denatured with 0.4 N NaOH for 15–20 min and capillary-transferred overnight onto nylon membrane (HybondN+, AmershamPharmacia Biotech, Buckinghamshire, UK) using alkaline transfer buffer, and the membrane was washed twice with 2 x SSC. DNA fixed to the membrane by UV crosslinking was hybridized to fluorescein-labeled probes using Gene Images Random Prime Labeling Kit (AmershamBiosciences). Hybridized signal was detected using Gene Image CDP-Star Detection Kit (Amersham-Biosciences). Genomic DNA from phenotypically normal individuals were used as controls.

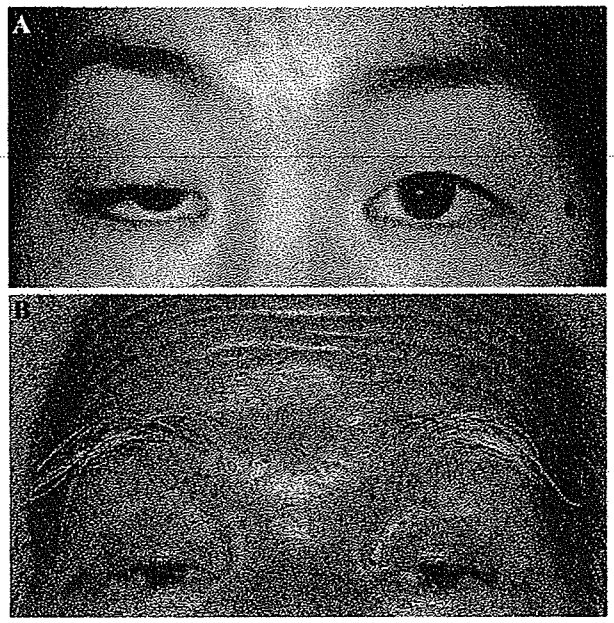

Fig. 2 Blepharopotosis of two affected members (V-5 and III-5). The proband (V-5) has unilateral blepharoptosis (**a**), and III-5 has bilateral ptosis (**b**). Both patients have overaction of the frontalis muscle

Methylation analysis of *ZFHX4*

DNA (1 μg) from patients and a control individual was modified with sodium bisulfite using CpGenome DNA modification Kit (CHEMICON, CA, USA). PCR primers specific for methylated or unmethylated DNA (Herman et al. 1996) were designed from sequences of two CpG islands of *ZFHX4* (Table 1), at which one island is located 3 kb upstream and the other 0.4 kb downstream of exon 1, respectively (Hemmi et al. 2006). Methylation-specific PCR was performed in a 20-μl reaction mixture containing 50 ng bisulfite-modified DNA/0.5 U AmpliTaq Gold (AppliedBiosystems)/200 μM dNTP/0.5 μM each primer/ 1× PCR buffer with a condition consisting of initial denaturation at 95°C for 10 min, 37 cycles of amiplification at 94°C, 30 s/60°C, 30 s/72°C, 30 s, and final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on 2.5% agarose gel. Because aberrant signals on Southern blot were observed, the bisulfite-modified DNA was also amplified with specific primers for the *Eco*RI site around exon12 of *ZFHX4*. PCR products were cloned using TOPO TA cloning Kit (Invitrogen, Carlsbad, CA, USA), and then cloned DNA was sequenced (Clark et al. 1994).

Results

Linkage and haplotype analyses

As the number of affected members in this family was small and penetrance might be considerably low, we set a cutoff LOD score ≥ 0.4 in all calculations. The two-point and multipoint analyses gave nine markers (D1S255, D1S484, D5S630, D7S669, D9S1776, D11S4046, D19S221, D19S226, and D14S276) that show such scores,

Table 1 Polymerase chain reaction (PCR) primers for methylation-specific PCR and bisulfite sequencing

Primer	Sequences 5′→3′	Anneal temperature	Products size (bp)
CpG1-MF	GTTTAGTCGTTCGGATCGCGTTCGT	60	182
CpG1-MR	AACTTAACCTCGAAACGCGCCAACG		
CpG1-UF	GTTAGGTTTAGTTGTTTGGATTGTGTTTGT	60	192
CpG1-UR	TCCAAAACTTAACCTCAAAACACACCAACA		
CpG2-MF	GGGTTTTGTTTTTTTCGCGAGTTTC	60	139
CpG2-MR	TACGAAAAACCAATCATCCCAATCG		
CpG2-UF	TTTTTGGGTTTTGTTTTTTTTGTGAGTTTT	60	149
CpG2-UR	ATAAATACAAAAAACCAATCATCCCAATCA		
Ex12-upF	GGAAAGTAAAGAAGTTGTTTTAAAA	56	180
Ex12-upR	ATAACAAAACAATACAACACAAATA		
Ex12-dwF	TGATAATGGTAGAAGGTAAAGTATT	56	200
Ex12-dwR	AACATATAAAACAAAAAAACCTCTA		

M methylated-specific, *U* unmethylated-specific, *F* forward primer, *R* reverse primer, *up* upstream restriction site, *dw* downstream restriction site

and the nonparametric analysis gave four candidate regions (3q22.1, 8q21.11, 12q24.32, and 20p13). The haplotype analysis at these loci revealed that all but 8q21.1, 12q24.3, and 14q22.3 regions (Fig. 1) were excluded, because some patients do not have putative disease-linked haplotypes (data not shown). Thus, we finally left these three regions commonly shared by the patients as candidates. Further refinement was impossible, because any more family samples were not available.

Mutation analysis of the *ZFHX4* gene

The direct sequencing of *ZFHX4* revealed one missense alteration (G12411T or L4137F) in exon 12 in the affected member and some normal control samples (data not shown), indicating that it is actually a single nucleotide polymorphism (SNP). Likewise, another mutation analysis for homologous sequences between the mouse and human in their promotor/enhancer region of *ZFHX4/Zfhx4* revealed no disease-associated mutation in the patient. Southern-blot analysis using a probe for exon 2 or exon 12 of *ZFHX4* detected a respective extra *Eco*RI-fragment in a patient (III-5) (Fig. 3) and an extra band for exon 12 in two other patients (III-13 and IV-12).There were no aberrant signals with other restriction enzymes. However, sequence analysis of these regions did not identify any genomic rearrangements and confirmed no restriction fragment length polymorphism (RFLP) around exon 2 or 12. This led us to try to detect the differential methylation status in the regions between patients and normal controls. However, methylation-specific PCR following a bisulfite treatment revealed no change of the methylation status in the patient's two CpG islands (data not shown).

Discussion

In this study, we performed linkage and haplotype analyses of a Japanese PTOS pedigree. Consequently, three regions, 8q21.11-q22.2, 12q24.32-qter, and 14q21.1-q23.2, were shown to be candidates. The two-point maximum LOD scores were considerably low, being 1.16, 0.47, and 0.72 at *D8S551*, *D12S1659*, and *D14S276* (Table 2), respectively, because some unaffected family members also had disease-linked haplotypes around the markers. It was obvious that the disease in the family shows incomplete penetrance. Although we diagnosed carefully many members of the family at the beginning of this study, we might have overlooked very mildly affected members.

Two regions responsible for autosomal PTOS were reported previously, i.e., 1p34.1-p32 for PTOS1 by linkage analysis (Engle et al. 1997) and 1p34.3/8q21.12 by analysis of a chromosomal translocation (McMullan et al. 2002). Three candidate loci we have detected include the translocation breakpoint 8q21.2 (McMullan et al. 2002) but do not contain 1p34.1-p32 (Engle et al. 1997). Therefore, the disease locus for our family could be associated with PTOS1. However, we failed to identify in our family any point mutation, genomic rearrangement, or methylation aberration involving *ZFHX4* that was disrupted at the 8q21.12 breakpoint of the translocation in a PTOS patient (McMullan et al. 2002).

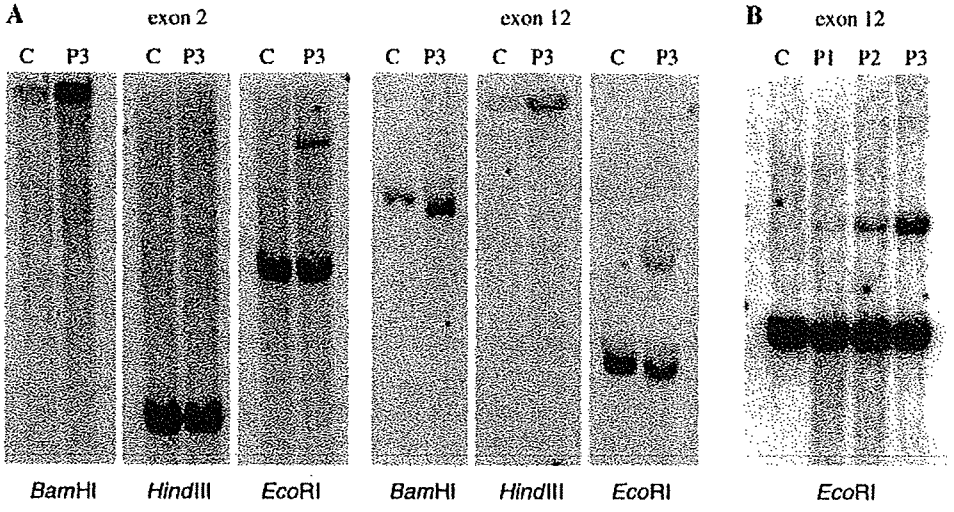

Fig. 3 Southern-blot analysis of patients (*P3*) and a control individual (*C*) after digestion with three different enzymes, using probes for exons 2 and 12 of *ZFHX4*. An extra band is seen in *Eco*RI fragments, including *ZFHX4* from a patient (III-5, P3) (**a**) and three patients (P1, III-13; P2, IV-12; and P3) (**b**)

