regulated kinase 1A, an enzyme defined as dual-substrate specific. A variety of substrates and interacting proteins has been described, suggesting that *DYRK1A* may participate in multiple biological pathways. Further analysis on the neurological outcomes resulted from the dosage effect of *DYRK1A* and biochemical impact of nutritional effects caused by duodenal stenosis/atresia are required.

In conclusion, we detected three patients with spastic quadriplegia and Down syndrome during nine years in our hospital. Even though the number of patients in our study was very limited in both normal controls with congenital duodenal stenosis/atresia and Down syndrome, the results indicated that congenital duodenal stenosis/atresia is a potential risk factor for spastic quadriplegia in Down syndrome patients. Presently, we are unable to present a detailed pathogenetic mechanism for the conditions. However, the rare neurological complications, which are serious and specific to Down syndrome, may provide us with another insight for consideration on the fragility of neuronal development in Down syndrome. Further analyses are required for improving the quality of life for patients with Down syndrome associated with severe complications.

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CASE REPORT

Sirenomelia with a de novo balanced translocation 46,X,t(X;16)(p11.23;p12.3)

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ABSTRACT **We report a female fetus with sirenomelia with 46,X,t(X;16)(p11.23;p12.3) de novo. Fluorescence *in situ* hybridization (FISH) with bacterial artificial chromosomes were employed for narrowing down the breakpoint regions. On chromosome 16, the breakpoint was mapped in the region of RP11-453F10 (19 920 640–20 118 153 bp from 16pter). On chromosome X, the breakpoint was mapped in the region of RP11-794A15 (47 333 744–47 524 066 bp from Xpter). This is the first case report of sirenomelia associated with translocations. The abnormal phenotype, associated with a balanced translocation, was caused by deletion or breakage of dosage-sensitive genes of the breakpoint, disruption of an imprinted gene, or uniparental disomy. Although the parental origin of normal 16 and der(16) remained undetermined, this case will provide insight into the pathogenetic mechanism of sirenomelia.**

Key Words: balanced translocation, blastogenesis, breakpoint, disruption, sirenomelia

INTRODUCTION

Sirenomelia is rare and usually comprises lethal congenital anomalies, with its characteristic fused lower limbs leading to a resemblance with the mythical mermaid. Its incidence is estimated to be about 1.5 to 4.2 per 100 000 births, and is higher in monozygotic twins than in dizygotic twins or singletons. Males are three times more often affected than females (Stocker and Heifetz 1987; Onyeije et al. 1998). The sequence of sirenomelia is characterized by a variable nature of lower limb abnormalities associated with severe gastrointestinal and urogenital malformations (Kampmeier 1927; Stocker and Heifetz 1987; Kjaer et al. 2003; Drossou-Agakidou et al. 2004; Duesterhoeft et al. 2007). Prenatal diagnosis of sirenomelia can be made by sonographic demonstration mostly during the second trimester, but often during the first trimester (Sepulveda et al. 1994; Schiesser et al. 2003; Van Keirsbilck et al. 2006).

The onset of embryological injury is relatively well defined (–28 days), and its pathogenesis is assumed to be a defect in blastogenesis (Opitz et al. 2002). However, several mechanisms have also been proposed to explain sirenomelia, such as gene mutations (Schreiner and Hoornbeek 1973; Orr et al. 1982) and teratogenic agents (Wei and Sulik 1996; Padmanabhan 1998), as demonstrated in animals. Most cases have normal karyotypes, and the recurrence risk for the same set of parents is negligible. In humans, detailed observation of anatomical dissection of sirenomelia cases has demonstrated a pattern of vascular abnormalities as the cause of complex anomalies (vascular steal) (Stevenson et al. 1986).

To our knowledge, no cases of sirenomelia associated with chromosomal aberration have yet been reported. Here, we report on a fetus with sirenomelia sequence with a reciprocal de novo translocation t(X;16). We refined the breakpoints of each derivative chromosome and discussed the etiological mechanisms.

CASE REPORT

A 32-year-old woman, gravida 2, para 0, was referred for a targeted ultrasound investigation at 20 weeks of gestation because of oligohydramnios. She had reduced renal function from her childhood, but further detailed information was obscure. On abdominal ultrasound, her right kidney was observed to be swollen but the left could not be identified, indicating a horseshoe kidney. Urinalysis revealed normal results, and creatinine clearance was 86.9 mL/min within the normal range. She had no manifestations of toxemia, neither hypertension nor edema. Serial transabdominal ultrasonographic examination revealed a singleton fetus with normal measurements in accordance with the gestational age. The brain and heart were normal. The lower extremities could not be differentiated from one another, and neither the kidneys nor bladder were detected. Based on these sonographic findings, sirenomelia with bilateral renal agenesis was diagnosed. The parents were counseled, and they opted for termination of the pregnancy. Labor was induced with vaginal gemeprost (Preglandin; Ono Pharmaceutical, Osaka, Japan) at 21 weeks of gestational age. The birth weight of the stillborn fetus was 294 g, and the length was 25.5 cm. Postmortem radiological examination (Fig. 1) revealed an abnormal bony pelvis and fused femur. At autopsy, the external genitalia were ambiguous and the anus was imperforate. Ovary-like immature tissue was detected; however, the uterus, kidneys, and bladder were absent. The gastrointestinal structure ended at the region of the sigmoid colon. The abdominal vasculature showed agenesis of renal arteries and inferior mesenteric artery.

MATERIALS AND METHODS

Cytogenetic analysis of chromosomes from phytohemagglutinin (PHA)-stimulated umbilical cord blood lymphocytes was performed according to standard protocols.

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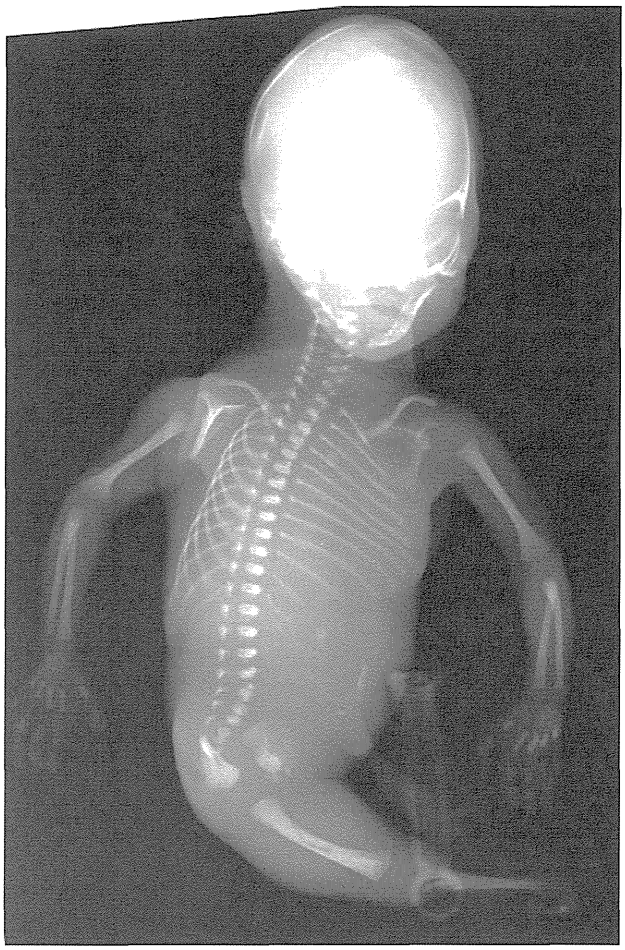

Fig. 1 Skeletal survey as a postmortem examination. The lower extremities were fused, but the upper extremities were of normal appearance. (Permission for the presentation of this picture was obtained from the patient's parents.)

