OBSTETRICS & GYNECOLOGY

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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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Date: Sep 17, 2020
To: "Lena Sagi-Dain" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-20-2223

RE: Manuscript Number ONG-20-2223

Isolated nuchal translucency of 3-3.4 mm warrants microarray analysis, which cannot be adequately replaced by NIPS

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).

Your paper will be maintained in active status for 14 days from the date of this letter. If we have not heard from you by Oct 01, 2020, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: The authors present data on microarray (CMA) abnormalities that would not have been detected with cell free DNA (cfDNA) in a population with mildly increased NT measurements.

The authors should definewhat is meant by isolated NT as an indication for testing. Did they perform ductus venosus or tricuspid valve Doppler? Did they evaluate the presence or size of the nasal bone?

The authors repeatedly refer to a comparison population, although it is not well characterized. There should be a table for population characteristics of the 2 study groups and the comparison group. Any significant differences need to be explained and controlled for an appropriate analysis. Specifically, was mean age the same in the groups? Did the frequency of abnormality vary in controls by indication? Was the frequency of abnormality artificially depressed by the large number (50%) who had no specific indication for invasive testing?

The repeated inclusion of both percentages and fractions is distracting, especially since those fractions are rounded, and most prenatal counseling uses odds, which are slightly different than the fractions presented.

The authors found that the risk of CMA abnormalities was higher with CVS, though that difference was not significant (p=.06) They fail to address the pregnancy losses that may have occurred between the time the two procedures are performed. How many miscarriages occurred after the NT but before amniocentesis? Were there any possible procedure-related losses?

Were there changes in the CMA platforms used during the 5 year study timespan, such as improved sensitivity? How do the authors address the constantly changing categorization of VOUS, since rapid advances in mapping VOUS to specific genes tends to reduce those still of unknown significance over time? How might that have influenced their results?

What is the true denominator in this study? That is how many women did not have invasive testing? Could there have been other factors that led these particular women to choose invasive testing? For the NT patients, what did their serum screening show and how did that inform their decisions to undergo diagnostic testing or not?

The authors conclude that the risk of CMA abnormality is increased for NT 3.0-3.4 mm. The data in Table 2 only support that to be true for 3.1-3.4 mm, since the 95% CI for 3.0 mm crosses unity.

Finally, we know that CMA has a higher yield than cfDNA for all women regardless of maternal age, NT or any other risk factors. Can the authors make a convincing argument that this is true for this NT cohort more than any other group? That will be a powerful argument, but will require more thorough statistical comparison than presented here.

Reviewer #2: This study examines the perplexing question of "should a patient with mildly elevated NT have invasive testing for CMA or is just NIPS adequate?" The study group is compared to another population who underwent invasive testing for a variety of reasons unrelated to NT.

Unfortunately, outcome data is unavailable. Ideally, the complication rate of invasive testing should be addressed, even if it is in terms of literature review. It would be quite interesting to have the outcomes of patients with thickened NT who did not have invasive testing versus those who did have invasive testing. The discussion at least deserves some commentary about the potential risks of CVS and amniocentesis.

Lichtenberg's study is cited and they appeared to conclude that it was a very small number of patients that the NT testing would have detected but the NIPS and u/s would have missed. However, they appear to not have done invasive testing on patients with normal NTs, so it is unclear if the detected abnormalities, like a triple X, could have been present in a normal NT group so it seems inappropriate to conclude that the NT was valuable. Holzer's review is quite sobering since it seemed like they only had 7 livebirths without ultrasound abnormalities out of 247 with thick NT. Here again, outcome data is extremely helpful.

The summary of the literature, acknowledging that some centers use a different NT cut-off compared to others, was quite excellent. The table that quantifies each mm increase of NT and its associations was also quite helpful.

Reviewer #3:

Abstract
The objective of our study was to examine the risk for clinically significant Chromosomal microarray findings in fetuses with NT between 3-3.4 mm.

Intro
The objective of our study were to examine in a large cohort the risk for clinically significant CMA results in pregnancies with isolated NT of 3-3.4 mm, and to define the yield

of NIPS in such pregnancies IS there a primary and secondary objective?

These objectives should completely align.