Table 2 Log of odds (LOD) scores of candidate loci of the third hereditary congenital ptosis locus (PTOS1)

Recombination fraction (θ)							Multipoint
Locus	0.00	0.05	0.10	0.20	0.30	0.40	LOD score
D8S551	0.95	1.12	1.16	1.01	0.68	0.29	1.46
D8S525	0.15	0.12	0.09	0.05	0.02	0.00	1.51
D8S270	−2.53	−1.33	−0.86	−0.36	−0.13	−0.02	1.39
D8S1772	0.33	0.31	0.29	0.23	0.15	0.08	1.22
D12S1679	−0.03	0.31	0.45	0.47	0.34	0.17	0.59
D12S1659	0.15	0.12	0.09	0.05	0.02	0.00	1.39
D12S97	−0.20	−0.11	−0.05	−0.00	0.00	0.00	1.61
D12S1599	−0.78	−0.43	−0.27	−0.11	−0.04	−0.01	1.63
D12S1723	0.15	0.25	0.27	0.23	0.14	0.06	1.79
D14S1057	0.62	0.64	0.61	0.51	0.37	0.20	−1.00
D14S276	0.46	0.65	0.72	0.65	0.44	0.19	1.31
D14S66	−1.65	−1.15	−0.83	−0.45	−0.25	−0.12	2.01
D14S274	−1.16	−0.30	−0.07	0.07	0.07	0.02	1.97
D14S1038	−2.15	−1.64	−1.27	−0.76	−0.43	−0.19	1.94

The human *ZFHX4* gene spans about 180 kb, contains 12 exons, is mapped at 8q13.3-q21.11, and has >90% homology to the mouse *Zfh4* and 52% to the human *ATBF1* (Hemmi et al. 2006). The *Zfh4* gene encodes a member of the zinc finger homeodomain family, which has four homeodomains and 22 zinc fingers (Sakata et al. 2000). *Zfh4* was highly expressed in developing muscle and brains of the mouse, especially in the midbrain and hindbrain (Kostich and Sanes 1995), and was also detected in adult rat oculomotor nucleus, which controls the levator palpebrae superioris (LPS) muscle (Nogami et al. 2005). The oculomotor nucleus exists in the mesencephalon. The oculomotor nerve innervates to LPS and the extraocular muscles, except for the lateral rectus and the superior oblique muscle, and also supplies parasympathetic preganglionic fibers to the ciliary ganglion through which postganglionic nerve controls the ciliary muscle and the sphincter muscle of the pupil. It was reported that *ZFHX4* was upregulated in postmitotic neurons and suggested that *ZFHX4* was influenced on neural differentiation including migration and axon outgrowth (Nogami et al. 2005; Hemmi et al. 2006). All these lines of evidence strongly suggest that *ZFHX4* is the responsible gene for a type of PTOS.

There are two opinions concerning the pathological change in LPS from congenital ptosis. Three reports approved that congenital ptosis is caused by primary dysgenesis or myodystrophy of LPS (Berke and Wadsworth. 1955; Isaksson and Mellgren 1961; Isaksson 1962; Stula 1988). On the other hand, Edmunds et al. (1998) argued against such a mechanism because there was no significantly different histology between LPS from PTOS patients and normal individuals. A light-microscope study of an LPS specimen from the proband in our family showed that the muscle fibers were displaced by fibrous and fatty tissues, and scanty atrophic striated muscles remained. This histological finding is consistent with dysgenesis of LPS. Therefore, if *ZFHX4* is relevant to congenital blepharoptosis, its abnormal expression might cause a failure of neuronal differentiation of the oculomotor nerve, leading to LPS dysgenesis.

In conclusion, we identified three possible regions candidate for PTOS1—8q21.11-q22.2, 12q24.32-qter, and 14q21.1-q23.2—by a whole-genome linkage analysis. These regions do not definitely overlap with a 1p34.1-p32 segment to which PTOS1 was mapped (Engle et al. 1997), and one of the regions we assigned includes a segment containing *ZFHX4* (McMullan et al. 2002). Thus, our data may support the existence of the PTOS1 locus. It remains to be seen whether other PTOS1 families have mutations in *ZFHX4*, another gene, or in a putative *ZFHX4* enhancer located at 8q21.11-q22.2.

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First case of liposarcoma arising from the fallopian tube: Case report and review of the literature

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Abstract

Liposarcoma is the most common soft tissue sarcoma in adults, and it typically occurs in either the retroperitoneum or the extremities. However, this malignant tumor is very rare in the female reproductive system. A 58-year-old woman presented with acute abdominal pain, and computed tomography (CT) scan detected multiple masses measuring 4–6 cm in size with a fatty density in her adnexal region. Laparoscopic evaluation revealed tumors of the left fallopian tube with a normal left ovary. Histopathological evaluation of the resected pelvic tumors showed lipocytes and lipoblasts of various sizes, leading to diagnosis of well-differentiated liposarcoma of the left adnexal region. This is the first known case of a liposarcoma arising from the fallopian tube. When a pelvic mass with fatty density that does not show typical findings of a mature cystic teratoma is detected by either CT or magnetic resonance imaging, the possibility of a liposarcoma should be considered.

Key words: fallopian tube, liposarcoma, mature cystic teratoma.

Introduction

Liposarcomas, which account for at least 20% of all sarcomas in adults, typically occur in either the retroperitoneum or the lower extremities. However, in the gynecological field, there have been very few reports of either retroperitoneal liposarcoma or intra-abdominal liposarcoma. To date, although several cases of liposarcoma from the uterine body have been reported, there is no case of a liposarcoma occurring in the adnexal region.

We report here the first known case of liposarcoma arising from stroma of the fallopian tube, which we mistakenly diagnosed to be a mature cystic teratoma before operation.

Case Report

A 58-year-old woman was transferred to our hospital due to acute onset of lower abdominal pain. She was taking medication for hypertension and diabetes mellitus, but was otherwise in good health prior to the onset of abdominal pain. A pelvic examination revealed severe tenderness in the left adnexal region, but a pelvic mass was not palpable. A complete blood count showed leukocytosis. Chest X-ray and electrocardiogram (ECG) findings were both normal. Emergency computed tomography (CT) scan of the abdomen detected multiple pelvic masses measuring 4–6 cm in size with a fatty density (Fig. 1a). In addition, magnetic resonance imaging (MRI) showed heterogeneous masses with a high signal intensity on T_1-weighted and T_2-weighted images (Fig. 1b–d). She was initially diagnosed with probable torsion of ovarian mature cystic teratomas, which are the most common ovarian neoplasms.

Laparoscopic surgery was carried out to remove the pelvic masses. Although no ascites was seen in the abdominal cavity, a normal left ovary and three yellowish smooth masses, with pedicles originating from the

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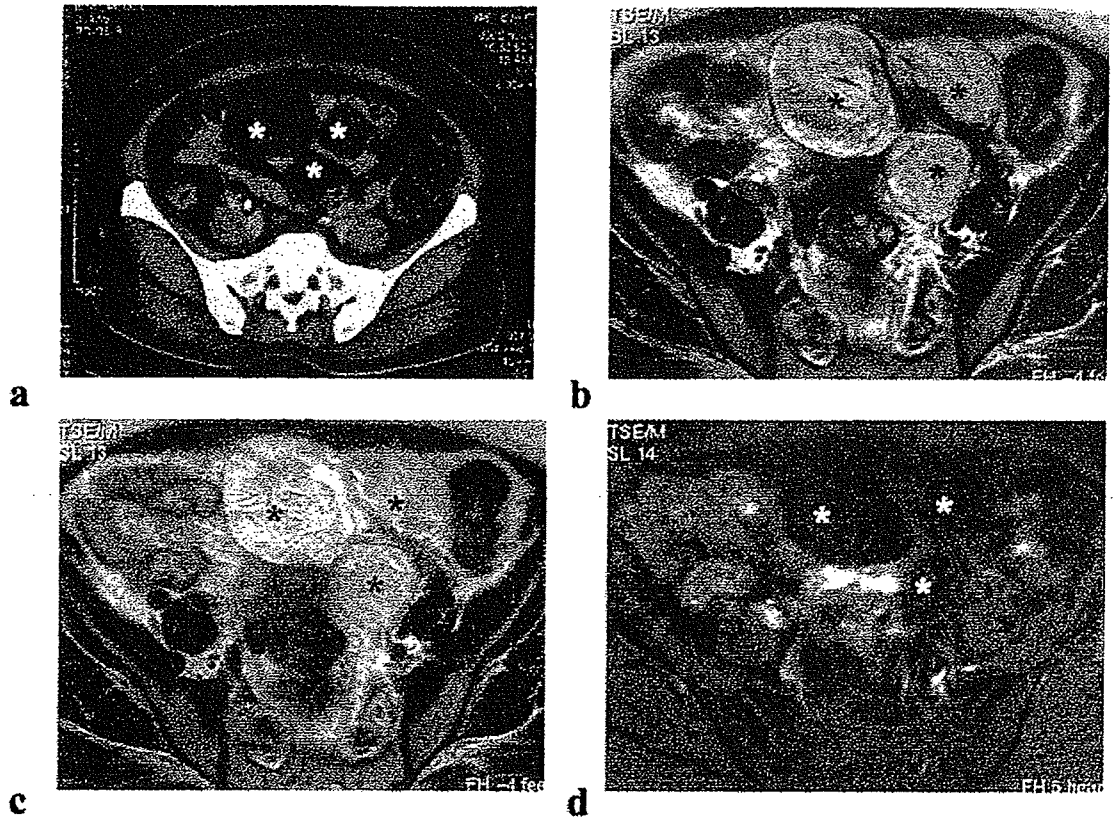