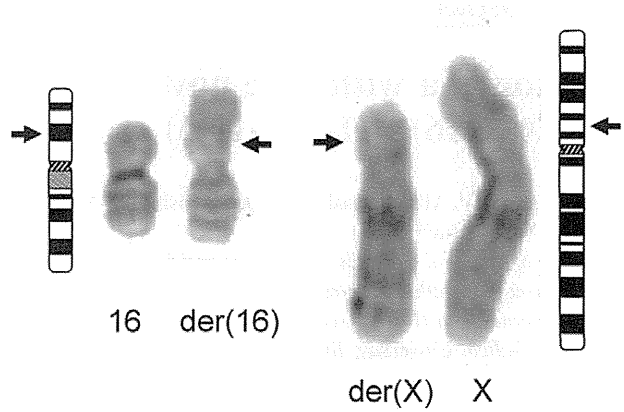

Fig. 2 Partial karyotype and ideogram showing the balanced translocation t(X;16)(p11.23;p12). Arrows indicate the breakpoints.

For fluorescence *in situ* hybridization (FISH) studies, bacterial artificial chromosome (BAC) clones were selected from the UCSC Genome Browser on Human March 2004 Assembly (http://genome.ucsc.edu/cgi-bin/hggateway). Bacterial stabs of BAC clones were streaked onto Luria-Bertani (LB) plates with the appropriate antibiotic. For probes, DNA was isolated from overnight cultures with the appropriate antibiotic using Qiagen QIAprep Spin Mini Prep Kit (Qiagen, Hilden, Germany). All DNA were labeled by nick translation according to the manufacturer's instructions (Nick Translation Mix; Roche Diagnostics, Basel, Switzerland). The probes were blocked with Cot-1 DNA (Roche Diagnostics) to suppress repetitive sequences. Centromere probes for chromosome 16 (D16Z3) and X (DXZ1) were used to confirm chromosome 16 and X (Vysis-Abbott). Slides were baked at 65°C for appropriate aging. Chromosomes and probes were denatured on a hotplate at 75°C for 3 min and then hybridized overnight at 37°C. The slides were then washed in 0.4xSSC and 0.3% NP-40 at 70°C for 2 min, washed in 0.2xSSC and 0.1% NP-40 at room temperature for 30 s, and stained with DAPI for 3 min. Slides were analyzed using completely motorized epifluorescence microscope (Leica DMRXA2), equipped with a CCD camera. Both the camera and microscope were controlled with the Leica CW4000 M-FISH software (Leica Microsystems Imaging Solutions, Cambridge, UK).

RESULTS

Karyotyping

Cytogenetic investigations (GTG banding) on 20 metaphases of the fetus revealed reciprocal translocation 46,X,t(X;16)(p11.3;p12) (Fig. 2). The parents had normal karyotypes, confirming that the origin of the translocation was de novo. Unfortunately, the results of the karyotyping of the fetus were obtained two weeks after labor; therefore, the specimen samples available from the fetus were limited for further analysis.

Breakpoint mapping of chromosome 16

FISH analysis with BAC-derived probes mapped to the regions of interest was performed to refine the breakpoints and the size of the translocated regions (Table 1). Serial hybridization led to the identification of two clones spanning the translocation breakpoints. Although no breakpoint-spanning signal constellation was observed with RP11-295I6 and RP11-594 K6, RP11-453F10 gave hybridization signals on both derivative chromosomes (Fig. 3A). The signal intensity of the translocated segment was stronger than that on the derivative X, which suggested that the breakpoint interval of chromosome 16 was narrowed to the centromeric portion of RP11-453F10. Based on the information from the UCSC Genome Browser (May 2004 build; http://genome.ucsc.edu/cgi-bin/hggateway), no known gene or anonymous expressed sequence tag (EST) has been assigned to the breakpoint interval of 16p12.3, except for GPR139, which is a G-protein coupled receptor 139 and functions as an orphan receptor expressed in the brain.

Breakpoint mapping of chromosome X

The breakpoint on chromosome X was narrowed to the BAC sequence of RP11-794A15 (Fig. 3B). Subsequent use of overlapping BACs further refined the breakpoint region. As the translocated segment of 190 kb BAC RP11-794A15 gave a reduced signal on der(16) and strong signal on der(X), whereas the closest BAC clones RP11-466C12 (translocated) and RP11-517F20 (retained) gave non-spanning signals, the breakpoint interval was assigned to the sequence of the distal region of one fourth of RP11-794A15.

Table 1 Fluorescence *in situ* hybridization (FISH) results around the breakpoints of t(X;16)

BAC clones	Locus	Position from pter	FISH results
CTD-3252I22	16p13.3	3 759 848–3 854 684	Translocation
RP11-91M7	16p13.12	14 350 355–14 529 445	Translocation
RP11-347K10	16p12.3	17 554 061–17 733 669	Translocation
RP11-306F17	16p12.3	19 135 483–19 136 116	Translocation
RP11-442P2	16p12.3	19 163 212–19 355 377	Translocation
RP11-313D3	16p12.3	19 391 011–19 589 554	Translocation
RP11-188I1	16p12.3	19 552 502–19 743 905	Translocation
RP11-295I6	16p12.3	19 775 528–19 971 626	Translocation
RP11-453F10	16p12.3	19 920 640–20 118 153	Breakpoint spanning
RP11-594K6	16p12.3	20 128 349–20 321 450	Retention
RP11-428N13	16p12.3	20 505 911–20 628 545	Retention
RP11-9A15	16p12.3	20 637 042–20 805 219	Retention
RP11-481A2	16p12.3	20 729 163–20 941 255	Retention
RP11-167K14	16p12.2	20 875 655–21 049 940	Retention
RP11-768I19	16p12.3	20 943 308–21 139 458	Retention
RP11-450G5	16p12.1	24 093 274–24 281 424	Retention
RP11-264M14	16p11.2	33 282 451–33 452 721	Retention
RP11-245M24	Xp11.3	45 232 841–45 290 934	Translocation
RP11-75A9	Xp11.3	45 954 371–46 105 963	Translocation
RP11-8G18	Xp11.3	46 957 829–46 958 118	Translocation
RP11-365I22	Xp11.23	46 573 742–47 734 370	Translocation
RP11-466C12	Xp11.23	47 277 752–47 473 803	Translocation
RP11-694N15	Xp11.23	47 204 444–47 382 703	Translocation
RP11-794A15	Xp11.23	47 333 744–47 524 066	Breakpoint spanning
RP11-517F20	Xp11.23	47 384 151–47 531 773	Retention
RP11-751K24	Xp11.23	47 469 880–47 615 141	Retention
RP11-416B14	Xp11.23	48 337 118–48 519 446	Retention
RP11-22B10	Xp11.22	51 265 319–51 453 343	Retention
RP11-698D7	Xp11.22	53 285 637–53 486 126	Retention
RP11-93M8	Xp11.1	57 060 483–57 176 843	Retention

BAC, bacterial artificial chromosome.

