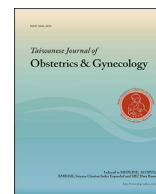




Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

A multicenter study of fetal chromosomal abnormalities in Chinese women of advanced maternal age



Yuning Zhu ^{a, b}, Shiming Lu ^{a, b, *}, Xuming Bian ^c, He Wang ^d, Baosheng Zhu ^e, Hua Wang ^f, Zhengfeng Xu ^g, Liangpu Xu ^h, Weihua Yan ⁱ, Yan Zeng ^j, Zhiyang Chen ^k, Shaohua Tang ^l, Guosong Shen ^m, Zhengyou Miao ⁿ

^a Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China

^b Prenatal Diagnosis Center of Zhejiang Province, Hangzhou, China

^c Department of Obstetrics and Gynecology, Academy of Medical Science, Chinese Peking Union Medical College, Peking Union Medical College Hospital, Beijing, China

^d West China Second University Hospital, Sichuan University, Prenatal Diagnosis Center of Sichuan Province, Chengdu, China

^e Genetic Diagnostic Center, the First People's Hospital of Yunnan Province, Kunming, China

^f The Maternal and Child Health Hospital of Hunan Province, Prenatal Diagnosis Center of Hunan Province, Changsha, Hunan, China

^g Center of Prenatal Diagnosis, Nanjing Maternity and Child Health Care Hospital, Nanjing, China

^h Center of Prenatal Diagnosis, Maternal and Child Health Hospital of Fujian Province, Fuzhou, China

ⁱ Center of Prenatal Diagnosis, Taizhou Hospital, Taizhou, China

^j Center of Prenatal Diagnosis, Shaoxing Maternity and Child Health Care Hospital, Shaoxing, China

^k Center of Prenatal Diagnosis, Ningbo Maternity and Child Health Care Hospital, Ningbo, China

^l Center of Prenatal Diagnosis, Wenzhou Central Hospital, Wenzhou, China

^m Center of Prenatal Diagnosis, Huzhou Maternity and Child Health Care Hospital, Huzhou, China

ⁿ Center of Prenatal Diagnosis, Jiaxing Maternity and Child Health Care Hospital, Jiaxing, China

ARTICLE INFO

Article history:

Accepted 5 January 2016

Keywords:

advanced maternal age
amniotic fluid
clinically significant chromosome abnormalities
genetic counseling
prenatal diagnosis

ABSTRACT

Objective: This study aimed to determine the rates of different fetal chromosomal abnormalities among women of advanced maternal age in China and to discuss the possible misdiagnosis risks of newer molecular techniques, for selection of appropriate prenatal screening and diagnostic technologies.

Materials and Methods: Second trimester amniocentesis and fetal karyotype results of 46,258 women were retrospectively reviewed. All women were ≥ 35 years old with singleton pregnancies. The rates of clinically significant chromosomal abnormalities (CSCAs), incidence of chromosomal abnormalities, and correlations with age were determined.

Results: From 2001 to 2010, the proportion of women of advanced maternal age undergoing prenatal diagnosis increased from 20% to 46%. The mean age was 37.4 years (range, 35–46 years). A total of 708 cases of CSCAs, with a rate of 1.53% were found. Trisomy 21 was the most common single chromosome abnormality and accounted for 55.9% of all CSCAs with an incidence of 0.86%. Trisomy 13, trisomy 18, and trisomy 21, the most common chromosome autosomal aneuploidies, accounted for 73.6% of all CSCAs, with a rate of 1.13%. As a group, the most common chromosomal aneuploidies (13/18/21/X/Y) accounted for 93.9% of all abnormalities, with a rate of 1.44%. The incidence of trisomy 21, trisomy 13/18/21 as a group, and 13/18/21/X/Y as a group was significantly greater in women aged 39 years and older ($p < 0.001$), but was not different between women aged 35 years, 36 years, 37 years, and 38 years.

Conclusion: These findings may assist in genetic counseling of advanced maternal age pregnant women, and provide a basis for the selection of prenatal screening and diagnostic technologies.

Copyright © 2016, Taiwan Association of Obstetrics & Gynecology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author. Women's Hospital, School of Medicine, Zhejiang University, Xueshi Road Number 1, Hangzhou 310006, China.

E-mail address: lushiminghz@163.com (S. Lu).