Figure 1 Computed tomography (CT) and magnetic resonance imaging (MRI) findings of liposarcoma. (a) White asterisk indicates one of the heterogeneous masses with fat density in the pelvic cavity detected by CT examination. (b) Black asterisk denotes well-defined masses with a high intensity by both T_1-weighted images of an MRI examination. (c) Black asterisk denotes well-defined masses with high intensity based on T_2-weighted images of an MRI examination. (d) White asterisk shows the masses with a low signal intensity based on T_1-weighted images with fat suppression.

left fallopian tube, were detected. These pedicles, consisting of three masses, had become intertwined with each other, thus most likely being the cause of the patient's abdominal pain. As the cut-surface appearance of these tumors, which we first resected by laparoscopic surgery, comprised a yellowish fatty and grayish white lobulated solid mass, malignant tumors were suspected even though the pelvic masses were not ovarian tumors (Fig. 2a,b). We therefore changed from laparoscopic surgery to a laparotomy in order to perform a complete resection, in addition to a total hysterectomy, bilateral salpingo-oophorectomy, partial omentectomy, and peritoneal washing cytology as the treatment for tubal cancer. No biopsies of the lymph nodes or peritoneal regions were carried out because no lymph node swelling or peritoneal dissemination was detected during the operation.

Pathological evaluation of the resected specimen showed the presence of well-differentiated liposarcoma, which was characterized by the proliferation of mature fat cells whose size ranged from small to large, and lipoblasts, which contained multiple vacuoles in the cytoplasm with enlarged, irregular, dense nuclei in fibrous tissue. The collagenous stroma was congested due to torsion of the pedicle (Fig. 3a,b). The resected tumors were diagnosed to be well-differentiated liposarcoma from the stroma including a tela subserosa between the lamina propria mucosae and tunica serosa (Fig. 3c). The mucosa of the fallopian tube was intact. The resection margin was negative and no metastasis was seen in any other specimens. An immunohistochemical study indicated that vimentin and S-100 protein were positive, whereas CD68 was negative. No malignant cells were observed in any peritoneal washing cytology specimens.

Neither chemotherapy nor any other adjuvant therapies were added, because the masses were considered

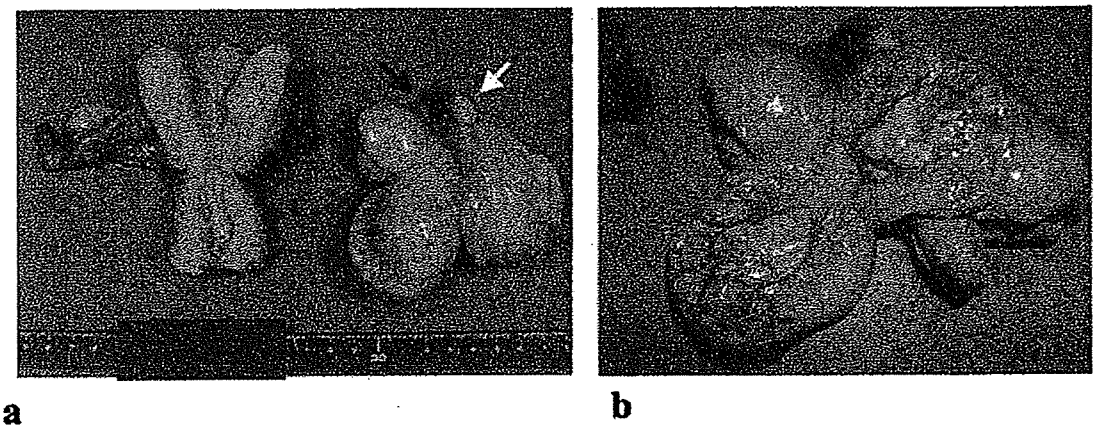

Figure 2 Macroscopic findings of the specimen. (a) Resected masses, uterus and bilateral adnexas. Black arrow indicates the fallopian tube and the white arrow indicates the left ovary. (b) The cut-surface appearance of the tumor was composed of a yellowish fatty and grayish-white lobulated solid mass.

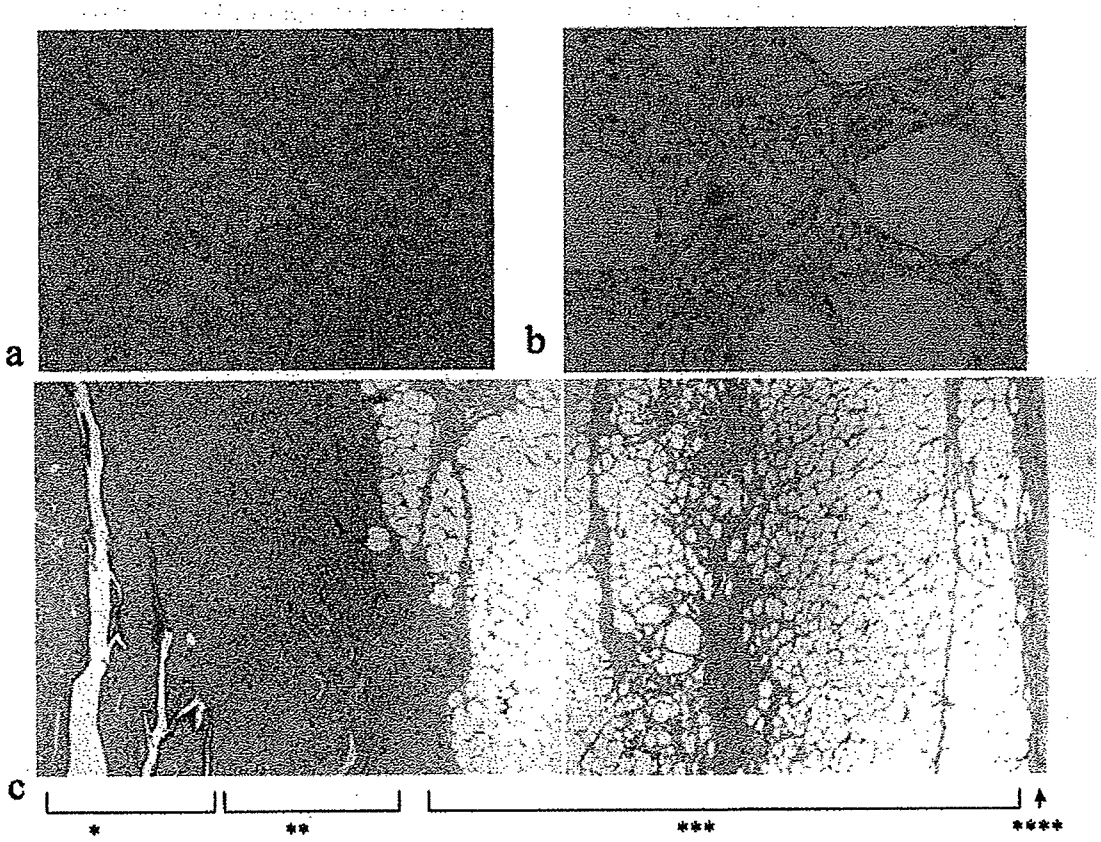

Figure 3 Pathological findings of liposarcoma arising from the stroma of the fallopian tube. (a) Low-power view of the tumor showing mature fat tissue and proliferation of fibrous tissue; H&E, original magnification ×40. (b) High-power view of the tumor cells. Several mature lipocytes and lipoblasts with vacuoles in the cytoplasm, enlarged hyperchromatic nuclei and prominent nucleoli were found. H&E, original magnification ×400. (c) Low-power composition view of the resected tumors which arose from the stroma of the fallopian tube showing the area of the epithelium (*), stroma (**), liposarcomas (***) and serosa (****) of the fallopian tube. H&E, original magnification ×40.

to have been adequately resected. The patient remains disease free 22 months after the surgery.