The breakpoint-spanning clone RP11-794A15 contains two validated genes. The coding sequence of Krueppel-type zing finger 81 (ZNF81), a candidate gene for X-linked mental retardation (Kleefstra et al. 2004), lies proximal to the breakpoint. A hypothetical gene AK094108, lies distal to the breakpoint region, with unknown functions.

DISCUSSION

We have characterized the chromosomal breakpoints of a fetus with sirenomelia sequence with a de novo X; autosome translocation t(X;16)(p11.23;p12.3). The chromosomal breakpoints on both the chromosomes did not disrupt the coding genes associated with early human development, especially with blastogenesis. To our knowledge, this is the first case report of sirenomelia associated with chromosomal aberrations. However, we could not assign any candidate genes in the region of the breakpoints.

In mice, sirenomelia anomalies (called as sirens) have been observed in crosses between specific strains. The first sirens model mouse was found in crosses between mutations at the T, Fused, or ur loci (Gluecksohn-Schoenheimer and Dunn 1945). Abu-Abed et al. (2001) and Sakai et al. (2001) reported sirenomelia caused by gene knockouts of the retinoic acid-degrading enzyme CYP26. More recently, sirens phenotypes in Tsg/Bmp7 compound mutants were reported by Zakin et al. (2005). Their molecular marker studies indicated that the sirenomelia phenotype is associated with defects in the formation of the ventroposterior mesoderm, and demonstrated that the dorsoventral patterning of the mouse posterior mesoderm is regulated by Bmp signaling, as is the case in other vertebrates. However, we could not find any association between the breakpoint regions in our case and the loci of human homologues of the genes for mutant mice with sirenomelia, such as Bmp7 on 20q13.31, Tsg (Twisted gastrulation) on 18p11.22, Bmp4 on 14q22.2, and Cyp26B1 on 2p13.3.

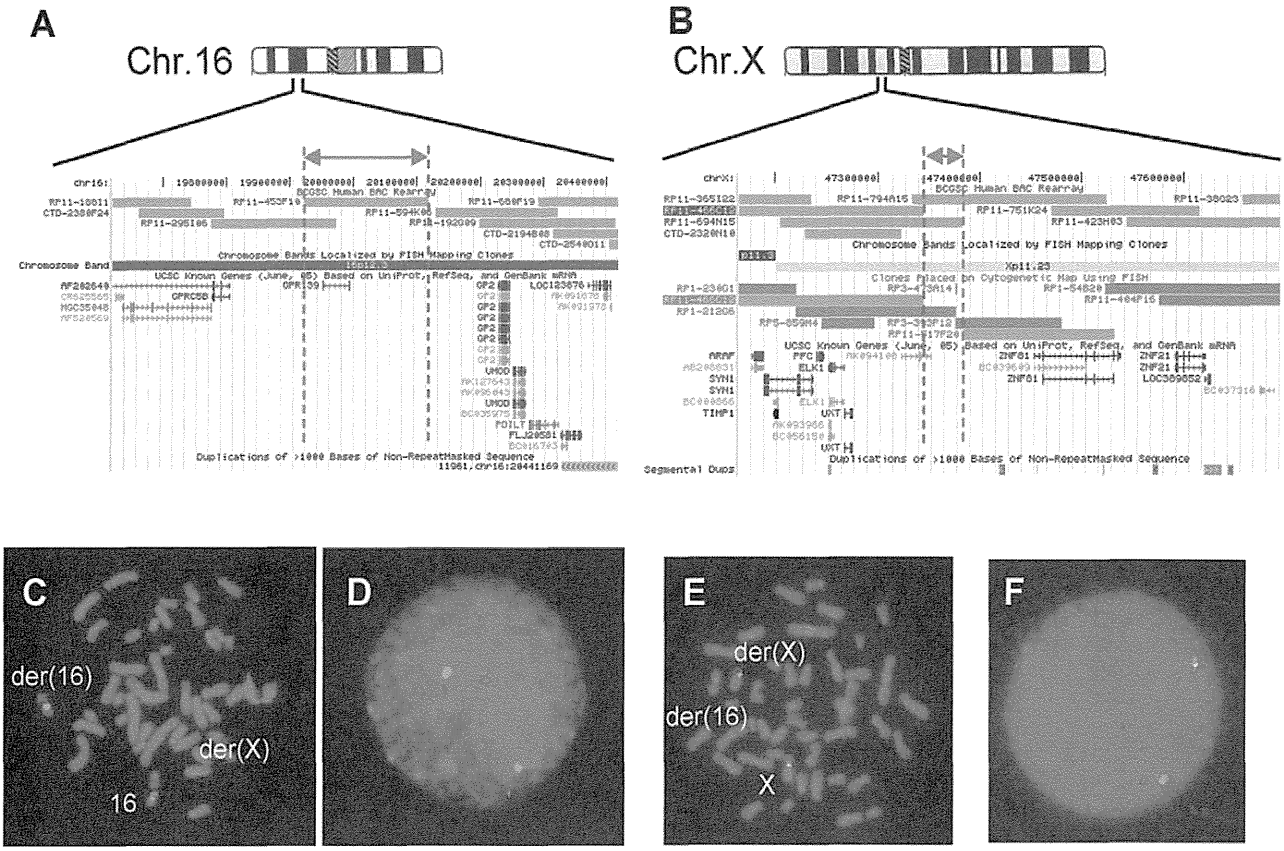

Fig. 3 Schematic representation of the 16p12.3 (A) and Xp11.23 (B) breakpoint regions. Breakpoint was refined by fluorescence *in situ* hybridization (FISH) with contig bacterial artificial chromosome (BAC) as the probes in the maps (http://genome.ucsc.edu). (C) Metaphase chromosomes after FISH with the BAC clone, showing breakpoint-spanning signals on both derivative chromosomes with RP11-453F10 (SpectrumOrange). The alpha-satellite probe D16Z3 (SpectrumGreen) was used as a control probe. (E) FISH with RP11-794A15 (SpectrumOrange) showed break-point signals on both derivative chromosomes. (D,F) Interphase nuclei showing split signals on both derivative chromosomes. The signal intensity was reduced on der(16), demonstrating that the breakpoint in RP11-453F10 could be on the centromere site. On the X chromosome, the breakpoint was noted in RP11-794A15 on the Xpter site.

