing platyspondyly, shortness of the limbs and dumbbell appearance (an arrow) of tubular bones. (b) Frontal image of MIP showing prominent broad metaphyses (an arrow) resulting from splaying, which strongly suggested a diagnosis of Kniest dysplasia. (c) Lateral image of MIP showing coronal cleft in the 4th lumber vertebral body (an arrow) and prominent broad metaphyses. Magnification of the coronal cleft is shown to the right of the arrow.

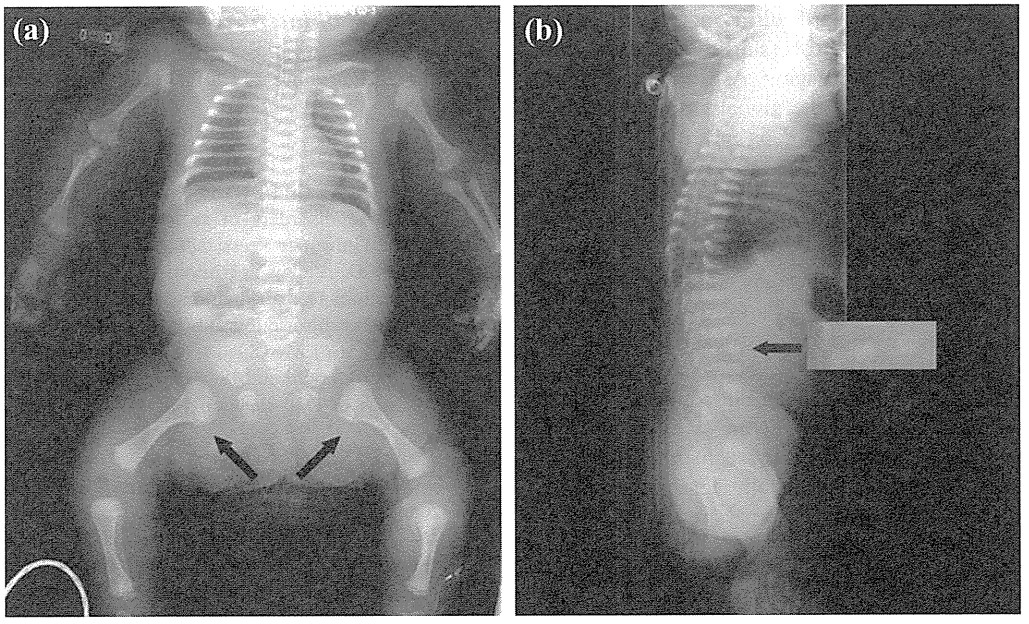

Figure 2. Standard radiographs after birth. (a) Frontal image showing relative broad thorax, platyspondyly, shortness of the limbs, short femora and dumbbell appearance (an arrow) of tubular bones, and broad ilia with hypoplasia of basilar portion (trefoil-shaped pelvis). (b) Lateral image showing prominent splaying of metaphyses of femora, coronal cleft in the 4th lumber vertebral body (an arrow). Magnification of the coronal cleft is shown to the right of the arrow.

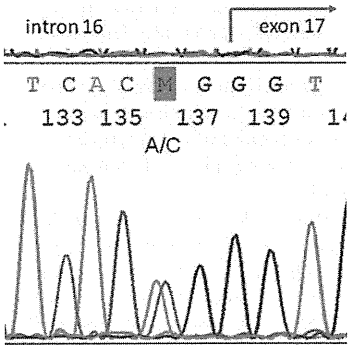

Figure 3. Sequencing analysis of the *COL2A1* gene showing AG to CG mutation in the splicing acceptor site of intron 16. In the absence of this mutation, exon 17 would start at the next codon G (blue arrow).

hypoplasia, presence of scapulae and clavicles, and decreased bone density (hypomineralization) in fetal skeletal dysplasias [10]. However, certain anatomical parts, such as the vertebral column and pelvic bones are difficult to evaluate, and several specific findings, such as stippled epiphyses, may be overlooked [9]. In the present case, ultrasonography displayed only moderate shortening of the limbs, mild thoracic hypoplasia, and abnormal facial profiles and we were not able to make a definitive diagnosis of Kniest dysplasia.

There have been very few reports on prenatal diagnosis of Kniest dysplasia. Only three cases have been reported in the literature [11–13]. Bromley et al. first reported the prenatal sonographic features of an affected fetus in the second trimester, but did not reach a diagnosis of Kniest dysplasia [11]. The diagnostic difficulty was attributed to the incomplete development of the skeletal alterations of the disorder in the second trimester, other than slightly short long bones. Kerleroux et al. also described the difficultly of making an antenatal diagnosis of Kniest dysplasia particularly because the disorder is similar to spondyloepiphyseal dysplasia congenita [12]. Cuiller et al. employed not only standard ultrasonography but also 3D ultrasonography, and they were able to conclude that the fetus had a severe, but non-lethal, skeletal dysplasia, most probably Kniest dysplasia, based on their findings with 3D ultrasonography [13]. However, they also commented on the diagnostic limitation of conventional ultrasonography.

