INTRODUCTION

The gestational period of human fetuses is approximately 40 weeks (or 280 days) from the last menstrual period, ranging from 37 0/7 to 41 6/7 weeks. Clinically, gestational age is subdivided into 3 periods, namely the first, second and third trimesters. The first trimester is defined as up to the first 14 weeks, from the fertilized egg to the fetus, in which organogenesis is complete and is considered the most critical period, when the genetic blueprint is determined and aberrations cause various malformations.

The quality of prenatal diagnosis of fetal abnormalities has advanced with improved resolution of ultrasound images and cytogenetic/molecular analysis. In this article, we briefly review the history of diagnosing fetal abnormalities and the current status of prenatal diagnosis during the first trimester (up to the first 14 weeks’ gestation), focusing especially on fetal malformations and chromosomal abnormalities. As for detectable morphological abnormalities, roughly half of all major structural anomalies including those in the central nervous system, cardiovascular system and gastrointestinal system can be detected, if not definitely diagnosed. For screening of chromosomal abnormalities, especially for trisomy 21, ultrasound soft markers such as increased nuchal translucency, maternal serum markers and their combinations have been implemented. More recently, non-invasive prenatal testing, by analyzing cell-free DNA in maternal serum, is now available to detect chromosomal abnormalities with higher predictability. Although invasive chorionic villus sampling offers definite diagnosis for chromosomal abnormalities during the first trimester, non-invasive diagnostic techniques are patient-friendly and promising in the future perspectives on prenatal diagnosis for chromosomal abnormalities.

ULTRASOUND DIAGNOSIS OF FETAL MALFORMATION

Prenatal ultrasound diagnosis of fetal malformation has been reported since the 1970s, when the implementation of real-time ultrasonography began in the clinical field [1-3]. With advances in the resolution of these ultrasound images, fetal malformations have been diagnosed more precisely, and earlier, in gestation. The timing of prenatal diagnosis depends on the characteristics of the malformation. The more deviated the morphological aberrations are, the earlier and easier diagnoses can be made. Another factor to be considered in diagnosis of malformations is the physiological development of the organs involved. For example, the diagnosis of anencephaly or acrania is
made through finding an absence of telencephalus and cranium (Fig. 1). This malformation is caused by the failure of the neural tube to close, occurring at 5 weeks’ gestation. However, definite diagnosis can only be made, at the earliest, at 11–12 weeks’ gestation (fetal crown-lump length of 4–5 cm) when normal ossification of the cranium should occur [4]. Another example is the diagnosis of omphalocele, in which a disturbance in the closure of the umbilical sac causes abdominal organs, mainly the liver, to be retained in the protruded sac. The closure of the umbilical sac is complete at 10–12 weeks’ gestation [5]. Until then, a “physiological” umbilical herniation is observed (Fig. 2A). Therefore the diagnosis of omphalocele becomes possible at 12 weeks’ gestation (Fig. 2B). It is estimated that a scan of the fetal anatomy in the first trimester allows for the detection of approximately half of all major structural anomalies, including those of the central nervous system, cardiovascular system and gastrointestinal system (Fig. 3), however the detection rates are dependent on various factors including the malformations themselves, patient conditions such as obesity, and the examiner’s skills and knowledge [6,7].

Another aspect of fetal morphological diagnosis is detecting findings which indicate fetal chromosomal abnormalities, called ultrasonographic soft markers for chromosomal abnormalities. The most notable is nuchal translucency (NT) which is observed as a subcutaneous edema in the nuchal region during the late first trimester (Fig. 4). NT itself is not always pathological, however, there are strong correlations between the thickening of NT and fetal chromosomal abnormalities such as trisomies 13, 18 and 21 and Turner syndrome [8,9]. Snijders et al. reported that fetuses with NTs of 3.5–4.5 mm have a 21.7% chance of chromosomal anomaly [10]. The likelihood ratio is elevated with an increase in NT, and the probability of chromosomal abnormalities in fetuses with NTs of >6.5 mm was 64.5%. Increased NTs are also associated with major structural anomalies (cardiovascular, gastrointestinal and musculoskeletal systems and other genetic syndromes [11-13]. To date, there are several soft markers such as absent or hypoplastic nasal bone, absent or reversed blood flow of the ductus venosus (Fig. 5) and regurgitant blood flow across the tricuspid valve (Fig. 6) [14,15]. Using a combination of these soft markers, the detection of chromosomal abnormality has become more accurate.