Introduction

Advanced maternal age (AMA) is an important risk factor for fetal chromosomal abnormalities [1]. Since the 1970s, amniocentesis and fetal karyotype analysis have generally been performed during the second trimester for AMA women [2]. In China, this strategy has been implemented for more than 10 years and has improved the detection rate of fetal chromosomal abnormality [3]. However, the population of China is over 1.3 billion, and in early 2014 the Chinese government launched its second-child policy, allowing some couples to have a second child [4]. This is expected to increase the number of AMA women as well as the demand for prenatal diagnoses. Owing to the associated risk of birth defects, AMA women are generally advised to undergo prenatal diagnosis directly in China, receiving fetal chromosomal analysis without necessarily undergoing prenatal screening.

Meanwhile, cytogenetic technical resources for prenatal diagnosis of fetal chromosomal abnormalities are already recognized as being insufficient, creating a bottleneck in prenatal diagnosis in China [5,6]. This shortage of resources for prenatal karyotype analysis will be exacerbated even further by the increased numbers of AMA women and increased demand for prenatal diagnoses. Birth defects and genetic diseases in China are increasing [3,5]. Therefore, in China, it has become critical to improve methods of prenatal diagnosis to help reduce the incidence of birth defects.

While karyotype analysis is accurate and reliable and is considered the gold standard for prenatal diagnosis of chromosomal abnormalities, it is technically demanding and labor intensive, and has a low throughput and long detection cycle (2–3 weeks). Newer rapid and effective molecular techniques may be alternatives to karyotype analysis for prenatal testing and diagnosis, including noninvasive prenatal testing (NIPT), quantitative fluorescent polymerase chain reaction (QF-PCR), fluorescence *in situ* hybridization (FISH), BACs-on-Beads (BoBs), multiplex ligation-dependent probe amplification (MLPA), chromosomal microarray analysis (CMA), and other technologies [7–11]. Some of these methods are able to reach a sensitivity of 98% or more with low false-positive rates [7–11]. However, risk may still exist. Although a newer molecular method (such as QF-PCR) provides accurate and reproducible results when compared with karyotype analysis, it only detects common trisomies, leaving a residual risk for a pregnancy to have a chromosomal abnormality detectable with conventional analysis methods [7,12]. The residual risk is reported to be 1%, which raises concerns as well as ethical issues [4]. Residual risk has not been confirmed in a mainland Chinese population or validated among AMA women in China.

Considering the increased demand for prenatal diagnosis and the current status of prenatal testing, an urgent need emerges for newer methods to assist in karyotype testing for prenatal diagnosis in China. Understanding the incidence of chromosomal abnormalities by age would provide a good basis for developing appropriate techniques and strategies for prenatal screening and diagnosis [7,13,14]. However, a few large-sample epidemiological studies of prenatal diagnostic strategies have been performed for AMA women in China [4]. We hypothesized that a large, multi-center, epidemiologic study of AMA women across China would help determine the incidence of clinically significant chromosomal abnormalities (CSCAs) in pregnant women referred for karyotype analysis solely because of AMA. Therefore, this study aimed to determine the rates of fetal chromosomal abnormalities in AMA women in China and also to evaluate the possible misdiagnosis risks of newer molecular techniques in clinical prenatal diagnosis. Results of this study may assist in genetic counseling of AMA pregnant women, and provide a basis for the selection of

appropriate prenatal screening and diagnostic technologies to augment present methods.

Methods

Participants

Data of 46,258 pregnant women who sought counseling because of AMA from January 2001 to December 2010 at 13 prenatal diagnosis centers in China were collected and analyzed retrospectively. Only patients with a naturally conceived, singleton pregnancy, with no personal or family history of chromosomal abnormalities and no fetal abnormalities detected by ultrasound or other indications of an unfavorable prenatal diagnosis, were included. Women with any family history of chromosomal abnormalities and any fetal abnormalities detected by ultrasound or other indications of an unfavorable prenatal diagnosis were excluded. The medical centers were informed of the inclusion and exclusion criteria, and the different centers individually compiled the data and forwarded it to the primary center for analysis. This study was approved by the Scientific Research Ethics Committee of Women's Hospital of Zhejiang University, Hangzhou, China and the Institutional Review Boards of the participating centers, and all patients provided informed consent.

