

OBSTETRICS & GYNECOLOGY



NOTICE: This document contains correspondence generated during peer review and subsequent revisions but before transmittal to production for composition and copyediting:

- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*
- Email correspondence between the editorial office and the authors*

**The corresponding author has opted to make this information publicly available.*

Personal or nonessential information may be redacted at the editor's discretion.

Questions about these materials may be directed to the *Obstetrics & Gynecology* editorial office:

obgyn@greenjournal.org.

Date: Dec 20, 2018
To: "Aaron Lazowitz" [REDACTED]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-18-2235

RE: Manuscript Number ONG-18-2235

Impact of genetic variants on steady-state etonogestrel concentrations among contraceptive implant users

Dear Dr. Lazowitz:

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 21 days from the date of this letter. If we have not heard from you by Jan 10, 2019, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

This is a prospective study using a candidate gene approach with known associated variants for hormonal metabolism and association with etonogestrel steady state levels in an implant cohort. This is a very pertinent and timely study related to pharmacogenetics and personalized medicine. The lack of data on one of the most commonly used medications, and related hormonal contraception, is over due. The meticulous study design and inclusion/exclusion criteria were well described and focused on the primary outcome of etonogestrel concentration levels with clinical correlation to possible efficacy and future research.

Abstract:

Line 33-34 The objective and technique of candidate gene approach is not clear to the general obstetrician and gynecologist. Although this is described in the introduction I would suggest a more generic objective stating enzymatic genetic variants and association with ENG levels.

Line 41-42 I would suggest deleting the demographics from the abstract to make it shorter. This is described in the results section and discussion. As stated without context it does not add to the abstract.

Line 51-52 The comment on possible efficacy for low dose OCP is not consistent with the rest of the results or objective. This should be left to the discussion section as noted in the manuscript.

Introduction:

Line 58-69 This is an excellent review of the topic of pharmacogenomics and the reason for this study. Further discussion of the process and review of the 35 drugs listed would help the reader understand the approach using candidate gene approach vs. genome wide association studies.

Line 72 There should be further discussion of therapeutic ranges for different hormonal contraceptives to put the 12 fold variation in context of efficacy. This is also relevant to generic medications.

Materials and methods:

Line 97 Why was 45 chosen as the inclusion age range? Later in the manuscript there is discussion of age and the impact on metabolism and hormone levels.

Line 101 What percent of participants had confirmed duration of use by medical records? This may be a significant confounder as stated in the recruitment of users between 1-3 years post insertion.

Line 102-104 There should be further description of the exact medications and confirmation that could impact the CYP3A4 enzymes along with more information on other confounders like Et-oh use.

Line 106 Why were low BMI patients excluded? There is also a known association with etonorgestrel levels with high BMI which was not excluded.

Line 131 The previous paragraph stated there were 14 gens not 13.

Line 135 Why were variants < 3% excluded? This may be important information to include for possible future association.

Line 153 Hardy-Weinberg equilibrium should be described in the context of principles associated with random selection and genetic frequency remaining stable.

Line 159 The technique of linear regression using a forward step-wise approach should be explained more. I am not familiar with the method but general criticisms reviewed have been related to data mining and need to verify the model on an independent data set such as as in the predicted residual error sum of squares (PRESS) statistic for cross validation. Was this done?

Line 161 Why was a 50% difference chosen for the power? Was this based upon avg. concentration levels and efficacy noted at an FDA level of 90? Explain.

Results:

Table 1 More specifics on demographics should be included specifically related to the enzymatic gene variants such as medications, Et-oh, and confirmation by medical records.

Figure 2 The figure with legend does not stand on it's own. The use of * and o in the upper quartiles are not explained.

Line 182 Explain what is meant by average call rate of 95.1% along with quality score cut offs.

Table 3

The ranges in addition to the median serum concentration should be listed.

Figure 3

The comparison of CYP3A7*1c to wild type shows significant difference in distribution with overlap across clinically relevant level of 90.