This is a retrospective look of all CMA tests performed due to an NT between 3 to 3.4 mm without additional sonographic anomaly information were retrospectively retrieved from the Israeli Ministry of Health computerized database between January 2013 and September 2018. This is simply a look in time without any follow-up of sonography or postnatal outcomes

Introduction is too long, I would transition your discussion of findings in other studies to the discussion section and spend more time talking about microarray and its possibilities for the general OBGYN reader

Current SMFM recommendation for an increased NT >3.0-3.5 is a karyotype and if normal a fetal echo. How many of the CNVs were associated with heart defects?

I appreciate the OMIN tables but the non genetic OBGYN (the vast majority) will need a bit more explanation about the different findings in the Tables especially those that contain

132 genes - 16 OMIM, 9 more explanation please

The risk for abnormal CMA findings was twice higher in pregnancies undergoing CVS (13/179, 7.3%) compared to amniocentesis (16/440 = 3.6%), approaching level of statistical

significance (p=0.060). Why do you think this is?? Thoughts?

STATISTICAL EDITOR COMMENTS:

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

Table 2: When the cohort is divided by increments of 0.1 mm, there were from 3 to 8 in each stratum. Need to provide CIs for each incidence estimate. That is, although the RRs are statistically significantly different from the referent (except for 3 mm group), the absolute proportion with abnormalities is small. Note also that among the control group, 1.4% or ~ 78 out of 5541 were abnormal. Thus, the absolute count of abnormals was >2.5x as many as among all of those with thickness in the 3-3.5 mm group. As compared to the referent of 1.4% among 5541, the 3 mm thickness cohort is not statistically different from that referent. Therefore, the statement that a threshold of 3 mm should be used as a basis for further testing is undermined by the Authors' own data analysis

lines 51-53: These rates are based on small counts and the differences are only nominal, ie, there is no statistically significant difference. The samples also lack sufficient power to generalize the NS difference. For example, using Fisher's test, the difference has p = 0.11. Put another way, the rate (1:73 has CI 1:34 to 1:200), while the rate (1:30 has CI 1:14 to 1:81).

EDITOR COMMENTS:

1. Please acknowledge in manuscript that at 3.0 mm, no increased risk.

2. The Editors of Obstetrics & Gynecology are seeking to increase 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:
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5. Your submission indicates that one or more of the authors is employed by a pharmaceutical company, device company, or other commercial entity. This must be included as a statement in the Financial Disclosure section on the title page.

6. Please submit a completed STROBE checklist.

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 links to the checklists are available at http://ong.editorialmanager.com. In your cover letter, be sure to indicate that you have followed the CONSORT, MOOSE, PRISMA, PRISMA for harms, STARD, STROBE, RECORD, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.

7. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-

informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

8. 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 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendixes) but exclude references.

9. 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).

10. Provide a short title of no more than 45 characters, including spaces, for use as a running foot.

11. 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.

12. 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.

13. 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.

14. 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.

If appropriate, please include number needed to treat for benefits (NNTb) or harm (NNTh). When comparing two procedures, please express the outcome of the comparison in U.S. dollar amounts.

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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16. Please review the journal's Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online here: http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.

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18. 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 confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter.

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 14 days from the date of this letter. If we have not heard from you by Oct 01, 2020, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Dwight J. Rouse, MD, MSPH

2019 IMPACT FACTOR: 5.524
2019 IMPACT FACTOR RANKING: 6th out of 82 ob/gyn journals

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To:
The Editor–in-Chief
Obstetrics and Gynecology
September 25th 2020

Dear Editors of Obstetrics & Gynecology,

We thank you for the opportunity to resubmit our manuscript entitled: "Isolated nuchal translucency of 3-3.4 mm warrants microarray analysis, which cannot be adequately replaced by NIPS" for consideration for publication in Obstetrics and Gynecology.

We are grateful 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, marked in red.

On behalf of all authors,
Lena Sagi-Dain.

Reviewer #1:

Our thanks to the reviewer for the time and effort expended on our behalf to enhance the presentation of our investigation. We appreciate the valuable comments and made the following additions and changes accordingly.

The authors present data on microarray (CMA) abnormalities that would not have been detected with cell free DNA (cfDNA) in a population with mildly increased NT measurements.