Counseling and genetic testing

All 46,258 women received genetic counseling, and then invasive prenatal diagnosis was performed after informed consent was obtained. Amniocentesis and karyotype analysis of amniotic fluid were performed at 18–22⁺⁶ weeks of gestation. The women's ages at due date, results of the karyotype analysis, gestational age of pregnancy at the time of amniocentesis, and other pertinent information were sent to the Prenatal Diagnosis Center of Zhejiang Province for analysis.

Chromosome abnormalities were divided into two categories: chromosomal abnormalities of clear clinical significance (CSCAs) and chromosomal abnormalities with a good or unclear prognosis. In this study, CSCAs included both common and rare abnormalities. Common chromosomal abnormalities included five common types of abnormalities affecting the number of chromosomes (13, 18, 21, X, and Y): autosomal aneuploidies (trisomy 21, trisomy 18, and trisomy 13), sex chromosome abnormalities (e.g., Turner syndrome, 47,XXY), polyploidies, and chimeras based on the type of involved chromosomal abnormality. Rare chromosomal abnormalities included imbalanced chromosomal structural abnormalities attributable to the addition or deletion of genetic material, such as partial chromosomal deletions or duplications.

The women were divided into seven groups based on their ages at their due dates: 35 years, 36 years, 37 years, 38 years, 39 years, and 40 years, and older than 40 years. The incidences of trisomy 21 and other CSCAs were calculated. Chromosomal abnormalities with a good or unclear prognosis, including balanced translocations, Robertsonian translocations, inversions, other balanced chromosome structural abnormalities, and marker chromosomes, were not analyzed in the present study. To examine the suitability of diagnostic techniques other than karyotype analysis, CSCAs were divided into three categories: (1) common autosomal aneuploidies, including trisomy 21, trisomy 18, and trisomy 13, which can be detected with NIPT, QF-PCR, FISH, and other techniques, as previously described [7,15]; (2) common chromosomal abnormalities involving five chromosomes (13, 18, 21, X, and Y), including trisomy 21, trisomy 18, trisomy 13, Turner syndrome, 47,XXX, 47,XXY, 47,XYY, 69,XXY, and 69,XYY, which can be detected using QF-PCR, FISH, BoBs, MLPA, CMA, and other technologies [7–9,15]; and (3)

rare chromosomal abnormalities, such as partial chromosome deletions and additions, and unbalanced translocations, which can be detected by CMA [9,16].

Statistical analysis

A Chi-square test was performed for comparison of the incidences of chromosomal abnormalities between groups. Pearson's correlation was used to analyze the correlations between the incidences of chromosomal abnormalities and age. A p value < 0.05 was considered statistically significant. SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

A total of 46,258 singleton pregnancies were included in the analysis; the details of the cases collected from the centers are shown in Table S1. The mean maternal age at the due date was 37.4 years (range, 35–46 years). From 2001 to 2010, the proportion of AMA women undergoing prenatal diagnosis increased from 20% to 46% (Figure 1). Among the 46,258 women, there were 708 cases of CSCAs (Table 1). The incidence of trisomy 21 was 0.86% (396/46,258), accounting for 55.9% of all CSCAs, and the incidence tended to increase with maternal age (Figure 2). The incidence of trisomy 21 did not differ in women aged 35 years, 36 years, 37 years, and 38 years ($p = 0.10$), and the mean incidence of trisomy 21 was 0.65% (224/34,287). The mean incidence of trisomy 21 in women aged 39 years and older was 1.44% (172/11,971), which was significantly different from that of the 35–38-year age group ($p < 0.001$) (Table 2). The incidences of trisomy 18, 47,XXX, and 47,XXY were significantly correlated with maternal age ($\gamma = 0.77$, $p = 0.042$; $\gamma = 0.93$, $p = 0.002$; and $\gamma = 0.85$, $p = 0.014$, respectively). The incidences of trisomy 13, Turner syndrome, and 47,XYY were not found to be significantly correlated with maternal age.

