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Chromosome Abnormalities Investigated by Non-Invasive Prenatal Testing Account for Approximately 50% of Fetal Unbalances Associated With Relevant Clinical Phenotypes

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During the past 20 years non-invasive screening tests have been increasingly utilized in prenatal diagnosis (PD) practice. Considerable effort has been exerted by multicenter consortia to evaluate the reliability of non-invasive screening tests in detecting those women with an increased risk of having a pregnancy affected by trisomies 21, 18, and 13, monosomy X, and triploidies. To what extent this group of abnormal karyotypes accounts for the total number of phenotypically relevant fetal chromosome abnormalities has, however, never been investigated. The present report is an attempt aimed to quantify this proportion. A retrospective analysis of a homogeneous survey of 115,128 consecutive invasive prenatal tests was undertaken. All cases were classified in accordance with the indication given for the invasive testing. Cytogenetic results regarding 96,416 karyotype analyses performed because of advanced maternal age (≥35 years) or gestational anxiety (<35 years) were considered since these are the patients who usually undergo non-invasive screening tests. We calculated the number of cases (T21, T18, T13, 45,X, and triploidy) that would have been detected by prenatal screening on the basis of the published detection rate of the combined-2 test or the quadruple test. Our findings indicate that the chromosomal abnormalities investigated by screening tests represent <50% of the fetal chromosomal abnormalities associated with an abnormal outcome ranging from intermediate-to-severe in women <35 years (45.8% and 39.6% in the first and second trimesters, respectively), and sensitivity >50% in women ≥35 years (65.1% and 61.8%, respectively). To conclude, approximately 50% of the phenotypically relevant abnormal karyotypes cannot be detected by non-invasive prenatal screening tests.

Key words: amniocentesis; chorionic villous sampling; prenatal screening

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INTRODUCTION

During the past 20 years screening tests have been increasingly utilized in prenatal diagnosis (PD) practice. Identifying the correlation between maternal serum markers combined with or without the nuchal translucency measurement has provided a complex multiple screening tool to single out women with an increased risk of having a pregnancy affected by a chromosomal abnormality [Saller and Canick, 2008].

Considerable efforts have been expended by different research consortia to evaluate the reliability of screening tests in detecting fetal chromosomal abnormalities. These are based on important

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investigations including the First and Second Trimester Evaluation of Risk (FASTER) Study carried out in the United States and the Serum Urine Ultrasound Study (SURUSS) done in the UK; other studies are the NIH-funded Blood Ultrasound Nuchal Study (BUN) and the One-Stop Clinic to Assess Risk (OSCAR) Study [Spencer et al., 2003; Wald et al., 2003; Wapner et al., 2003; Ball et al., 2007; Breathnach et al., 2007].

The major chromosomal abnormality investigated by screening tests is trisomy 21 (T21); in this case the SURUSS and FASTER reported detection rates (dr) ranging from 82% to 85% using the combined-2 test (maternal age + measurements of nuchal translucency, free β-hCG and PAPP-A) and from 81% to 83% using the quadruple test (maternal age + measurements of AFP, free β-hCG, uE_3, and inhibin-A) with a 5% false-positive rate (fpr).

Pregnancies affected by trisomy 18 (T18) have a specifically low-level analyte pattern, enabling a detection rate of 100% (fpr = 0.3%) with a quadruple test and 82% (fpr = 6%) using the combined-2 in the first trimester [Canick et al., 1990; Breathnach et al., 2007]. Even if T18 does not meet the criteria for an effective screening test because of its low frequency and high mortality rate, using the same markers with an alternative algorithm makes it highly cost effective and safe.

In order to evaluate if the screening algorithms targeted for T21 and T18 could be adopted to detect other aneuploidies and polyploidies, the FASTER trial was performed for trisomy 13 (T13), monosomy X (45,X), and triploidy (69 chromosomes) [Breathnach et al., 2007]. A 76% cumulative detection rate (fpr = 6%) using the combined-2 test and a 44% detection rate (fpr = 8.9%) applying the quadruple screening was reported.

The SURUSS and FASTER trials were exhaustive, detailed studies aiming to determine the actual reliability of various options offered by different combinations of the screening tests that are available. How many unbalances out of the total number of phenotypically relevant fetal chromosome abnormalities that are detectable by prenatal screening has, however, never been evaluated. The present report, designed around data collected over 14 years of clinical practice in fetal karyotyping, aimed to quantify the proportion of chromosome changes associated with clinically abnormal phenotypes, ranging from moderate to severe [Drugan et al., 1990; Evans et al., 1996; Clementi et al., 2006; Wallerstein et al., 2006] that were undetected by prenatal screening tests.

MATERIALS AND METHODS

Sample Collection, Procedures, and Cytogenetic Analysis

During the 14-year collection period, our laboratory (TOMA Advanced Biomedical Assays S.p.A.) processed 115,128 invasive PD samples: 30,658 of these were chorionic villous samples (CVS) and 84,470 were amniotic fluid samples (AFS). Each prenatal sample arrived no later that 24 hr after it was taken at one of the 85 public and private PD centers located in Northern Italy, representing about 8–10% of all Italian prenatal centers, and providing a homogeneous population of women receiving qualitatively medium-to-high medical care. Each sample was accompanied by requisition forms, including a concise and complete description of the fetal phenotypic features at ultrasound and the indication for PD that, in all cases, was ascertained by the physician who performed the invasive procedure.

