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- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

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Questions about these materials may be directed to the Obstetrics & Gynecology editorial office: obgyn@greenjournal.org.
Can NIPS adequately replace conventional maternal serum screening? A retrospective cohort study and meta-analysis.

Dear Dr. Sagi-Dain:

Your manuscript has been reviewed by the Editorial Board and by special expert referees. Although it is judged not acceptable for publication in Obstetrics & Gynecology in its present form, we would be willing to give further consideration to a revised version.

If you wish to consider revising your manuscript, you will first need to study carefully the enclosed reports submitted by the referees and editors. Each point raised requires a response, by either revising your manuscript or making a clear and convincing argument as to why no revision is needed. To facilitate our review, we prefer that the cover letter include the comments made by the reviewers and the editor followed by your response. The revised manuscript should indicate the position of all changes made. We suggest that you use the "track changes" feature in your word processing software to do so (rather than strikethrough or underline formatting).

Please be sure to address the Editor comments (see "EDITOR COMMENTS" below) in your point-by-point response.

Your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jan 25, 2022, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: These authors present a cohort study in which they examined the prevalence of clinically significant chromosome abnormalities on microarray in a women with a normal ultrasound examination who underwent amniocentesis. The compared the prevalence of such results in women with normal maternal serum screening and in those with an increased risk maternal serum screening result. They also examined the prevalence of chromosome abnormalities in those with abnormal screening results by the magnitude of the risk and the maternal age. In this examination, their primary objective was to determine the residual risk of a clinically significant chromosome abnormality if cell-free DNA screening had been performed as the primary screening modality as opposed to maternal serum screening. Based on the results of the amniocentesis with microarray, they modeled the performance of cell-free DNA screening and subtracted the abnormalities that they felt would have been detected by cell-free DNA (applying a number of different strategies) to arrive at an estimate for the residual risk. The also attempted to review the published literature on this subject to perform a systematic review and estimate of the residual risk. Overall they reported that 559 amniocenteses were performed for an abnormal maternal serum screen in women with a normal ultrasound and 3.8% had a significant microarray abnormality. After removing those cases which the authors deemed detectable by cell-free DNA screening, they estimated that the residual risk for a chromosome aberration is 2%.

1. Overall this is an important concept that is often overlooked and not sufficiently acknowledge. The amount of information in this study is voluminous and at times difficult to really comprehend and keep straight because the authors are trying to cover so many different areas in the same manuscript. Either the manuscript needs to be restructured in the results to present in subsections the risks in abnormal MSS vs normal MSS, then among the abnormal MSS by age, then by MSS result overall and with age included or some of the subgroup data should be removed and presented in a separate manuscript.

2. When the authors discuss the risk categories of the MSS, is this for trisomy 21 only? This should be clearly stated. The type of MSS included should be specified—first trimester, integrated, sequential, quad?

3. Which of the cell-free DNA strategies did the authors use in the primary analyses? As opposed to potentially including all as part of the consideration, one strategy should be used as the primary analysis and then consideration can be given to performance of a sensitivity-type analysis in which things can be evaluated further to say for example, "even if genome-wide cell-free DNA screening were performed, the residual risk would still have been...". Since only the common trisomies and the sex chromosomes are the standard approach utilized, this should be the basis of the primary analysis.

4. Were there any cases of triploidy? Would the CMA used detect it?
5. In the paragraph that begins on line 187, as opposed to providing all the detail of the abnormalities noted in the text, it would be clearer to refer to the table and then highlight in the text only those things that cell-free DNA screening would not have detected.

6. The rates of clinically significant findings associated with higher MSS risks is among the most important in the study but gets lost. This needs to be better emphasized and explained.

7. While the authors point about residual risk is pertinent and key, the authors should acknowledge in the discussion that the ability of an abnormal MSS to identify the additional chromosome abnormality is contingent upon the willingness of the patient to have a diagnostic procedure with microarray. In many locales, following an abnormal MSS and a normal ultrasound, even without cell-free DNA, the uptake of amniocentesis is low.