The overall incidence of CSCAs was 1.53% (708/46,258), and showed a tendency to increase with age (Figure 3). The combination of trisomies 13, 18, and 21 (T21/18/13 group), with a detection rate of 1.13% (521/46,258), accounted for 73.6% (521/708) of all CSCAs. The common chromosomal abnormalities of five chromosomes (13, 18, 21, X, and Y; 21/18/13/X/Y group), with a detection rate of 1.44% (665/46,258), accounted for 93.9% (665/708) of all CSCAs. Rare chromosomal abnormalities, including partial chromosome imbalance and unbalanced translocations, with a detection rate of 0.09% (43/46,258), accounted for 6.1% (43/708) of all CSCAs. There were no significant differences in the incidence of the

T21/18/13 and 21/18/13/X/Y groups among women aged 35 years, 36 years, 37 years, and 38 years ($p > 0.05$). However, the incidence of the T21/18/13 and 21/18/13/X/Y groups in women aged 39 years, 40 years, and older than 40 years was significantly different from that in the 35–38-year age group ($p < 0.001$). The incidence of rare chromosomal abnormalities did not increase with age, and their relative proportion of CSCAs tended to decrease with age (Table 3).

Discussion

Results of the present large-scale epidemiologic study of AMA pregnant women in China who underwent second trimester amniocentesis and fetal karyotyping showed that the need for fetal karyotype testing has increased from 20% to 46% over the past decade. The five most common types of chromosomal abnormalities (21/18/13/X/Y group) accounted for 93.9% of all CSCAs, with trisomy 21 accounting for over half of these. The incidence of trisomy 21 was similar between women aged 35–38 years, but increased significantly in those aged 39 years and older. Prior to the present study, no detailed information was available on the relative incidence of specific chromosomal anomalies in AMA pregnancies in China. Results of this large multicenter investigation suggest that alternatives to karyotype analysis are applicable in China and can help meet the increased demand for prenatal diagnoses among an increasing population of AMA women. This work complements the large national effort to reduce the incidence of birth defects, and results may contribute to the development of national policies on prenatal diagnosis.

The proportion of AMA women undergoing prenatal diagnostic testing in China is increasing along with the demand for prenatal diagnosis. This trend is consistent with the increases noted in Western countries [17–20]. In China, the “second-child policy” is placing an additional burden on already overburdened cytogenetic technical resources. Thus, rapid, noninvasive, and cost-effective diagnostic techniques are needed urgently, and data on the frequency of chromosomal abnormalities with respect to maternal age should help determine which alternatives to karyotype analysis are suitable.

Incidence of specific chromosomal abnormalities

In the present study, the overall incidence of CSCAs was 1.53%, which is lower than that reported in previous studies, including 2.31% in Taiwan [21], 2.4% in Spain [13], and 4.3% in Turkey [22]. The reason for this lower number may be that the current study included only abnormalities with clear clinical significance. The discrepancy may also be related to the age distribution of the participants since the frequency of some CSCAs is directly associated with maternal age [23].

In China, the incidence of trisomy 21 among AMA women in the second trimester was 0.86%, accounting for over half of all CSCAs. This highlights the importance of prenatal diagnosis of Down syndrome for older pregnant women, especially because it is substantially higher than the 0.125% reported for the general population of pregnant women [24]. The incidence of trisomy 21 was also found to increase with maternal age; however, there were differences from other reports [23,24]. The current study showed a similar incidence between women aged 35–38 years, while it increased significantly in those aged 39 years and older. Results of the present study also showed that the incidences of trisomy 18, 47,XXX, and 47,XXY correlated closely with maternal age, but correlations were not seen with trisomy 13, Turner syndrome, and 47,XYY, which is also consistent with other reports [23,24].

Results of the present study suggest that different strategies may be used in pregnant women 35–38 years of age and in those

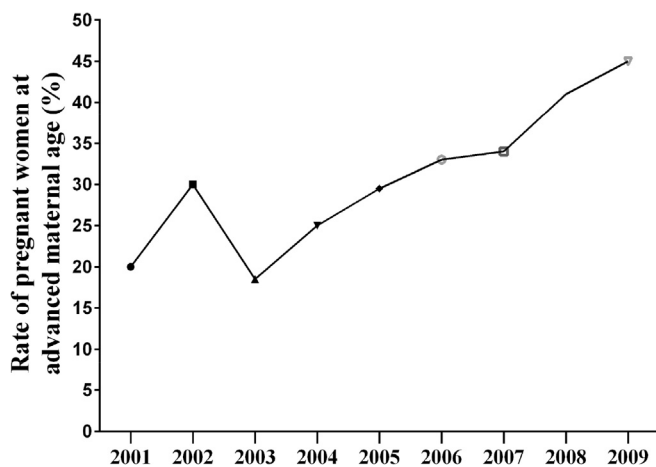


Figure 1. Change in the relative number of pregnancies in which mothers were older than 35 years, during 2001–2010.