All of the samples underwent similar procedures using homogeneous evaluation criteria. A total of at least 30 metaphases were analyzed for each CVS, combining the semi-direct method and long-term culture preparations. A condition of CV mosaicism was defined as the presence of at least two cells showing the same chromosomal alteration (trisomy or a structural rearrangement) or at least three cells in the case of a monosomy. Whenever there were CV mosaicisms, a subsequent amniocentesis was performed for confirmation.

Amniocytes karyotyping was performed by analyzing at least 15 metaphases from a minimum of 10 colonies taken from more than one culture. True fetal mosaicism (TFM) was defined as the presence of the same abnormality previously observed in a CVS or after an identical chromosome change in cells from one or more colonies from a minimum of two different culture vessels was found.

Data Collection and Analysis

Information concerning each case (maternal age, indication for PD, gestational age in weeks (gw) at the invasive sampling, and the fetal karyotype) were collected in an in-house database. All cases considered by our survey were classified according to the indication for invasive PD provided by the obstetrician (Table I): fetal US abnormality (n = 4,723), previous affected fetus/child (n = 1,869), balanced chromosomal abnormality in one of the parents (n = 420), supernumerary marker or chromosome mosaic in one of the parents (n = 58), increased risk for a chromosomal abnormality after the screening test (n = 8,470), confirmatory amniocentesis after CV mosaicism (n = 692), one or more spontaneous abortions (n = 233), chromosomal abnormality in a relative (n = 1,190), and miscellaneous reasons including a suspected infection during pregnancy, a high risk for a fetal monogenic disease, that is, hemophilia, fragile X, and thalassemia, or exposure of one of the parents to mutagenic agents (n = 1,057). The remaining and more consistent two groups included pregnant women who decided to undergo invasive PD because of advanced maternal age (≥35 years; n = 68,489) or anxiety (<35 years; n = 27,927). The principle aim focused on the last two groups of women (hereafter referred to as ≥35 and <35 years) as they are the patients who normally undergo non-invasive screening tests. In this study the traditional division between women younger or older than 35 was maintained because it is a widely accepted custom in most countries outside of the United States and for historical reasons. It is not recommended by the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) and is under discussion in some European countries.

The abnormal karyotypes found in this study were the following:

(a) trisomies 21 (T21), 18 (T18), and 13 (T13);
(b) monosomy X (45,X);

(c) triploidies (69,XXX/XXY/XYY);
(d) autosomal aneuploidies other than T21, T18, and T13 (i.e., T9, T22, and double aneuploidies such as 48,+18,+21; 48,XXY,+21; 46,X,+13);
(e) complex unbalanced karyotypes;
(f) de novo non-satellited and satellited supernumerary markers (SMCs) containing euchromatic material proven by FISH (47,+mar);
(g) unbalanced structural rearrangements;
(h) TFM involving autosome aneuploidy or a 45,X cell line (in CVS were included cases with a 45,X cell line >30% at the confirmatory amniocentesis);
(i) de novo inversions;
(j) de novo apparently balanced reciprocal translocations;
(k) mos46/45,X (in CVS were included both 46,XX and 46,XY cases with a 45,X cell <30% at the confirmatory amniocentesis);
(l) de novo and familial Roberstonian translocations;
(m) inherited inversions;
(n) inherited or de novo satellited SMCs without euchromatic material;
(o) apparently balanced reciprocal translocations inherited from a normal parent;
(p) homogeneous and mosaic sex chromosome anomalies other than 45,X or mos46/45,X (i.e., 47,XXX, 47,XXY, 47,XYY, and X–Y dicentric chromosomes).

All the chromosomal abnormalities (a–p) were divided into three main groups based on their phenotypic effects affecting the decision of pregnancy termination in agreement with previous criteria reported by Drugan et al. [1990], Evans et al. [1996], Clementi et al. [2006], and Wallerstein et al. [2006]:

(i) Group A (high risk [HR]): Unbalanced karyotypes associated with a high risk or with a relevant fetal abnormal phenotype (a–h).
(ii) Group B (intermediate risk [IR]): Chromosomal abnormalities associated with an intermediate risk or clinical phenotype (i–k).
(iii) Group C (low risk [LR]): Abnormal karyotypes without or with a minor phenotypic effect or associated with negligible risk of an abnormal fetal phenotype (l–p).