8. In the subgroup analysis by MSS results was there adequate power to evaluate the differences in risk in each of the different groups examined?

9. Given the heterogeneity of the different studies that examined the issue of residual risk, the information presented seems more suited for inclusion in the discussion section than presentation as a systematic review in the results since there really is no synthesis of the data into one cohesive estimate.

10. In the discussion, it is important to emphasize the population of this study is in women with normal second trimester ultrasound examinations.

11. The sentence from lines 281-286 that discusses the risk of abnormal CMA in women 35 or older with or without abnormal MSS is confusing and as written can be misinterpreted. It should be clarified.

12. Table 3 does not make sense. As the extent of what is screened increases the RR for residual defects should decrease not increase. This should be looked at for accuracy and if accurate then there should be an explanation why this is the direction of risk.

Reviewer #2: The authors set out to conduct a retrospective cohort study and systematic review of the use of chromosomal microarray (CMA) in pregnancies with abnormal maternal serum screening (MSS). They sought to understand whether non-invasive prenatal screening could replace CMA in pregnancies with abnormal MSS. Additionally, they wanted to understand how maternal age impacted utility of CMA.

Introduction
While the authors detail basic maternal serum screening, NIPS and CMA in the introduction the case they make for pursuing these aims does not appear evidence based. The sentence where authors mention "limited" data (lines 78-79) have no references. Please include what is known and what is not known to clarify the gap in literature.

Methods
Overall, I found the methods somewhat confusing. The attempt to incorporate both a retrospective analysis, a cohort study and support it with the unpublished data that has to be explained is complex for the reader.

1. Could the authors clearly describe genetic screening patterns in their country for reference. Do all patients start with MSS or do some go straight to NIPS?

2. Authors only include pregnancies that underwent amniocentesis to potentially "ensure absence of sonographic anomalies on early ultrasound." Why not include pregnancies undergoing CVS and amniocentesis and then exclude if there is an anomaly. While CVS is performed sooner, obvious abnormalities may be detected at the time of this early ultrasound. Additionally, the retrospective nature of the study would allow for determination.

3. The findings in lines 128-136 are confusing and were not well set up. Please describe the use of the control

4. Is there a reason why a third party/author was not consulted when disagreements arose (line 176)?

Results
5. Please frame the results into sections with headers for the retrospective analysis and the systematic review.

6. Why was the estimated risk for Down syndrome calculated for only 410 pregnancies? (line 183) How was this risk derived?

7. In line 246 please define cFTS.
Discussion

8. The statement in lines 286-287 "suggests that invasive testing should be performed in all pregnancies of women older than 35.." is far reaching. Would recommend removing the word "should".

STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

Table 2: Need to include CIs for the columns related to "Clinically significant CMA results following ..." Based on the sample sizes in the "n(%)" column and the overall sample sizes. Also, it is not clear from the Table what the referent is for the various RRs.

Table 4: Need to include CIs for the row entries in "Residual risk following theoretically normal NIPS". For the present study, the estimate of 1:50 has 95% CI = 1:28 to 1:100.

Figs 1 and 2: Both require CIs for the estimates of abn MSS rates. In Fig 1, the estimates of 7.9 and 3.7 (based on 1:100) have wide CIs and are NS different, as are the estimates of 4.9 and 1.7 (1:101-1:200) and the 2.7 and 0 (1:200-1:380). The same holds for Fig 2, the point estimates have wide CIs and none of the pairs (1:100, 1:101-200, 1:201-380) are statistically significant in their respective differences.

EDITOR COMMENTS:

1. The Editors of Obstetrics & Gynecology have increased transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:

A. OPT-IN: Yes, please publish my point-by-point response letter.
B. OPT-OUT: No, please do not publish my point-by-point response letter.