Table 1
Clinically significant chromosomal abnormalities by age group.

Age (y)	No. of cases	Trisomy 21	Trisomy 18 ^a	Trisomy 13 ^b	Turner syndrome ^b	47,XXX ^c	47,XXY ^d	47,XYY ^b	Other abnormalities ^e
35	9946	77 (0.77)	13 (0.13)	5 (0.05)	13 (0.13)	3 (0.03)	8 (0.08)	2 (0.02)	9 (0.09)
36	9923	51 (0.51)	12 (0.12)	4 (0.04)	10 (0.10)	5 (0.05)	7 (0.07)	1 (0.01)	10 (0.10)
37	8012	58 (0.72)	17 (0.21)	1 (0.01)	8 (0.10)	4 (0.05)	9 (0.11)	1 (0.01)	8 (0.10)
38	6406	38 (0.59)	20 (0.31)	3 (0.05)	5 (0.08)	4 (0.06)	8 (0.12)	0	7 (0.11)
39	4459	39 (0.87)	14 (0.31)	1 (0.02)	3 (0.07)	5 (0.11)	9 (0.20)	1 (0.02)	5 (0.11)
40	3276	36 (1.10)	19 (0.58)	2 (0.06)	3 (0.09)	3 (0.09)	10 (0.31)	4 (0.12)	2 (0.06)
>40	4236	97 (2.29)	13 (0.31)	1 (0.02)	2 (0.05)	5 (0.12)	9 (0.21)	2 (0.05)	2 (0.05)
Total	46,258	396 (0.86)	108 (0.23)	17 (0.04)	44 (0.10)	29 (0.06)	60 (0.13)	11 (0.02)	43 (0.09)

Data are presented as *n* (%), unless otherwise indicated.

^a The incidence of trisomy 18 was significantly associated with maternal age ($R = 0.77$, $p = 0.042$).

^b The incidences of trisomy 13, Turner syndrome, and 47,XYY were not associated with maternal age ($p > 0.05$).

^c The incidence of 47,XXX was significantly associated with maternal age ($R = 0.93$, $p = 0.002$).

^d The incidence of 47,XXY was significantly associated with maternal age ($R = 0.85$, $p = 0.014$).

^e Other chromosomal abnormalities of clinical significance includes abnormalities with imbalanced genetic material and poor prognosis other than the five common types of chromosomal aneuploidy (13, 18, 21, X, and Y), such as partial chromosome deletions and additions, and unbalanced translocations.

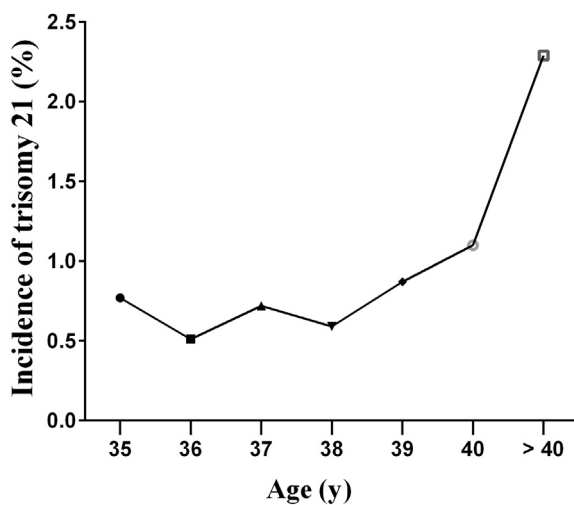


Figure 2. Relationship between the incidence of trisomy 21 during the second trimester and maternal age.

who are older. Unlike in previous reports [23,24], in this study, the incidence of trisomy 21 was not found to increase significantly with age within a range of 35–38 years. However, in women aged 39 years and older, the incidence of trisomy 21, and that of other CSCAs, increased significantly with age. The difference with other reports may be due to population differences. In the present study, the 35–38-year-old age groups accounted for 74.12% of all women, but the incidence of Down syndrome was 0.65%, significantly lower than the 1.44% in the 25% of women aged 39 years and older. Based on the fact that the detection rate of the current prenatal screening technology can exceed 90% [25], American Congress of Obstetricians and Gynecologists (ACOG) guidelines recommend routine prenatal screening for older pregnant women [2]. Based on the results of the present study and the ACOG guidelines, prospective studies on prenatal screening for AMA pregnant women 35–38 years of age should be conducted to determine whether these methods can be viable alternatives to karyotype analysis in this age group.