Discussion

The presence of a fatty component in pelvic masses usually indicates a mature cystic teratoma, as they are the most common ovarian neoplasm found in women. However, the possibilities of fat necrosis, carcinosarcoma or liposarcoma should also be considered. In particular, a small number of cases of retroperitoneal liposarcoma or intra-abdominal liposarcoma have been reported and, therefore, pelvic masses with a fatty component should be managed as potentially malignant tumors. The CT and MRI findings in our patient showed three distinct masses consisting of fat with septations, which are characteristic of well-differentiated liposarcoma.[1] We therefore could have made a diagnosis of liposarcoma before the operation if we had included the possibility of liposarcoma in the differential diagnosis. The poor prognosis of liposarcoma depends on several factors including age older than 60 years, tumor size greater than 5 cm, and high-grade histology.[2]

The histological cell type of liposarcoma, which is classified as being either well differentiated, myxoid/round cell, pleomorphic, or dedifferentiated, is the most important factor predicting survival rates for cases of liposarcoma. The 5-year survival rate for well-differentiated subtypes is 90%, whereas myxoid liposarcomas have a 5-year survival probability of 70–90% and both round cell liposarcoma and pleomorphic liposarcoma have a high metastatic potential and a 5-year survival probability of only 20–50%.[3] According to the American Joint Committee (AAJC) staging protocol for sarcoma of the soft tissue, the stage is determined by the histological grade, the presence of lymph node metastasis and distant metastasis, the tumor size and its location.[4] In this case of a 58-year-old woman, the tumor was an intra-abdominal mass measuring more than 5 cm in size but without any metastasis, and the histological grade of the tumor was a low grade (well-differentiated type). Therefore, this case was classified as stage I disease by AAJC staging and stage Ia by FIGO staging.

The recommendation of the National Cancer Institute for the treatment of adult soft tissue sarcoma of patients with stage I disease is surgical excision. If the tumor is unresectable, preoperative radiation therapy and/or postoperative radiation therapy may be used. In addition, chemotherapy is not carried out in cases of tumors with a low metastatic potential.[5] In the present case: (i) a total hysterectomy, a bilateral salpingo-oophorectomy and a partial omentectomy were all carried out; (ii) no metastases were detected by chest X-ray, CT and MRI examinations; (iii) the peritoneal washing cytology findings were negative; and (iv) her histological diagnosis was well-differentiated liposarcoma with a minimal metastatic potential. Accordingly, neither radiation therapy nor chemotherapy was carried out as adjuvant treatment. She is being followed up clinically and by serial chest X-rays and CT scans of the chest, abdomen and pelvis, and no relapse of symptoms has been observed.

This is the first reported case of liposarcoma arising from a fallopian tube. As the normal treatment protocol for tubal cancer was selected, we performed a total hysterectomy, a bilateral salpingo-oophorectomy and a partial omentectomy for the liposarcoma arising from the stroma of the fallopian tube.

When multiple pelvic masses with a fatty density are detected by either CT or MRI examinations which do not correspond to the typical findings of mature cystic ovarian teratomas, then the possibility of a liposarcoma should be taken into consideration and included in the differential diagnosis.

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Management of prenatal ovarian cysts

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KEYWORDS
Fetal ovarian cycsts;
Spontaneous resolution;
Postnatal surgery

Abstract

Objectives: The aim of the present study was to analyze the antenatal and postnatal outcome of fetal ovarian cysts in relation to their ultrasonographic pattern and size.
Methods: Sixteen fetal ovarian cysts were diagnosed in 16 fetuses and followed with serial ultrasonograms in utero and after birth until spontaneous or surgical resolution.
Results: Eleven fetal ovarian cysts were simple cysts at first prenatal scan but 3 of the 11 became complex cysts at last prenatal scan and required postnatal laparoscopic surgery. Seven of the 11 simple cysts (63%) disappeared on follow-up imaging by ultrasonograms or MRI during pregnancy or within 2 months after birth. The rate of spontaneous resolution of simple cysts was higher than that of complex cysts (40.0%). The mean maximum diameter of the ovarian cysts before delivery that were subsequently excised surgically at postnatal period (50±13.4 mm) was not different from that of ovarian cysts that resolved spontaneously (42.8±12.8 mm, $P=0.2918$).
Conclusion: In our study, cyst size did not predict the risk of ovarian loss. The opportunity of laparoscopic exploration versus conservative management needs to be investigated because some complex cysts resolved spontaneously in the postnatal period.

1. Introduction

The rate of detection fetal ovarian cysts has increased since the advent of routine antenatal sonography [1]. The etiology of fetal ovarian cysts is not entirely clear. Maturation of the hypothalamus–pituitary–ovary axis commences from the 29th week of gestation under elevated levels of fetoplacental estrogen [2]. An immature hypothalamus–pituitary–ovarian feedback is thought to be responsible for gonadal hyperstimulation in premature fetuses. After delivery, reduction in hormonal stimulation can lead to spontaneous resolution of the ovarian cyst; therefore, a conservative management of uncomplicated cysts has been suggested, although some cysts carry the risk of subsequent complication that might require postnatal surgery [3–5]. Various complications of ovarian cysts have been described, such as compression of neighboring viscera, rupture of the cyst, hemorrhage and ovarian torsion [6–9]. However, there is no standard treatment of fetal ovarian cysts and their management varies widely among different centers. The present retrospective study

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was conducted to analyze the antenatal and postnatal outcome of fetal ovarian cysts in relation to their ultrasonographic pattern and size.

2. Materials and methods

A total of 16 fetal ovarian cysts were diagnosed in 16 fetuses between 30 and 37 weeks of gestation on routine sonography conducted between 1989 and 2006 in our hospitals. The detection of a cystic structure in the fetal abdomen with otherwise normal anatomy of the gastrointestinal and urinary tracts in a female fetus was highly suggestive of an ovarian cyst. This assumption was based on the following fact: other anomalies that can be mistaken as ovarian cysts, such as mesenteric cysts, urachal cysts, enteric duplication, and dilated bowel, are very rare. The shape of enteric bowel duplication is generally tubular, and the difference is a two layered muscle wall seen on antenatal ultrasonography in bowel duplication. But duplications of the gastrointestinal tract are mimick fetal ovarian cysts. Duodenal atresia has a typical double bubble appearance. In cases with a suspected ovarian cyst, a thorough sonographic assessment with/without MR imaging of the entire fetus was performed to exclude other associated anomalies.

The cysts were classified as either complex or simple according to Nussbaum et al. [1]. A cyst that was completely anechoic and had a thin wall was defined as simple, while an echogenic cyst was defined as complex (Fig. 1). We measured the maximum antenatal size of the ovarian cyst, and the associated complications and outcome.

After diagnosis, the mothers were followed by serial ultrasound scans every 2 weeks until delivery, and the newborn was followed postnatally until spontaneous resolution or surgical excision. Spontaneous vaginal delivery took place in all cases except when cesarean section was indicated for obstetrical reasons not related to the fetal ovarian cyst. All study protocols were approved by the Committee for Ethical Issues on Human Genome and Analysis of Nagasaki University.

3. Results

The mean diameter of the 16 fetal ovarian cysts was 42.6 ± 12.3 mm (±SD) at diagnosis. All cysts were first detected in the third trimester. Eleven fetal cysts were simple and five were complex when first detected. All 16 fetuses were born at term, with 14 by normal vaginal delivery and 2 by Cesarean section because of breech presentation or cephalopelvic disproportion.

Spontaneous resolution during pregnancy was noted in 2 of the 11 (18.2%) simple cysts. Five simple cysts diminished in size 6 months after delivery and 4 of these 5 disappeared spontaneously within 2 months after birth, while surgery was performed for enucleation of one simple cyst due to its large size (62 mm) at last prenatal diagnosis (Table 1, Case 3). We didn't aspirate for this case because we took into consideration the possibility of fetal ovarian tumor. Histopathological examination of this cyst showed a simple cyst with no specific epithelial findings.

With regard to the remaining 4 (36.4%) simple cysts, their size did not change or increase at late pregnancy. Three of these simple cysts that did not change in size became the complex type during the prenatal follow-up scans. Repeat sonograms showed a fluid level in the cyst or a solid pattern. Accordingly, surgery was performed within 10 months after birth due to suspected torsion or hemorrhage (Table 1). One of these three cysts showed evidence of torsion at surgery (Table 1, Patient 9) and histopathological examination indicated a necrotic simple cyst with calcification. The other two cysts showed evidence of intracystic hemorrhage but not that of torsion (Table 1, Patients 10 and 11). Histopathological analysis indicated two simple cysts with bleeding with or without calcification. One simple cyst that increased in size later showed spontaneous resolution within

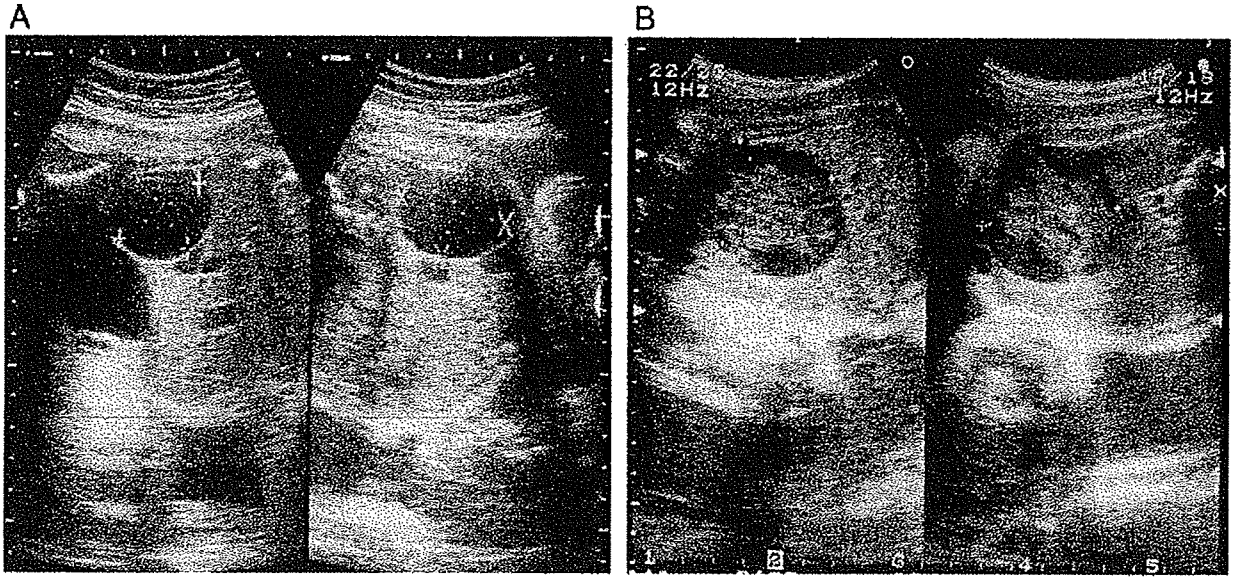

Figure 1 Fetal cystic tumors identified by ultrasonography in the third trimester. (A) A representative example of a simple cyst. The cyst was completely anechoic. Note the surrounding thin wall. (B) A representative example of an echogenic complex cyst.