1) The authors should define what is meant by isolated NT as an indication for testing. Did they perform ductus venosus or tricuspid valve Doppler? Did they evaluate the presence or size of the nasal bone?

The following was added to "Methods" section, describing the NT measurement routine in Israel:

There are no binding guidelines for measurement of ductus venosus or tricuspid valve Doppler, or for evaluation of the presence or size of the nasal bone.

And to the limitations paragraph:

We had no data regarding the presence or size of the nasal bone at the time of NT measurement, or Doppler of ductus venosus or tricuspid valve.

2) The authors repeatedly refer to a comparison population, although it is not well characterized. There should be a table for population characteristics of the 2 study groups and the comparison group. Any significant differences need to be explained and controlled for an appropriate analysis. Specifically, was mean age the same in the groups? Did the frequency of abnormality vary in controls by indication? Was the frequency of abnormality artificially depressed by the large number (50%) who had no specific indication for invasive testing?

We thank the reviewer for noticing this important point, which lead to a major change in the selected control population. Our original control cohort of pregnancies with normal ultrasound included women with advanced maternal age, abnormal screening for Down syndrome and soft markers. Mean maternal age in this group was 36.0 ± 3.9 years, significantly higher than the age in the study group of pregnancies with increased NT ($p<0.0001$). Thus, we have changed the control cohort to a subgroup of the original controls – 2752 pregnancies with normal ultrasound, in which invasive testing was performed due to maternal request. In this group, 21 (0.76%) clinically significant CMA findings were noted, and the mean maternal age was 31.4±2.1 years, not significantly differing from the study group.

The following was added to the Methods section:
The relative risk (RR) for clinically significant CMA results in pregnancies with increased NT was calculated, compared to a historical local control population of 2752 low-risk pregnancies (i.e. younger than 35 years with normal ultrasound), undergoing CMA due to maternal request (13). Mean maternal age in this control population was 31.4±2.1 years. In this group, CMA testing yielded 21 clinically significant results (0.76%), 4 of these NIPS-detectable.
And to the Results section:
Mean maternal age was 31.6±4.9 years, not significantly different from the control population (p= 0.1121).
The calculations of the relative risks and the 95% confidence intervals were changed accordingly.

3) The repeated inclusion of both percentages and fractions is distracting, especially since those fractions are rounded, and most prenatal counseling uses odds, which are slightly different than the fractions presented.
Actually, the fractions were presented to reflect (and oppose) the results of previous studies exploring the issue (PMID 25754604, 30901484). Nevertheless, we tried to decrease the presentation of both percentages and fractions throughout the manuscript.

4) The authors found that the risk of CMA abnormalities was higher with CVS, though that difference was not significant (p=.06) They fail to address the pregnancy losses that may have occurred between the time the two procedures are performed. How many miscarriages occurred after the NT but before amniocentesis? Were there any possible procedure-related losses?
Unfortunately, and as we have mentioned in the Limitations paragraph, "the main drawback is lack of data regarding further pregnancy follow-up, such as second trimester anatomical survey or a possible subsequent fetal demise". Our data were acquired from Ministry of Health database, comprised only of CMA tests performed due to various sonographic anomalies, with no further data.

5) Were there changes in the CMA platforms used during the 5 year study timespan, such as improved sensitivity? How do the authors address the constantly changing categorization of VOUS, since rapid advances in mapping VOUS to specific genes tends to reduce those still of unknown significance over time? How might that have influenced their results?
No major changes were executed in CMA platforms over the five years. Indeed, categorization of VOUS findings could have changed over the years. Therefore, all CMA findings in the current study as well as in the control cohort (including VOUS variants) were

re-evaluated by the same author (Dr. Idit Maya). Thus, the change in categorization of VOUS findings would be expected to be performed in both cohorts, and thus is not expected to influence the results.

6) What is the true denominator in this study? That is how many women did not have invasive testing? Could there have been other factors that led these particular women to choose invasive testing? For the NT patients, what did their serum screening show and how did that inform their decisions to undergo diagnostic testing or not?
As our study was based on data reported to the Ministry of Health of all Israeli pregnancies undergoing invasive testing due to sonographic anomalies, we did not have the data regarding women eligible for invasive testing but electing not to perform it. Thus, we have no data regarding the characteristics of these two cohorts, as well as factors affecting the women's decisions to undergo invasive testing.
This point was added to the limitations paragraph.