To define the proportion of fetal chromosomal abnormalities with a significant phenotypic effect that go undetected by prenatal screening, only the high-risk and intermediate risk abnormal karyotypes were taken into account with the exclusion of the low -risk abnormal ones. Genotypic imbalances are implicated in the natural selective elimination of the genetically abnormal conceptuses [Semprini and Simoni, 2000]. The type and the frequency of chromosome abnormalities in the first trimester are consequently somewhat different from those of the second trimester. This is the biological explanation for which we reported fetal karyotyping results in a separate way for the first and the second trimester. The SURUSS and FASTER studies were used as sources for detection rate values of the screening tests: in the first trimester, the best detection rate with combined-2 were 85% for T21, 82% for T18, and 76% for T13, 45,X, and triploidy together (hereafter referred to as other aneuploidies [OA]) [Ball et al., 2007; Breathnach et al., 2007]. In the second trimester, the quadruple test reached the highest detection rate for T21 (83%) [Wald et al., 2003]; T18 had a detection rate of 100%; and the OA detection rate was 44% [Ball et al., 2007; Breathnach et al., 2007]. The cumulative amount of fetal chromosomal abnormalities with a significant phenotypic effect that might be identified by prenatal screenings (Σ detectable) was calculated subtracting the portion that would be undetected due to the specific detection rate of the screening test (false negatives) from the observed amounts of T21, T18, and OA (Σ observed). This value was then compared to the total phenotypically relevant chromosomal abnormalities. The following formulas were applied, depending on the trimester:

$$\sum \text{detectable (comb2)} = \left\{ \frac{[(\text{no. T21obs.} \times 85/100) + (\text{no.T18obs.} \times 82/100) + (\text{no. OA obs.} \times 76/100)]}{\text{no. total abnormal cases}} \right\} \times 100$$

$$\sum \text{detectable (quad)} = \left\{ \frac{[(\text{no. T21obs.} \times 83/100) + (\text{no.T18obs.} \times 100/100) + (\text{no. OA obs.} \times 44/100)]}{\text{no. total abnormal cases}} \right\} \times 100$$

in which: comb2 and quad = combined-2 and quadruple tests; no. T21/T18/OA obs. = number of T21/T18/OA cases observed in the survey; no. total abnormal cases = total cases with an abnormal karyotypes observed (depending on which chromosomal abnormalities are considered to be associated with a significant phenotypic effect).

RESULTS

The incidence of HR + IR abnormal karyotypes in the survey was 4.23% (1,297/30,658) in CVS and 1.59% (1,344/84,470) in AFS (Table I). Pregnancies with the highest percentages of HR + IR chromosome changes were those with a fetal US abnormality (27.87% on CVS and 11.46% on AFS), one or more spontaneous abortions (18.60% on CVS and 3.16% on AFS), a balanced chromosomal abnormality in one of the parents (11.28% on CVS and 0.89% on AFS), an increased risk on a screening test (7.61% on CVS and 2.28% on AFS) and a supernumerary marker or mosaic in one of the parents (4.35% on CVS and 2.86% on AFS; Table I).

Focusing on women ≥35 years (advanced maternal age; n = 68,489) and <35 years (gestational anxiety; n = 27,927), we found that the incidences of HR + IR karyotypes were 2.42% and 1.22% in CVS and 1.42% and 0.61% in AFS, respectively. As expected, T21 was the most common chromosomal abnormality, appearing at a higher frequency in older pregnant women. The incidence was 1:270 (20/5,400) in <35 years and 1:75 (274/20,502) in ≥35 years in CVS and 1:425 (53/22,527) in <35 years and 1:120 (399/47,987) in ≥35 years in AFS. The distributions at different maternal ages (≤29, 30, 31,. . .,37, and ≥38 years) in CVS and AFS, together with the

TABLE I. HR + IR Chromosomal Abnormalities Detected in Chorionic Villi (CV) and in Amniotic Fluid (AF) According to the Indication for Invasive Prenatal Diagnosis

Chromosomal abnormality	<35 years	≥35 years	US fetal abnorm.	Bal. chr. abn. in a parent	sSMC or mosaic in a parent	Previous affected child or fetus	Increased risk at screening test	Confirmatory amniocentesis after CVS abn.	One or more spontaneous abortions	Chr. abn. in a relative	Other	Subtotal (no. cases)
CV												
Total number of cases analyzed	5,400	20,502	2,297	195	23	1,034	473	—	43	165	526	30,658
No. of HR + IR karyotypes	66	496	640	22	1	22	36	—	8	2	4	1,297
Frequency of HR + IR karyotypes (%)	1.22	2.42	27.86	11.28	4.35	2.13	7.61	—	18.60	1.21	0.76	4.23
AF												
Total number of cases analyzed	22,527	47,987	2,426	225	35	835	7,997	692	190	1,025	531	84,470
No. of HR + IR karyotypes	137	682	278	2	1	6	182	35	6	9	6	1,344
Frequency of HR + IR karyotypes (%)	0.61	1.42	11.46	0.89	2.86	0.72	2.28	5.06	3.16	0.88	1.13	1.59

US fetal abnorm., ultrasound fetal abnormalities; Bal. chr. abn., balanced chromosome abnormalities; sSMC, small supernumerary chromosome marker; CV, chorionic villi; AF, amniotic fluid; HR, high risk; IR, intermediate risk.

expected distributions [Snijders et al., 1999], are depicted in Figure 1. In the second trimester, in women ≤29 years, there was an incidence of 1:766 that progressively increased to 1:678 in those 30 years of age and to 1:332 in women 33 years of age. In older pregnant women, it increased from 1:258 at 34 years to 1:75 in ≥38 years. CVS data were discontinuous since the number of samples analyzed in each age group was smaller than that in the AFS ones. Nevertheless, T21 appeared more frequently in the first trimester in each age group than it does in the second trimester.