2. When you submit your revised manuscript, please make the following edits to ensure your submission contains the required information that was previously omitted for the initial double-blind peer review:
   * Include your title page information in the main manuscript file. The title page should appear as the first page of the document. Add any previously omitted Acknowledgements (ie, meeting presentations, preprint DOIs, assistance from non-byline authors).
   * Funding information (ie, grant numbers or industry support statements) should be disclosed on the title page and in the body text. For industry-sponsored studies, the Role of the Funding Source section should be included in the body text of the manuscript.
   * Include clinical trial registration numbers, PROSPERO registration numbers, or URLs at the end of the abstract (if applicable).
   * Name the IRB or Ethics Committee institution in the Methods section (if applicable).
   * Add any information about the specific location of the study (ie, city, state, or country), if necessary for context.

3. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA), which must be completed by all authors. When you uploaded your manuscript, each co-author received an email with the subject, "Please verify your authorship for a submission to Obstetrics & Gynecology." Please check with your coauthors to confirm that they received and completed this form, and that the disclosures listed in their eCTA are included on the manuscript’s title page.

4. Responsible reporting of research studies, which includes a complete, transparent, accurate and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), observational studies using ICD-10 data (ie, RECORD), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and
5. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 5,500 words. Stated word limits include the title page, précis, abstract, text, tables, boxes, and figure legends, but exclude references.

6. Titles in Obstetrics & Gynecology are limited to 100 characters (including spaces). Do not structure the title as a declarative statement or a question. Introductory phrases such as "A study of..." or "Comprehensive investigations into..." or "A discussion of..." should be avoided in titles. Abbreviations, jargon, trade names, formulas, and obsolete terminology also should not be used in the title. Titles should include "A Randomized Controlled Trial," "A Meta-Analysis," or "A Systematic Review," as appropriate, in a subtitle. Otherwise, do not specify the type of manuscript in the title.

7. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:

* All financial support of the study must be acknowledged.
* Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly.
* All persons who contributed to the work reported in the manuscript, but not sufficiently to be authors, must be acknowledged. Written permission must be obtained from all individuals named in the acknowledgments, as readers may infer their endorsement of the data and conclusions. Please note that your response in the journal's electronic author form verifies that permission has been obtained from all named persons.
* If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting).
* If your manuscript was uploaded to a preprint server prior to submitting your manuscript to Obstetrics & Gynecology, add the following statement to your title page: "Before submission to Obstetrics & Gynecology, this article was posted to a preprint server at: [URL]."

8. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limit for Original Research articles is 300 words. Please provide a word count.

9. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

10. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.

11. In your Abstract, manuscript Results sections, and tables, the preferred citation should be in terms of an effect size, such as odds ratio or relative risk or the mean difference of a variable between two groups, expressed with appropriate confidence intervals. When such syntax is used, the P value has only secondary importance and often can be omitted or noted as footnotes in a Table format. Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context than citing P values alone.

Please standardize the presentation of your data throughout the manuscript submission. For P values, do not exceed three decimal places (for example, "P = .001"). For percentages, do not exceed one decimal place (for example, 11.1%)

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15. Figures 1 and 2 may be resubmitted as-is with the revision.

16. Each supplemental file in your manuscript should be named an "Appendix," numbered, and ordered in the way they are first cited in the text. Do not order and number supplemental tables, figures, and text separately. References cited in appendixes should be added to a separate References list in the appendixes file.

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* A point-by-point response to each of the received comments in this letter. Do not omit your responses to the Editorial Office or Editors’ comments.

If you submit a revision, we will assume that it has been developed in consultation with your co-authors and that each author has given approval to the final form of the revision.

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jan 25, 2022, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Jason D. Wright, MD
Editor-in-Chief

2020 IMPACT FACTOR: 7.661
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To:
Editorial Office
Obstetrics & Gynecology
January 23th, 2022

Dear Editors,

We thank you for the opportunity to resubmit our manuscript entitled: "Can NIPS adequately replace conventional maternal serum screening? A retrospective cohort study and systematic review" for consideration for publication in Obstetrics & Gynecology.

We are very grateful to the editors and the reviewers for their thoughtful, supportive comments and suggestions. The manuscript has been substantially reformatted and modified based on these comments. We have addressed the issues that were raised, and point-by-point responses to each comment are marked in red.