As in the present case, abnormal facial and head profiles with a short and flat nose, no frontal bossing, normal BPD, and mild to moderate shortening of the limbs are common ultrasound findings of Kniest dysplasia in previous reports. By contrast, straight or bent long bones, absence or presence of polyhydramnios, and normal to mild hypoplastic thorax are variable among reported cases. To ascertain the diagnosis of Kniest dysplasia, it is necessary to rely on the skeletal hallmarks of the disorder; i.e., coronal clefts, platyspondyly, severe metaphyseal flaring (a dumbbell-shaped appearance) of the long bones, and broad ilia with hypoplasia of the basilar portion (trefoil-shaped pelvis). In our case, we were able to identify a coronal cleft on the fetal 3D-CT, which pointed to a diagnosis of Kniest dysplasia. Coronal clefts are observed not only in Kniest dysplasia (63%) but also in other bone dysplasias; e.g., in atelosteogenesis (88%), chondrodysplasia punctata (79%), dyssegmental dysplasia (73%), and short rib polydactyly syndrome (73%) [14]. However, 3D-CT clearly delineated other skeletal hallmarks of Kniest dysplasia and eventually led to a definitive diagnosis. In general, ultrasonography often fails to identify platyspondyly even when the examination was conducted with experienced ultrasound operators [10]. Identification of coronal clefts of the vertebral body and exact assessment of pelvic deformity may also be beyond the diagnostic capability of ultrasonography.

We have already reported on the usefulness of fetal 3D-CT, where based on short limbs, hypoplastic lung and macrocephaly, it was possible to diagnose thanatophoric dysplasia [6]. Fetal 3D-CT can also identify fetal skeletal dysplasias more

accurately (73–94%) than standard ultrasonography [8,9]. MIP and 3D-VR are the image-processing techniques most commonly used current 3D-CT imaging. As shown in Figure 1, 3D-VR is helpful in observing the global structure of the skeleton. On the other hand, MIP is useful in precise observation of each bone. In our case, MIP imaging was similar to postnatal radiographs (Figures 1b and 2a). The use of both techniques is recommended since this result in exquisite imaging and reliable interpretation. Two-dimensional multiplanar reformatted imaging (MPR) may also be done to preclude overlooking subtle changes in a fetus. However, we have to emphasize that image smoothing associated with image processing may underestimate certain skeletal changes, such as deformity of the vertebral bodies, as exemplified in our case.

In the present case, we needed to make a differential diagnosis between Kniest dysplasia and dyssegmental dysplasia Rolland–Desbuquois type both prenatally and postnatally. Dyssegmental dysplasia is an autosomal recessive (AR) disorder caused by mutations of the perlecan gene. The radiological hallmarks, including platyspondyly with multiple coronal clefts and dumbbell-shaped tubular bones, overlap with those of Kniest dysplasia. Yet, coronal clefts are more severe in dyssegmental dysplasia than those in Kniest dysplasia [1]. In addition, the vertebral bodies in dyssegmental dysplasia are often irregular in shape and size, the finding of which is termed anisospondyly. In our case irregularly shaped vertebral bodies were somewhat prominent. Since differential diagnosis between dyssegmental dysplasia and Kniest dysplasias is important for genetic counseling because of different modes of inheritance (AR *vs.* AD), we performed a molecular analysis to detect mutations in *COL2A1* in the present child. Heterozygous mutations of *COL2A1* cause several clinical entities collectively termed type II collagenopathies, including Kniest dysplasia, Stickler dysplasia type 1, and spondyloepiphyseal dysplasia with variable severity [15]. Most cases of Kniest dysplasia are caused by exon skipping due to splice-site mutations in the triple helical region of *COL2A1* [15]. The mutation of A–C transversion in the splicing acceptor site of intron 16 found in the present patient was novel and presumed to cause exon 17 skipping (Figure 3).

In conclusion, we have reported a sporadic case of Kniest dysplasia successfully diagnosed *in utero* with fetal 3D-CT. The diagnosis was confirmed by genetic testing after birth. Fetal 3D-CT is a powerful tool in the diagnosis of fetal skeletal dysplasias when ultrasound diagnosis is inconclusive. In addition to the 3D-VR technique commonly utilized with 3D-CT, the MIP technique is essential to evaluate anatomical details in congenital skeletal dysplasias.

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RESEARCH LETTER

Low prevalence of genetic prenatal diagnosis in Japan

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KEY WORDS: prenatal diagnosis; maternal serum marker screening; amniocentesis; chorionic villus sampling

Advances in molecular genetics, cytogenetics, and ultrasound diagnosis have revealed many abnormalities of fetuses *in utero*. In addition to technical advances and economic aspects, ethical considerations influence how prenatal diagnosis is used in communities. The outcome of prenatal diagnoses may be associated with trends in babies born with congenital disorders. We surveyed the number of genetic prenatal diagnoses to examine the trends in prenatal diagnosis in Japan.