7) The authors conclude that the risk of CMA abnormality is increased for NT 3.0-3.4 mm. The data in Table 2 only support that to be true for 3.1-3.4 mm, since the 95% CI for 3.0 mm crosses unity.
Following thorough discussion with the co-authors, the following was added to the Discussion section:
It must be noted that in the subgroup of 198 pregnancies with NT of 3 mm the risk for abnormal CMA results was not significantly increased. However, as it is possible that this group also included fetuses with slightly lower NT, which was rounded to 3 mm to receive eligibility for invasive testing, we believe the 3 mm cutoff should still be included in the recommended cutoff.

8) Finally, we know that CMA has a higher yield than cfDNA for all women regardless of maternal age, NT or any other risk factors. Can the authors make a convincing argument that this is true for this NT cohort more than any other group? That will be a powerful argument, but will require more thorough statistical comparison than presented here.
We have examined this fascinating point, and have added the following to the Results:
NIPS aimed at the five common aneuploidies could have theoretically detected 17 cases (58.6%), ranging between 33.3% to 75% by each tenth of millimeter (Table 2). a significantly higher rate compared to the 4/21 (0.15%) NIPS-detectable findings in the control population (p=0.0085).
And to the Discussion:

It was interesting to note that detection rate of NIPS aimed at five common aneuploidies in fetuses with NT 3-3.4 mm was significantly higher than in low-risk pregnancies, probably reflecting the known association of increased NT with trisomy 21.

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:

This study examines the perplexing question of "should a patient with mildly elevated NT have invasive testing for CMA or is just NIPS adequate?" The study group is compared to another population who underwent invasive testing for a variety of reasons unrelated to NT.

Unfortunately, outcome data is unavailable. Ideally, the complication rate of invasive testing should be addressed, even if it is in terms of literature review. It would be quite interesting to have the outcomes of patients with thickened NT who did not have invasive testing versus those who did have invasive testing.

Indeed truly unfortunately, and as we have mentioned in the Limitations paragraph, "the main drawback is lack of data regarding further pregnancy follow-up, such as second trimester anatomical survey or a possible subsequent fetal demise".

Our data were acquired from Ministry of Health database, comprised only of CMA tests performed due to various sonographic anomalies, with no further data. Due to this method of data acquisition, we cannot estimate the outcomes of patients with thickened NT who did not have invasive testing and compare them to those who did. Likewise, we do not have data regarding complications rate of invasive testing.

The discussion at least deserves some commentary about the potential risks of CVS and amniocentesis.

The following was added to the Discussion section:

Thus, previous studies do not offer clear evidence that NIPS with subsequent sonographic survey can adequately replace NT measurement with CMA in abnormal cases. Indeed, CVS and amniocentesis are associated with increased risk for miscarriage; however, according to recent studies, this risk is estimated to be negligible (26581188, 31124209), especially in population at an increased risk for trisomy 21 (30120476).

Lichtenberg's study is cited and they appeared to conclude that it was a very small number of patients that the NT testing would have detected but the NIPS and u/s would have missed. However, they appear to not have done invasive testing on patients with normal NTs, so it is unclear if the detected abnormalities, like a triple X, could have been present in a normal NT group so it seems inappropriate to conclude that the NT was valuable.

Holzer's review is quite sobering since it seemed like they only had 7 livebirths without ultrasound abnormalities out of 247 with thick NT. Here again, outcome data is extremely helpful.

This point was added to the Discussion (to paragraph discussing the limitations of previous studies exploring this subject, "no comparison to pregnancies with normal ultrasound was done").

The summary of the literature, acknowledging that some centers use a different NT cut-off compared to others, was quite excellent. The table that quantifies each mm increase of NT and its associations was also quite helpful.

We thank the reviewer for the kind words about our paper and wish to express our gratitude for the very careful and detailed critique of our submission.

Reviewer 3:

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:

Abstract

The objective of our study was to examine the risk for clinically significant Chromosomal microarray findings in fetuses with NT between 3-3.4 mm.