On behalf of all authors,
Lena Sagi-Dain.
Reviewer #1:
We wish to thank the reviewer for the in depth analysis of our work and for raising several important points that needed clarification. We appreciate the time and effort expended on our behalf. We addressed each issue that was raised as follows:

These authors present a cohort study in which they examined the prevalence of clinically significant chromosome abnormalities on microarray in a women with a normal ultrasound examination who underwent amniocentesis. The compared the prevalence of such results in women with normal maternal serum screening and in those with an increased risk maternal serum screening result. They also examined the prevalence of chromosome abnormalities in those with abnormal screening results by the magnitude of the risk and the maternal age. In this examination, their primary objective was to determine the residual risk of a clinically significant chromosome abnormality if cell-free DNA screening had been performed as the primary screening modality as opposed to maternal serum screening. Based on the results of the amniocentesis with microarray, they modeled the performance of cell-free DNA screening and subtracted the abnormalities that they felt would have been detected by cell-free DNA (applying a number of different strategies) to arrive at an estimate for the residual risk. The also attempted to review the published literature on this subject to perform a systematic review and estimate of the residual risk. Overall they reported that 559 amniocenteses were performed for an abnormal maternal serum screen in women with a normal ultrasound and 3.8% had a significant microarray abnormality. After removing those cases which the authors deemed detectable by cell-free DNA screening, they estimated that the residual risk for a chromosome aberration is 2%.

1. Overall this is an important concept that is often overlooked and not sufficiently acknowledge. The amount of information in this study is voluminous and at times difficult to really comprehend and keep straight because the authors are trying to cover so many different areas in the same manuscript. Either the manuscript needs to be **restructured in the results to present in subsections** the risks in abnormal MSS vs normal MSS, then among the abnormal MSS by age, then by MSS result overall and with age included or **some of the subgroup data should be removed** and presented in a separate manuscript.

We have added subheadings and slightly restructured the results, by presenting the abnormal MSS results overall, then by subgroups, then the comparison to the control
cohort, and then the residual risks following normal MSS. We hope that the results are more clear now.

2. When the authors discuss the risk categories of the MSS, is this for trisomy 21 only? This should be clearly stated. The type of MSS included should be specified—first trimester, integrated, sequential, quad?

The risk categories of the MSS indeed refer to trisomy 21 only. This was now highlighted in Methods and Results sections.

The information about the specific MSS type for each case was unavailable.

This was not highlighted in the limitations paragraph:

“Finally, the specific MSS risk was not uniformly recorded, and the information about the MSS method (whether first trimester, triple test, quad test or integrated screening) was unavailable”.

3. Which of the cell-free DNA strategies did the authors use in the primary analyses?

As opposed to potentially including all as part of the consideration, one strategy should be used as the primary analysis and then consideration can be given to performance of a sensitivity-type analysis in which things can be evaluated further to say for example, "even if genome-wide cell-free DNA screening were performed, the residual risk would still have been…". Since only the common trisomies and the sex chromosomes are the standard approach utilized, this should be the basis of the primary analysis.

Indeed, the NIPS technique used for primary analysis was the standard approach (by omission of trisomies 13, 18 and 21, as well as sex chromosomal aberrations). This was highlighted in text (in several places).

4. Were there any cases of triploidy? Would the CMA used detect it?

The CMA technique used in our medical center is based on 750,000 CGH and SNP probes (able to detect triploidy). No cases of triploidy were noted.

5. In the paragraph that begins on line 187, as opposed to providing all the detail of the abnormalities noted in the text, it would be clearer to refer to the table and then highlight in the text only those things that cell-free DNA screening would not have detected.

Actually, it was problematic to present only the non-NIPS detectable findings, since these findings differ by various NIPS types. In this paragraph we tried to summarize the clinically significant CMA findings detailed in Table 1, to facilitate the
understanding of the readers.

6. The rates of clinically significant findings associated with higher MSS risks is among the most important in the study but gets lost. This needs to be better emphasized and explained.

Does the reviewer refer to the last sentence in the first paragraph of the Results section?