In 2002, we conducted a survey on genetic prenatal diagnoses performed in Japan during the previous 5-year period (Sago *et al.*, 2005). This report was the first to explore the trends in prenatal diagnosis in Japan. The study surveyed clinical laboratories nationwide and was assumed to cover most cases of genetic prenatal tests performed in Japan. Adding the results of a recent 6-year survey, we have now completed an 11-year survey for the period between 1998 and 2008. Based on the methods in the previous survey, we sent questionnaires to both commercial and academic clinical laboratories thought to be performing prenatal diagnostic tests and obtained answers regarding the numbers of genetic tests of maternal serum markers, amniocentesis, and chorionic villus sampling (CVS) conducted between January 2003 and December 2008. We combined the data from this new 6-year survey with that of the previous survey conducted between 1998 and 2002 and examined the trends over this 11-year period. Of the 59 responding laboratories, 25 laboratories had actually conducted some sort of prenatal genetic test. Yearly changes in the numbers of maternal serum marker screenings, amniocentesis procedures, and CVS procedures are shown in Table 1. The annual number of maternal serum marker screenings reached a maximum in 1998 and then decreased markedly until 2001, after which time it increased. The annual number of amniocentesis procedures was about 10 000 between 1998 and 2002, increasing by approximately 30% in 2008. On the other hand, the number of CVS procedures was very low.

According to a report by the Ministry of Health, Labor, and Welfare, the Japanese population in 2008 was 127.7 million and the total number of births in 2008 was 1.09 million. The total number of prenatal genetic tests was about 32 000 in 2008. Consequently, only 3% of all pregnant women received prenatal diagnoses based on maternal serum marker screening or chromosome analysis; however, some pregnant women received both maternal serum marker screening and chromosome analysis (i.e. some overlap existed). The rate of prenatal genetic diagnosis is extremely low in Japan, compared with the rates in other advanced countries where the maternal ages are also high, similar to the situation in Japan.

Japanese law permits abortions for maternal economic or health problems but not for fetal abnormalities. Actually, abortions because of fetal abnormalities are, in many cases, performed with maternal health problems given as the reason. Pregnant women may be deterred from receiving a prenatal diagnosis because abortions for fetal abnormalities are not permitted legally and many people believe that abortions are unethical even if a fetus has serious abnormalities.

Maternal serum marker screening was introduced to Japan in 1994 and soon began to be used clinically; however, insufficient genetic counseling for this test facilitated anxiety in pregnant women, creating a social problem. Therefore, in 1999, the Expert Committee on Prenatal Diagnosis of the Sciences Council for Evaluating Advanced Medical Techniques of Japan published the 'View on Prenatal Serum Marker Screening'. This view stated that physicians were not required to give information on this test to pregnant women vigorously and that physicians should not recommend this test. Thereafter, the number of maternal serum screening tests decreased for a few years and remained at about 15 000 annually. However, the number began to increase in 2003, albeit slightly, and reached 18 000 in 2008. A nuchal translucency scan at 11–13 weeks of gestation was also rarely performed, although ultrasonography is performed during the first trimester to check the fetal heartbeat and growth in almost all pregnant women in Japan. The lack of information on prenatal diagnosis provided by doctors

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Table 1—The number of prenatal genetic tests and liveborns in Japan 1998–2008

Year	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Liveborn infant[a]	1 203 147	1 177 669	1 190 560	1 170 662	1 153 855	1 123 610	1 110 835	1 062 530	1 092 674	1 089 818	1 091 150
Pregnant women of ≥35 years[b]	127 442	130 497	141 653	142 784	147 636	157 369	169 516	173 754	192 905	211 711	228 444
Maternal serum screening	21 708	18 312	15 927	15 308	15 627	16 591	16 613	16 279	17 558	17 333	18 209
Amniocentesis	10 419	10 516	10 627	10 070	9 926	11 284	11 261	11 557	11 703	12 458	13 402
Chorionic villus sampling	76	58	96	120	129	65	80	43	36	36	46

[a] The number of liveborn infants has been significantly decreasing (number of infants = 1.200.079.13312 × (year-1998), ($R^2 = 0.85$).
[b] Advanced age pregnancies has been significantly increasing (proportion of over 35 years = 0.10 + 0.01 × (year-1998), $R^2 = 0.96$).

Table 2—Prenatal screening policies and screening rates in industrialized countries

Countries	Screening policies	Total fertility rates[a] (2000/2008)	Screening rates
Australia	W	1.76/1.97	98% (2007)[b]
Denmark	W	1.77/1.89	84.4% (2006)[c]
England and Wales	W	$1.64/1.90_{(2007)}$	88% (2009)[d]
France	W	1.88/2.00	—
Germany	W	1.38/1.38	—
Italy	W	1.26/1.41	—
Switzerland	W	1.50/1.48	—
USA	W	$2.06/2.12_{(2007)}$	—
Netherlands	S	1.72/1.78	—
Spain	S	1.23/1.46	—
Japan	S	1.36/1.37	3%(2008)

W, prenatal screening offered in whole country; S, no national policy but some form of screening in some area.
[a] From United Nations Statistics Division, 2008.
[b] From Genetics Education in Medicine Consortium, 2007.
[c] From Ekelund *et al.* (2008).
[d] From National Down Syndrome Cytogenetic Register, 2009.

is thought to be one of the reasons why relatively few pregnant women receive genetic prenatal diagnosis.