Approximately 1 in 2000 newborns has a de novo balanced chromosomal rearrangement. Clinical studies of these de novo apparently balanced translocations have demonstrated that the abnormal phenotype is present in 6.1% of translocation carriers. Several mechanisms for the abnormal phenotypes have been considered as follows: direct breakage of a dose-sensitive gene, complex rearrangements at the breakpoint, a mutation in the second allele causing an autosomal recessive phenotype, disruption of an imprinted gene causing an apparent imprinting disorder, and uniparental disomy (UPD). Chromosomal abnormalities, such as Robertsonian and reciprocal translocations or supernumerary marker chromosomes, increase the occurrence of nondisjunction, resulting in UPD. According to the literature, maternal UPD16 cases showed clinical abnormalities, such as body stalk anomaly, intrauterine growth retardation, imperforate anus, congenital heart disease, and spontaneous abortion (Kalousek et al. 1993; Vaughan et al. 1994; O'Riordan et al. 1996; Abu-Amero et al. 1999; Chan et al. 2000; Kondo et al. 2004). Body stalk anomaly is one of the defects of blastogenesis in human development and can be considered to have a common mechanism with the sirenomelia sequence. Unfortunately, the specimen sample and cells available were extremely limited in our case, and further analysis on the parental origin of the normal and derivative chromosome 16 could not be performed.

In conclusion, we have presented here a sirenomelia fetus associated with balanced translocation t(X;16)(p11.23;p12.3) and assigned the breakpoints on chromosome X and 16. We could not identify the genes associated with early human development around the breakpoint sequences. Further analysis is important for elucidating the etiology of sirenomelia from a genetic perspective.

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CASE REPORT

Ehlers-Danlos syndrome, vascular type: A novel missense mutation in the *COL3A1* gene

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ABSTRACT **We report a 34-year-old Japanese female with the vascular type of Ehlers-Danlos syndrome. She had thin translucent skin, extensive bruising, toe joint hypermobility, left lower extremity varicose veins, and chronic wrist, knee and ankle joint pain. She also had dizziness caused by autonomic dysfunction. Magnetic resonance angiography showed tortuous vertebral and basilar arteries, mild left carotid canal bulging, and right anterior tibial artery hypoplasia. Electron microscopic examinations of a skin biopsy revealed extremely dilated rough endoplasmic reticulum in dermal fibroblasts and wide variability of individual collagen fibril diameters. A molecular analysis using a conventional total RNA method and a high-resolution melting curve analysis using genomic DNA revealed a novel missense mutation within exon 48 of the *COL3A1* gene, c.3428G>A, leading to p.Gly1143Glu.**

Key Words: *COL3A1*, Ehlers-Danlos syndrome, high-resolution melting curve analysis, novel missense mutation, vascular type

INTRODUCTION

The vascular type of Ehlers-Danlos syndrome (vEDS; OMIM#130050) is an autosomal dominant disorder caused by abnormal type III collagen resulting from heterogeneous mutations of the type III procollagen gene (*COL3A1*). An early and definitive diagnosis of the disease may improve the management of life-threatening complications, such as arterial, intestinal, and uterine rupture (Pepin et al. 2000; Oderich et al. 2005). Here, we describe a 34-year-old female with vEDS associated with a novel missense mutation in the *COL3A1* gene.

CASE REPORT

The proposita was the second child of non-consanguineous parents. At the time of her birth, her mother was 30 years of age. She had had extensive bruising on the palm and sole since 10 years of age, when she carried heavy baggage and walked a long distance. Blood examinations, including coagulation factors, showed normal results at 26 years of age. She gave birth to three sons and experienced massive postpartum arterial bleeding during the last delivery at the age of 32 years. She had no abortions or stillbirths. At 33 years of age, she was referred to the Division of Medical Genetics, Department of Pediatrics, Kawasaki Medical School by the Department of Hematology and Oncology, Okayama University Hospital, with suspicion of vEDS because of extensive bruising, positive family history, and dizziness occasionally associated with syncope during the previous 3 months. In the previous hospital, magnetic resonance angiography (MRA) of the brain showed tortuous vertebral and basilar arteries and mild bulging of 4-mm width suspected to be an aneurysm at the left carotid canal. Magnetic resonance imaging (MRI) of the brain and cervical MRA revealed normal results. The patient's bruising worsened when a vasopressor was given for treatment of the dizziness.

The patient presented with thin translucent skin, extensive bruising, hypermobility of the toe joints, and varicose veins of the left lower extremity. Genetic counseling prior to the genetic test including a skin biopsy was provided by a certificated expert clinical geneticist, and informed consent was obtained from the patient. Genetic counseling for the couple, including the result of the genetic test and the recurrence risk of vEDS for their children, was provided by a certificated expert clinical geneticist and a certified genetic counselor. At 34 years of age, the patient had chronic pain of the wrist, knee, and ankle joints. MRA of the brain showed that the bulge remained at the same size as 1 year previously (Fig. 1a). MRA of the chest and abdomen showed no remarkable changes. MRA of the legs revealed hypoplasia of the right anterior tibial artery, but no stenosis or obstruction (Fig. 1b). Although MRI of the lumbar spine and cord showed dural ectasia, liquorrhea was not detected (Fig. 1c). The blood pressure of the right arm at rest was 100/60 mmHg and the pulse at rest was a regular 70 bpm. The most plausible cause of the dizziness was autonomic dysfunction based on otorhinolaryngological examinations.

Family history

The mother of the proposita had extensive bruising since childhood, hyperthyroidism under treatment, and a past history of hysteromyomectomy. She died of a ruptured thoracic aortic aneurysm at 48 years of age. The proposita was told that angiorrhaphy on the mother was difficult for the surgeon to perform because of her vascular fragility. The mother's parents, three elder brothers, and two elder sisters had no history of extensive bruising or arterial/intestinal/uterine rupture.