“The rates of abnormal findings increased with higher estimated MSS risk – from 1.4% in 1:201-1:380 MSS risk, to 3.3% in 1:101-1:200 MSS risk, and up to 5.5% in pregnancies with risk of 1:100 or greater (Figure 1)”? As no statistical significance was found between these rates, and as the presentation of these rates were not the main scope of our study, these findings indeed were not emphasized.

This sentence was changed to:

“The rates of abnormal findings increased with higher estimated MSS risk – from 1.4% in 1:201-1:380 MSS risk, to 3.3% in 1:101-1:200 MSS risk, and up to 5.5% in pregnancies with risk of 1:100 or greater (Figure 1), a non-statistically significant difference”.

In addition, confidence intervals were added for the proportions presented in Figure 1, to better represent lack of statistical significance.

If the reviewer did not refer to this sentence but rather to other statements, we would readily correct these.

7. While the authors point about residual risk is pertinent and key, the authors should acknowledge in the discussion that the ability of an abnormal MSS to identify the additional chromosome abnormality is contingent upon the willingness of the patient to have a diagnostic procedure with microarray. In many locales, following an abnormal MSS and a normal ultrasound, even without cell-free DNA, the uptake of amniocentesis is low.

This was added to the discussion.

8. In the subgroup analysis by MSS results was there adequate power to evaluate the differences in risk in each of the different groups examined?

As subgroup analysis led to relatively low numbers in each group, the power is not adequate (lower than 80% in all subgroups, except for the overall analysis). This is
represented by the wide confidence intervals of the relative risks.

9. Given the heterogeneity of the different studies that examined the issue of residual risk, the information presented seems more suited for inclusion in the discussion section than presentation as a systematic review in the results since there really is no synthesis of the data into one cohesive estimate.

As we aimed to present all available evidence examining the yield of chromosomal microarray in pregnancies with abnormal MSS, a formal systematic review was performed, in full accordance with MOOSE guidelines. Thus, we went over 174 potentially relevant manuscripts and summarized the available evidence. According to MOOSE guidelines, the standard methodology for the systematic review has to be presented in the Methods section, and the evidence be summarized in the Results. We are afraid that inclusion of the relevant studies in the Discussion only might lead the readers to falsely think that we decided to selectively present only some of the studies, rather than all available evidence, undermining our results and conclusions. However, we have highlighted the heterogeneity of the studies, in the results and discussion, and have changed the title from “meta-analysis” to “systematic review”.

10. In the discussion, it is important to emphasize the population of this study is in women with normal second trimester ultrasound examinations.

This was added to the first paragraph of the Discussion section:

“The residual risk for clinically significant CMA findings in pregnancies with high-risk MSS and normal second trimester ultrasound, following omission of common theoretically NIPS detectable aberrations, is 2.0%, or one in 50 pregnancies, and is significantly increased compared to normal pregnancies”.

11. The sentence from lines 281-286 that discusses the risk of abnormal CMA in women 35 or older with or without abnormal MSS is confusing and as written can be misinterpreted. It should be clarified.

We hope the paragraph is more clear now:

Interestingly, none of the previous studies exploring the yield of CMA in pregnancies with abnormal MSS has examined the influence of maternal age in this cohort. Thus, although the risk for abnormal CMA in the overall cohort was significantly increased compared to control population, in a subgroup analysis by maternal age and MSS risk categories we showed that in a subgroup of women older than 35 years, the risk for abnormal CMA results in general, or the risk for Trisomy 21 in specific, was not increased compared to the baseline risk (in AMA pregnancies with normal MSS
results). This finding probably reflects the a priori increased risk for abnormal CMA results in AMA pregnancies (10), and suggests that invasive testing might be performed in all pregnancies of women older than 35 years, regardless of the MSS. It was interesting to note that all abnormal microarray results in the AMA group with known MSS risk were found only in pregnancies in which the MSS has increased the baseline risk by maternal age only. These findings imply that in AMA pregnancies, the MSS might point for increased risk for abnormal CMA only in case it increases the baseline risk; however, the relatively small.

12. Table 3 does not make sense. As the extent of what is screened increases, the RR for residual defects should decrease not increase. This should be looked at for accuracy and if accurate then there should be an explanation why this is the direction of risk.