A comparison of the status of prenatal diagnosis between Japan and other advanced countries is difficult because nationwide data are scarce (Boyd *et al.*, 2008). Available data of screening policies and screening rates in industrialized countries are shown in Table 2. Many industrialized countries have a national policy to offer first or second trimester screening for all pregnant women. More than half of pregnant women are assumed to undergo genetic prenatal screening tests in advanced countries (Genetic Education in Medicine Consortium, 2007; Ekelund *et al.*, 2008; National Down Syndrome Cytogenetic Registry, 2009). On the other hand, only 3% of all pregnant women receive genetic prenatal screening or diagnostic tests in Japan and most chromosomal analyses were performed using amniocentesis in the 11-year survey.

Recently, the number of births in Japan has been decreasing yearly and the number of advanced-age pregnancies has been increasing markedly (Table 1). This situation raises the concern that the number of babies born with congenital disorders related to maternal age, such as Down syndrome, may increase. Kajii reported that if the current situation continues, the number of babies born with Down syndrome would reach 3000 per year in 2011, representing a 50% increase over the previous 5-year period (Kajii, 2008).

The present study clarified the genetic prenatal diagnosis trends in Japan over the past 11 years. Although the number of prenatal tests being performed has increased slightly, the rate of prenatal testing in pregnant women remains extremely low at about 3%, and amniocentesis was the main invasive procedure used to confirm a diagnosis. We should carefully observe how these results affect society in terms of the number of

babies born with congenital disorders. The data presented herein provides valuable information for assessing the effect of medical care on society.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

TRPV4-Pathy Manifesting Both Skeletal Dysplasia and Peripheral Neuropathy: A Report of Three Patients

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Heterozygous missense mutations of transient receptor potential vanilloid 4 channel (*TRPV4*) cause a spectrum of skeletal disorders, including brachyolmia, spondylometaphyseal dysplasia Kozlowski type, metatropic dysplasia, parastremmatic dysplasia, and spondyloepimetaphyseal dysplasia Maroteaux type. Similarly, heterozygous missense mutations of *TRPV4* cause a spectrum of peripheral neuropathy, including hereditary motor and sensory neuropathy type IIC, congenital spinal muscular atrophy, and scapuloperoneal spinal muscular atrophy. There are no apparent differences in the amino acid positions affected or type of change predicted by the *TRPV4* mutations responsible for the two disease spectrums; nevertheless, no fundamental phenotypic overlap has been shown between the two spectrums. Here, we report on three patients who had both skeletal dysplasia and peripheral neuropathy caused by heterozygous *TRPV4* missense mutations. The skeletal and neurologic phenotypes of these patients covered the wide spectrum of reported TRPV4-pathies (disease caused by *TRPV4* mutations). The molecular data are complementary, proving that "neuropathic" mutations can cause skeletal dysplasia but also the "skeletopathic" mutations can lead to neuropathies. Our findings suggest that pathogenic mechanisms of TRPV4-pathies in skeletal and nervous systems are not always mutually exclusive and provide further evidence that there is no clear genotype–phenotype correlation for either spectrum. Co-occurrence of skeletal dysplasia and degenerative neuropathy should be kept in mind in clinical practice including diagnostic testing, surgical evaluation, and genetic counseling. © 2012 Wiley Periodicals, Inc.

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INTRODUCTION

TRPV4 (transient receptor potential vanilloid 4 channel; OMIM 605427) is a calcium permeable non-selective cation channel involved in many different cell functions [Everaerts et al., 2010]. *TRPV4* is expressed in diverse tissues [Everaerts et al., 2010]. *TRPV4* mutations have been implicated in autosomal dominant diseases in two systems—skeletal and peripheral nervous [Dai et al., 2010a]. Skeletal dysplasias caused by *TRPV4* mutations include brachyolmia (OMIM 113500), spondylometaphyseal dysplasia Kozlowski type (SMD-K; MIM 184252), metatropic dysplasia (OMIM 156530), parastremmatic dysplasia (OMIM 168400), and spondyloepimetaphyseal dysplasia[Q1] Maroteaux type (SEMD-M; OMIM 184095) [Rock et al., 2008; Krakow et al., 2009; Nishimura et al., 2010]. Peripheral neuropathies caused by *TPRV4* mutations included hereditary motor and sensory neuropathy type IIC (HMSN2C; OMIM 606071) [Auer-Grumbach et al., 2010; Chen et al., 2010; Deng et al., 2010; Landoure et al., 2010; Berciano et al., 2011; Klein et al., 2011], congenital distal spinal muscular atrophy (OMIM 600175) [Auer-Grumbach et al., 2010], and scapuloperoneal spinal muscular atrophy (OMIM 181405) [Auer-Grumbach et al., 2010; Deng et al., 2010].