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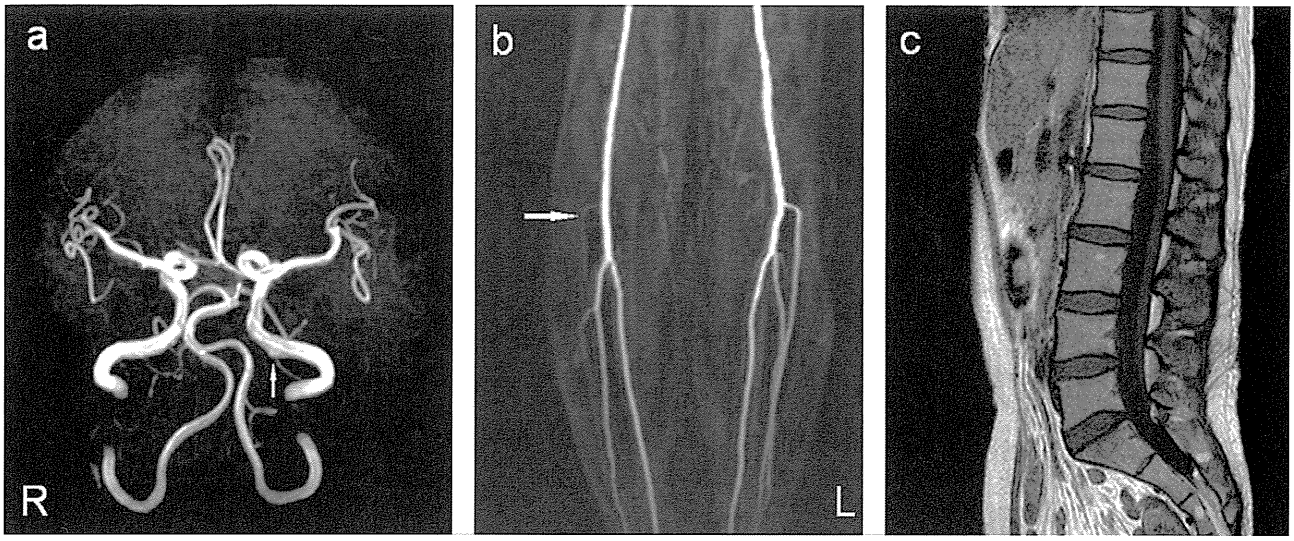

Fig. 1 (a) Magnetic resonance angiograph of the brain showing tortuous vertebral and basilar arteries and a mild bulging of 4-mm width suspected to be an aneurysm at the left carotid canal (arrow). (b) The arrow indicates hypoplasia of the right anterior tibial artery without stenosis or obstruction. (c) Magnetic resonance image of the lumbar spine and cord showing dural ectasia.

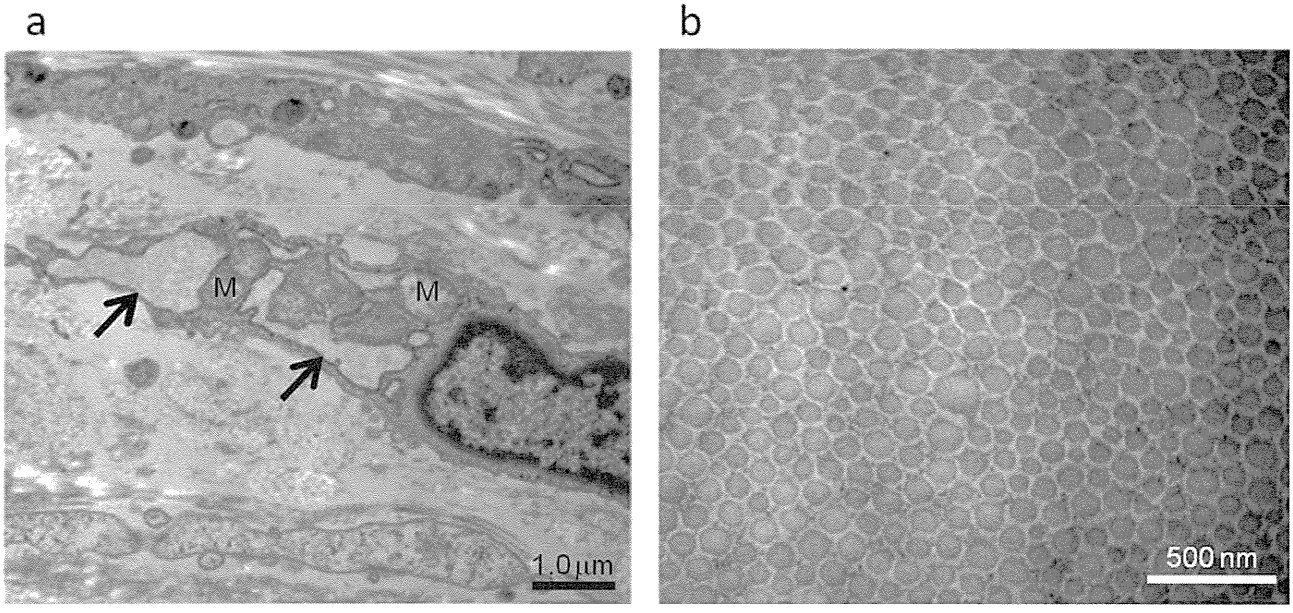

Fig. 2 Ultrastructure of collagen fibrils in the dermis. (a) Fibroblast with extremely dilated rough endoplasmic reticulum (arrows). M, mitochondria. Bar, 1 μm. (b) Collagen fibrils showing wide variability of individual fibril diameters, with a mean diameter of 103 ± 18 nm. Bar, 500 nm.

The elder brother of the proposita was healthy and had a past history of Achilles tendon fragmentation. The eldest son (9 years of age) of the proposita had hypermobility of the finger and elbow joints and recently presented with extensive bruising. The second (5 years of age) and third (2 years of age) sons were in good health.

Laboratory analysis

This study was approved by the Research Ethics Committee of Kawasaki Medical School and Hospital.

Cell culture

Dermal fibroblasts were obtained from explants of a skin biopsy specimen taken from the left upper arm, after appropriate informed consent had been given.

Light and electron microscopy, and image analysis

Light microscopic examinations of the skin biopsy specimen showed a decreased collagen content and a relative increase in elastin, representing non-diagnostic results. Electron microscopic