Intro

The objective of our study were to examine in a large cohort the risk for clinically significant CMA results in pregnancies with isolated NT of 3-3.4 mm, and to define the yield of NIPS in such pregnancies IS there a primary and secondary objective? These objectives should completely align.

Our primary objective was to evaluate the risk for clinically significant CMA results in pregnancies with isolated NT of 3-3.4 mm.

Secondary objective was to define the yield of NIPS in such pregnancies.

This was corrected in Abstract and in the Introduction.

This is a retrospective look of all CMA tests performed due to an NT between 3 to 3.4 mm without additional sonographic anomaly information were retrospectively retrieved from the Israeli Ministry of Health computerized database between January 2013 and September 2018. This is simply a look in time without any follow-up of sonography or postnatal outcomes. Introduction is too long, I would transition your discussion of findings in other studies to the discussion section and spend more time talking about microarray and its possibilities for the general OBGYN reader

The following was added as an opening paragraph of the introduction:

Chromosomal microarray analysis (CMA) is currently the recommended method of genetic testing in prenatal diagnosis (1). In pregnancies with structural sonographic anomalies, CMA detection rate of clinically relevant deletions or duplications is 6% higher compared to traditional karyotyping (2). In pregnancies with normal ultrasound this test can detect clinically significant findings in about 1% (3, 4).

Description of findings in other studies in the Introduction was shortened and partially removed to the Discussion section.

Current SMFM recommendation for an increased NT >3.0-3.5 is a karyotype and if normal a fetal echo. How many of the CNVs were associated with heart defects?

As the reviewer has righteously noted, our study reflects a look in time without any follow-up of sonography or postnatal outcomes. As fetal echocardiography is performed in Israel at 22-24 weeks of gestational age, we did not have the results of this test.

I appreciate the OMIN tables but the non genetic OBGYN (the vast majority) will need a bit more explanation about the different findings in the Tables especially those that contain 132 genes - 16 OMIM, 9 more explanation please

The following was added to the Methods section:

Using the American College of Medical Genetics (ACMG) metric, copy number variants scoring 0.99 points or higher are defined as "pathogenic", and between 0.90 and 0.98 points as "likely pathogenic" (18). Several parameters significantly affect the pathogenicity score; for instance, partial or whole inclusion of over 35 protein-coding genes in the copy-number loss imparts the variant a score of 0.9. Most cytogenetically visible alterations (>3–5 Mb) are usually defined as pathogenic (19).

And to the Table 1 footnote:

OMIM - Online Mendelian Inheritance in Man, a continuously updated catalog of human genes and genetic disorders and traits. OMIM-morbid gene – gene associated with clinical disorders.

The risk for abnormal CMA findings was twice higher in pregnancies undergoing CVS (13/179, 7.3%) compared to amniocentesis (16/440 = 3.6%), approaching level of statistical significance (p=0.060). Why do you think this is?? Thoughts?

As routine pregnancy management in Israel includes 14-16 weeks anatomic sonograohic survey (described in Methods), we believe this explains the marginally increased rate of abnormal CMA results in pregnancies undergoing CVS (in which later ultrasound survey could detect additional sonographic anomalies).

The following was added to the Discussion section:

…. This might also explain the marginally increased rate of abnormal CMA results in pregnancies undergoing CVS in our study (in which later ultrasound survey could detect additional sonographic anomalies).

Statistical editor comments:

We are grateful to the Statistical Editor for the valuable in-depth analysis of our paper and for the important comments and suggestions for enhancing it. We addressed each of them as follows:

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

Table 2: When the cohort is divided by increments of 0.1 mm, there were from 3 to 8 in each stratum. Need to provide CIs for each incidence estimate. That is, although the RRs are statistically significantly different from the referent (except for 3 mm group), the absolute proportion with abnormalities is small.

We have added the CIs for each incidence estimate (Table 2).

Note also that among the control group, 1.4% or ~ 78 out of 5541 were abnormal. Thus, the absolute count of abnormals was >2.5x as many as among all of those with thickness in the 3-3.5 mm group.