Discussion:

Line 224 although there was a 23% reduction in levels of etonogestrel to wild type the distribution and mean in the wild type is significantly greater. Looking at figure 3 it looks like the difference in medians is 19% not 23%. This needs to be clarified why the mean is used instead of median.

Lines 237 What percent of wild type from figure 3 fell below the threshold of 90 pg/ml? This needs to be stated as well. Also discuss FDA and ACOG Committee Opinion 375 related to The average blood level deviation from the brand vs generic must be in the range of 80-125%.

Overall the limitations not previously mentioned were acknowledged along with suggest future studies.

Reviewer #2: The authors present work that they expect to lay the foundation for pharmacogenomics studies that may eventually dramatically alter the field of women's health, given the breadth of indications for steroid hormones throughout a woman's life-span.

Major suggestions for improving the presentation of this work are:

1. identifying the potential clinical relevance of this work in settings which are not routinely genotyping patients. Perhaps having a genetic predisposition to lower hormone levels should be included on the differential diagnosis of reasons why women may experience an undesired pregnancy despite reporting perfect adherence to a contraceptive pill or ring? Currently, most clinicians assume such women are just over-reporting their medication adherence, which may adversely affect clinician-patient relationships
2. clarifying in the abstract the hormone level that has been identified as the threshold for implant effectiveness and whether any subjects were found to have levels below this threshold (eg the data presented on lines 236-238 perhaps belongs in the abstract)

More minor issues:

1. Line 36- has an extra "and"
2. Line 41-"most" is not the best word for 41%
3. Line 90-missing word?

Reviewer #3: The primary objective of this manuscript was to identify genetic variants that influence steady-state ENG concentrations among ENG implant users, 12-36 months after implant insertion. I found this to be a very novel and important study and had only a couple of minor comments.

- 1) Results: I was confused by Lines 208-9 on page 12: "Both genetic variants remained significant with equivocal β -coefficients when self-reported participant race/ethnicity was factored into the regression model." What do you mean by the word "equivocal" here? You say that the variants remained significant; do you mean that their β -coefficients in the expanded model were similar to the β -coefficients in the prior model? Since you don't show the β -coefficients for the variants in the expanded model, and I thought that "equivocal" was synonymous with "uncertain", I couldn't figure out what Lines 208-9 were saying. It would be helpful if you expanded Table 2 to show the β -coefficients for both the final and final expanded models, so that we could easily compare them, and/or added the β -coefficients for the expanded model to the text.
- 2) Results, Lines 209-211: I think it would be also helpful to add the word "decreased" in front of the words "serum ENG concentrations" so that the reader doesn't have to think too much about the β -coefficients to determine directionality of the association. Not all readers of this journal will be familiar with β -coefficients and how to interpret them. On a related note, I think you should also specify directionality of the associations with the variations, duration of implant use, and BMI in the previous sentences as you currently have to look at the β -coefficients in Table 2 to find that information.

STATISTICAL EDITOR COMMENTS:

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

line 47: An R^2 for the model may be statistically significant, but may not have any clinically useful predictive ability

lines 166-169: This statistical analysis would apply had the hypothesis been limited to one comparison, but there were 120-19, or 101 SNPs tested in 14 genes. Therefore using an inference threshold of $< .05$ is too high and likely to include apparent associations that were spurious, due to multiple hypothesis testing. Apart from the multiple SNPs, simply testing 14 candidate genes would have required an inference threshold of .0035. Therefor both the NR1I2(PXR) and CYP3A7*1C do not achieve the needed threshold.

On the other hand, months of implant use and BMI do appear to have significant negative associations with etonogestrel.

From the figures, it appears that serum ENG levels have significant skewing to higher values. Perhaps a transformation of the data (?log) would result in a more normal distribution and better overall model fitting.

EDITOR COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. In addition to the comments from the reviewers above, you are being sent a notated PDF that contains the Editor's specific comments. Please review and consider the comments in this file prior to submitting your revised manuscript. These comments should be included in your point-by-point response cover letter.