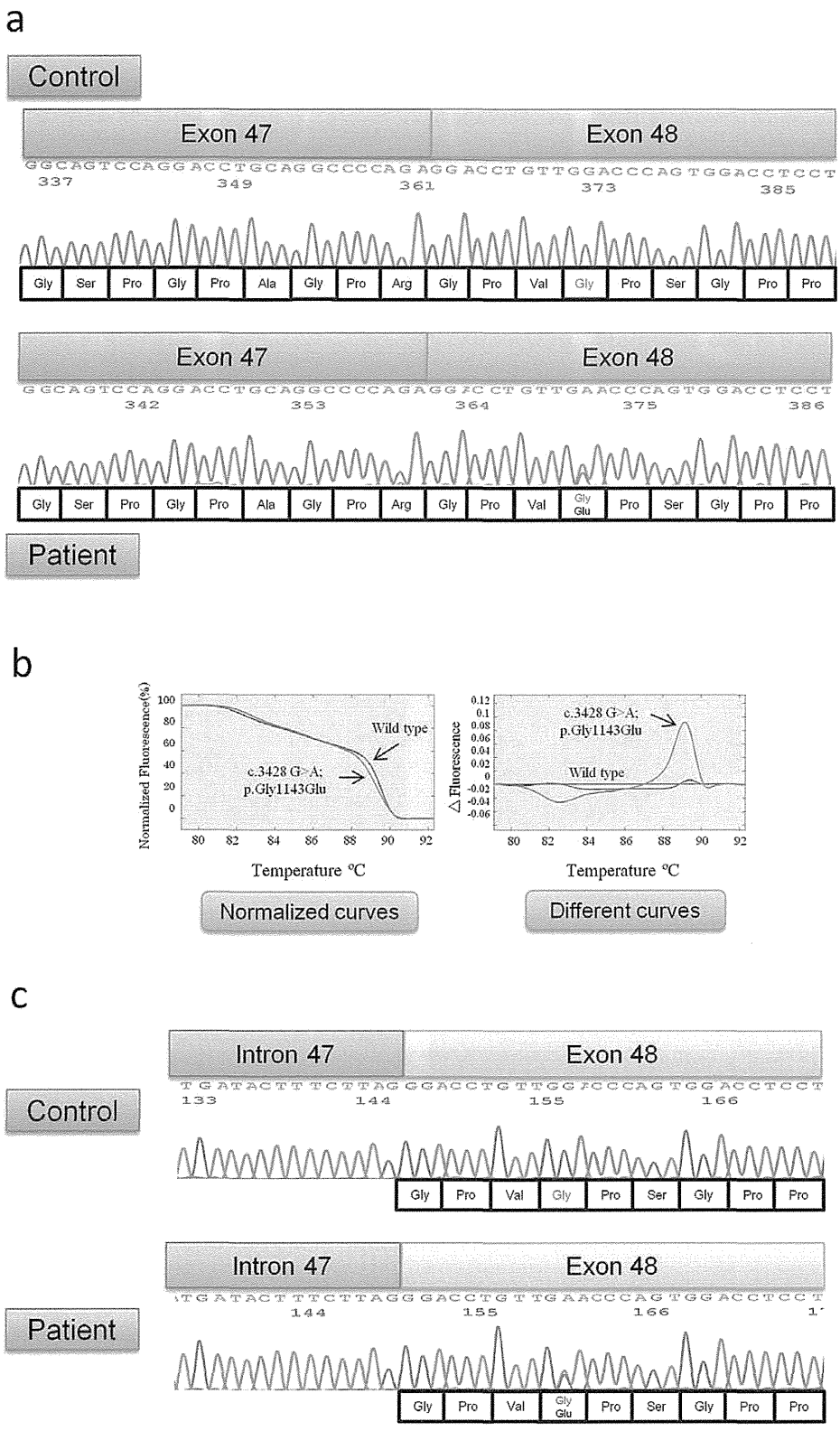

Fig. 3 (a) *COL3A1* gene mutation detected using a cDNA. A heterozygous mutation was found within exon 48 of the *COL3A1* gene in the proband c.3428G>A, leading to p.Gly1143Glu. (b) A high-resolution melting curve analysis using *COL3A1* genomic DNA clearly differentiated between the wild-type and the patient's melting curves in the exon 48 amplicon. (c) *COL3A1* gene mutation detected using genomic DNA. The same mutation was identified as in the cDNA analysis.

examinations revealed the presence of fibroblasts with extremely dilated rough endoplasmic reticulum (Fig. 2a). Electron micrographs of cross-sections of collagen fibrils showed wide variability of individual fibril diameters (Fig. 2b). Measurements of more than 900 fibril diameters by the image analyzer software IP Lab for Windows (BD Biosciences, Franklin Lakes, New Jersey, USA) showed that the mean diameter of the collagen fibrils was 103 ± 18 nm.

Mutation identification

Total cellular RNA was extracted from cultured dermal fibroblasts, and a cDNA of the *COL3A1* gene was synthesized by priming with random hexamers. The polymerase chain reaction (PCR) fragments were directly sequenced as described previously (Watanabe et al. 2007). In addition, a mutation screening of the 52 exons by high-resolution melting curve analysis (HRM analysis) was performed using genomic DNA from peripheral blood (Naing et al. 2011). To confirm the identified mutation, the region of interest was amplified from genomic DNA and directly sequenced.

We identified a heterozygous mutation within exon 48 of the *COL3A1* gene in the proband c.3428G>A, leading to p.Gly1143Glu (Fig. 3a). This mutation has not previously been reported. We could clearly differentiate between the wild-type and the patient's melting curves in the exon 48 amplicon using HRM analysis (Fig. 3b). The same mutation found in the cDNA analysis within exon 48 was detected using genomic DNA (Fig. 3c).

HRM analysis of the *TGFBR1* and *TGFBR2* genes in the genomic DNA showed normal results (data not shown).

DISCUSSION

There are two major types of mutations of the *COL3A1* gene. About two-thirds of the reported mutations of the *COL3A1* gene result in substitution of other amino acids for glycine residues in the $[Gly\text{-}X\text{-}Y]_{343}$ triplets of the triple helical domain of the gene, as in the present patient. The remaining mutations affect splice sites in the *COL3A1* gene with a marked preference for the 5′ (donor) splice site in an intron (Pepin et al. 2000). In 29 Japanese patients, the former type of mutation was found in 15 patients and the latter type in 14 patients (Watanabe and Shimada, 2008; Shimaoka et al. 2010). Recently, a 1-bp deletion and nonsense mutation that could not be determined by the conventional total RNA method, were detected by HRM analysis using genomic DNA (Naing et al. 2011). Using this method, *COL3A1* gene mutations can be screened easily and rapidly without an invasive skin biopsy and cell culture. There is intra- and inter-familial variable expressivity. Although no apparent genotype-phenotype correlation between missense and splicing mutations was observed (Pepin et al. 2000), Leistritz et al. (2011) reported that null mutations had reduced penetrance compared with missense and splicing mutations, and the phenotype seemed to be limited almost entirely to vascular events.

Gly1143 in the present mutation is located in one of the Gly-X-Y triplets of the triple helical domain of the gene, which is essential for ensuring the proper assembly of the alpha monotrimers. As a previous report suggested that any glycine replacement in the triple helix region of the *COL3A1* gene will cause disease (Persikov et al. 2004), this mutation is most likely to be responsible for the disease in the present patient.

The present patient had extremely dilated rough endoplasmic reticulum in her dermal fibroblasts and wide variability of individual collagen fibril diameters on electron microscopic examinations. The former findings were similar to those reported by Smith et al. (1997). They also reported that small collagen fibrils in the dermis were more common for mutations near the carboxyl-terminal end of the triple helix, whereas variable fibril diameters reflected mutations toward the amino-terminal of the triple-helical domain. In contrast, the present patient had a mutation near the carboxyl-terminal end, but showed variable collagen fibril diameters. Two unrelated men in their thirties with the same mutation, G373R, showed collagen fibril diameters of 86 ± 16 and 120 ± 12 nm, respectively (Smith et al. 1997). Thus, the correlation between the size and distribution of collagen fibril diameters and the mutation site might not be adequate.