Actually, the control population was changed doe to reviewer's comments (trying to match for the main confounder of maternal age). Our original control cohort of pregnancies with normal ultrasound included women with advanced maternal age, abnormal screening for Down syndrome and soft markers. Mean maternal age in this group was 36.0 ± 3.9 years, significantly higher than the age in the study group of pregnancies with increased NT ($p<0.0001$). Thus, we have changed the control cohort to a subgroup of the original controls – 2752 pregnancies with normal ultrasound, in which invasive testing was performed due to maternal request. In this group, 21 (0.76%) clinically significant CMA findings were noted, and the mean maternal age was 31.4±2.1 years, not significantly differing from the study group.

The following was added to the Methods section:

The relative risk (RR) for clinically significant CMA results in pregnancies with increased NT was calculated, compared to a historical local control population of 2752 low-risk pregnancies (i.e. under the age of 35 years with normal ultrasound), undergoing CMA due to maternal request (13). Mean maternal age in this control population was 31.4±2.1 years. In this group, CMA testing yielded 21 clinically significant results (0.76%), 4 of these NIPS-detectable.

And to the Results section:

Mean maternal age was 31.6±4.9 years, not significantly different from the control population ($p= 0.1121$).

The calculations of the relative risk were changed accordingly.

As compared to the referent of 1.4% among 5541, the 3 mm thickness cohort is not statistically different from that referent. Therefore, the statement that a threshold of 3 mm should be used as a basis for further testing is undermined by the Authors' own data analysis.
This is an important point, and the following was added to the Discussion section:
It must be noted that in the subgroup of 198 pregnancies with NT of 3 mm the risk for abnormal CMA results was not significantly increased. However, as it is possible that this group also included fetuses with slightly lower NT, which was rounded to 3 mm to receive eligibility for invasive testing, we believe the 3 mm cutoff should still be included in the recommended cutoff.

lines 51-53: These rates are based on small counts and the differences are only nominal, ie, there is no statistically significant difference. The samples also lack sufficient power to generalize the NS difference. For example, using Fisher's test, the difference has p = 0.11. Put another way, the rate (1:73 has CI 1:34 to 1:200), while the rate (1:30 has CI 1:14 to 1:81).
The data were presented to demonstrate the expected rates of "missing out" abnormal results in NIPS, and we did not intend to perform statistical differences. We have added the CIs to all rates to further emphasize the small counts of the samples.

EDITOR COMMENTS:

We appreciate the valuable comments of the Editor and made the following additions and changes accordingly.

1. Please acknowledge in manuscript that at 3.0 mm, no increased risk.

The following was added to the Discussion section:

It must be noted that in the subgroup of 198 pregnancies with NT of 3 mm the risk for abnormal CMA results was not significantly increased. However, as it is possible that this group also included fetuses with slightly lower NT, which was rounded to 3 mm to receive eligibility for invasive testing, we believe the 3 mm cutoff should still be included in the recommended cutoff.

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This will be performed following the resubmission process.

4. In order for an administrative database study to be considered for publication in Obstetrics & Gynecology, the database used must be shown to be reliable and validated. In your response, please tell us who entered the data and how the accuracy of the database was validated. This same information should be included in the Materials and Methods section of the manuscript.

The following was added to the Methods section:

Data collection for this study was performed by retrospective search of the Ministry of Health computerized database (data entered by head of Community Genetics, Public Health Services).
Each CNV classification was reviewed by one author (IM) and reclassified as needed.

5. Your submission indicates that one or more of the authors is employed by a pharmaceutical company, device company, or other commercial entity. This must be included as a statement in the Financial Disclosure section on the title page.
Actually, none of the authors is employed by a pharmaceutical company, device company, or other commercial entity. If the Editors could point out the specific author, we will readily explain the affiliation.

6. Please submit a completed STROBE checklist.
In your cover letter, be sure to indicate that you have followed the CONSORT, MOOSE, PRISMA, PRISMA for harms, STARD, STROBE, RECORD, CHEERS, SQUIRE 2.0, or CHERRIES guidelines, as appropriate.
A complete STROBE checklist was added.

7. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

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The phrase "we describe the largest cohort" was changed to "the one of the largest cohorts".

16. Please review the journal's Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online here: http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.

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