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ORIGINAL PAPER

Proportion of malformations and genetic disorders among cases encountered at a high-care unit in a children's hospital

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Abstract Genetic disorders and birth defects account for a high percentage of the admissions in children's hospitals. Congenital malformations and chromosomal abnormalities are the most common causes of infant mortality. So their effects pose serious problems for perinatal health care in Japan, where the infant mortality is very low. This paper describes the reasons for admissions and hospitalization at the high-care unit (HCU) of a major tertiary children's referral center in Japan. We retrospectively reviewed 900 admission charts for the period 2007–2008 and found that genetic disorders and malformations accounted for a significant proportion of the cases requiring admission to the HCU. Further, the rate of recurrent admission was higher for patients with genetic disorders and malformations than for those with acquired, non-genetic conditions. Over the past 30 years, admissions attributed to genetic disorders and malformations has consistently impacted on children's hospital and patients with genetic disorders and malformations form a large part of this facility. These results reflect improvements in medical care for patients with genetic disorders and malformations and further highlight the large proportion of cases with genetic disorders, for which highly specialized management is required. Moreover, this study emphasizes the need for involvement of clinical geneticists in HCUs at children's hospitals.

Keywords Malformation · Genetic disease · High-care unit · Children's hospital · Mortality

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Introduction

Genetic disorders and birth defects account for a high percentage of the admissions to children's hospitals [4, 13]. In 2008 [5], the Ministry of Health, Labor and Welfare in Japan reported that congenital malformations, chromosomal abnormalities, and genetic diseases are the leading causes of death in children during the first year of life. As per that report, 999 infants under the age of 1 year died of congenital malformations and chromosomal abnormalities; this corresponds to 35.7% of the total number of deaths in this age group. Since 1985, congenital malformations and chromosomal abnormalities have remained the leading causes of infant mortality in Japan [5]. Indeed, in USA it

has been found that patients with genetic disorders had a greater need for hospital admission and were hospitalized for longer durations than were those without genetic disorders [14].

However, recent advances in treatment are likely to improve the survival of individuals with congenital malformations, which, in turn, is likely to increase the rates of readmission to pediatric intensive care units (PICUs) [16]. Several studies have assessed the role of genetic disorders in pediatric mortality and hospitalization [2, 6, 7, 16]. Congenital malformations and chromosomal abnormalities pose serious challenges for perinatal health care in this country, as they are the leading contributors to the infant mortality rate in Japan.

In this study, we assessed the reasons for admissions and hospitalization to the high-care unit (HCU) of a major tertiary children's referral center in Kanagawa Prefecture, Japan, and compared our findings to those of a study of this unit 30 years ago. To elucidate the impact and contribution of birth defects and genetic diseases on pediatric hospitalization, we studied the reason for hospitalization, underlying diagnoses, and duration of hospitalization in this children's hospital in Japan.

Materials and methods

Permission for the study was obtained from the Ethical Committee of our medical center.

We retrospectively analyzed the cases of children hospitalized at the HCU of Kanagawa Children's Medical Center (KCMC) between June 2007 and December 2008. KCMC is a major tertiary children's referral center for pediatric cardiology, surgery, and cancer cases and serves a large area in Kanagawa Prefecture, Japan. It has an institute for the severely handicapped, a PICU, a neonatal intensive care unit, and an HCU. In contrast to the PICU, which admits patients who have undergone cardiovascular or neurosurgery, the HCU specializes in pediatric patients with other acute conditions. All of the patients were included if they were admitted to the HCU from the emergency room, operating room, or inpatient ward. KCMC, with 419 beds, is the only specialized pediatric hospital in Kanagawa Prefecture, where the total number of births is 80,000 annually [8, 9]. About 8,500 patients (male/female, 1:1) were admitted to KCMC in 2007, and the average of hospital stay was 15.3 days.

We summarized and reviewed the medical charts of all patients admitted to the HCU. The charts and summaries were reviewed for age, sex, duration of hospitalization, underlying disease, and reason for admission. Subcategories were created for the underlying diseases and reason for admission.

The underlying disease was classified into two main categories: genetic conditions and acquired (non-genetic) conditions. Genetic conditions were considered to include chromosomal abnormalities, recognizable malformation and dysplasia, multiple malformations, isolated malformations (e.g., those related to the heart, central nervous system (CNS), and respiratory and gastrointestinal tracts), other single-gene defect-related conditions, mitochondrial diseases, and metabolic disorders (Table 1). All cases of chromosomal abnormalities and multiple malformations were examined using standard karyotyping. Cases of recognizable malformation/dysplasia were ascertained by clinical dysmorphologists (H.Y., N.F., and K.K.). Acquired conditions were considered to include perinatal complications, trauma, neoplasm, and sequelae of severe infectious conditions.

The reasons for admission were classified as problems of the respiratory system, CNS, heart, gastrointestinal tract, kidneys and urinary tract, infectious diseases, post-operative management, and unknown condition. Those cases that did not fall into these categories were placed into a category called "others."

Statistical analyses were performed to compare the duration of hospitalization and the age distribution, using StatView version 5.0 (SAS Institute, Inc; Cary, NY). Categorical data were reported as counts and percentages, and continuous data as mean (SD) or median values. Statistical differences for categorical variables were determined by using chi-squared analyses. Median differences were compared by Mann–Whitney *U* test.

Results

A total of 900 admissions, consisting of 687 individual cases with 200 recurrent admissions, were reviewed. Sixteen admissions were excluded from the study because of insufficient information regarding the underlying causes for admission.