Recently, 20 Japanese patients (mean age, 26 years) with vEDS confirmed by molecular analysis were characterized by thin and translucent skin with extensive bruising and hypermobility of the small joints in about 90% of the patients, while the prevalence of serious complications was relatively low (30%) (Shimaoka et al. 2010). Although the present patient has had no serious complications, early detection of complications and management of emergencies by a multidisciplinary team approach is imperative, because more than 80% of such patients have had at least one complication by 40 years of age (Pepin et al. 2000). Prophylactic treatment for cardiovascular events with the beta blocker celiprolol (Ong et al. 2010) might be difficult in the present patient because of her dizziness. Genetic counseling for her sons is also important in the future.

ACKNOWLEDGMENT

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特集　不妊と周産期医療

多科連携における不育症への小児科の役割

—出生前対応を含む—

升野 光雄

はじめに

不育症は，2回以上の流産・死産，あるいは早期新生児死亡の既往と定義されている(平成20～22年度厚生労働省研究班)。これらの事例の約半数は偶発的流産で，特別な治療を行わなくても次回妊娠予後は良好であるが，残りの半数に子宮形態異常や凝固異常，夫婦いずれかの染色体異常などの共通のリスク因子が認められる。妊娠歴のある35～79歳の女性のうち，3回以上の流産は0.9%，2回以上の流産は4.2%で，38%が1回以上の流産を経験していることが明らかとなり，海外のデータとほぼ同様である[1,2]。

小児科医のかかわりとしては，流産胎児組織あるいは新生児が染色体不均衡型構造異常をもつ場合が想定される。その際，夫婦のいずれかが染色体均衡型構造異常(転座あるいは逆位)の保因者の可能性がある。遺伝学的検査である染色体検査による保因者診断や出生前診断に際しては，日本医学会のガイドライン[3]，日本産科婦人科学会の見解[4]に準拠し，検査前後の遺伝カウンセリングが必須となる。

染色体異常からみた不妊症・不育症・先天異常

ゲノムの量的不均衡をもたらす染色体異常は，その生体に与える影響の程度により不妊症・不育症・先天異常のいずれの原因ともなり得る[5]。

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反復流産と染色体異常

妊娠第1三半期の自然流産胎児の50～70%が染色体異常であり，多くは数の異常である。不均衡型・均衡型構造異常は2%であり，その半数は親の均衡型構造異常に由来している。この傾向は反復流産でも同様である[5]。

「研究班の成績でCGHアレイ法で染色体の異常を検討すると流産胎児の80%に染色体異常が検出されていた。そのため流産回数が2回，3回，4回の場合，計算上，リスク因子がなく偶発的に(胎児染色体異常による)流産をくり返しただけの人が64%，51%，41%存在する。医師ならびに患者はリスクがなくても偶発的に流産をくり返している症例が多いことを認識すべきである」[1]

反復流産既往のある夫婦33,442人(16,721組)で，いずれかが均衡型構造異常の保因者は，相互転座1.3%，Robertson型転座0.6%，逆位0.2%であり，新生児集団調査より高い頻度であった[6]。国内の多施設共同研究では，反復流産既往のある夫婦2,382組のうち相互転座3.0%，Robertson型転座0.6%，逆位1.0%であった。いずれかが均衡型相互転座保因者夫婦の63%が，診断後の初回妊娠で不均衡型相互転座ではない生児を得ている。ともに正常核型の夫婦では，78.7%が診断後の初回妊娠で生児を得ている[7]。いずれかが均衡型構造異常保因者夫婦の診断後の累積生児獲得率は83%であり，非保因者夫婦の84%と差がないと報告されている[8]。

染色体均衡型構造異常の保因者診断

「厚労研究班の研究成果を基にした不育症管理に関する提言」からの抜粋を示す。

夫婦染色体検査[スクリーニングとしては保険診療外]

「染色体や遺伝子などの遺伝情報を取り扱う際には，検査の実施前から充分な遺伝カウンセリングが必要である。不育症に関する医学的・心理的サポートを含む多面的なカウンセリングに加え，遺伝情報に関しては特に専門的な遺伝カウンセリングが求められる。検査の実施にあたって，検査の意義，情報を知ることの長所と問題点，結果の開示の内容や方法についても事前から相談する必要がある。また，遺伝情報は重要な個人情報であるために，その取り扱いに関しても厳格に管理されなければならない。検査結果を開示する際にも充分な遺伝カウンセリングが必要となる。結果によっては一方の配偶者がそれによって不利益を得ないように配慮が必要である。本来の遺伝情報は，クライエント本人に開示することが原則であるが，不育症に対する染色体検査の結果を開示する際に夫婦のどちらかが染色体の構造異常を有している場合に，どちらかを特定せずに染色体均衡型構造異常の保因者であることを知らせる選択肢について予め意思の確認をすることが望まれる。不育症への対応策を考えるうえで，夫婦のどちらかを特定することは必ずしも夫婦にとって長所につながらないからである」[1)]

夫婦の染色体均衡型構造異常の保因者診断における遺伝カウンセリングについて，相互転座を例にして述べる。

1．均衡型相互転座保因者検査前の遺伝カウンセリング

説明内容の具体例

1）児の不均衡型相互転座についての説明：異常を認める染色体の部分モノソミーと由来不明の部分トリソミーの可能性がある。
2）夫婦のいずれかが均衡型相互転座保因者である可能性は約50％である。
3）その場合，児の部分トリソミーの染色体由来も判明する。
4）保因者についての説明：本人の生殖以外の健康には影響がなく，400人に1人(200組の夫妻に1組)の頻度でみられ，保因者であることは誰の責任でもない。
5）均衡型相互転座保因者は，不均衡型相互転座による流産や先天異常をもつ子どものリスクがあるが(**図**)[9)]，累積生児獲得率は80％に近い。
6）染色体検査で得られた遺伝情報は血縁者で共有していることがあり，検査を受けた人だけに影響がとどまらない可能性がある。
7）検査結果の伝え方：夫婦の染色体検査結果は，原則として2人同時に伝える。いずれかが相互転座をもつ場合，どちらが均衡型相互転座保因者であるかを特定せずに結果を伝えるという選択肢も含め，検査前に夫婦の意思確認をしておくことが望まれる。

上記のように，夫婦にとって染色体検査は，以後の妊娠や血縁者に不均衡型相互転座による流産や先天異常を生じる可能性についての情報を得るために行われることを十分理解してもらう必要がある。

2．保因者検査後の遺伝カウンセリング

1）夫婦がともに正常核型の場合

反復流産既往のある夫婦がともに正常核型の場合は，その後の初回妊娠で78.7％が生児を得ていることを伝える[7)]。

2）いずれかが均衡型相互転座をもつ場合

「厚労研究班の研究成果を基にした不育症管理に関する提言」からの抜粋を示す。

染色体異常

「夫婦のどちらかに均衡型転座などの染色体構造異常が発見されたら，充分な遺伝カウンセリングを行う。その際，累積生児獲得率は染色体正常カップルと比べても決して低くないこと，流産を回避する目的で着床前診断を行う選択肢があることなどを説明する。なお現在のところ，着床前診