We have examined the accuracy of Table 3, and this is correct, since the comparison was made to the control population. As the extent of NIPS increases, its detection in the general population increases as well, thus the residual risk for non-NIPS detectable findings has decreased in the control cohort also (which constituted the denominator in the formula of relative risk). This is the reason that the relative risk for residual defects have actually increased with increasing extent of NIPS.
Reviewer #2:
Thank you for the time and effort invested to improve our manuscript. The points that were raised were well taken and we have revised the original document accordingly. Our point-by-point responses are as follows:

The authors set out to conduct a retrospective cohort study and systematic review of the use of chromosomal microarray (CMA) in pregnancies with abnormal maternal serum screening (MSS). They sought to understand whether non-invasive prenatal screening could replace CMA in pregnancies with abnormal MSS. Additionally, they wanted to understand how maternal age impacted utility of CMA.

Introduction
While the authors detail basic maternal serum screening, NIPS and CMA in the introduction, the case they make for pursuing these aims does not appear evidence based. The sentence where authors mention "limited" data (lines 78-79) have no references. Please include what is known and what is not known to clarify the gap in literature.
Actually, the evidence “examining the effect of maternal age and the specific MSS risk, as well as the residual risk for clinically significant CMA results following normal NIPS in pregnancies with abnormal MSS” is not “limited”, as was conservatively stated in our study, but rather “completely missing” (according to our systematic review on the subject). This is the reason no references were mentioned; however, we tried to avoid the absolute statement prior to presenting the results of the systematic review in the “results” section.

This was corrected to:
However, there is lack of evidence examining the effect of maternal age and the specific MSS risk, as well as the residual risk for clinically significant CMA results following normal NIPS in pregnancies with abnormal MSS is limited.

Methods
Overall, I found the methods somewhat confusing. The attempt to incorporate both a retrospective analysis, a cohort study and support it with the unpublished data that has to be explained is complex for the reader.
Meanwhile the unpublished data regarding the control cohort was published (reference 20), thus we hope it will facilitate the appreciation of the data.
1. Could the authors clearly describe genetic screening patterns in their country for reference. Do all patients start with MSS or do some go straight to NIPS?

We thank the reviewer for this important note.

The following description of the current pregnancy management was added as a first paragraph of Methods section:

Routine pregnancy management in authors’ country includes first and second trimester screening, as well and first (14-16 weeks) and second (20-24 weeks) sonographic surveys. Pregnancies with abnormal serum screening (at 1:380 cut-off) or with sonographic anomalies are referred to diagnostic prenatal testing. NIPS is selectively performed according to maternal request and is partially funded by health maintenance organizations.

2. Authors only include pregnancies that underwent amniocentesis to potentially "ensure absence of sonographic anomalies on early ultrasound." Why not include pregnancies undergoing CVS and amniocentesis and then exclude if there is an anomaly. While CVS is performed sooner, obvious abnormalities may be detected at the time of this early ultrasound. Additionally, the retrospective nature of the study would allow for determination.

Actually, in our study we tried to answer the question “what is the residual risk for abnormal CMA in pregnancies with normal NIPS and normal ultrasound” (as detection of sonographic anomalies is by itself a recommendation for invasive testing).

We decided not to include pregnancies undergoing CVS due to the concern of bias which would falsely elevate the rates of abnormal CMA results. After all, in most cases, detection of abnormal CMA results would lead to termination of pregnancy. If the genetic anomaly is detected early enough, before the performance of any sonographic survey, these pregnancies would be considered as “sonographically normal”, despite the reasonable chance to develop sonographic anomalies later on due to abnormal genetics. In other words, by inclusion of pregnancies undergoing amniocentesis (performed in Israel at 17 weeks and higher), following a normal first sonographic survey (performed at 14-16 weeks), we tried to ensure that these pregnancies are sonographically normal in at least one survey.