It is intriguing that dominant *TRPV4* mutations produce phenotypes in two different systems following different modes of development: Enchondral ossification process of the skeleton and degenerative disorder of the peripheral nerve. Even more intriguing is that there are no fundamental differences in the positions and pattern of amino acid changes of the *TRPV4* mutations in the two disease spectrums [Dai et al., 2010a]. In both groups of disorders, the phenotypes are variable within the respective spectrum. However, to our knowledge, no patient has been reported to have both skeletal dysplasia and peripheral neuropathy caused by a *TRPV4* mutation. In none of the *TRPV4*-related skeletal dysplasias is peripheral neuropathy a recognized feature, despite the fact that numerous patients have been reported and many of them received detailed neurological examinations.

Here, we report on three patients manifesting both skeletal dysplasia and peripheral neuropathy as a consequence of heterozygous *TRPV4* mutations.

CLINICAL REPORTS

Patient 1

The girl was a product of normal full-term pregnancy from non-consanguineous marriage of Korean parents. Her birth weight was 3,600 g. She first presented with metatarsus adductus deformity of the right foot at age 6 months. A skeletal survey at age 1 year suggested a diagnosis of a form of spondylometaphyseal dysplasia (SMD). Speech, mental, and fine motor development were within normal limits; however, she was unable to walk independently until age 3 years.

At 4 years of age, she was 100 cm tall (27th centile). A skeletal survey showed platyspondyly with scoliosis, flaring of iliac wings, flat acetabular roofs, and mild metaphyseal widening of the long bones without epiphyseal dysplasia. These findings were compatible with a diagnosis of SMD-K (Fig. 1). She presented with marked waddling gait, seemingly due to pelvic girdle muscle weakness. Gross motor power of the upper extremities was normal. Lower extremity muscles were all atrophic, most visible in the distal muscle groups. Quadriceps power was grade 4/5 by the Medical Research council grading, ankle dorsi-flexor grades 2/5 (right) and 3/5 (left), and ankle plantar flexor grades 3/5 (right) and 4/5 (left). Knee and ankle jerks were physiologic and Babinski sign was absent. At the latest follow-up at age of 6 years, her waddling gait persisted though partially improved but running remained impaired. She did not show any problem in voiding, breathing, or phonation. Whole spine MRI examination did not show any spinal cord lesions or cauda equina compression.

Radiologic findings in her mother at age 33 years included platyspondyly, flaring of iliac bones, and precocious degenerative osteoarthritis of the knees, which were compatible with skeletal dysplasia caused by *TRPV4* mutations (Fig. 2). Her height was 145 cm (below 1st centile). She suffered from intermittent back pain and mild sciatica. The right knee pain developed due to a meniscus tear, which improved after arthroscopic meniscectomy. The activities of daily living were not limited. The father of proposita was 170 cm tall (28th centile) and athletically active without any physical symptom. Apart from the proposita and her mother, there was no family history of skeletal and/or neurologic abnormalities.

Nerve conduction and needle electomyographic studies were performed in the lower extremities, as well as a muscle biopsy as a clinical diagnostic work-up. The motor nerve conduction velocity was definitely decreased in the lower limbs, as the compound motor action potential was absent in both common peroneal nerves. The sensory nerve conduction was normal. Needle electromyography revealed a neurogenic pattern with abnormal spontaneous activities, large amplitude motor unit action potentials in the bilateral biceps femoris, tibialis anterior, and the left gastrocnemius. A histological examination showed grouped atrophy of some fascicles with several scattered atrophic angulated fibers, highly suggestive of neurogenic cause (Fig. 3). The rectus femoris was hypotrophic but remained fleshy in appearance. The vastus lateralis muscle was replaced with fibro-fatty tissue.

Patient 2

A 14-year-old Japanese girl presented with left hip pain after prolonged walking starting at age 13. She was the product of an uncomplicated pregnancy and delivery with birth weight of 3,250 g and birth length of 49 cm. Family history was unremarkable. Her psychomotor development was normal in the first decade of life. Her height was 158 cm (50th centile), and weight 48 kg. Her intelligence was normal. She had reduced visual acuity, but no hearing loss. Physical examination revealed brachydactyly, restriction of the hip movements, and genu valgum. The upper extremities were grossly normal.