Abnormal karyotypes with a phenotypic effect from moderate to severe (HR + IR) in CVS were 562: 66 in <35 years and 497 in ≥35 years (Table II); the Σ observed amount of T21, T18, and OA accounted for 56.06% (37/66) and 78.02% (387/496), respectively (Table III). The HR + IR karyotypes in AFS were 819 (137 in <35 years and 682 in ≥35 years; Table II) and the Σ observed were 51.09% (70/137) and 74.93% (511/682), respectively (Table III). The Σ detectable calculated values are reported in Table III: 45.79% in <35 years and 65.10% in ≥35 years in CVS; 39.61% in <35 years and 61.78% in ≥35 years in AFS.

DISCUSSION

Prenatal screening tests are useful tools for identifying pregnant women with an increased risk for fetal chromosomal abnormality. Considerable effort has been expended by multicenter consortia to calculate the reliability of prenatal screening tests in detecting pregnancies affected by trisomies 21, 18, and 13, monosomy X, and triploidies [Spencer et al., 2003; Wald et al., 2003; Wapner et al., 2003; Ball et al., 2007; Breathnach et al., 2007]. There are little data, however, indicating what percentage these unbalances represent out of the total number of phenotypically relevant fetal chromosomal abnormalities. The present study, based on the data of a single center survey analyzing a total of 115,128 invasive PD samples, aimed to quantify the portion of fetal chromosomal abnormalities associated with a high-intermediate risk of an abnormal outcome that are undetected by prenatal screening tests.

We initiated our studies making two assumptions: (i) chromosomal abnormalities without risk or with a negligible risk of an abnormal fetal outcome (LR) would not be considered; (ii) only pregnant women with advanced maternal age (≥35 years) or gestational anxiety (<35 years) as the sole indications for invasive testing would be taken into consideration for our analysis since they are the usual patients for non-invasive screening tests. As a result only 96,416 out of the 115,128 cases surveyed were considered (68,489 of ≥35 years and 27,927 of <35 years), and the abnormal karyotypes included were those of the HR (a–h) and IR (i–k) groups [Drugan et al., 1990; Evans et al., 1996; Clementi et al., 2006; Wallerstein et al., 2006].

This study reflects 14 years of karyotyping practice in a PD laboratory receiving samples from a number of medical centers. The main weaknesses are that it was not a population study since it was based on a survey of pregnancies with an increased risk of fetal chromosomal abnormalities. In addition, the indications for invasive PD outlined in the requisition forms were determined exclusively by the obstetrician and never verified by us even if there is a direct contact between the two services. Finally, it should be remembered that the indications for prenatal testing, the