The median age at admission was 3.5 years (range, 1 day–32.5 years), and the sex ratio was 1.36 (396 males and 291 females). The median lengths of hospitalization in the HCU were 4 days. Table 2 shows the distribution of the 884 admissions across the different categories of causes for admission. Most patients were admitted for common medical problems, including respiratory problems, post-operative management, and CNS problems. Of the 298 admissions for respiratory problems, most cases involved respiratory infection, including pneumonia and bronchitis. Admissions for post-operative management accounted for 30.7% cases (271 of 884 admissions), while CNS problems such as convulsions, encephalitis, and meningitis accounted for 16.3% (144 of 884 admissions).

Table 1 Definitions of categories

Category	Examples
Chromosomal syndromes	Down syndrome, trisomies 13 and 18, cri du chat syndrome, and Wolf–Hirschhorn syndrome
Recognizable malformation/dysplasia	22q11.2 deletion syndrome, CHARGE syndrome, and VATER association, Lowe syndrome, achondroplasia, Crouzon syndrome, Noonan syndrome, and Treacher–Collins syndrome
Multiple malformations	
Isolated malformations	
Congenital heart diseases	VSD ASD, AVSD, TGA, and DORV
Central nervous system malformations	Schistorrhachis, hydrocephalus, and meningoencephalocele
Gastrointestinal malformations	Diaphragmatic hernia, biliary atresia, and congenital intestinal obstruction
Respiratory system malformations	CCAM and tracheal stenosis
Other isolated malformations	Cleft palate and cleft lip
Single-gene defect	Metabolic diseases, spinal muscular atrophy, and spinocerebellar degeneration
Mitochondrion	

The classification of the underlying conditions of the 687 patients is shown in Table 3. In 13 cases, the data for identifying the underlying disease were insufficient (e.g., charts were missing). These cases were categorized as "unknown condition." Of the total 687 patients, 372 (54.1%) had genetic disorders and the remaining 302 (44.0%) had acquired conditions unrelated to genetic disorders, including perinatal complications, neoplasm, and trauma. Among the 372 patients with genetic disorders, 72 had chromosomal abnormalities, with Down syndrome (29 cases) being the most common underlying disorder. Seventy patients had recognizable malformations and dysplasia, with conditions such as osteogenesis imperfecta, 22q11.2 deletion syndromes, CHARGE syndrome, and VATER association. Multiple malformations with unrecognizable patterns were present in 38 cases while isolated malformations, including CNS malformation, congenital heart disease, and gastrointestinal malformation were present in 160 cases.

We also summarized the reasons for the total of 884 admissions, according to the underlying condition (genetic or acquired). Of these admissions, 200 were readmissions. Patients with genetic disorders and malformations had a greater tendency to be hospitalized repeatedly as compared with those with acquired conditions (Fig. 1). In both genetic and acquired condition categories, respiratory disease, post-operative management, and CNS problems were the major medical problems leading to admission.

We further compared age distribution and the lengths of hospitalization between the groups with genetic and acquired disorders (Table 4). The patients with genetic

Table 2 Medical problems for admission (N=884)

Causes for admission	Number	Percent
Respiratory problems	298	33.7
Post-operative management	271	30.7
CNS problems	144	16.3
Gastrointestinal problems	35	4.0
Cardiac diseases	23	2.6
Other infectious state	23	2.6
Examination	21	2.4
Kidney and urinary tract problems	14	1.6
Other	55	6.2
Total	884	100.0

Table 3 Classification of underlying diseases in 678 patients

Underlying diseases	Number	Percent
Genetic disorders and malformations (subtotal)	372	54.1
Chromosomal abnormalities	(72)	10.5
Recognizable malformation/dysplasia	(70)	10.2
Multiple malformations	(38)	5.5
Isolated malformations	(subtotal:160)	23.3
Central nervous system malformation	(71)	10.3
Congenital heart disease	(35)	5.1
Gastrointestinal malformation	(32)	4.7
Respiratory system malformation	(9)	1.3
Other isolated malformations	(13)	1.9
Single-gene defect	(26)	3.8
Mitochondrion	(6)	0.9
Acquired non-genetic conditions (subtotal)	302	44.0
Perinatal complications	(66)	9.6
Neoplasm	(38)	5.5
Trauma(non-accidental and accidental)	(27)	3.9
Infection	(16)	2.3
Other	(155)	22.6
Unknown	13	1.9
Total	687	100.0

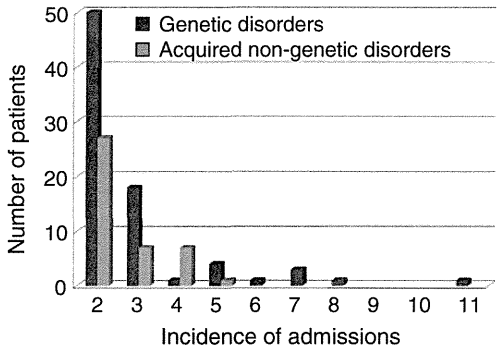

Fig. 1 Comparison of the incidence of admission between the groups with genetic disorders and acquired disorders. In both groups, a total of 200 patients were readmitted. The group with genetic disorders generally required frequent readmission

disorders were significantly younger than those with acquired conditions (median age, 2.0 vs. 4.9 years; $P<0.0001$). There is no significant difference in the length of hospitalization between the patients with genetic disorders and those with acquired conditions (median, 4 vs. 4 days; $P=0.26$), but some patients with genetic disorders had much longer hospitalization (mean, 13.0 vs. 7.0 days; $P=0.007$; range, 1–979 days). Among the reasons for admission, respiratory problems tended to have a longer duration of hospitalization for patients with genetic disorders than for those with acquired conditions (median, 7 vs. 5 days; $P=0.17$).