Radiographic examination revealed skeletal changes compatible with SEMD-M (Fig. 4). There were slightly flattened vertebral bodies with irregular vertebral plates and narrowing of the intervertebral disc spaces. The hips had dysplastic changes, including

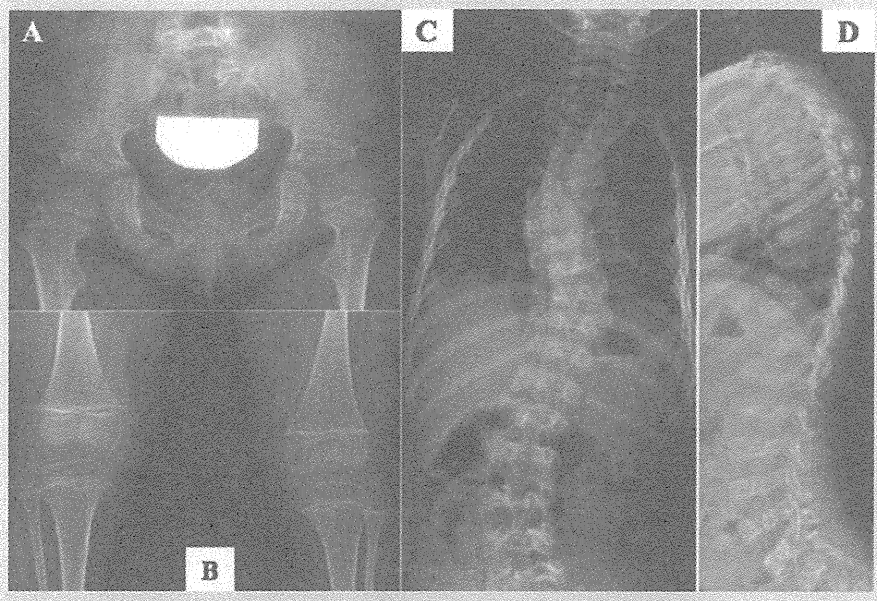

FIG. 1. Radiographs of Patient 1 at age 4 years. A: Pelvis A-P. Flared and short ilia and horizontal acetabulum with supra-acetabular notches. The femoral necks are short and broad with mild metaphyseal irregularities. The femoral heads are ossified well. B: Knee A-P. Mild flaring of the metaphyses of the long bones. The epiphyses are spared. C,D: Anteroposterior (C) and lateral (D) projections of the spine. Scoliosis, marked platyspondyly with overfaced pedicles. These findings are compatible with spondyloepipyseal dysplasia, Kozlowski type.

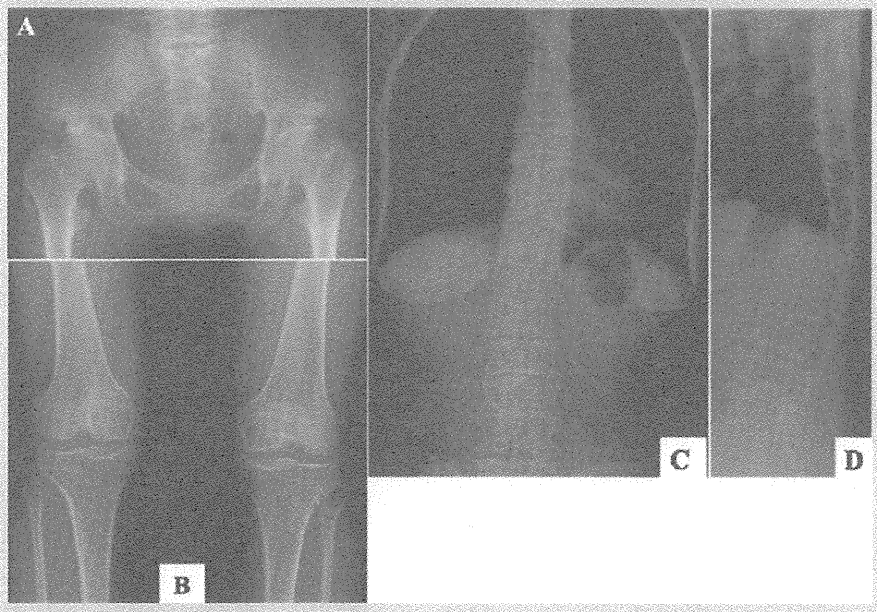

FIG. 2. Radiographs of the mother of Patient 1 at age 33 years. A: Pelvis shows marked flaring of the ilia drooped down to the acetabulum with supra-acetabular notches. The femoral necks are short. B: Knee shows mild flaring of the distal femur and proximal tibial metaphyses. At the medial compartment, the distal femur is flat and the tibial plateau is depressed. C,D: Anteroposterior and lateral projections of the spine show thoracolumbar kyphosis, overfaced pedicles, platyspondyly with elongated vertebral bodies. Degenerative spondylosis ensues uniform narrowing of the intervertebral disc spaces.

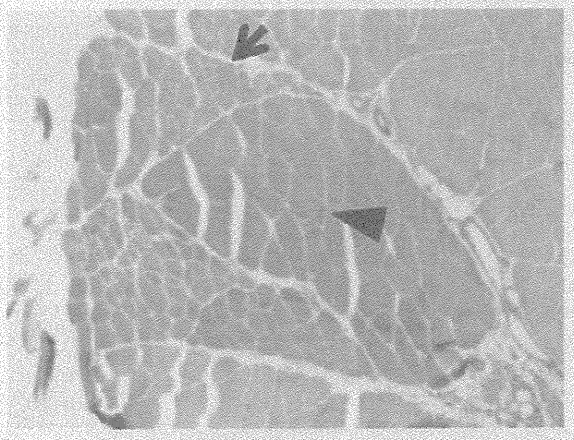

FIG. 3. A histology of muscle biopsy specimen from the rectus femoris of Patient 1 (NADH-TR staining). Grouped angulated fibers (arrow) with scattered atrophied angulated fibers (arrow head) implicate neurogenic muscle atrophy.

short femoral necks with flattened capital femoral epiphyses. The epiphyses of the knees were dysplastic, flattened, and irregular. There were hypoplastic/dysplastic changes in the fingers and toes.