**CHORIONIC VILLOUS SAMPLING**

For the definite diagnosis of fetal chromosomal abnormalities, sampling of fetal tissue for karyotyping is necessary. For this purpose, chorionic villus sampling (CVS) is performed at 11–14 weeks’ gestation (Fig. 7) and amniocentesis is performed after 15 weeks’ gestation. There are two approaches for CVS: transvaginal and transabdominal, however, because of the potential risk of infection after sampling, the transvaginal approach tends to be avoided as of late. The risk of abortion in CVS is reported as 0.5–1.0% [16]. More recently, with the advent of non-invasive prenatal testing (NIPT) as mentioned below, the use of invasive prenatal testing for diagnosing chromosomal abnormalities especially amniocentesis has decreased because of the potential risk of miscarriage [17].

**MATERNAL SERUM MARKER TEST**

The history of using maternal serum markers for detecting fetal abnormalities dates back to the 1960s. Alpha-fetoprotein (AFP) produced exclusively in the fetal liver is detected in the maternal serum during pregnancy. It is well-known that maternal serum AFP levels are elevated when fetuses have neural tube defects such as anencephaly and spina bifida. Since the fetal central nervous system is directly exposed to amniotic fluid, high levels of AFP drain into the maternal bloodstream. Therefore, assay of the maternal AFP level has been applied for screening of fetal neural tube defects [18]. In 1980, it was reported that maternal serum AFP levels of women with fetuses having trisomy 21 (T21) were lower compared with those carrying normal fetuses [19]. In addition to AFP, abnormal concentrations (either decreased or elevated) of human chorionic gonadotropin (hCG) and unconjugated estriol and inhibit A were also associated with fetuses having T21. The quadruple test has been available as a screening test during the second trimester (after 15 weeks’ gestation) for T21 screening. To date, a combined test in which the probabilities of having fetal T21 are calculated based on NT measurement, assay of pregnancy-associated plasma protein-A (PAPP-A) and free β subunit of hCG, in the first trimester (10–13 weeks’ gestation) is the widely used method for screening [20,21]. PAPP-A is lower and free β-hCG is elevated in the maternal serum of women carrying fetuses with T21. Other options are the integrated and sequential tests (Fig. 8). The sequential test consists of two examinations; the first step is a combined test. When the test result is positive (the
Fig. 1. Ultrasonographic images of a case with anencephaly at 11 weeks' gestation. White arrows indicate defects of the cranium and telencephalus. Sp, spine.

Fig. 2. Ultrasonographic images of a case of “physiological” umbilical herniation at 11 weeks' gestation (A), and a case with omphalocele at 13 weeks' gestation (B).

Fig. 3. B mode and color Doppler images of a normal four-chamber view of a normal fetus at 13 weeks (A, B) and a case with single atrium and ventricle at 13 weeks' gestation (C, D). Sp, spine, RA, right atrium, RV, right ventricle, LA, left atrium, LV, left ventricle, SA, single atrium, SV, single ventricle.
probability of T21 is more than approx. 1/200), CVS is performed for the definite diagnosis. When the test result is negative (less than approx. 1/200), the next step is the quadruple test at 15–21 weeks’ gestation. In the case of a positive result, amniocentesis is chosen. Alternatively, in the integrated test, NT measurement and assay of PAPP-A in the first trimester and the quadruple test in the early second trimester are done in tandem. When false positive rates are set at 5%, the sensitivities (diagnostic rates) for detecting T21 were 72–83%, 83–90%, 93% and 94% in quadruple, combined, integrated and sequential tests, respectively [22].

NON-INVASIVE PRENATAL TESTING

Fetal blood is segregated from maternal blood because fetal blood cannot permeate robust barriers, i.e., the placenta. However, the fact that fetal cells exist in the maternal bloodstream has been known for more than a century. Fetal cells are a fascinating source for extracting fetal genetic information, and extensive studies isolating fetal nucleated red blood cells have been done [23]. Fetal cells are sparse in maternal blood (roughly 10 cells out of 1 mL of maternal blood), however it has been demonstrated that cells derived from fetuses persisted for more than one year after birth, possibly leading to erroneous results in future pregnancies [24].

In 1997, Lo, et al. reported that DNA sequences originating from the Y-chromosome existed in the serum of pregnant women, indicating the existence of fetal cell-free DNA (cfDNA) in the maternal serum [25]. Fetal cfDNA in maternal serum originated mainly from trophoblasts by apoptosis, and are short fragments of which 80% are <193 base pairs in length and composed of approximately 10% of total cfDNA [26-28]. Fetal cfDNA is detected in maternal serum at 5 weeks’ gestation and disappears soon after delivery [29].