Table 2
Incidence of trisomy 21 in women 35–38 years of age and those ≥ 39 years of age.

Age group (y)	No. of cases	Trisomy 21 cases	Incidence of trisomy 21 (%) [*]	Positive predictive value
35–38	34,287	224	0.65	1:154
≥ 39	11,971	172	1.44	1:69

^{*}A significant difference was observed in the incidence of trisomy 21 between the groups ($p < 0.001$).

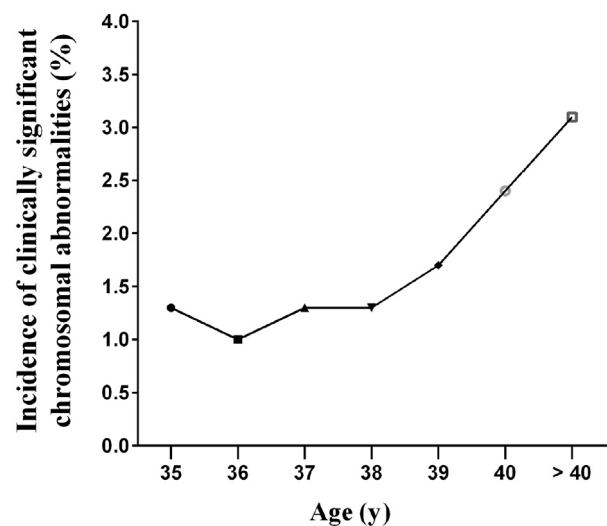


Figure 3. Relationship between the incidence of clinically significant chromosomal abnormalities and maternal age.

New technologies as alternatives to karyotype analysis

The current analysis indicates that the most common chromosomal anomalies in China can be detected using newer prenatal screening technologies, including the analysis of free fetal DNA in peripheral blood (NIPT), QF-PCR, CMA, and other technologies that have matured and offer high sensitivities and low false-negative rates [7–11,13,15,26]. The detection rates of the three most common chromosomal abnormalities, trisomy 21, trisomy 18, and trisomy 13, using NIPT are above 98%, with a false-positive rate under 0.5% [27]. Based on these values, ACOG has included AMA as an indication for noninvasive DNA testing [15]. However, the accuracy of detecting chromosomal abnormalities other than trisomy 21, trisomy 18, and trisomy 13 is not as high. In the present study, for example, NIPT would have detected 73.6% of CSCAs, but may have missed 20.3% of sex chromosome abnormalities and 6.1% of other

Table 3
Incidence of three types of chromosomal abnormalities by age group.

Age group (y)	Rate of all abnormalities	Rate of three autosomal aneuploidies ^a (out of all abnormalities)	Rate of five common chromosome aneuploidies ^b (out of all abnormalities)	Rate of rare abnormalities ^c (out of all abnormalities)
35	1.30	0.95 (73.1)	1.21 (93.1)	0.09 (6.9)
36	1.01	0.68 (67.0)	0.91 (90.0)	0.10 (10.0)
37	1.32	0.95 (71.7)	1.22 (92.5)	0.10 (7.5)
38	1.33	0.95 (71.8)	1.22 (91.8)	0.11 (8.2)
39	1.73	1.21 (70.1)	1.62 (93.5)	0.11 (6.5)
40	2.41	1.74 (72.2)	2.35 (97.5)	0.06 (2.5)
>40	3.09	2.62 (84.7)	3.04 (98.4)	0.05 (1.5)
Total	1.53	1.13 (73.6)	1.44 (93.9)	0.09 (6.1)

Data are presented as %, unless otherwise indicated.

^a The incidences of trisomy 21, trisomy 18, and trisomy 13 were comparable among women aged 35 years, 36 years, 37 years, and 38 years ($p = 0.133$), but began to significantly increase in those aged 39 years and older ($p < 0.001$).

^b The incidences of trisomy 21, trisomy 18, trisomy 13, Turner syndrome, 47,XXX, 47,XXY, 47,XYY, 69,XXY, and 69,XYY were comparable among women aged 35 years, 36 years, 37 years, and 38 years ($p = 0.124$), but began to significantly increase in those years 39 years and older ($p < 0.001$).