Together with progressive hip pain, she also complained of leg fatigability and had suffered frequent falls. She was referred for neurologic examination and found to have objective muscle weakness. The neck and upper limb muscles were normal. Iliopsoas and quadriceps muscles were grade 4/5, and the other lower limb muscles were grade 5/5. She could not sense vibration but her position sense was normal. Nerve conduction studies revealed that F wave conduction velocity and F wave occurrence were reduced in the tibial nerve (42.1 and 3/16 m/s, respectively), while those in the median and ulnar nerves were unremarkable; sensory nerve conduction velocity (SCV) was also reduced in the sural nerve (39.2 m/s). These data confirmed the presence of a peripheral motor and sensory neuropathy dominant in the lower limbs.

Patient 3

A 5-year-old Argentine girl was referred to one of us because of bilateral clubfoot deformities and for evaluation of skeletal dysplasia. Her 38-year-old father and 36-year-old mother were consanguineous. Her three brothers and two sisters were healthy. The family history included three prior miscarriages and a cousin with dystonia. During pregnancy the mother noticed poor fetal movements. She was delivered at 38 weeks by cesarean due to breech presentation. Her birth weight was 3,600 g. At birth bilateral clubfoot were noted. She had motor developmental delay. She was able to walk at the age of 18 months but showed a waddling gait. She had difficulty in rising from the floor and climbing stairs. Her development was otherwise normal. A skeletal survey during infancy showed findings compatible with SMD-K (Fig. 5).

At the age of 5 years, her height was 103 cm (25th centile) and her weight was 15.0 kg (10th centile); she had normal teeth and palate and mild scapula alta. Her cognitive development was normal. Neurological examination showed no ophthalmoparesis, but slight facial weakness. She had a waddling gait and positive Gowers' sign. The neck muscles, upper limbs, proximal predominantly, and lower limbs were weak (all grade 3/5). Hypotrophy of the shoulder girdle and the lower limb distal to the knees as well as distal hyperlaxity were observed. Achilles tendon reflexes were decreased bilaterally. Brain and spine MRIs did not show any lesions. Serum CPK was normal.

A needle electromyography showed fibrillation and positive sharp-wave potentials at rest, signs of denervation, and spontaneous activity. There was generalized reduction of voluntary recruitment of motor unit action potentials and potentials of increased amplitude and duration (>4 mV). Motor and sensory nerve conduction studies for four limbs were normal with normal action potential latencies and amplitudes.

METHODS

TRPV4 Mutation Analysis

Genomic DNA was extracted by standard procedures from peripheral blood of the patients and/or their family members after informed consent. The exon sequences of *TRPV4* and their flanking intronic sequences (The GenBank reference sequence: NM_021625.3) were amplified by PCR from genomic DNA. PCR products were directly sequenced using ABI Prism automated sequencers (PE Biosystems). Primer sequences and PCR protocols are previously reported [Dai et al., 2010b]. The study was approved by the ethical committee of RIKEN and participating institutions.

In silico Protein Analysis

The atomic coordinates of the X-ray crystal structure of chicken TRPV4 protein [residues 132–383; Landoure et al., 2010], and human TRPV2 protein [residues 69–319; McCleverty et al., 2006] were obtained from the Protein Data Bank (pdb 3jxiA and pdb 2f37A); a model of the N-terminal cytoplasmic domain (residues 146-396) of human TRPV4 was obtained using the semi-automated modeling server SWISS-MODEL [Schwede et al., 2003] as described previously [Lausch et al., 2009]. TRPV protein sequences were obtained from Ensembl (http://www.ensembl.org) and alignments were generated with clustalW [Thompson et al., 1994].

RESULTS

Patient 1 and her mother had a heterozygous c.832G > A (p.E278K) mutation as previously reported [Dai et al., 2010b]. Dosage tests by the multiplex ligation-dependent probe amplification for exons 7 and 8 of the *SNM1* gene were performed in Patient 1 to exclude possibility of associated spinal muscular atrophy. No deletions involving exons 7 and 8 of *SNM1* were found.

Patient 2 had a heterozygous c.2396C > G (p.P799R) mutation. The mutation has previously been identified in two patients with metatropic dysplasia [Dai et al., 2010b].