Discussion

Our study shows that genetic disorders and malformations account for a significant proportion of cases requiring admission to the HCU. Additionally, the rate of recurrent admission was higher among patients with genetic disorders and malformations than among those with acquired non-genetic conditions. This finding is in agreement with those of previous reports for other countries [4, 13].

Several studies from different countries have previously suggested that genetic conditions and malformations and the associated mortality and morbidity have a significant impact on the cost burden for society and the patients' families. Cunniff et al. reported that 19% of deaths in a PICU were in cases of heritable disorders [1]. Stevenson and Carey reported that the 34.4% of deaths in a children's hospital were due to malformations and genetic disorders [15]. On the basis of a population-based study, Yoon et al. reported that the overall rate of hospitalization was related to birth defects and genetic diseases, and varied with age and race/ethnicity [16]. McCandless et al. reported the enormous impact of genetic disease on inpatient pediatrics and the health care system in both admission rates and the total hospital charges [11]. These studies emphasize the importance of understanding the impact that genetic diseases have on mortality and healthcare strategies [15]. Furthermore, it is also clear that early recognition of the underlying disorders is necessary for optimal management of patients with genetic disorders.

Our study highlights another aspect related to the impact of genetic disorders and malformations. In 1981, Matsui et al. analyzed the cases of 18,736 children of total admission during 1975–1979 to KCMC and found that 44% had genetic disorders and malformations [10]. Although our study period and ward are limited to those in the HCU, the patients with genetic disorders and malformations had consistently significant impact in KCMC during the ensuing three decades. Further, it emphasizes that medical care for acute conditions and surgical procedures frequently requires highly specialized knowledge of unusual disease conditions and should be provided in consultation with specialists such as clinical geneticists.

Table 4 Comparison of patients with genetic disorder vs. acquired condition on ages at admission and lengths of stay

	Genetic disorders		Acquired conditions		
	Median (range)	*n*	Median (range)	*n*	*P*
Ages	2.0 years (1 day–27.0 years)	372*	4.9 years (9 days–32.5 years)	302*	<0.0001
Length of hospitalization (days)					
Respiratory problem	7 (1–979)	182	5 (1–97)	109	0.17
CNS	4 (1–54)	73	4 (1–207)	68	0.61
Cardiovascular	4 (2–11)	13	4 (2–24)	8	0.94
Gastrointestinal	5.5 (1–37)	22	5 (2–15)	12	0.60
Kidney and urinary tract	3 (2–12)	5	8 (2–12)	9	0.32
Sepsis	3.5 (2–9)	14	7 (2–20)	9	0.19
Post-operative care	2 (1–49)	174	2 (1–62)	93	0.18
Total	4 (1–979)	518	4 (1–207)	366	0.26

*For the patients who have recurrent admissions, the only first admission was calculated

Although the strategies for management of respiratory infection, by means of newly developed antibiotics and mechanical ventilators, and surgical intervention for infants with malformations, have improved, the general strategies for the medical treatment of genetic disorders and malformations remain to be clarified. Hall commented on the report by Yoon et al. [16] and emphasized the significance of basic research on the human genome and developmental genetics [3]. As shown in Table 2, genetic disorders and malformations include rare diseases, which, although uncommon, remain an important public-health issue and a challenge for the medical community [12].

Our study had the limitations of genetic studies and evaluation in cases with multiple malformations and other isolated malformations. The underlying conditions of most patients in this study were ascertained by clinical geneticists, but high-resolution genome analysis with arrays using comparative genomic hybridization was applied in only limited cases. Recently, research attention has focused to a large extent on rare genetic disorders and Mendelian diseases, because of their significant effect on human health, with the aim of identifying disease-related genetic variations. Re-evaluation and classification of underlying disorders, especially in the case of multiple congenital anomalies in undiagnosed patients, are required for further analysis.

Another limitation of our study is estimation of the financial burden of the group of patients with a genetic background. McCandless et al. showed that the disorders with genetic determinant account for 81% of the total hospital charges [11]. Their results are consistent with those of Hall et al. in 1978 [4]. Further analysis of financial burden in our study may provide useful information for improvement of health care systems.

In conclusion, we report here the proportion of genetic disorders and malformations among cases encountered at the HCU of a tertiary children's medical center in Japan. Over 30 years, the proportion of admissions attributed to genetic disorders and malformations has impact and currently accounts for more than half of admissions to this facility. These results firstly indicate improvements in medical care for patients with genetic disorders and malformations and further highlight the large proportion of cases with genetic disorders. As these cases require highly specialized management, the involvement of clinical geneticists in HCUs at children's hospitals is crucial. Eventually, a better fundamental understanding of genetic disorders and malformations may lead to further improvements in medical care and may reduce the impact of these conditions on the patients and their families.

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Conflict of interest The authors declare no conflict of interest.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Trends in Occurrence of Twin Births in Japan

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The rise in the rate of multiple births since the 1980s is due to the effect of advanced maternal age and increased use of assisted reproductive technology (ART). To determine the trends of prevalence in twin births, we studied the data of a population-based birth defects monitoring system during 26 years in Kanagawa Prefecture, Japan. A total of 15,380 twins from 7,690 deliveries were ascertained from 990,978 births in the Kanagawa Birth Defects Monitoring Program (KAMP) during 1981–2008. From the start of KAMP in 1981, the incidence of twin births had been consistently increasing from 57.0 to 98.6 per 10,000 deliveries until 2003, but after this time, the incidence declined to 78.5 in 2007. While the rate of monozygotic twins has been stable (~40 per 10,000 deliveries) after 1990, that of dizygotic twins increased from 25.3 to 57.3 per 10,000 deliveries until 2002, and recovered to 40.1 in 2007. These results showed the most recent tendency of twin births and indicated that the single embryo transfer method can provide protection and reduction of perinatal risk caused by multiple births.