Table 1 Perinatal appearances of fetal ovarian cysts in relation to prenatal ultrasound pattern and size

Case	1st prenatal scan	Size at prenatal diagnosis (mm)	Last prenatal scan	Maximum diameter (mm)	Outcome in pregnancy	Postnatal outcome	Pathology
1	S	45	S	45	Reduced	S.R.	
2	S	48	S	48	Reduced	S.R.	
3	S	75	S	75	Reduced	Surgery	Simple cyst
4	S	32	S	32	Reduced	S.R.	
5	S	31	S	34	S.R.	–	
6	S	23	S	23	S.R.	–	
7	S	41	S	41	Reduced	S.R.	
8	S	44	S	66	Increased	S.R.	
9	S	50	C	56	No change	Surgery	Simple cyst with necrosis, calcification and torsion
10	S	40	C	41	No change	Surgery	Simple cyst with calcification and hemorrhage
11	S	49	C	49	No change	Surgery	Simple cyst with hemorrhage
12	C	32	C	32	No change	Surgery	Simple cyst with torsion and adhesion between bowel and ovarian cyst
13	C	42	C	46	No change	Surgery	Simple cyst with necrosis, calcification and torsion
14	C	46	C	51	No change	Surgery	Simple cysts with calcification and hemorrhage
15	C	40	C	40	No change	S.R.	
16	C	55	C	55	Reduced	S.R.	

S=Simple cyst; C=complex cyst; S.R. =Spontaneous resolution.

6 months after birth (Table 1). What happened to the ovary after the cyst resolved remains unknown, because we did not perform the second look observation by laparoscopy.

With regard to the five complex cysts, four of them showed no change in size during prenatal ultrasound monitoring. Postnatally, three (75%) of 4 infants underwent surgery for persistence of the cyst or postnatal evidence of torsion or evidence of neoplastic changes (Table 1, Patients 12, 13 and 14). Histopathological examination showed two simple cysts with torsion and one simple cyst with intracystic hemorrhage. The other two complex cysts disappeared on follow-up imaging within 11 months after birth as confirmed by follow-up ultrasonography or other imaging. We don't know whether the ovary after the cyst resolved leaved a normal ovary or disappeared on follow-up imaging because the laparoscopic second look observation was not performed in all infants.

Surgery was performed for excision of 7 of 16 cysts. The procedure included oophorectomy, sometimes with enucleation. Oophorectomy was performed to the cases that were detected the ischemic necrosis and self-amputation of the fetal ovarian cyst. Microscopic examination indicated that all seven cysts were simple cysts (Table 1). Three out of the seven cysts were simple cysts with torsion, but adhesion between the bowel and ovary was noted in one case during surgery. The other three cysts showed intracystic hemorrhage and one cyst was simple cyst without specific epithelial findings. The histopathological finding was benign and neoplastic tumor was not detected in our study. But Pietro Bagolan et al. recommended the surgery of the ovarian cysts with an ultrasound pattern of torsion which persisting after birth [8]. We also think that fetal ovarian complex cysts should be managed carefully after birth because 3 of 6 complex cysts that surgeries were performed were cysts with torsion and 1 of 3 cysts with torsion showed the adhesion between the bowel and the cyst.

The mean maximum diameter of the ovarian cysts before delivery that were subsequently excised surgically at postnatal period (50±13.4 mm) was not different from that of ovarian cysts that resolved spontaneously (42.8±12.8 mm, P=0.2918).

4. Discussion

The majority of fetal ovarian cysts are considered to result from excessive hormonal stimulation [10,11] and resolve spontaneously after birth and that they are of no clinical significance. The detection of ovarian cysts in utero has increased lately, with the increased use of routine ultrasonography, raising questions on the perinatal management of these anomalies. After the diagnosis of fetal ovarian cyst, serial ultrasound examinations are necessary in order to detect any torsion. It has been suggested that such complication depends on the size of the fetal ovarian cyst [6]. However, there was no such correlation in our series, as we did not detect any difference in the mean size of cysts with and without torsion at diagnosis. The mean maximum diameter of ovarian cysts with torsion was 44.6±12.1 mm, while that without torsion was 44.2±12.8 mm.

Interestingly, 7 of the 11 simple cysts (63.6%) disappeared spontaneously during pregnancy or within 2 months after birth. The rate of spontaneous resolution of simple cysts was higher than that of complex cysts (40.0%).

Surgery was performed for excision of seven cysts. Indications for surgeries in our hospital were the following: 1) large fetal ovarian cysts that did not diminish in size at postnatal scan, 2) complex cysts that showed evidence of torsion at postnatal scan, and 3) cysts suspected to be neoplastic tumor. In our experience, cyst size did not predict the risk of ovarian loss; the mean of maximum prenatal diameter of ovarian cysts that were subsequently excised surgically was not different from that of cysts that resolved spontaneously.

We did not carry out any ovarian cyst aspiration during fetal and neonatal life during the study period. Although aspiration of large simple cysts might prevent serious complications, we didn't aspirate them because we took into consideration the possibility of fetal malignant ovarian tumor. And the selection of cases to undergo aspiration is difficult because 7 of the 11 simple cysts (63.6%) disappeared spontaneously during pregnancy or within 2 months after birth. After the diagnosis of fetal ovarian cyst is established, we recommend a conservative approach that includes serial ultrasound examinations and follow the course of the anomaly until spontaneous resolution.

Complex cysts should be managed carefully because they may represent entities such as intestinal duplications or tumors. Fetal cystadenomas and granulosa cell tumors have been reported in the literature [14,15]. It is possible that intracyst hemorrhage could result in fetal anemia or torsion of the cyst could cause bowel obstruction [8,12,13].

In our study, two out of eight complex cysts resolved spontaneously. A cyst with an echo pattern of torsion that disappears spontaneously is clinically important, but knowledge of the real nature of such cyst is difficult. Gohar et al, in their report of the case of spontaneously disappearing cyst, suggested that it could be represented by a cyst that underwent initial torsion and subsequent spontaneous detorsion [16]. What happened to the ovary after the cyst resolved remains unknown, because we did not perform the second look observation by laparoscopy, but we also think that there is a possibility of the spontaneous detorsion of the fetal ovarian tumor. Pietro Bagolan et al. reported the case that spontaneously disappeared at ultrasound, and said the possibility of the self-amputation of the ovary and adhesion to other organs [8].

Furthermore, laparoscopic surgery was performed to remove four out of seven fetal ovarian cysts. Since the number of cases in this report was small, it is difficult to determine the conditions that dictate a requirement for, and a timing of any surgical intervention. However, from the point of view of minimally invasive management, laparoscopic exploration or conservative management may be necessary as the first line intervention in terms of diagnosis and therapy of the fetal ovarian cysts.

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Giant cystic meconium peritonitis associated with a cloacal anomaly: case report

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Cloacal anomaly;
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Hydrocolpos

Abstract This report describes a case of giant cystic meconium peritonitis (GCMP) associated with a cloacal anomaly. Antenatal ultrasonography and magnetic resonance imaging demonstrated persistent fetal ascites, bilateral hydronephrosis, and 3 pelvic cystic structures. The baby girl showed duplicated hydrocolpos and a single orifice of the cloaca with a long common channel inducing a urinary outflow obstruction. After constructing a diversion colostomy, a cutaneous vesicostomy was necessary to prevent recurrent urinary tract infections. These findings are consistent with a prenatal diagnosis of cloacal anomalies, thus suggesting an association with severe obstruction of lower urinary tract and meconium peritonitis.Most of reported cases of meconium peritonitis associated with the cloaca show fibroadhesive types with scattered intraperitoneal calcifications and adhesions. However, the present case showed a rare GCMP suggesting continuous urinary influx via the fallopian tubes until the later stage of intrauterine life.

Meconium peritonitis (MP) is aseptic peritonitis occurring because of a spill of meconium in the abdominal cavity through one or several intestinal perforations, which has taken place during intrauterine life [1]. Commonly, MP is complicated with an intestinal obstruction, such as intestinal atresia and meconium ileus. Several pediatric surgeons have described patients with a cloacal anomaly showing intraperitoneal calcification or a dense plastic peritonitis associated with hydrometrocolpos or hydrosalpinx [2-6].