Patient 3 had a heterozygous c.649G > T (p.A217S) transversion that was not found in her parents nor in 121 controls of mixed ethnicities. This novel mutation predicts an amino acid substitution in the N-terminal cytoplasmic domain of the TRPV4 protein,

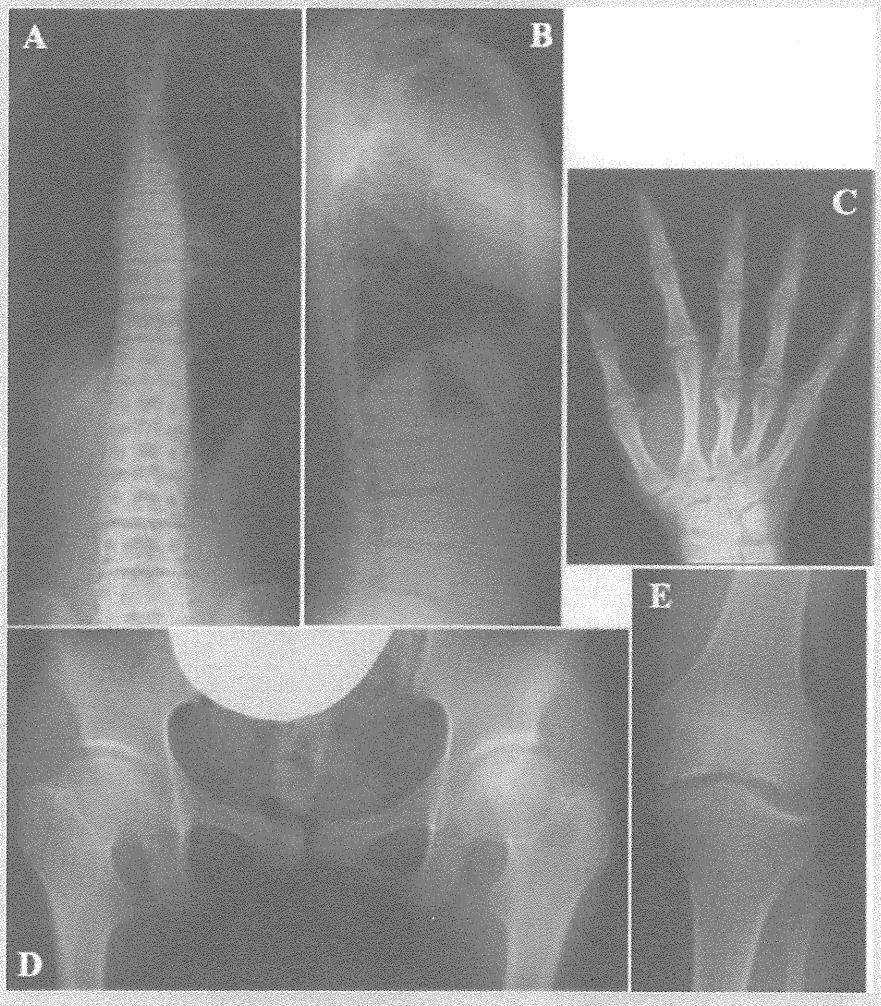

FIG. 4. Radiographs of Patient 2 at age 14 years compatible with spondyloepimetaphyseal dysplasia Maroteaux type. A,B: Spine A-P (A) and lateral (B). Slightly flattened vertebral bodies with irregular vertebral plates and narrowing of the intervertebral disc spaces. C: Hand A-P. brachydactyly. D: Pelvis A-P. Dysplastic changes in the hips, including short femoral necks with flattened capital femoral epiphyses. E: Knee A-P. The epiphysis is dysplastic, flattened, and irregular.

affecting the second ankyrin repeat domain, just 16 amino acids N-terminal to the stretch where all but one reported "neuropathic" TRPV4 mutations cluster (Fig. 6A). The simulation in a model based on the crystal structure of the TRPV4 protein (amino acids 146–396, pdb 3jxiA) indicated a disruption of a short helical stretch by the non-polar to polar substitution (Fig. 6B). Alanine 217 is conserved through evolution of TRPV4 from fish to human (not shown) and is even highly conserved among TRPV family proteins (Fig. 6C). Changing this residue causes misfolding of the whole N-terminal cytoplasmic domain including the six ankyrin repeats, and thus is likely to alter TRPV4 function.

DISCUSSION

A full radiographic survey and detailed neurologic examinations of Patient 1 indicated the diagnosis of SMD-K and axonal type peripheral neuropathy compatible with HMSN type II for the skeletal and neurologic phenotypes, respectively. The neurologic phenotype in the patient did not completely match with any neuropathies previously reported in association with *TRPV4* mutations [Auer-Grumbach et al., 2010; Berciano et al., 2011; Chen et al., 2010; Deng et al., 2010; Klein et al., 2011; Landoure et al., 2010]. However, TRPV4-associated neuropathies are diverse in manifestation. Hence, it is probable that the axonal type peripheral neuropathy in the proposita was caused by the *TRPV4* mutation.

In the family of Patient 1, skeletal dysplasia associated with *TRPV4* was inherited from mother to daughter, but the peripheral neuropathy was present only in the daughter. This may be due to incomplete penetrance which has been previously described in TRPV4-associated neuropathy [Berciano et al., 2011]. In contrast, penetrance of the skeletal dysplasia phenotype seems high,