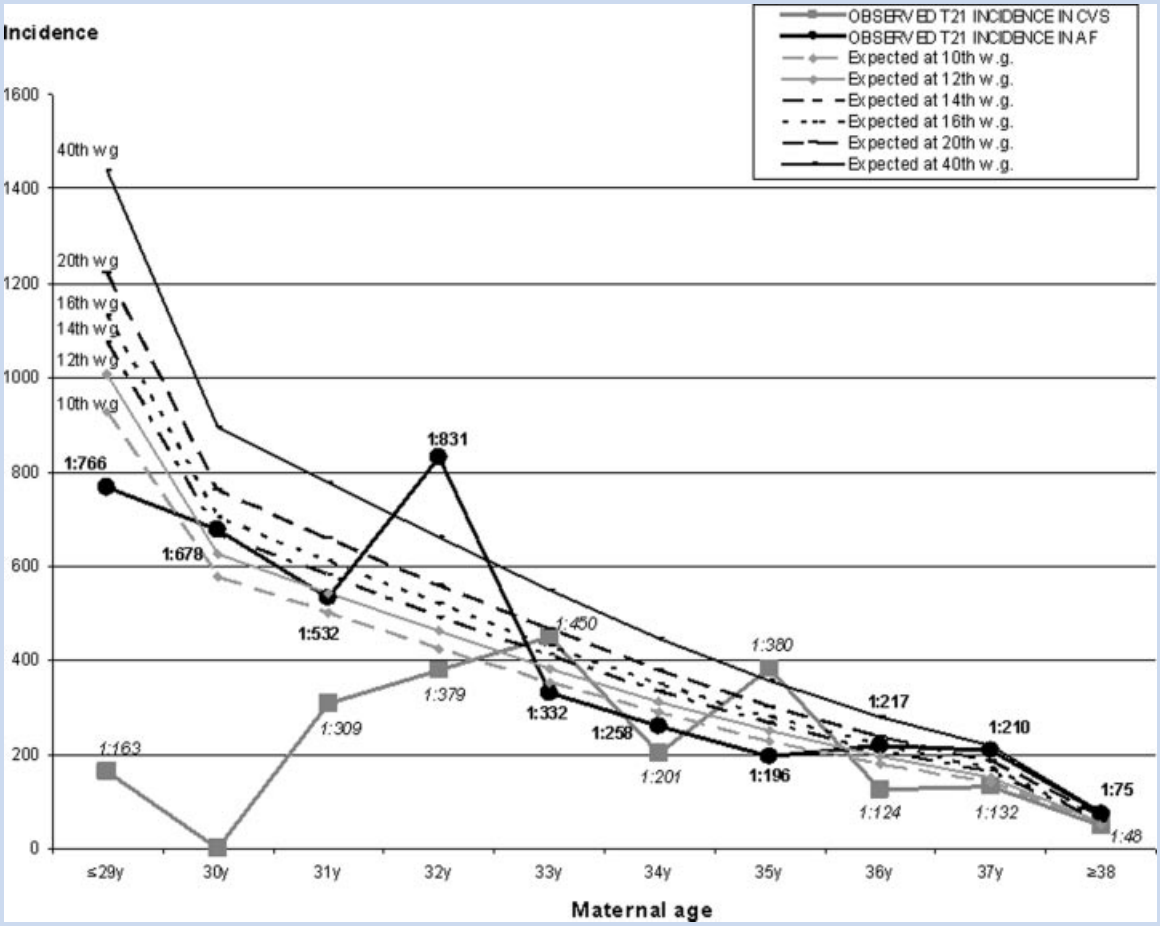

FIG. 1. Incidence of trisomy 21 (T21) according to maternal and gestational ages in pregnant women with advanced maternal age (≥35 years) and gestational anxiety (<35 years) as the sole indications for invasive prenatal diagnosis. The distribution of the incidence of T21 observed at different maternal ages (≤29, 30, 31,. . .,37, and ≥38 years) on CVS and AFS, together with the expected distribution according to Snijders et al. [1999], is depicted.

techniques utilized, and antenatal medical knowledge in general have rapidly progressed over this time span.

The study's strengths were the large number of the samples analyzed and, being a single center survey, the standardization of protocols and methods. In addition, the results from such a wide number of samples arriving from a large number of medical centers lessened parameter bias (i.e., misinterpretation of the indication for invasive PD).

In this study, the traditional division between women younger and older than 35 was maintained since it is a widely accepted custom in most countries. In both age groups the detected incidence of T21 was slightly higher than expected, and more so in the younger women in whom a 1:425 in AFS instead of 1:662–1:700 (14th–16th wg) was found [Snijders et al., 1999]. These data might be explained if the prevalence at different maternal ages is investigated. A trend with a substantial progressive increase of the incidence of T21 can be observed starting from 1:776 in ≤29 years to 1:75 in ≥38 years. This distribution indicates that T21 is more frequent than expected at each maternal age. Interestingly, in women 33 and 34 years of age, there were frequencies of 1:332 and 1:258, respectively, which are values that might have contributed to the unexpected higher incidence of T21 in the group of pregnant women <35 years. This bias might reflect the absence of a standardized Italian policy and of a commonly accepted rule regarding the interpretation of the maternal cut-off age defining the increased risk of an unbalanced fetal chromosome pattern. Our findings indicate that the maternal cut-off point beginning with which there is an increased risk of having T21 fetus was 34 years at the time of conception (1:258). In addition, our data are in agreement with those of previous studies demonstrating that serum testing has a lower detection rate in younger women and that maternal age influences the detection rates of serum screening in both trimesters [Reynolds et al., 1993; Spencer, 2001].

Since the reliability of prenatal screening is generally not 100% and they investigate only a limited group of relevant unbalances, in the first trimester the Σ detectable amount of unbalances associated with a intermediate-to-severe abnormal outcome accounts for 45.8% in <35 years and 65.1% in ≥35 years of the total HR + IR. In the second trimester, these percentages decreased to 39.6% and 61.8%, respectively. These results indicate that if only

TABLE II. The Relative Frequencies of Each HR and IR Chromosomal Abnormality Detected in Chorionic Villi (CV) and Amniotic Fluid (AF) in Pregnant Women <35 and ≥35 years

		CV				AF			
		<35 years		≥35 years		<35 years		≥35 years	
Chromosomal abnormality	Type	# cases	RF (%)	# cases	RF (%)	# cases	RF (%)	# cases	RF (%)
Trisomy 21 (T21)	HR	20	30.30	274	55.24	53	38.69	399	58.50
Trisomy 18 (T18)		5	7.58	69	13.91	5	3.65	73	10.70
Trisomy 13 (T13)		5	7.58	24	4.84	5	3.65	28	4.11
Monosomy X (45,X)		5	7.58	9	1.81	7	5.11	8	1.17
Triploidy		2	3.03	11	2.22		0.00	3	0.44
Autosomal aneuploidies other than T21, T18, T13		1	1.52	22	4.44	1	0.73	3	0.44
Complex unbalanced karyotypes			0.00	2	0.40	1	0.73	5	0.73
Non-satellited and satellited de novo SMC containing euchromatic material		1	1.52	5	1.01	2	1.46	14	2.05
Unbalanced structural rearrangements		2	3.03	9	1.81	23	16.79	28	4.11
True fetal mosaicism (TFM) (autosomes and mos46/45,X >30%)		10	15.15	45	9.07	27	19.71	83	12.17
De novo inversion	IR	10	15.15	7	1.41	6	4.38	13	1.91
Apparently balanced de novo reciprocal translocation		4	6.06	14	2.82	6	4.38	18	2.64
TFM 46/45,X (45,X<30%)		1	1.52	5	1.01	1	0.73	7	1.03
Familial Robertsonian translocation	LR	7		13		16		47	
De novo Robertsonian translocation		2		7		6		14	
Inherited inversion		9		32		27		56	
Inherited and satellited de novo SMC without euchromatic material				4		2		8	
Apparently balanced familial reciprocal translocation		3		18		10		29	
Sex chr. anomalies other than 45,X (mosaic and homogeneous)		11		72		39		119	
No. of HR + IR karyotypes		66		496		137		682	

HR, high risk; IR, intermediate risk; SMC, supernumerary chromosome marker; RF, relative frequency of each phenotypically relevant chromosomal abnormality compared to the total HR + IR cases.

prenatal screenings are utilized, on average about 50% of the total significant fetal chromosomal abnormalities would go undetected (Fig. 2). This portion includes not only T21, T18, and T13, 45,X, and triploidies that are missed by screening tests due to their sensitivity (false negatives) but also the chromosomal abnormalities that cannot be identified by the screenings.