This report documents a case of giant cystic meconium peritonitis (GCMP) associated with a cloacal anomaly. To the

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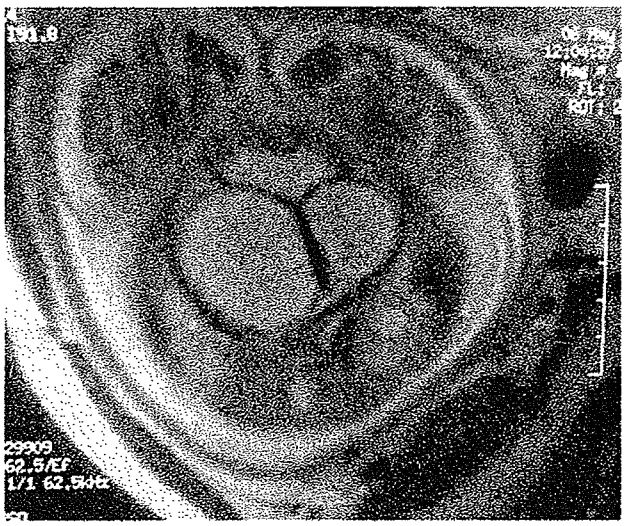

Fig. 1 Demonstration of duplicated hydrocolpos behind the bladder on magnetic resonance imaging.

best of our knowledge, there are no similar reports showing GCMP associated with cloacal malformations. The pathophysiology of this rare condition is herein discussed.

1. Case report

A 32-year-old female, gravida 0 para 0, was referred to the Department of Obstetrics and Gynecology of our University Hospital (Nagasaki University Hospital, Nagasaki, Japan) at 31 5/7 weeks of gestation for evaluation of fetal ascites. A fetal ultrasound examination and magnetic resonance imaging showed fetal ascites, bilateral hydronephrosis, and 3 abdominal cystic masses (Fig. 1). At 33 6/7 weeks of gestation, a cesarean delivery was performed because of an apparent decrease in the amnion and nonreassuring fetal heart rate pattern. The neonate weighing 2506 g and Apgar scores of 5 and 6 after 1 and 5 minutes, respectively, was delivered. A physical examination revealed generalized edema, abdominal distension, ambiguous genitalia, and anal atresia with a single perineal opening. By applying contrast medium during cystoscopy, the bladder and duplicated hydrocolpos were revealed. Despite of the passage of urine, distension of upper abdomen persisted. A laparotomy revealed a large cystic mass occupying the upper abdomen, which was incised and viscous fluid was drained. The bowel could be seen through the posterior wall of the cyst and was exposed after an incision. Multiple adhesions were present in the peritoneal cavity, but there was no evidence of perforation of the bowel. A transverse colostomy was performed for fecal diversion, and a drain was inserted into the cyst. The postoperative course was uneventful, and there was no discharge from the drain. Histologically, the contents of the cyst consisted of amorphous eosinophilic material, containing inflammatory cells such as neutrophils, eosinophils, macrophages, and lymphocytes as well as a small number of erythrocytes. Intestinal epithelial cells were not evident. Genitography showed dilated duplicated hydrocolpos, bladder, and rectum communicating with long, narrow common channel. There was no spillage of contrast medium into the peritoneal cavity (Fig. 2). The patient underwent a cutaneous vesicostomy for a recurrent urinary tract infection 4 months after birth. Further reconstructive surgery for the cloacal malformation and imperforate anus is planned to be attempted at a later date.

2. Discussion

In the present case, the operative findings indicated giant cystic type of MP; nevertheless, there was no evidence of bowel perforation and obstruction. Lorimer and Ellis [7] described 3 major types of MP—fibroadhesive, generalized, and cystic. Among the cystic type, GCMP is relatively unusual. The entire peritoneal cavity is converted into a large meconium-filled cyst lined by a thick membrane containing

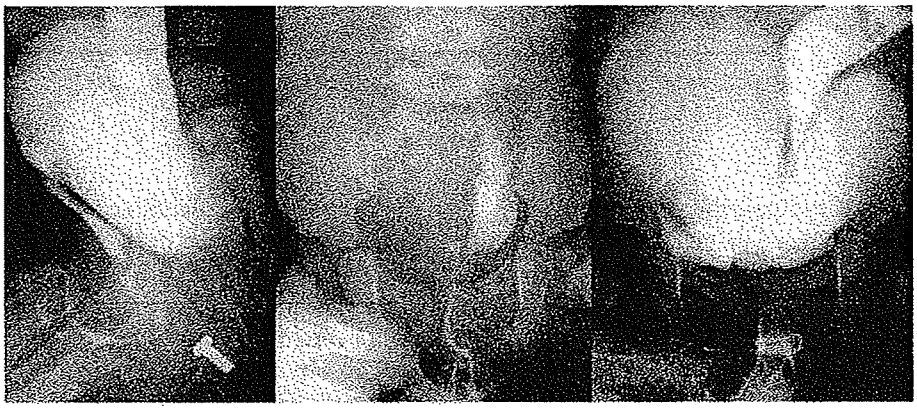

Fig. 2 Genitography demonstrated duplicated hydrocolpos behind the bladder and rectum as a narrow fistula. The level of confluence was observed between the pubococcygeal line and the midline. The common channel was narrow and it measured 3.5 cm in length. The distance from the bladder neck to confluence was 1.6 cm. Spillage of contrast media into the peritoneal cavity was not observed.

multiple calcium deposits and plaques, and the intestine are collapsed and compressed against the posterior abdominal wall [8].

A *cloacal anomaly* is defined as the junction of the rectum, vagina, and urethra into a single common channel [9], which is because of an earlier embryonic arrest during development in a female neonate [10]. In the presence of the lower urinary tract obstruction, urine might drain from the fetal bladder into the vagina and rectum, and the mixture of urine and meconium can occur. Regarding MP associated with cloacal anomaly, most authors have speculated that the route of the urine and meconium leakage is via the fallopian tubes. As urine accumulates in the vagina, a large hydrocolpos could develop. Urine containing a meconium component escapes from hydrocolpos via the fallopian tubes into the peritoneal cavity and causes intrauterine peritonitis [4,5,11-14]. In a rare case, Stephenson et al [3] reported a case of MP associated with cloacal malformation and perforated hydrocolpos.

The pathophysiology of hydrocolpos in this case is thought to be secondary to a disturbance of urinary efflux and reflux into the vagina probably because of a long and narrow cloacal channel. Prenatal continuous urinary back-flow into the vagina may increase intravaginal pressure resulting in a hydrocolpos. The route for an influx of meconium into the peritoneal cavity might have occurred via regurgitation through the fallopian tubes, or a perforation of hydrocolpos or bowel may also have been possible. Of these 2 hypotheses, the former might be more likely in this case because neither a perforation of hydrocolpos nor the bowel was observed. In addition, the intrauterine healing of a perforated hydrocolpos or bowel may also be possible.

In the present case, the type of MP was quite unusual; most of reported cases of MP associated with cloacal anomaly generally display fibroadhesive type [2,3,11,15]. Petrikovsky et al [16] suggested that urine enters the abdominal cavity via the fallopian tubes at an early stage, causing "transient" fetal ascites, however, chronic urinary and meconium irritation of the tubal mucosa may cause tubal obstruction, which may lead to the subsequent development of hydrocolpos and the compression of the ureters and bladder, which leads to hydronephrosis at a later stage. Transient fetal ascites in a cloacal anomaly may indicate the formation of a fibroadhesive type of MP. In the present case, however, it appeared that the peritoneal cavity developed adhesion because of the initial inflow of urine mixed with meconium, and the subsequent continuous influx and a late obstruction of fallopian tubes because of inflammation might thus have led to the formation of a giant pseudocyst.

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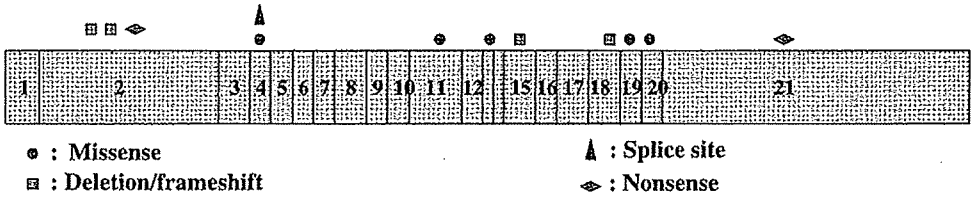

Fig. 1 Distribution of new mutations on the *ATP7B* gene

with WD, ten out of 19 of the novel mutations found were localized on exons 14, 16, and 19, which belong to the group of exons previously found to be the site of many frequent, as well as rare, WD-causing mutations. The genetic analysis of 56 Saudi patients with WD revealed that 50% of them had mutations in three exons (8, 19, and 21) of the *ATP7B* gene. Mutations in exon 19 and 21 were unique for Saudi patients (Takeshita et al. 2002). A novel deletion mutation, c.4193delC, in exon 21 of the *ATP7B* gene mutation, appears to be unique to Saudi patients and is found frequently in this ethnic group (Al Jumah et al. 2004; Majumdar et al. 2000, 2003). This is in contrast to our study, in which the novel mutations detected were randomly distributed all over the *ATP7B* gene, including exons (2, 4, 5, 7, 11, 13, 15, 17–21; Fig. 1).