Key words: assisted reproductive technology (ART); twin; Kanagawa Birth Defects Monitoring Program (KAMP); zygosity

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INTRODUCTION

Multiple births including twin births have several implications for maternal and child health care. Twin pregnancy is associated with an increased incidence of anomalies [Bahtiyar et al., 2007; Glinianaia et al., 2008; Hardin et al., 2009a], a higher risk of perinatal mortality, and preterm births with low birth weight [Helmerhorst et al., 2004; McDonald et al., 2005] compared with singleton pregnancy. A tendency for an increasing rate of twin delivery has been observed in 14 out of 16 countries in Europe, Canada, Australia, Singapore, and Hong Kong [Imaizumi 1998]. This tendency has also been observed in Japan [Imaizumi 2000]. The rise in the rate of multiple births is due to the effect of advanced maternal age and increased use of assisted reproductive technology (ART) [Bondel and Kaminski, 2002]. In the USA and Europe, between 20 and 30% of deliveries following ART are twin births compared with 1% following spontaneous conception [Andersen et al., 2008; Wright et al., 2008]. However, the rate of twin pregnancies in the USA has stabilized at 32 per 1,000 births in 2006 [Chauhan et al., 2010]. In Australia, recent data indicated that the proportion of twin deliveries decreased in 2006 [Wang et al., 2008].

To determine the trends of prevalence in twin births, we studied the data of a population-based birth defects monitoring system during 26 years between 1981 and 2008 in Kanagawa Prefecture, Japan. Kanagawa Prefecture, which is adjacent to Tokyo, includes Yokohama City with a total population 3,687,000. To investigate the effects of ART, we analyzed the data of twins according to the zygosity during the study period.

MATERIALS AND METHODS

A total of 15,380 twins from 7,690 deliveries were ascertained from 990,978 births in the Kanagawa Birth Defects Monitoring Program (KAMP). This program has been in operation since October 1981 as the first population-based monitoring system in Japan. Details of KAMP are described elsewhere [Kuroki et al., 1982; Kuroki and Konishi, 1984, 1992; Kuroki, 1988; Kurosawa et al., 1994; Yuan et al., 1995]. KAMP covers one-half of the total births (40,000 births annually) in Kanagawa Prefecture. All live births and stillbirths are screened for 44–48 marker malformations (only surface anomalies), arranged in 10–11 groups, and they are examined by general obstetricians or occasionally by general pediatricians within 7 days after birth. During the study period between 1981 and 2008, the KAMP was divided into four stages according to a minor modification in marker anomalies and registration systems. The first two stages, for 1981–1983 and 1984–1988, had total birth registration systems including all the malformed infants, normal

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infants, and all multiple births. However, in the last two stages, 1989–2000 and 2001–2008, all malformed infants as well as all multiple births were registered with two consecutive normal infants. Information on zygosity is not available in the KAMP, and therefore, we used Weinberg's differential rule for zygosity estimation [Fellman and Eriksson, 2006; Hardin et al., 2009b]. The incidence of twin births was defined as the number of twin pairs per total deliveries.

RESULTS

During the period of analysis, the incidence of malformed infants was 0.88% in live births and 17.24% in stillbirths. The sex ratio was 1.05. From the start of KAMP at 1981, the incidence of twin births had been consistently increasing from 57.0 to 98.6 per 10,000 deliveries until 2003 (Fig. 1). This tendency is consistent with the results of previous studies [Imaizumi, 1998, 2000]. The incidence of twin births peaked at 98.6 per 10,000 deliveries in 2003, but after this time, the incidence declined to 78.5 per 10,000 deliveries in 2007. The incidence of monozygotic twins fluctuated during the first 10 years, but after 1990 the incidence was stable at 40 per 10,000 deliveries. The incidence of dizygotic twins increased from 25.3 to 57.3 per 10,000 deliveries in 2002, but rapidly decreased to 40.1 in 2007, while the incidence of monozygotic twins was stable (Fig. 2). These results indicated that the incidence of twins is directly affected by the rate of dizygotic twins, and that the incidence of dizygotic twin births has already reached its peak, at least in the urban area of Japan.

DISCUSSION

Our study found that during the last 20 years, the incidence of twin births increased from 57 to 98 per 10,000 deliveries, but after it reached a peak in 2003, it recovered to 78.5 per 10,000 deliveries in 2007. Our study demonstrated that the trend in twin births was affected by the incidence of dizygotic twins. The incidence of monozygotic twins was stable at 40 per 10,000 deliveries, while that of dizygotic twin births attained a peak in 2002 with 57.3 per 10,000 deliveries, and it declined to 40.1 after this time. To the best

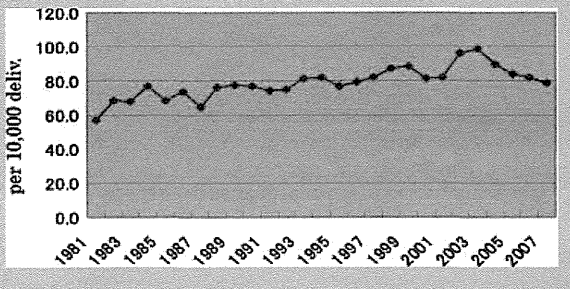

FIG. 1. Trends of the incidence of total births of twin pairs per total deliveries in Japan. The rate of twin births consistently increased from 57.0 per 10,000 deliveries in 1981 to 98.6 in 2003, but after this time, the rate recovered to that of the 1990s.