The notated PDF is uploaded to this submission's record in Editorial Manager. If you cannot locate the file, contact Randi Zung and she will send it by email - rzung@greenjournal.org.

- In general, we avoid the word "impact" as it implies a force or blow. Quirky green journal thing. Could you substitute "association of" or "Influence of"?

- Slightly longer precis than it should be. its efficacy in women with this genotype, not the genotype itself, isn't

it? Could you make the precis less specific? The precis is used in the TOC to draw readers in to paper--for the average clinician, this precis is likely not appealing as the reference to the gene name at the beginning may make them flip past it. Could you say something more general like "Some genetic variants such as those influencing cytochrome P450 function may decrease efficacy of steroid-hormone contraception"

- Please consult the Instructions for Authors regarding the use of abbreviations, and what constitutes an acceptable abbreviation. This is not an acceptable abbreviation. Please spell the words out throughout the manuscript. On line 36, you use a virgule (/). The Journal style doesn't use the virgule (/) except in numeric expressions. Please edit here and in all instances

- how were these women selected?

- please read instructions for authors and follow guidelines for organization of your paper. For instance, we don't use 1.0 type of headings.

- did you measure height and weight or go by patient report?

- why did you select 50%?

- where were these women recruited from? GYN clinics? Community advertising?

2. Please pay special attention to the Statistical Editor's concerns.

Given the number of hypotheses you are testing (the panel of candidate genes, the histories and demographics of the women) you need to use a stricter threshold to determine significance. Unfortunately, it seems that by so doing, only BMI and duration of time with the implant will end up being significant and that the presence or absence of a mutation will not.

Specific note from Editor-in-Chief:

As it appears that there is a serum threshold of etonogestrel below which the efficacy of the implant declines.

Please include the following:

1. Of the 17 women with the mutation that is associated with lower steroid hormone levels, what % had levels below the effective level. This would need to be in the abstract.

2. What percent of women in each group were below this level? How were BMI, time with the implant associated with this?

While we are very interested in personalized medicine as a topic, our assessment of the results of your paper with the proper statistical analysis is that the genetic mutation presence is not going to be a very good predictor of lack of efficacy. If so, this could by itself be a very important thing to report and a decision about acceptance or rejection of your paper does not hinge on whether or not they are left being significant but these analysis are required for us to proceed with the paper further.

3. 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, as well as subsequent author queries. 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:

1. OPT-IN: Yes, please publish my response letter and subsequent email correspondence related to author queries.
2. OPT-OUT: No, please do not publish my response letter and subsequent email correspondence related to author queries.

4. As of December 17, 2018, Obstetrics & Gynecology has implemented an "electronic Copyright Transfer Agreement" (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Any author agreement forms previously submitted will be superseded by the eCTA. During the resubmission process, you are welcome to remove these PDFs from EM. However, if you prefer, we can remove them for you after submission.

5. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained."

*The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please

ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission in Editorial Manager.

6. 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), 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, 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 and gynecology data definitions at <https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize>. 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 26 typed, double-spaced pages (6,500 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 limits for different article types are as follows: Original Research articles, 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. 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.

15. Figures

Figure 1: The n values in the first exclusion box total 32. Are items not mutually exclusive?

Figures 2-4: May be resubmitted as-is.

16. Authors whose manuscripts have been accepted for publication have the option to pay an article processing charge and publish open access. With this choice, articles are made freely available online immediately upon publication. An information sheet is available at <http://links.lww.com/LWW-ES/A48>. The cost for publishing an article as open access can be found at <http://edmgr.ovid.com/acd/accounts/ifaauth.htm>.

Please note that if your article is accepted, you will receive an email from the editorial office asking you to choose a publication route (traditional or open access). Please keep an eye out for that future email and be sure to respond to it promptly.

17. If you choose to revise your manuscript, please submit your revision via Editorial Manager for Obstetrics & Gynecology at <http://ong.editorialmanager.com>. It is essential that your cover letter list point-by-point the changes made in response to each criticism. Also, please save and submit your manuscript in a word processing format such as Microsoft Word.