The risk of an abnormal pregnancy outcome (mental retardation and/or congenital anomalies) after prenatal detection of an apparently balanced de novo rearrangement is 6.1–8.9% for reciprocal non-Robertsonian translocations and 9.4–12.1% for inversions [Warburton, 1991]. Based on these data, the balanced de novo reciprocal translocation has a pathologic outcome only in a relatively small number of cases. Hence, a re-evaluation of the Σ detectable percentage could be carried out considering this rearrangement as belonging to the LR group (48.74% in <35 years and 67.00% in ≥35 years in the first trimester; 41.43% in <35 years and 63.45% in ≥35 years in the second trimester; Table III). The recent application of the array comparative genomic hybridization in cases with de novo reciprocal translocations, considered balanced by conventional cytogenetics, displayed a cryptic deletion either at one of the breakpoints or elsewhere [De Gregori et al., 2007] and provide the biological explanation of the previously estimated risk of 8% of an abnormal pregnancy outcome [Warburton, 1991]. On this basis, we suggest that de novo reciprocal translocations should be included in the group of significant chromosomal abnormalities.

In contrast to the apparently balanced de novo reciprocal translocations, although the severity and consequences are unpredictable with any certainty, prenatal detection of a TFM is generally associated with a HR of an abnormal phenotype that varies depending on the chromosome involved and on the percentage and localization of the abnormal cells [Hsu et al., 1997; Wallerstein et al., 2000].

Regardless of the risk of an abnormal outcome associated with the IR abnormal karyotypes, if only unbalances belonging to the HR group are taken into consideration, the Σ detectable amount in the first trimester was 59.25% in <35 years and 68.71% in ≥35 years of the total severe chromosomal abnormalities. In the second trimester, these percentages decreased to 43.77% and 65.42%, respectively (Table III and Fig. 2).

Σ detectable values referred to the entire survey of 115,128 cases considering HR + IR karyotypes were 68.94% in the first trimester and 60.76% in the second trimester. They increased to 70.15% and 62.15%, excluding balanced de novo reciprocal translocations, and to 71.76% and 63.60% when only HR karyotypes were considered.

TABLE III. Fetal Chromosomal Abnormalities With a Relevant Phenotypical Effect Detectable by Prenatal Screening Tests

	Group	% T21 obs.	% T21 det.	% T18 obs.	% T18 det.	% OA obs.	% OA det.	Σ % obs.	Σ % det.	Δ% (Σ % obs. − Σ % det.)
Combined-2 test DR: T21 = 85%; T18 = 82%; OA = 76%										
% on (HR + IR)	Whole survey	48.50	41.22	17.66	14.48	17.42	13.24	83.58	68.94	14.63
	<35	30.30	25.76	7.58	6.21	18.18	13.82	56.06	45.79	10.27
	≥35	55.24	46.96	13.91	11.41	8.87	6.74	78.02	65.10	12.92
% on [(HR + IR) − (BDNRT)]	Whole survey	49.33	41.93	17.96	14.73	17.73	13.47	85.02	70.13	14.89
	<35	32.26	27.42	8.06	6.61	19.35	14.71	59.68	48.74	10.94
	≥35	56.85	48.32	14.32	11.74	9.13	6.94	80.29	67.00	13.29
% on (HR)	Whole survey	50.48	42.91	18.38	15.07	18.14	13.78	87.00	71.76	15.23
	<35	39.22	33.33	9.80	8.04	23.53	17.88	72.55	59.25	13.29
	≥35	58.30	49.55	14.68	12.04	9.36	7.11	82.34	68.71	13.63
Quadruple test DR: T21 = 83%; T18 = 100%; OA = 44%										
% on (HR + IR)	Whole survey	51.71	42.92	12.80	12.80	11.46	5.04	75.97	60.76	15.21
	<35	38.69	32.11	3.65	3.65	8.76	3.85	51.09	39.61	11.48
	≥35	58.50	48.56	10.70	10.70	5.72	2.52	74.93	61.78	13.15
% on [(HR + IR) − (BDNRT)]	Whole survey	52.89	43.90	13.09	13.09	11.72	5.16	77.70	62.15	15.55
	<35	40.46	33.58	3.82	3.82	9.16	4.03	53.44	41.43	12.01
	≥35	60.09	49.88	10.99	10.99	5.87	2.58	76.96	63.45	13.50
% on (HR)	Whole survey	54.13	44.93	13.40	13.40	11.99	5.28	79.52	63.60	15.92
	<35	42.74	35.48	4.03	4.03	9.68	4.26	56.45	43.77	12.69
	≥35	61.96	51.42	11.34	11.34	6.06	2.66	79.35	65.42	13.92

T21/18, trisomies 21 and 18; OA, trisomy 13 (T13), monosomy X (45,X), and triploidy together; DR, detection rate; %T21/18/OA obs., observed incidence of T21/18/OA; %T21/18/OA det., detectable portion of T21/18/OA by screening test; BDNTR, balanced de novo reciprocal translocations; Σ% obs., cumulative observed incidence of T21, T18, and OA; Σ% det., cumulative portion of T21, T18, and OA detectable by screening tests; Δ% = Σ % obs. − Σ % det.