In the laboratory setting, the fetal diagnoses for Rh genotyping and single gene aberrations such as congenital myotonic dystrophy, as well as aneuploidies,
using maternal serum, were reported in the 1990s [30-33]. After the epoch-making event of the determination of DNA sequences of human whole genome in 2003 and the advent of a next-generation sequencer, the diagnosis of fetal chromosomal anomalies has become commercially available. To date, there are four types of analyses of fetal chromosomal anomalies: massive parallel sequencing (MPS), targeted massive parallel sequencing (t-MPS) in which specific chromosomes are selected, single nucleotide polymorphism (SNP)-based analysis and array-based analysis [34].

In MPS, after extracting DNA from the maternal serum, DNA sequencing is determined for the DNA terminal to identify the origins of the chromosomes. The percentages of cfDNA content for each chromosome per total cfDNA content corresponds to the length of the individual chromosome. For example, in theory, fetal cfDNA content originating from chromosome 21 in cases with fetuses having T21 (3 sets of chromosome 21) should be 1.5 times higher than cases of normal fetuses (2 sets of chromosome 21). Therefore, the ratio of cfDNA content of chromosome 21, of maternal and fetal origin, per total cfDNA content, in cases of fetal T21 are slightly higher than those in cases of fetuses with a normal karyotype (T21:1.42% vs. normal fetus:1.3%) (Fig. 9) [35]. Initial validation studies performed on patients at high-risk for aneuploidy (such as advanced maternal age) showed high detection rates and low false positive rates (T21, 94.4-100.0%, 0.0-0.94%, T18, 87.5-100.0%, 0.0-0.22%, T13, 40.0-100.0%, 0.0-0.25%, respectively) [35,36]. In Japan, non-invasive prenatal testing (NIPT) for T21, 18 and 13 has been available since 2013. According to the report by Sago, et al., a total of 7,740 cases were examined in Japan in 2013, with a median maternal age of 38.3 years old [34]. Among them, 95.4% of subjects had an advanced maternal age of ≥35 years old. There were 142 positive cases (1.8%), comprised of 79 cases of T21, 50 cases of T18 and 13 cases of T13. Among 126 cases who underwent amniocentesis or CVS for karyotyping, 13 cases (10.3%) were normal (false positive) [37]. More recently, data collected from two large studies in the US demonstrated that NIPT for low risk women showed a positive predictive value (PPV) for T21 of 50-81% [38,39]. Nonetheless, NIPT is superior to standard screening, having a PPV of 3.4-4.2%.

Since the total cfDNA contents of both maternal and fetal origins were combined and counted, there are several reasons for false positive results including banishing twin (early phase abortion of one of twin embryos), placental or fetal mosaicism, maternal acquired genetic alterations (e.g. bone marrow or organ transplant, malignancy, benign hematologic conditions) and copy number variation of fetal or maternal chromosome [40-43].

NIPT should be an informed patient choice, with an underlying foundation of shared decision making, that fits the patient’s clinical circumstances, values,
interests and goals [37,44,45]. The Japan Society of Obstetrics and Gynecology recommends that the test not be widely introduced into general obstetric practice in Japan until a system is in place for specialists of obstetrics, with knowledge of clinical genetics, to provide appropriate genetic counseling to pregnant women who require it [37]. On the other hand, in the United States, the American College of Medical Genetics and Genomics recommends that all pregnant women be informed that NIPS is the most sensitive screening option for traditionally screened aneuploidies including T21, 18 and 13 [46].

**ETHICAL ISSUES**

Prenatal diagnosis for fetal malformations and chromosomal aberrations has become a part of routine obstetric care in developed countries, especially, in Europe and North America, and helps to provide pregnant women with various management options including termination of pregnancy. Medical care providers involved in this field should recognize the significance and limitations of prenatal diagnosis. Inaccurate and uncertain information has the potential to cause anxiety and may lead to erroneous decision-making by the patients and their families. A multidisciplinary team is necessary for quality assurance in making prenatal diagnoses and providing sufficient information and psychological support for those who receive undesired results.

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**Fig. 8.** Diagnostic charts for the detection and diagnosis of fetal trisomy 21 during the 1st and 2nd trimester. NT, nuchal translucency, β-hCG, beta-human chorionic gonadotropin, PAPP-A, pregnancy-associated plasma protein-A, AFP, alpha-fetoprotein, uE3, unconjugated estriol.

**Fig. 9.** The principle of massive parallel sequencing for counting cell-free DNA contents derived from individual chromosomes and the cell-free DNA contents of chromosome 21 in cases of fetuses with both normal karyotype and trisomy 21.
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