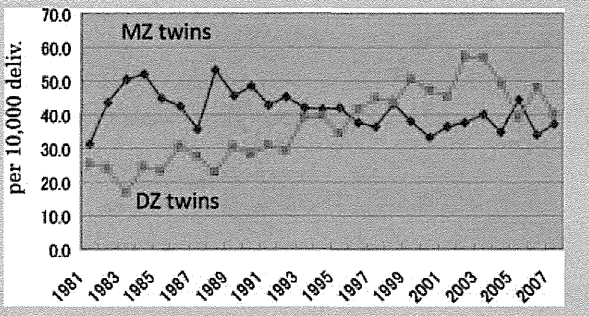

FIG. 2. Comparison of the rate of twin births between monozygotes and dizygotes. In the first 10 years, the fluctuation was remarkable in the rate of births in monozygotic twins for unknown reasons, but during the last 20 years, the rate was stable at approximately 40 per 10,000 deliveries. However, the rate of dizygotic twins consistently increased to reach a peak in 2003, and it then rapidly decreased.

of our knowledge, this is the first report describing the trends of a decrease in the rate of twin births in Japan. Because the rates of monozygotic twins are thought to be constant throughout the world, our results on the tendency of the rates of monozygotic twins have implication of the accuracy of the study. In the USA, between 1980 and 2006, the rate of twin pregnancies consistently increased from 18.9 to 32.1 per 1,000 births [Chauhan et al., 2010]. However, the rapid rise appeared to end in 2004 and the rate stabilized in 2006. A rise in the prevalence of twin births has also been observed in Austria, Finland, Norway, Sweden, Canada, Australia, Hong Kong, Israel, and Singapore [Imaizumi, 1997]. The rate of twin births in these countries stabilized between 2004 and 2006, and recent trends of a decreasing rate has been reported in some countries [Wang et al., 2008].

Clearly, the use of ART has contributed to the changes in the rate of twin pregnancies [Wright et al., 2008; Hansen et al., 2009]. ART twins have a greater risk of adverse perinatal outcome including preterm birth, low birth weight, and cerebral palsy compared with spontaneously conceived twins and singletons [Hansen et al., 2009]. The use of single embryo transfers reduces multiple birth rates and the risks of these adverse outcomes following ART. According to a report from the European Society of Human Reproduction and Embryology, compared with the number of cycles in 2003, fewer embryos were transferred in Germany in 2004, but there were still huge differences between countries [Andersen et al., 2008]. This transfer policy had a considerable impact in Belgium, Finland, Sweden, and several other countries [Andersen et al., 2008], and therefore, a reduced rate of twin births may be observed within a few years in these countries. In the case of Japan, the reduction of the rate was rapid, but a stable rate was not observed at the end of the study period. The rate of twin births may be stabilized when there is a balance between maternal age distribution in reproductive generation and establishment of technical standardization of single embryo transfer. Further analysis on the rates of multiple births based on the population-based monitoring system is required to determine the impact of ART.

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death for type II and type III patients was 10.7 months and 17.6 months, respectively.[6] Our patient had a very severe form of the disease and died when she was 8 months old. Immunoblot analysis of DBP revealed the absence of the 79 and 45 kDa bands of DBP with trace amounts of the 35 kDa component which strongly suggests that the proband can be classified as a type 1 deficient patient.

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Miller syndrome with novel dihydroorotate dehydrogenase gene mutations

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Key words dihydroorotate dehydrogenase (*DHODH*) gene, Miller syndrome, postaxial acrofacial dysostosis.

Miller syndrome (postaxial acrofacial dysostosis; OMIM #26375) was described by Miller *et al.* in 1979;[1] it is characterized by postaxial limb deficiency, cup-shaped ears, and malar hypoplasia. The etiology of this syndrome, which is the mutation of the dihydroorotate dehydrogenase (DHODH) gene, was established in 2010.[2] Here we report a Japanese girl with Miller syndrome, probably the first case in Japan, with novel compound heterozygous mutations of the *DHODH* gene.

Case report

The patient, a 2-year-old Japanese girl, was the first child of nonconsanguineous healthy parents. The pregnancy course was uneventful. The mother and father were 20 and 25 years old, respectively, when the girl was born. She was born at 38 weeks of gestational age by normal vaginal delivery with weight and length of 3480 g (+1.0 SD) and 50.0 cm (+0.6 SD), respectively. Her weight was 10.5 kg (+1.0 SD) and height 77.2 cm (mean) at the age of 15 months. At the age of 2 years and 5 months, her weight was 13.0 kg (+ 0.7 SD), height 87.6 cm (mean), and head circumference 46.7 cm (–0.7 SD). She was referred to a pediatric department because of her limb anomalies.

She had mild micrognathia, mild malar hypoplasia, sparse eyebrows and eyelashes, hypertelorism, down-slanting short palpebral fissures, lower eyelid clefts, protruding and low set small, cup-shaped ears, long philtrum, conical teeth, and ankyloglossia (Fig. 1a). Her cleft palate was not seen. She also had pectus excavatum and an accessory nipple near her left underarm. Postaxial limb deficiencies included absence/hypoplasia/dysplasia of the fifth digits in all limbs, without short forearms (Fig. 1b–e). Bone X-rays of her hands showed the absence of