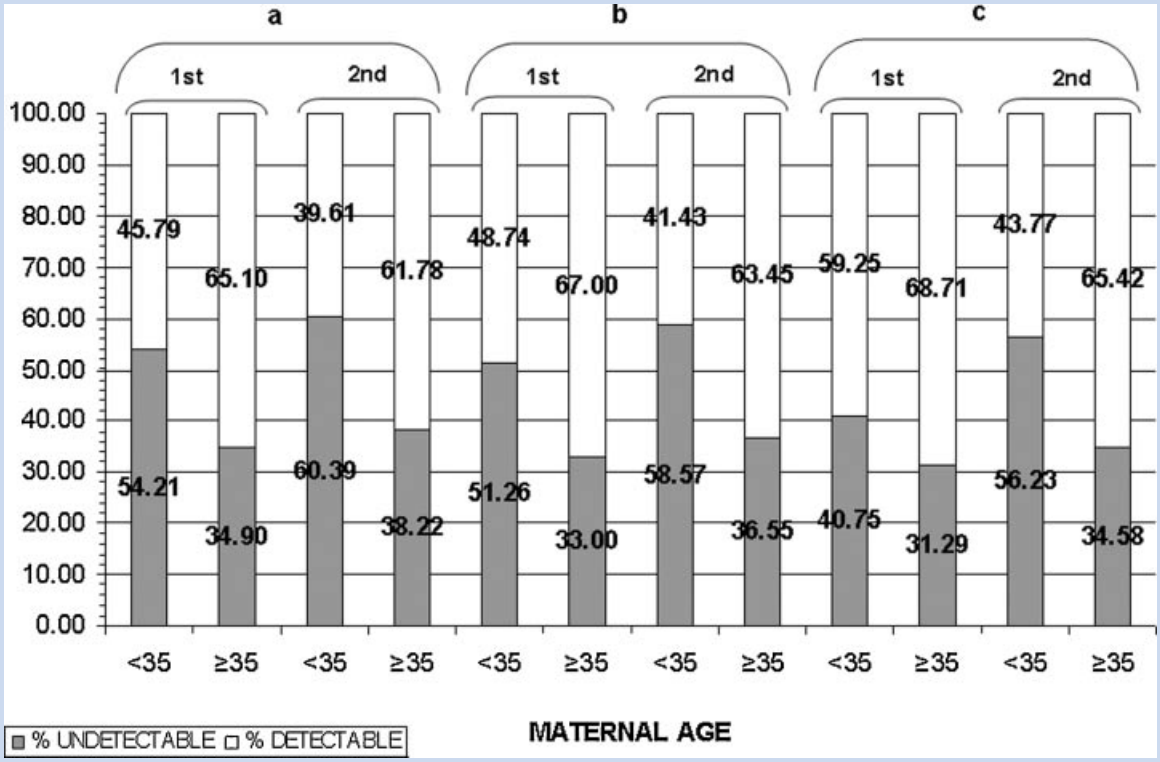

FIG. 2. Histograms representing the detectable and undetectable proportion of fetal chromosomal abnormalities by screening tests in women with advanced maternal age (≥35 years) and gestational anxiety (<35 years) as the sole indications to invasive prenatal diagnosis. Percentages referring to both trimesters (first and second) are reported considering (a) HR + IR, (b) (HR + IR)-balanced de novo reciprocal translocation (BDNRT), and (c) only HR chromosomal abnormalities as the total phenotypically relevant fetal chromosomopathy.

CONCLUSION

In conclusion, the amount of phenotypically relevant (HR + IR) fetal chromosomal abnormalities that are undetected by prenatal screenings because of their intrinsic detection rates and their ability to unmask only a specific group of chromosome unbalances has been quantified by the present study. It is evident from these data that a large proportion of chromosome abnormalities associated with a relevant clinical phenotype cannot be detected by non-invasive prenatal screening tests. Interestingly, in pregnant women <35 years, who undergo non-invasive PD, the quadruple test detects <50% portion.

It should be remembered that both the ACOG and the ACMG no longer use the 35-year cut-off and in fact recommend offering invasive testing to every pregnant woman.

Considering the false-negative rates for a large number of significant chromosomal abnormalities, the present findings support this approach. As recently pointed out by Driscoll and Gross [2009], a woman may decide to bypass the screening in order to know if the fetus has a chromosomal anomaly.

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REFERENCES

Ball RH, Caughey AB, Malone FD, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Gross SJ, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Emig D, D'Alton ME. 2007. First and Second Trimester Evaluation of Risk (FASTER) Research Consortium. First- and second-trimester evaluation of risk for Down syndrome. Obstet Gynecol 110:10–17.

Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Tripp T, Bianchi DW, D'Alton ME. 2007. First and Second Trimester Evaluation of Risk (FASTER) Research Consortium. First- and second-trimester screening: Detection of aneuploidies other than Down syndrome. Obstet Gynecol 110:651–657.

Canick JA, Palomaki GE, Osathanondh R. 1990. Prenatal screening for trisomy 18 in the second trimester. Prenat Diagn 10:546–548.

Clementi M, Di Gianantonio E, Ponchia R, Petrella M, Andrisani A, Tenconi R. 2006. Pregnancy outcome after genetic counselling for prenatal diagnosis of unexpected chromosomal anomaly. Eur J Obstet Gynecol Reprod Biol 128:77–80.

De Gregori M, Ciccone R, Magini P, Pramparo T, Gimelli S, Messa J, Novara F, Vetro A, Rossi E, Maraschio P, Bonaglia MC, Anichini C, Ferrero GB, Silengo M, Fazzi E, Zatterale A, Fischetto R, Previderé C, Belli S, Turci A, Calabrese G, Bernardi F, Meneghelli E, Riegel M, Rocchi M, Guerneri S, Lalatta F, Zelante L, Romano C, Fichera M, Mattina T, Arrigo G, Zollino M, Giglio S, Lonardo F, Bonfante A, Ferlini A, Cifuentes F, Van Esch H, Backx L, Schinzel A, Vermeesch JR, Zuffardi O. 2007. Cryptic

deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: A study of 59 patients. J Med Genet 44:750–762.

Driscoll DA, Gross S. 2009. Clinical practice. Prenatal screening for aneuploidy. N Engl J Med 360:2556–2562.

Drugan A, Greb A, Johnson MP, Krivchenia EL, Uhlmann WR, Moghissi KS, Evans MI. 1990. Determinants of parental decisions to abort for chromosome abnormalities. Prenat Diagn 10:483–490.

Evans MI, Sobiecki MA, Krivchenia EL, Duquette DA, Drugan A, Hume RF Jr, Johnson MP. 1996. Parental decisions to terminate/continue following abnormal cytogenetic prenatal diagnosis: "What" is still more important than "when". Am J Med Genet 61:353–355.

Hsu LY, Yu MT, Neu RL, Van Dyke DL, Benn PA, Bradshaw CL, Shaffer LG, Higgins RR, Khodr GS, Morton CC, Wang H, Brothman AR, Chadwick D, Disteche CM, Jenkins LS, Kalousek DK, Pantzar TJ, Wyatt P. 1997. Rare trisomy mosaicism diagnosed in amniocytes, involving an autosome other than chromosomes 13, 18, 20, and 21: Karyotype/phenotype correlations. Prenat Diagn 17:201–242.

Reynolds TM, Nix AB, Dunstan FD, Dawson AJ. 1993. Age-specific detection and false positive rates: An aid to counseling in Down syndrome risk screening. Obstet Gynecol 81:447–450.

Saller DN Jr, Canick JA. 2008. Current methods of prenatal screening for Down syndrome and other fetal abnormalities. Clin Obstet Gynecol 51:24–36.

Semprini AE, Simoni G. 2000. Not so inefficient reproduction. Lancet 356:257–258.

Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. 1999. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 13:167–170.

Spencer K. 2001. Age related detection and false positive rates when screening for Down syndrome in the first trimester using fetal nuchal translucency and maternal serum free G-hCG and PAPP-A. Br J Obstet Gynaecol 108:1043–1046.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: A review of three years prospective experience. Br J Obstet Gynecol 110: 281–286.

Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. 2003. SURUSS Research Group: First and second trimester antenatal screening for Down's syndrome: The results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 7:1–77.

Wallerstein R, Strmen V, Durcan J, White S, Bar-Lev A, Pruski-Clark J, Wallerstein D, McCarrier J, Twersky S. 2006. Factors in decision making following genetic counseling for pre-natal diagnosis of de novo chromosomal rearrangements. Clin Genet 69:497–503.

Wallerstein R, Yu MT, Neu RL, Benn P, Lee Bowen C, Crandall B, Disteche C, Donahue R, Harrison B, Hershey D, Higgins RR, Jenkins LS, Jackson-Cook C, Keitges E, Khodr G, Lin CC, Luthardt FW, Meisner L, Mengden G, Patil SR, Rodriguez M, Sciorra LJ, Shaffer LG, Stetten G, Van Dyke DL, Wang H. 2000. Common trisomy mosaicism diagnosed in amniocytes involving chromosomes 13, 18, 20 and 21: Karyotype–phenotype correlations. Prenat Diagn 20:103–122.

Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, Platt L, Mahoney M, Johnson A, Hogge WA, Wilson RD, Mohide P, Hershey D, Krantz D, Zachary J, Snijders R, Greene N, Sabbagha R, MacGregor S, Hill L, Gagnon A, Hallahan T, Jackson L. 2003. First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. First-trimester screening for trisomies 21 and 18. N Engl J Med 349:1405–1413.

Warburton D. 1991. De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: Clinical significance and distribution of breakpoints. Am J Hum Genet 49: 995–1013.