Again, the majority of mutations detected in this study were found in the homozygous state (42 patients from 26 independent families), whereas in the study of Loudianos et al. (1999), most mutations detected were in the compound heterozygous state, and in only four cases, homozygosity was present. This may be explained by the high percentage of consanguinity in our study (75%) compared with consanguinity in the Egyptian population (32–35%).

Although homozygous mutations were found in 42 patients from 26 independent families, six of those patients from six different families were the result of nonconsanguineous mating. Also heterozygous mutations were found in six patients, two were the result of consanguineous mating. This unexpected finding, together with the finding of one patient homozygous for both p.N1270S and p.T1434M mutations, necessitates a larger-scale population study for the carrier frequency and the origins of these mutations in different families in this community.

Conclusion

The mutational spectrum of *ATP7B* among Egyptian children presenting with WD is very heterogenous, which mandates a larger-scale population screening to identify the carrier rate in this community. Frameshift and nonsense mutations may result in earlier presentation of the disease at childhood. Despite mutation heterogeneity in Egyptian patients, genotype–phenotype correlation analysis seems to be promising in this population, as many patients carry homozygous mutations.

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ORIGINAL ARTICLE

Does increased nuchal translucency indicate a fetal abnormality? A retrospective study to clarify the clinical significance of nuchal translucency in Japan

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Abstract The results of a chromosomal test by genetic amniocentesis in 58 cases with an increased nuchal translucency (NT; ≥3 mm thickness) revealed 47 cases showing a normal karyotype (81%) and 11 cases (19%) showing an abnormal karyotype. However, the cases of a normal karyotype with increased NT also included those with fetal abnormalities. Among the 49 cases in which NT was observed during the first trimester and then subsequently disappeared, chromosomal abnormalities were observed in five, and fetal abnormalities other than chromosomal abnormalities were observed in two. Meanwhile, all nine cases in which an increased NT remained or in which NT continued to increase in size during the second trimester were diagnosed as having cystic hygroma, and chromosomal abnormalities were found in six cases (67%). It should be noted that the shape of increased NT includes NT with a notch (notched NT) and NT without a notch (smooth NT). Among the 20 cases of notched NT, chromosomal abnormalities were observed in eight (40%), and cystic hygroma was observed in nine (45%). On the other hand, among the 38 cases of smooth NT, chromosomal abnormalities were observed in three (7.9%), but no cystic hygroma was observed. Our results confirm that increased NT does not always indicate a fetal abnormality. Whether NT thickness should be measured as a screening tool for fetal abnormalities remains controversial. However, increased NT may be detected by chance, because a maternal–fetal medical examination using ultrasonography is usually performed in Japan. It is therefore considered to be extremely important to establish a system in which cases are referred to obstetricians who are licensed clinical genetic specialists to obtain appropriate genetic counseling whenever increased NT is clinically observed.

Keywords Nuchal translucency · Prenatal diagnosis · Genetic counseling · Ultrasonography · Obstetrics · Genetic amniocentesis · Screening marker

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Introduction

Nuchal translucency (NT) is a low-intensity area observed in the fetal posterior cervical region upon ultrasonography at 11–14 weeks of gestation. NT itself is a finding inherent in all fetuses and is not necessarily an abnormal finding. The relationship between increased NT and chromosomal abnormalities was first reported by Nicolaides et al. (1992). Since then, the relationship with diseases other than chromosomal abnormalities, such as cardiac, genetic, and urinary system diseases has been described in various reports (Nicolaides et al. 1992; Souka et al. 2005; Westin et al. 2006). However, although there are many reports on the usefulness of NT measurement as a marker for chromosomal abnormalities, maternal age and the degree of increased NT vary in the medical literature, and the frequency of chromosomal abnormalities in cases of increased NT ranges from 11% to 88%, which also indicates differences. Therefore, no consistent viewpoint has emerged (Pandya et al. 1995; Brambati et al. 1995; Szabo et al. 1995).

It is known that even when increased NT is observed in the first trimester, in most cases, it later spontaneously

disappears. However, even in cases in which it disappears, the frequency of observing chromosomal abnormalities and other fetal abnormalities has been reported to be higher than that in the general population (Müller et al. 2004). In addition, there are cases in which the increased NT observed during the first trimester lso remains into the second trimester, and it is then diagnosed as cystic hygroma. However, it is difficult to diagnose this abnormality as cystic hygroma during the first trimester.

In Europe and the United States, a One-Stop Clinic for the Assessment of Risk (OSCAR) has been implemented, and a screening test according to maternal age with a serum marker for Down syndrome in addition to NT is conducted. In addition, NT measurement has is one of the items of the screening test for Down syndrome (Bindra et al. 2002).

On the other hand, the situation in Japan is that ultrasonography is almost universally used as part of the normal maternal health checkup. Therefore, whereas the correlation between increased NT and chromosomal abnormalities has been reported in Europe and the United States, NT measurement became widely used in Japan without sufficient understanding of the clinical significance of such measurement and how to accurately interpret such findings. It is therefore common for increased NT to be identified by chance during ultrasonographic examinations, and a serious issue has recently arisen in which artificial abortion often tends to be selected when increased NT is observed in a fetus, because sufficient genetic counseling has not been conducted.

Therefore, to clarify the clinical significance of NT in Japan, we studied the frequency of chromosomal abnormalities in cases of increased NT, the frequency of fetal abnormalities in cases of a normal karyotype with increased NT, the frequency of fetal abnormalities in cases with disappearance of NT, and the relationship between NT shape and fetal abnormalities.

Materials and methods

Subject

To obtain data of chromosomal karyotype, we included 171 pregnant women who received genetic amniocentesis at 16 weeks of gestation between February 1998 and May 2007 at the Department of Obstetrics and Gynecology at Nagasaki University Hospital. This included 102 cases with advanced maternal age (≥35 years old), 58 with NT thickness of at least 3 mm (increased NT), five with a history of delivering children with chromosomal abnormalities, and six others (four of a request for a chromosomal test, one with a history of delivery with Noonan syndrome, and one with a history of delivering a child with a cardiac abnormality). All cases were managed at regional private clinics and referred to Nagasaki University Hospital for genetic counseling. For all cases, genetic counseling was performed by obstetricians who were licensed clinical genetic specialists. Patient consent before measuring NT was obtained, and NT thickness was measured between 11 and 14 weeks of gestation in 171 cases of single pregnancy.

NT measurement by ultrasonography

NT was measured by an ultrasound specialist according to the method stipulated by the Fetal Medicine Foundation (Nicolaides et al. 1999). NT thickness of ≥3 mm was defined as increased NT, because the frequency of abnormal karyotypes in fetuses with NT thickness ≥3 mm at 11–14 weeks of gestation was higher than the respective number expected on the basis of maternal age (Pandya et al. 1995). Once fetuses with increased NT were detected at 11–14 weeks of gestation, they were followed as cases of increased NT until the end of pregnancy. We measured NT thickness before genetic amniocentesis at 16 weeks of gestation. We also performed a fetal screening of structural abnormalities by ultrasonography at 20 weeks of gestation. When increased NT at 11–14 weeks of gestation was not detected visually or had decreased to <3 mm at 16 weeks of gestation, those cases were defined as cases of NT disappearance.

Classification of the shape of increased NT

Cases in which NT had a smooth surface were defined and classified as smooth NT (s-NT group; Fig. 1a), and those in which the NT surface had a notch-like dent were classified as notched NT (n-NT group; Fig. 1b). The frequency of chromosomal abnormalities and the frequency of cases diagnosed as cystic hygroma in the second trimester were compared between the groups.

Statistical analysis

For statistical analysis, the Mann–Whitney U test was used. A value of $P < 0.05$ was determined to indicate a significant difference.

Results

Frequency of fetal chromosomal abnormalities in fetuses with increased NT

As a result of genetic counseling at our hospital, among the 171 cases in which subjects had undergone genetic

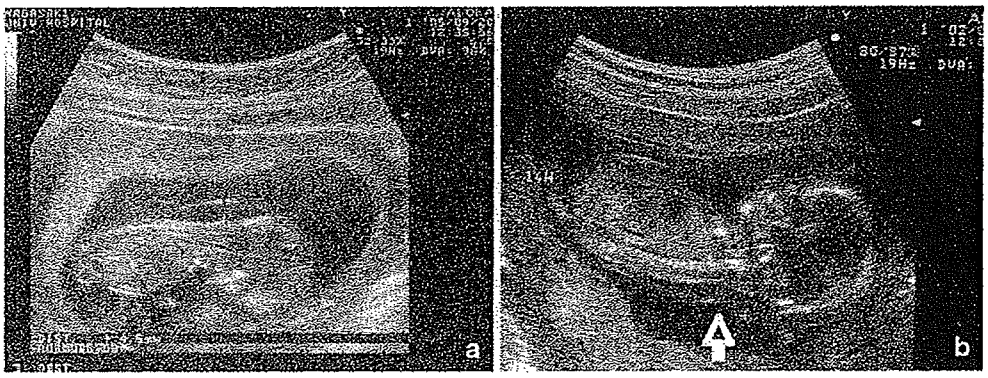

Fig. 1 Classification according to the shape of nuchal translucency (NT). **a** NT with a smooth surface was classified as smooth NT, and **b** NT with a notch (*arrow*) was classified as notched NT

amniocentesis, an increased NT was observed in 58 cases. Among the 58 cases with increased NT, chromosomal abnormalities were observed in 11(19%), including five of trisomy 21, for of trisomy 18, one of monosomy X, and one of mosaic (Fig. 2).