^c Except for the five common abnormalities in the number of chromosomes, the incidence of other clinically significant chromosomal abnormalities (such as partial chromosomal deletions and unbalanced translocations) did not increase with age ($p > 0.05$), and their relative incidence of total chromosomal abnormalities showed a decreasing tendency with age ($p < 0.05$).

CSCAs, and these missed diagnoses would have accounted for 0.40% of AMA women who had undergone prenatal diagnosis. It must be noted, however, that NIPT has only been available in China since 2012, and given the length of the study period, this may have influenced the results.

In cases in which there are no ultrasound abnormalities, less than two ultrasound soft markers, no genetic history of chromosomal rearrangements, and no nuchal translucency abnormalities, rapid molecular diagnostic techniques such as QF-PCR and FISH can detect approximately 99.9% of CSCAs [7,12,28]. Our results showed that the five most common chromosomal abnormalities accounted for 93.9% of all CSCAs. If QF-PCR and FISH miss 6.1% of other CSCAs, these missed diagnoses would account for 0.09% of all AMA pregnant women seeking prenatal screening. In other words, a normal QF-PCR or FISH result would indicate a 99.9% chance of no CSCAs, which is consistent with other reports [4].

Although CMA can be used to analyze genome copy number, and is capable of detecting chromosomal abnormalities and copy number variations (CNVs) [9,16], it is expensive and not always interpretable. The present study suggested no particular advantage of CMA in pregnant women older than 35 years.

The present study has certain limitations. Amniocentesis and karyotype analysis of amniotic fluid were performed at 18–22⁺6 weeks of gestation, and AMA was the sole reason for genetic testing. We did not examine first trimester testing and screening methods, so results may be different in a patient population with other risk factors for fetal chromosomal anomalies. The 93.9% rate that we found for the five most common types of chromosomal anomalies is lower than the rates reported in the literature [7,13,23]. This may be because the majority of women in this study did not undergo regular ultrasound screening during the second trimester before prenatal diagnosis, and some cases may have shown abnormalities if ultrasound had been performed.

Conclusion

Results of this study showed that the rates of fetal chromosomal abnormalities among AMA women in China are different from those in other populations and that newer technologies may be appropriate as alternatives to karyotype analysis. These findings may assist in the genetic counseling of AMA pregnant women, and provide a basis for the selection of prenatal screening and diagnostic technologies, and for the rational allocation of resources for prenatal diagnosis.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This work was supported by the National 11th Five-year Plan of the Science and Technology Support Program (No. 2006BAI05A10) and the Program for Zhejiang Leading Team of Scientific and Technological Innovation, P.R. China (No. 2011R50013-15).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tjog.2016.01.002>.

References

- [1] Heffner LJ. Advanced maternal age—how old is too old? *N Engl J Med* 2004;351:1927–9.
- [2] ACOG Committee on Practice Bulletins. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. *Obstet Gynecol* 2007;109:217–27.
- [3] Ministry of Health, People's Republic of China. Report on the prevention and treatment of newborn defects for 2012. Sept.12,2012. Available at: <http://www.gov.cn/gzdt/att/att/site1/20120912/1c6f6506c7f811bacf9301.pdf>.
- [4] Liao C, Yi CX, Li FT, Li DZ. The prevalence of non-detectable chromosomal abnormalities by QF-PCR in amniocentesis for certain referral indications: experience in a mainland Chinese hospital. *Arch Gynecol Obstet* 2014;289:75–8.
- [5] Bian XM, Guo Q, Qi QW. Current situation and development of prenatal diagnosis in China. *Front Med China* 2010;4:271–4.
- [6] Wang D, Shi Y, Stewart D, Chang C, Li CY. Factors associated with the utilization and quality of prenatal care in western rural regions of China. *Health Educ* 2012;112:4–14.
- [7] Ogilvie CM, Lashwood A, Chitty L, Waters JJ, Scriven PN, Flinter F. The future of prenatal diagnosis: rapid testing or full karyotype? An audit of chromosome abnormalities and pregnancy outcomes for women referred for Down's syndrome testing. *BJOG* 2005;112:1369–75.
- [8] Vialard F, Simoni G, Gomes DM, Abourra A, De Toffol S, Bru F, et al. Prenatal BACs-on-Beads™: the prospective experience of five prenatal diagnosis laboratories. *Prenat Diagn* 2012;32:329–35.
- [9] Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosome microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012;367:2175–84.
- [10] Wellesley DG, Lucassen A. Prenatal diagnosis of chromosomal imbalances. *Arch Dis Child Fetal Neonatal Ed* 2014;99:F338–41.
- [11] Norwitz ER, Levy B. Noninvasive prenatal testing: the future is now. *Rev Obstet Gynecol* 2013;6:48–62.
- [12] Speevak MD, McGowan-Jordan J, Chun K. The detection of chromosome anomalies by QF-PCR and residual risks as compared to G-banded analysis. *Prenat Diagn* 2011;31:454–8.
- [13] Mademont-Soler I, Morales C, Clusellas N, Soler A, Sánchez A. Group of Cytogenetics from Hospital Clínic de Barcelona. Prenatal cytogenetic diagnosis in Spain: analysis and evaluation of the results obtained from amniotic fluid