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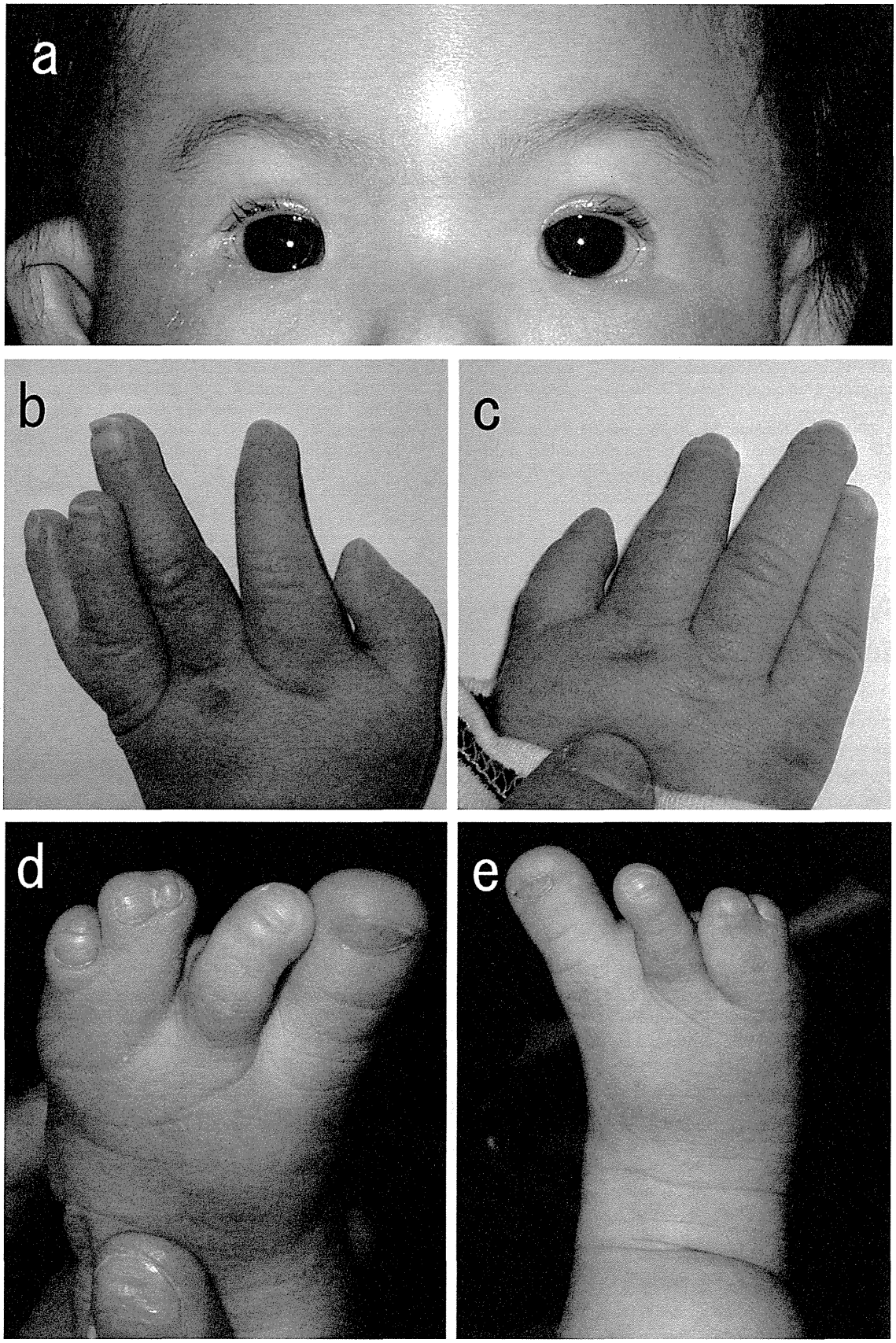

Fig. 1 (a) Front facial image. The patient has short palpebral fissures, ectropion of lower lids, and cupped ears. (b) The left hand with split hand and syndactyly. (c) Absence of the fifth digit of the right hand. (d, e) Bilateral syndactylies involving the third to fifth toes.

fifth metacarpal bone with fusion of the fourth and the fifth proximal phalanges on the left and complete absence of the metacarpal to phalangeal bones on the right (Fig. 2a,b). X-rays of both her feet showed four metatarsal bones, a lack of the fifth metatarsal bone, and cutaneous syndactyly of toes III to V (Fig. 2c). Furthermore, she had bilateral congenital conductive hearing impairment. She had bilateral narrow auditory canals. Auditory brainstem response (ABR) showed mild hearing loss bilaterally.

Brain computed tomography (CT) and echocardiography revealed no abnormal findings. Abdominal ultrasonography showed hypoplasia of the right kidney without abnormal renal function. We tested development using the Japanese ordinary developmental quotient test, known as the Enjoji Infant Developmental Scale. Her developmental quotient at the age of 2 years and 5 months was 110 in motor, 120 in social, and 72 in the verbal field. Her mild verbal disturbance was more serious in recognition than in speech.

Her chromosomal analysis showed normal karyotype, 46XX. Genomic DNA was extracted from the peripheral blood of the patient and her parents. To investigate copy number change (CNC) of the patient, we performed microarray-based copy number analysis using Cytogenetics Whole-Genome 2.7 M Array and Chromosome Analysis Suite software (Affymetrix, Santa Clara, CA, USA). Copy number analysis revealed that 33 CNC, including 18 copy number gains and 10 losses were negligible as benign copy number variants (CNV), because they were registered in our original Japanese CNV database (unpublished) and/or Database of Genomic Variants (http://projects.tcag.ca/variation/). Real-time polymerase chain reaction (PCR) analysis of the three samples using Universal Probe Library (Roche, Basel, Switzerland) for reconfirmation of the results of the microarray studies revealed that all of remaining CNC, including four copy number gains and one loss, were inherited from one of the parents. The patient's *DHODH* gene was studied by PCR amplification and direct sequencing. Primers of all exons of the *DHODH* gene were designed according to Ng *et al.*[2] PCR-direct sequence method was performed according to ordinary procedure. The patient was found to be compound heterozygote for missense mutations in her *DHODH* gene, L28P (T→C) in exon 2 and A347T (G→A) in exon 8. These mutations were novel. Her mother and father were heterozygous for L28P in exon 2 and A347T exon 8, respectively.