Frequency of chromosomal abnormalities in fetuses demonstrating NT measuring less than 3 mm

The relationship between increased NT and chromosomal abnormalities has been reported, but some cases without an increased NT also have chromosomal abnormalities. Therefore, the frequency of fetal abnormalities was studied in 113 cases with an NT thickness <3 mm. Indications for undergoing a chromosomal test were advanced maternal age in 102 cases, history of delivery with chromosomal abnormalities in five, history of delivery with Noonan syndrome in one, history of delivering a child with a cardiac abnormality in one, and request for a chromosomal test in four. Among these 113 cases, chromosomal abnormalities were observed in six (5%), including four of trisomy 21 and two of trisomy 18. One case of single atrium and single ventricle (1/107 cases; 0.9%) was detected in 107 fetuses with normal karyotype and normal NT.

Abnormalities in fetuses with normal karyotype and increased NT

As various fetal abnormalities other than chromosomal abnormalities have been reported for cases with increased NT, we studied which of the possible causes of increased NT were present. The screening of structural abnormalities by ultrasonography was performed at 20 weeks gestation. As a result of the amniotic fluid chromosomal test, among the 58 cases with NT measuring at least 3 mm, 47 (81%) demonstrated normal karyotype. Among the 47 normal karyotype cases with NT measuring at least 3 mm, fetal abnormalities were observed in five (5/47 cases; 10.6%), including one of cardiac abnormality, two of fetal hydrops, one of fetal pleural effusion, and one of diaphragmatic hernia (Fig. 2).

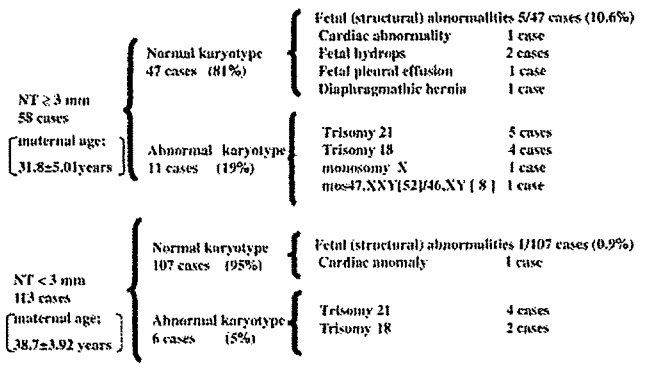

Fig. 2 Nuchal translucency (NT) thickness, fetal chromosomal abnormalities, and fetal structural abnormalities. A flow chart of 58 cases with an increased NT of at least 3 mm observed in the first trimester and the 113 cases with an NT measuring less than 3 mm

Outcomes in the cases of increased NT that later disappeared

It is known that most cases of increased NT observed in the first trimester disappear as pregnancy progresses. We therefore investigated the frequency of fetal abnormalities in such cases. The average NT thickness in cases of increased NT that later disappeared was 4.1 ± 1.4 (mm). Among the 58 cases in which increased NT was observed in the first trimester, 49 (84%) showed a disappearance of NT in the second trimester. In five of those cases, chromosomal abnormalities (three of trisomy 21 and two of trisomy 18) were observed; and in another two, congenital abnormalities (one each of diaphragmatic hernia and single atrium and single ventricle) were observed (Fig. 3).

Subsequent outcomes in cases of increased NT that did not later disappear

Meanwhile, among the 58 cases in which NT was observed in the first trimester, nine (16%) did not show NT disappearance, even during the second trimester. All of these cases were diagnosed to have cystic hygroma, and chromosomal abnormalities were observed in six (67%) of the nine cases. The breakdown includes two cases of trisomy

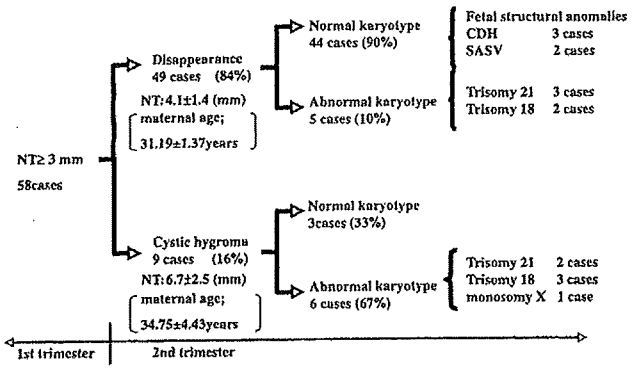

Fig. 3 Outcome of nuchal translucency (NT) observed in the first trimester. The outcomes of 58 cases with increased NT measuring at least a 3 mm in the first trimester are shown in the flow chart. In the second trimester, increased NT disappeared in 49 cases (84%) but remained in nine (16%) who were later diagnosed to have cystic hygroma. *CDH* congenital diaphragmatic hernia, *SASV* single atrium and single ventricle

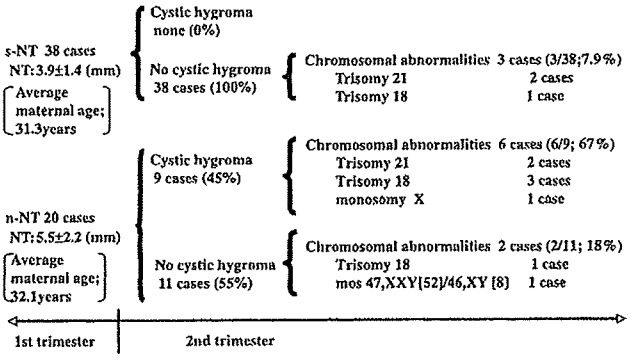

Fig. 4 Relationship between nuchal translucency (NT) patterns and fetal abnormalities. The smooth NT (s-NT) group consisted of 38 cases, whereas the notched NT (n-NT) group consisted of 20 cases. In the s-NT group, three cases (7.9%) had chromosomal abnormalities. In the n-NT group, eight cases (40%) had chromosomal abnormalities. The frequency of chromosomal abnormalities in the n-NT group was significantly higher than that in the s-NT group ($P = 0.0027$)

21, three of trisomy 18, and one of monosomy X (Fig. 3). The average NT thickness in the nine cases of cystic hygroma was 6.7 ± 2.5 (mm).

Relationship between NT shape and fetal abnormalities

The average NT thickness in 38 cases of s-NT was 3.9 ± 1.4 (mm), whereas that in 20 cases of n-NT was 5.5 ± 2.2 (mm). There was significant difference between the groups ($P < 0.0006$). All nine cases diagnosed as cystic hygroma colli in the second trimester demonstrated increased NT with a notch in the first trimester. Maymon et al. (2001) reported a notch in 62% of NT cases that showed increased NT in the first trimester and that were later diagnosed with Down syndrome. It was thus indicated that NT with a notch is a marker more closely related to Down syndrome. As described above, because cystic hygroma has a high frequency of being accompanied by chromosomal abnormalities, we analyzed the relationship between NT with a notch observed in the first trimester and chromosomal abnormalities as well as cystic hygroma, and we studied whether NT with a notch observed in the first trimester can be regarded as a marker for fetal abnormalities and cystic hygroma.

As a result of classifying 58 cases of increased NT according to the presence or absence of a notch, the s-NT group consisted of 38 cases, whereas the n-NT group consisted of 20 cases (Fig. 4). In the s-NT group, three cases had chromosomal abnormalities (7.9%; two of trisomy 21, one of trisomy 18), and in the n-NT group, eight (40%) had chromosomal abnormalities. The frequency of chromosomal abnormalities in the n-NT group was significantly higher than that in the s-NT group ($P = 0.0027$). Among the eight cases in the n-NT group with chromosomal abnormalities indicated, six were diagnosed to have cystic hygroma in the second trimester, with two of trisomy 21, three of trisomy 18, and one of monosomy X. The remaining two were diagnosed as one case each of mosaic (mos 47,XXY[52]/46,XY[8]) and 18 trisomy. In addition, nine cases of cystic hygroma in the second trimester were classified as belonging to the n-NT group, but none were classified as belonging to the s-NT group ($P = 0.0001$).

Discussion

Among the 58 cases with increased NT, 11 (19%) had chromosomal abnormalities. The frequencies of chromosomal abnormalities in cases with increased NT varies in reports by different authors, but it was 19.2% and 16.2%, respectively, in one study of 11,315 cases (Kagan et al. 2006) and one of 1,015 cases (Pandya et al. 1995), and our study results were also similar. Indeed, the frequency of accompanying chromosomal abnormalities is high in cases with increased NT, but conversely, 81% of the cases with increased NT demonstrate a normal karyotype. This means that most cases are normal karyotype fetuses despite increased NT. The presence or absence of chromosomal abnormalities cannot be determined according to NT thickness, as performing NT measurements is only a screening test. It is therefore necessary to be aware that a chromosomal test is also required to verify a diagnosis of chromosomal abnormalities.

Among 113 cases with NT measuring <3 mm, chromosomal abnormalities were observed in six (5%). As the 113 cases included 102 cases (90%) of mothers with advanced maternal age (i.e., >35 years of age) and all six cases with chromosomal abnormalities were included in such advanced maternal-age cases, it is believed that chromosomal abnormalities cannot be ruled out, even if NT