- samples during the last decade. *Eur J Obstet Gynecol Reprod Biol* 2011;157:156–60.
- [14] Qi QW, Jiang YL, Zhou XY, Liu JT, Yin J, Bian XM. Genetic counseling, prenatal screening and diagnosis of Down syndrome in second trimester in women of advanced maternal age: a prospective study. *Chin Med J (Engl)* 2013;126:2007–10.
- [15] American College of Obstetricians and Gynecologists. Practice bulletin. Invasive prenatal testing for aneuploidy. *ACOG Pract Bull* 88. *Obstet Gynecol* 2007;110:1459–67.
- [16] Lo JO, Shaffer BL, Feist CD, Caughey AB. Chromosomal microarray analysis and prenatal diagnosis. *Obstet Gynecol Surv* 2014;69:613–21.
- [17] Bornstein E, Lenchner E, Donnenfeld A, Barnhard Y, Seubert D, Divon MY. Advanced maternal age as a sole indication for genetic amniocentesis; risk-benefit analysis based on a large database reflecting the current common practice. *J Perinat Med* 2009;37:99–102.
- [18] Resta RG. Changing demographics of advanced maternal age (AMA) and the impact on the predicted incidence of Down syndrome in the United States: implications for prenatal screening and genetic counseling. *Am J Med Genet A* 2005;133:31–6.
- [19] Chang YH, Chen PY, Li TY, Yeh CN, Li YS, Chu SY, et al. Discrepancy of cytogenetic analysis in Western and eastern Taiwan. *Pediatr Neonatol* 2013;54:161–5.
- [20] Cogulu O, Ozkinay F, Akin H, Onay H, Karaca E, Durmaz AA, et al. Reasons for adult referrals for genetic counseling at a genetics center in Izmir, Turkey: analysis of 8965 cases over an eleven-year period. *J Genet Couns* 2011;20:287–93.
- [21] Tseng JJ, Chou MM, Lo FC, Lai HY, Chen MH, Ho ES. Detection of chromosome aberrations in the second trimester using genetic amniocentesis: experience during 1995–2004. *Taiwan J Obstet Gynecol* 2006;45:39–41.
- [22] Danisman N, Kahyaoglu S, Celen S, Kahyaoglu I, Candemir Z, Yesilyurt A, et al. A retrospective analysis of amniocenteses performed for advanced maternal age and various other indications in Turkish women. *J Matern Fetal Neonatal Med* 2013;26:242–5.
- [23] Driscoll DA, Gross S. Clinical practice. Prenatal screening for aneuploidy. *N Engl J Med* 2009;360:2556–62.
- [24] Forabosco A, Percesepe A, Santucci S. Incidence of non-age-dependent chromosomal abnormalities: a population-based study on 88965 amniocenteses. *Eur J Hum Genet* 2009;17:897–903.
- [25] Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005;353:2001–11.
- [26] Song Y, Liu C, Qi H, Zhang Y, Bian X, Liu J. Noninvasive prenatal testing of fetal aneuploidies by massively parallel sequencing in a prospective Chinese population. *Prenat Diagn* 2013;33:700–6.
- [27] Gil MM, Akolekar R, Quezada MS, Bregant B, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 2014;35:156–73.
- [28] Hills A, Donaghue C, Waters J, Waters K, Sullivan C, Kulkarni A, et al. QF-PCR as a stand-alone test for prenatal samples: the first 2 years' experience in the London region. *Prenat Diagn* 2010;30:509–17.