Discussion

Miller syndrome is a rare autosomal recessive acrofacial disorder including peculiar facies such as severe micrognathia, cleft lip and/or palate, coloboma of the eyelids, supernumerary nipples, and hypoplasia or aplasia of the postaxial elements of the limbs.[3] Facial features are similar to Treacher Collins syndrome, Goldenhar syndrome and Nager syndrome, thus differential diagnosis is necessary.

Coloboma is present in the lower eyelid in Treacher Collins syndrome, Nager syndrome, and Miller syndrome. On the other hand, Goldenhar syndrome has coloboma in the upper eyelid.[4] The above syndromes are distinguished from Miller Syndrome by the limb anomalies. Postaxial limb deficiency is a cardinal feature in Miller syndrome. Treacher Collins syndrome and Goldenhar syndrome usually have no limb anomalies. Nager syndrome shows preaxial limb anomalies. Our patient had postaxial limb deficiencies, but her facial features were not typical. These were mild for Miller syndrome. She did not have respiratory problems, the cleft palate seen in 90% of patients with Miller syndrome,[3] in-curving arms, or abdominal findings needing surgical intervention. The most distinctive facial features of our patient were lower eyelid clefts, short palpebral fissures, and small and low set ears. These could be cardinal key points for diagnosis of first and second branchial arch-related disorders. We could clinically diagnose her with Miller syndrome with limb anomalies and mild facial features.

The cause of her verbal developmental delay is unclear, because most of patients with Miller syndrome have normal intelligence/development. Re-examinations showed mild hearing impairment, however, this created few obstacles in daily life. We will follow her developmental course carefully.

Treacher Collins syndrome and Nager syndrome are generally considered to be autosomal dominant disorders.[5,6] Some patients of Nager syndrome an reveal autosomal recessive trait.[7] Treacher Collins syndrome is caused by mutations in the *TCOF1* gene located on 5q32-q33.1. Haploinsufficiency of the *TCOF1* gene in Treacher Collins syndrome patients results in the inhibition of production of properly modified mature rRNA in addition to inhibition of rDNA gene transcription, which consequently affects proliferation and proper differentiation of specific embryonic cells during development.[8] On the other hand, disruption of DHODH activity in the fetuses of mice causes a wide range of limb and craniofacial defects. The *DHODH* dysfunction inhibits NF-κB activity directly, and the interruption of NF-κB signaling during development can result in disrupted cell migration, diminished cellular proliferation, and increased apoptosis. These observations suggest that the malformations observed in individuals with Miller syndrome could be caused by perturbed NF-κB signaling due to loss of the *DHODH* function.[2] *TCOF1* and *DHODH* genes are quite different; however, mutations in either gene can cause similar dysfunctions of cell proliferation, migration, and differentiation. So, these mutations would lead to similar phenotypes.

Miller syndrome had been hypothesized to be an autosomal recessive disorder. The genetic cause of Miller syndrome, the *DHODH* gene was discovered using exome sequencing.[2] The *DHODH* gene is located on 16q22 and composed of 9 exons. DHODH is a monofunctional protein which, in most eukaryotic organisms, is located on the outer surface of the inner mitochondrial membrane, and catalyzes the fourth enzymatic step in de novo pyrimidine biosynthesis. The human *DHODH* gene, which is reported as the causable gene of Miller syndrome, was cloned in 1992.[9] This gene exists in various species. Our patient has compound hetero mutations in 28 L and 347A in the transmembrane domain and in the β7~α11 region, respectively. Her parents had one of these mutations each. The 28 L region was conserved from zebrafish and 347A from drosophila (Fig. 3).[10] These regions are essential for the preservation of various species. Thus,