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

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 10, 2019, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2017 IMPACT FACTOR: 4.982
2017 IMPACT FACTOR RANKING: 5th out of 82 ob/gyn journals

In compliance with data protection regulations, please contact the publication office if you would like to have your personal information removed from the database.



January 8th, 2019

Dear Editors and reviewers,

Thank you for your review and consideration of our original research article “Influence of genetic variants on steady-state etonogestrel concentrations among contraceptive implant users.” We are excited to resubmit the edited manuscript for publication in *Obstetrics & Gynecology*.

We greatly appreciate the Editor’s and reviewers’ time and the valuable feedback provided. We have edited the manuscript based on these comments and have included our responses to these comments in this cover letter. Our responses are bolded below.

Of note, this manuscript contains a reference to unpublished data that was presented at a scientific meeting and is currently under review for publication (Lines 183-184). We will add a citation to this manuscript as soon as the data has been accepted for publication.

This manuscript is not under consideration elsewhere and will not be submitted elsewhere until a final decision is made by the editors of *Obstetrics & Gynecology*. **The lead author [Dr. Aaron Lazorwitz] affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.** This study was approved by the Colorado Multiple Institutional Review Board and all participants gave written informed consent at enrollment. This study is registered on clinicaltrials.gov (ID: NCT03092037).

Dr. Teal has served on scientific advisory boards of Allergan and Bayer Healthcare, and serves on a Data Monitoring Board for a study funded by Merck and Co. Dr. Teal and Dr. Lazorwitz receive research funding from Merck and Co. for an Investigator Initiated Study on drug-drug interactions with the etonogestrel contraceptive implant. The University of Colorado Department of Obstetrics and Gynecology has received research funding from Bayer, Agile Therapeutics, Merck and Co, and Medicines360. Dr. Guiahi’s time was supported by the Society of Family Planning Junior Investigator Career Grant SFPRF10-JI1. The authors have no other conflicts of interest to disclose.

I appreciate the opportunity to resubmit our manuscript and look forward to your response. All authors have fulfilled the requirements for authorship and confirmed submission.

Thank you,

A handwritten signature in black ink, appearing to read 'Aaron Lazorwitz', written in a cursive style.

Aaron Lazorwitz, MD, MSCS
Principal Investigator

REVIEWER COMMENTS and **Author responses:**

Reviewer #1:

This is a prospective study using a candidate gene approach with known associated variants for hormonal metabolism and association with etonogestrel steady state levels in an implant cohort. This is a very pertinent and timely study related to pharmacogenetics and personalized medicine. The lack of data on one of the most commonly used medications, and related hormonal contraception, is over due. The meticulous study design and inclusion/exclusion criteria were well described and focused on the primary outcome of etonogestrel concentration levels with clinical correlation to possible efficacy and future research.

Thank you for your review and comments.

Abstract:

Line 33-34 The objective and technique of candidate gene approach is not clear to the general obstetrician and gynecologist. Although this is described in the introduction I would suggest a more generic objective stating enzymatic genetic variants and association with ENG levels.

We have removed “candidate gene approach” from the Objectives to help simplify this per your recommendation. (Lines 35-36)

Line 41-42 I would suggest deleting the demographics from the abstract to make it shorter. This is described in the results section and discussion. As stated without context it does not add to the abstract.

We have removed the demographics from the abstract. We have combined the first two sentences of the Results section of the abstract. (Lines 42-43)

Line 51-52 The comment on possible efficacy for low dose OCP is not consistent with the rest of the results or objective. This should be left to the discussion section as noted in the manuscript.

Thank you for your suggestion. We have removed the comment regarding possible efficacy for low dose OCP and replaced it with a more generic comment on how CYP3A7 proteins could be involved in the metabolism of all steroid hormones. (Lines 53-55)

These lines state: “Women with this variant may potentially have increased metabolism of all steroid hormones, as 27.8% (5/18) of CYP3A7*1C carriers had serum etonogestrel concentrations that fell below the threshold for consistent ovulatory suppression (<90pg/mL).”