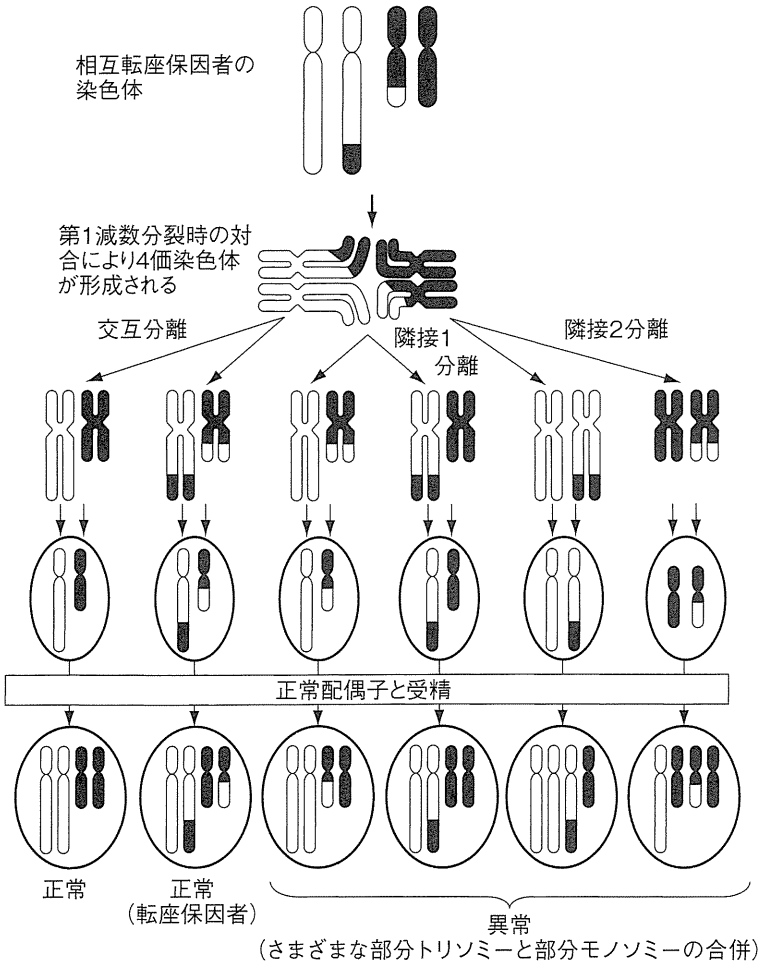

図　均衡型相互転座保因者から生じる不均衡型構造異常(福嶋, 2003 より引用一部改変)[9]

均衡型相互転座保因者の配偶子形成期(第1減数分裂時)に転座にかかわる2組4本の染色体が対合した4価染色体の2:2分離の一例を示す。隣接1分離あるいは隣接2分離では受精後，部分トリソミーと部分モノソミーをもつ接合子となり，流産や先天異常の原因となる。さらに，相同染色体間の組換えや3:1分離などもあり，派生する不均衡型構造異常の種類は20を超える

断を行った方が自然妊娠より生児獲得率が高くなるというエビデンスはない。着床前診断の適応と運用に関しては日本産科婦人科学会の見解を遵守し，倫理審査を経た上で実施する」[1]

均衡型相互転座保因者夫婦への出生前診断

出生前診断の遺伝カウンセリングは妊娠前が望ましい。先に述べたように，均衡型相互転座保因者夫婦の流産率は高まるが，累積生児獲得率は正常核型の夫婦と大差がないことを理解してもらう。羊水染色体検査の時期まで妊娠が継続した場合には，不均衡型相互転座をもつ胎児のリスクは0.8～2.9%である[8,10]。これは，不均衡型相互転座をもつ生児を発端者とする保因者夫婦における不均衡型相互転座をもつ生児の経験的再発率(5～10%)に比べて低頻度である。

検査前の遺伝カウンセリングでは，羊水染色体検査で不均衡型相互転座や偶然に数の異常が判明した場合に妊娠を継続するか否かを，検査前に夫婦で決めておくことが大切である。性染色体異常など表現型に影響のない結果が出る可能性も伝える。染色体異常以外にも医療を要する先天異常をもつ子どもが生まれるリスクは，どの夫婦にも3%ほどあることも伝える。

日本医学会ガイドラインには「未成年者に対する非発症保因者の診断は，原則として本人が成人し自律的に判断できるまで実施を延期すべきで，両親等の代諾で検査を実施すべきではない」[3]とあるが，羊水染色体検査では均衡型相互転座保因者も対象に実施することは避けられない。出生後，均衡型相互転座保因者本人が生殖年齢に達したら，時期をみて本人に結果を伝える必要がある。保因者本人に遺伝情報が伝わらないことを避

けるために，本人が18歳になった時点で，生殖におけるリスクを伝えることの必要性と遺伝カウンセリングの紹介を両親に喚起する手紙を送るという取り組みもある(不均衡型相互転座をもつ生児を発端者とする保因者診断に関連した論文ではある)[11]。

均衡型相互転座保因者夫婦への着床前診断

日本では2006年から重篤な遺伝性疾患に加え，均衡型染色体構造異常に起因すると考えられる習慣流産(反復流産を含む)も着床前診断の対象になり，症例ごとに日本産科婦人科学会で審査されている。承認は約200例を数えるが，大半が習慣流産症例である[12]。「遺伝カウンセリングは，着床前診断実施者が所属する診療部門以外の第三者機関もしくは診療部門において，実施者以外の臨床遺伝専門医または認定遺伝カウンセラー等の遺伝医療の専門家がこれを行う」としている[13]。

妊娠20週より前に2回以上の流産既往のある夫婦で，いずれかが均衡型相互転座保因者夫婦のその後の妊娠転帰についてのシステマティックレビューでは，自然妊娠の60%が診断後の初回妊娠で不均衡型相互転座ではない生児を得ており，男性保因者では75%，女性保因者では52%であった。累積生児獲得率は74%であった。一方，着床前診断された35%が診断後の初回妊娠で不均衡型相互転座ではない生児を得ており，男性保因者と女性保因者で差は認めていない。累積生児獲得率も同様に35%であった。しかし，自然妊娠と着床前診断例の両者のエンドポイントが異なるため，妊娠転帰を直接比較することは困難であり，両者の比較を可能にする研究が待たれるとある[14]。

おわりに

遺伝学的検査である染色体検査による保因者診断や出生前診断に際しては，検査前後の遺伝カウンセリングが必須となる。理想的には，産科主治医とは倫理的に独立し，染色体不均衡型構造異常をもつ小児の診療に精通し，自然歴や社会資源も含めた情報提供が可能な臨床遺伝専門医である小児科医や認定遺伝カウンセラーが遺伝カウンセリングを担当することが望ましい。

所属医療機関に臨床遺伝専門医がいない場合は，紹介可能な近隣医療機関を臨床遺伝専門医一覧から参照されたい。特に生殖補助医療領域の知識に習熟した「生殖医療に関する遺伝カウンセリング相談受入れ可能な臨床遺伝専門医」も明示されている[15]。

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