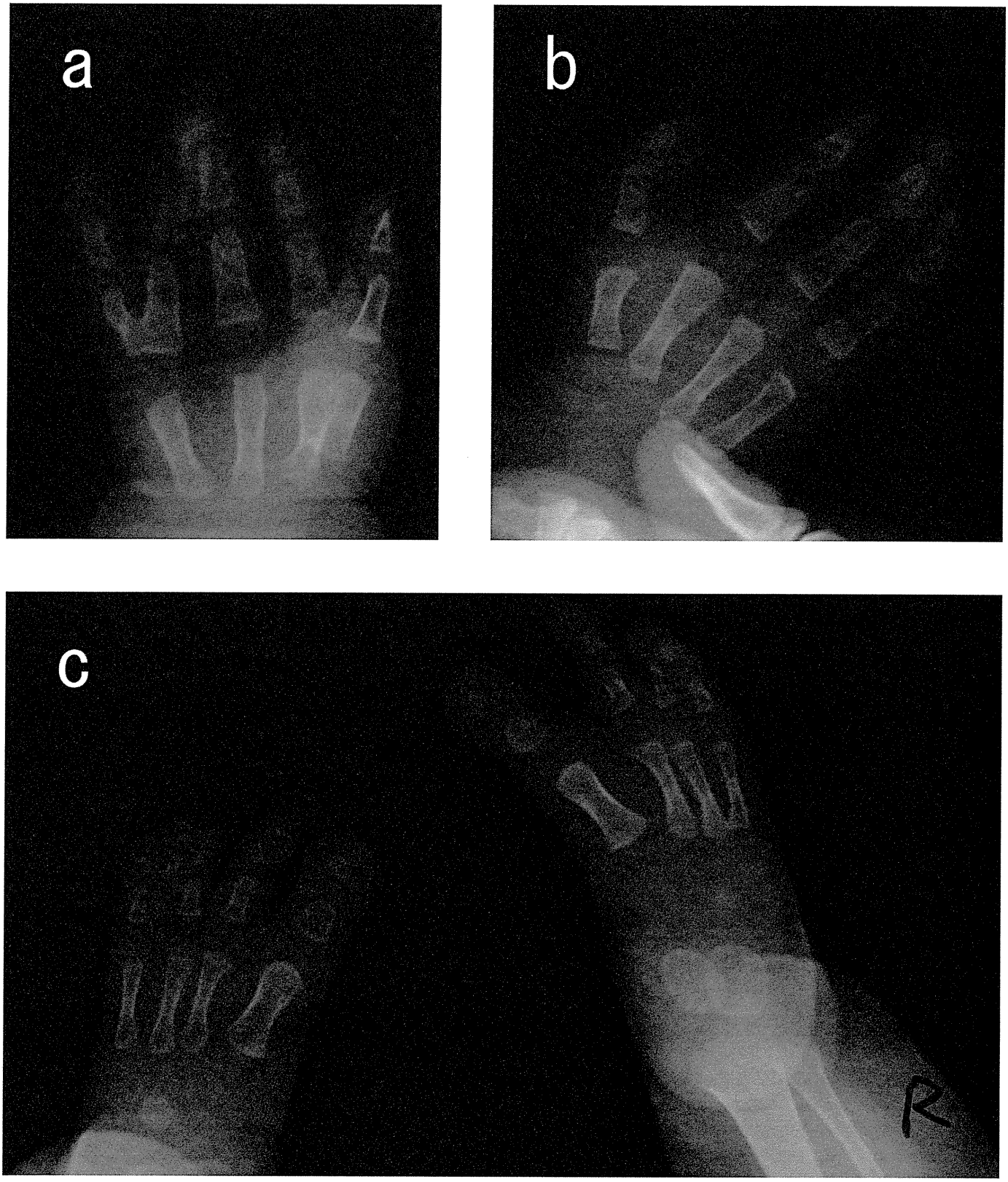

Fig. 2 (a) X-ray findings of left hand. The fifth metacarpal bone is absent, and the fourth and fifth proximal phalanges are fused. (b) Aplasia of the fifth digit in the right hand. (c) Both feet showing absence of the fifth metatarsal bone.

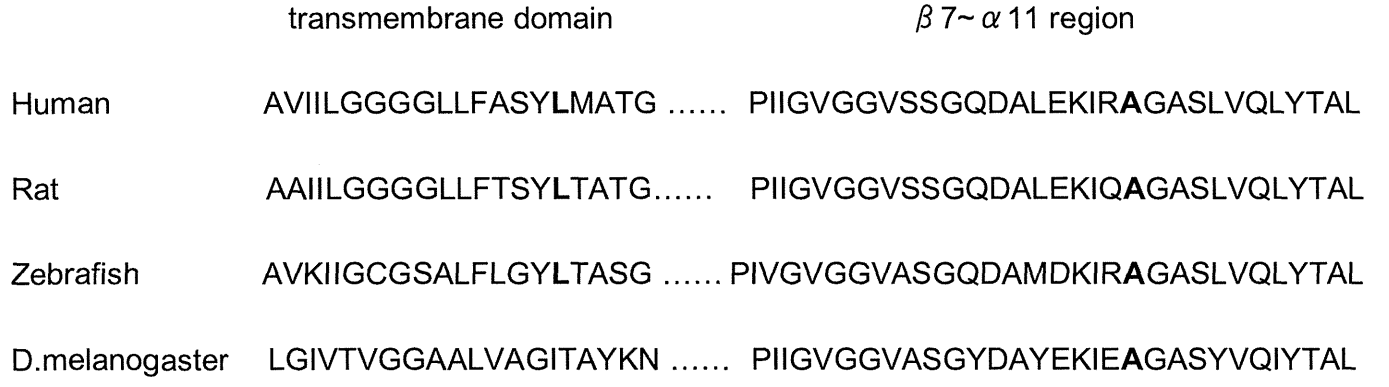

Fig. 3 Homology of the amino acid sequence in the *DHODH* gene. Transmembrane domain and β7~α11 region are shown. 28 L and 347A in the human genome are in bold.

missense mutations of these regions may be a significant etiological mechanism.

A total of 13 mutations of the *DHODH* gene are reported in Miller syndrome including our case, with a spread from exon 2 to exon 9. Human *DHODH* mutations have not yet been registered on the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/) except those reported by Ng *et al.*[2] The mutations of our patient were not found in the Miller patients described by Ng *et al.* Therefore, we think the mutation is novel. Further study is needed to elucidate the genotype/phenotype correlation.

In summary, we report a girl with Miller syndrome who was compound heterozygote of novel missense mutations in the *DHODH* gene. Facial features may not always be typical in this syndrome. Some patients with Miller syndrome have developmental delay, so a close follow-up system is needed for development as well as limb anomalies.

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Congenital anterior neck cysts classified as 'thyroglossal anomalies'

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Key words demoid cyst, Sistrunk operation, thyroglossal duct cyst.

The most frequent congenital anterior neck cyst is the thyroglossal duct remnant cyst and the second most frequent is the dermoid cyst.[1,2] A dermoid cyst in the anterior neck is considered to have arisen from abnormal invagination of the surface ectoderm that forms the face and neck.[3] A thyroglossal duct remnant cyst is caused by failure of obliteration of the thyroglossal duct when it descends from the foramen cecum to the infrahyoid region in early embryologic life. In this context, both congenital lesions are etiologically distinct but are considered by some to have a close relationship, and such lesions are sometimes collectively called 'thyroglossal anomalies'.[4–7]

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