Introduction:

Line 58-69 This is an excellent review of the topic of pharmacogenomics and the reason for this study. Further discussion of the process and review of the 35 drugs listed would help the reader understand the approach using candidate gene approach vs. genome wide association studies.

Thank you for your comment. We have added two lines to this paragraph of the introduction to help readers understand the candidate gene approach vs genome wide association studies and how these studies are used to develop CPIC clinical guidelines. (Lines 69-73)

These lines state: “Drug-gene research often consists of both candidate gene approaches, selecting specific genetic variants to study based on physiologic plausibility, and genome wide association studies, searching for novel associations across the whole genome. If well-

designed, both of these approaches can provide high quality evidence for the development of clinical guidelines (6)."

Line 72 There should be further discussion of therapeutic ranges for different hormonal contraceptives to put the 12 fold variation in context of efficacy. This is also relevant to generic medications.

We have added a sentence to explain the concept of bioequivalence commonly used for generic medications to help put the 12 -fold variation into better context (Lines 79-82)

These lines state: "This wide inter-individual variability is often beyond the accepted criteria for bioequivalence (95% confidence interval of the mean parameter values within 80-125% of the accepted standard), which may put some women outside the therapeutic range of their hormonal contraceptive method (11, 12)."

Materials and methods:

Line 97 Why was 45 chosen as the inclusion age range? Later in the manuscript there is discussion of age and the impact on metabolism and hormone levels.

We chose 45 as the maximum age for inclusion based on the both the age group that utilizes hormonal contraception (excluding minors) and the desire to avoid potential altered metabolism due to aging effects that may be found in women older than 45 years. We have included a short line in the methods to introduce this potential effect of age. (Lines 113-114) This line states: "We excluded women over the age of 45 years due to potential altered drug metabolism from aging effects (18)."

Line 101 What percent of participants had confirmed duration of use by medical records? This may be a significant confounder as stated in the recruitment of users between 1-3 years post insertion.

Approximately 75% of participants had confirmed duration of use by medical records. We unfortunately did not record which participants had confirmed duration of use by medical records or self-report at the time of enrollment. We did not include this percentage as it is an approximation and we are unable to provide an exact percentage at this time.

Line 102-104 There should be further description of the exact medications and confirmation that could impact the CYP3A4 enzymes along with more information on other confounders like Et-oh use.

We have added a line to explain how medication history was collected and the exact screening process we used to look for CYP3A4 inducers or inhibitors. (Lines 116-118) We have included a citation for the list of medications as it is quite extensive.

These new lines state: "We reviewed concomitant medications through both participant report and medical record review and screened for any medications included in the U.S. Food and Drug Administration list of known CYP3A4 inducers or inhibitors (19)."

We did not screen for alcohol use as we are not aware of an effect of alcohol and CYP3A4, or between alcohol and CYP enzymes known to be involved in steroid hormone metabolism. Chen & Anderson (Clin Pharmacokinet 2014) describe an interaction between alcohol use and CYP2E1 function, but this specific CYP is not known to be involved in steroid hormone metabolism.

Line 106 Why were low BMI patients excluded? There is also a known association with etonogestrel levels with high BMI which was not excluded.

Low BMI has been suggested to broadly alter drug metabolism, and therefore, we excluded women who met the CDC definition for underweight (BMI <18.5kg/m²). This explanation is included in the Methods within lines 119-122: “As low body mass index (BMI) has been associated with abnormal etonogestrel metabolism, we measured a height and weight for each participant and excluded women with a measured BMI less than 18.5 kg/m².”

The association between etonogestrel levels and high BMI is actually not conclusive based on the available literature. The two largest available studies on the association between high BMI and etonogestrel levels (McNicholas et al, AJOG 2017 and Morrell et al, Contraception 2016) actually disagree on the direction of the association between high BMI and etonogestrel levels. We, therefore, decided to not have an upper BMI cut-off and instead to factor BMI into our analysis.