3. The findings in lines 128-136 are confusing and were not well set up. Please describe the use of the control

This was better described now (facilitated by the fact that the manuscript describing the control population has meanwhile been published):
Relative risk (RR) for clinically significant CMA findings with 95% confidence intervals (CI) was calculated using comparison to the recently described baseline rate of abnormal CMA results in normal pregnancies (REF). This control cohort of 7,235 pregnancies with normal MSS and sonography, undergoing amniocentesis in the same clinical laboratory, included 4,048 CMA analyses in AMA pregnancies, as well as 3,187 pregnancies of women younger than 35 years (undergoing invasive testing due to parental request). In this control group, 87 (1.2%) clinically significant CMA findings were noted: 63 (1.56%) in the AMA cohort (seven cases of Trisomy 21, one Trisomy 18, 18 sex chromosome anomalies, three 22q11.2 microdeletions and one additional genome-wide NIPS-detectable finding, i.e., sized over 7Mb), and 24 (0.75%) in younger cohort (including two cases of Trisomy 21, two sex chromosome anomalies and three additional genome-wide NIPS detectable findings).

4. Is there a reason why a third party/author was not consulted when disagreements arose (line 176)?

We have corrected the sentence, since an additional author was involved in case of disagreement (LB). This was added to the “methods” section.

Results

5. Please frame the results into sections with headers for the retrospective analysis and the systematic review.

We have added the relevant headers.

6. Why was the estimated risk for Down syndrome calculated for only 410 pregnancies? (line 183) How was this risk derived?

The risk was derived from the results of routine first/second trimester screening, due to which the women were referred to invasive testing. Unfortunately, this risk was not registered for all pregnancies in our electronic database, thus only 410 of 559 pregnancies could be analyzed.

This was better clarified in Methods and Results:

Methods:
The registered data included maternal age, risk for Down syndrome according to the MSS result (when noted), and CMA result (coordinates and classification).

Results (first paragraph):
The estimated risk for Down syndrome was noted in 410 of the 559 pregnancies.
7. In line 246 please define cFTS.

This was corrected to “combined first trimester screening”.

Discussion

8. The statement in lines 286-287 "suggests that invasive testing should be performed in all pregnancies of women older than 35.." is far reaching. Would recommend removing the word "should".

The word “should” was replaced with “might”.
STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

Table 2: Need to include CIs for the columns related to "Clinically significant CMA results following ..." Based on the sample sizes in the "n(%)" column and the overall sample sizes. Also, it is not clear from the Table what the referent is for the various RRs.

95 confidence intervals were added to all proportions and ratios in Table 2.
A footnote was added explaining that the RRs are a product of comparison to the control cohort of pregnancies with normal ultrasound and maternal serum screening.

Table 4: Need to include CIs for the row entries in "Residual risk following theoretically normal NIPS". For the present study, the estimate of 1:50 has 95% CI = 1:28 to 1:100.

95-confidence intervals were added to all proportions and ratios in Table 4.

Figs 1 and 2: Both require CIs for the estimates of abn MSS rates. In Fig 1, the estimates of 7.9 and 3.7 (based on 1:100) have wide CIs and are NS different, as are the estimates of 4.9 and 1.7 (1:101-1:200) and the 2.7 and 0 (1:200-1:380). The same holds for Fig 2, the point estimates have wide CIs and none of the pairs (1:100, 1:101-200, 1:201-380) are statistically significant in their respective differences.

95-confidence intervals were added to all percentages in both Figures.
EDITOR COMMENTS:

1. The Editors of Obstetrics & Gynecology have increased transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:

A. **OPT-IN: Yes, please publish my point-by-point response letter.**
B. **OPT-OUT: No, please do not publish my point-by-point response letter.**

12. **Line 335: Your manuscript contains a priority claim. We discourage claims of first reports since they are often difficult to prove.** How do you know this is the first report? If this is based on a systematic search of the literature, that search should be described in the text (search engine, search terms, date range of search, and languages encompassed by the search). If it is not based on a systematic search but only on your level of awareness, it is not a claim we permit.

   Actually, as our study included a systematic review on the subject, the priority claim is evidence based. However, if the statement is inappropriate, we can readily dismiss that.