We have added a sentence to the Methods to explain why women with high BMIs were not excluded (Lines 122-124):

“We did not exclude women with high BMIs, as the present literature is inconclusive about the existence of an association between high BMI and serum etonogestrel concentrations (9, 20).”

Line 131 The previous paragraph stated there were 14 gens not 13.

We apologize for this error. This line has been corrected to state 14 genes. (Line 148)

Line 135 Why were variants < 3% excluded? This may be important information to include for possible future association.

We excluded variants with prevalence <3% as we would not enough power to adequately capture these variants in our sample size of 350. We have included this information in the manuscript to help explain this cut-off. (Lines 151-154):

“We excluded any genetic variants with a frequency of less than 3% in either Caucasian or African populations, the two predominant racial groups in our local population, due to inadequate power to find associations with these rare variants.”

Line 153 Hardy-Weinberg equilibrium should be described in the context of principles associated with random selection and genetic frequency remaining stable.

We have added a definition of Hardy-Weinberg equilibrium to explain the principle in the context you have described. (Lines 174-176)

This line states: “...Hardy-Weinberg equilibrium, which is based on the principle that genetic frequencies will remain constant across generations when solely inherited by random selection and barring outside influences.”

Line 159 The technique of linear regression using a forward step-wise approach should be explained more. I am not familiar with the method but general criticisms reviewed have been related to data mining and need to verify the model on an independent data set such as as in the predicted residual error sum of squares (PRESS) statistic for cross validation. Was this done?

Thank you for your comment and suggestion. Based on your feedback and the Statistical Reviewer’s suggestion, we have changed to a generalized linear model approach using a

backward step-wise methodology. We chose a generalized linear model as this is better suited for a dependent variable that is not normally distributed. We also decided on a backward step-wise approach due to the concerns regarding the forward approach you highlighted. The backward step-wise approach places all variables into the model first and then we using Akaike's Information Criterion to find the best fit model. As Akaike's Information Criterion penalizes models for including more variables, this approach avoids 'data mining' and is best suited to our analytic approach.

We have added explanations for this revised analytic approach in Lines 178-190:

"Given that serum etonogestrel concentrations do not follow a normal distribution (9), we used a generalized linear model, which allows for multivariable linear regression analysis regardless of the distribution of the dependent variable (24). In this generalized linear model, we included all genetic variants found to be significantly associated with serum etonogestrel concentrations in simple linear modeling, and also included the variables of months of implant use and BMI based on our prior findings (Lazorwitz et al, work presented at the 2018 North American Forum on Family Planning). We utilized a backward-stepwise approach to create our final linear model where all variables were entered into the model initially. We then sequentially removed variables without significant associations ($p < 0.05$) until we obtained a model with the minimal Akaike's Information Criterion value (25). Given the multiple hypothesis testing performed using simple linear regression, we determined a Bonferroni corrected p-value based on the number of simple linear regression analyses performed. We used this corrected p-value to determine overall significance for the variables included in our final generalized linear model."

Line 161 Why was a 50% difference chosen for the power? Was this based upon avg. concentration levels and efficacy noted at an FDA level of 90? Explain.

We chose a 50% difference as this would cause mean serum ENG concentrations to fall below 90pg/mL. We have added a line to clarify this reasoning. (Lines 193-195)

"We selected a 50% difference as this would cause the mean serum etonogestrel concentration to fall below 90pg/mL, which is the manufacturer's reported threshold for maintain ovulatory suppression with the implant (13)."

Results:

Table 1 More specifics on demographics should be included specifically related to the enzymatic gene variants such as medications, Et-oh, and confirmation by medical records.

We are unclear as to what you mean by "medications" here. We did exclude any women taking CYP3A4 inducers or inhibitors, which included 3 women as described in the Study Flow Diagram (Figure 1). We did not collect any information regarding alcohol use per the reasoning outlined above.

Figure 2 The figure with legend does not stand on it's own. The use of * and o in the upper quartiles are not explained.

We have added an explanation to the Legend for Figure 2 to explain the difference between the * and ° markings in the figure. The ° denotes outliers with values between 1.5 and 3 times the interquartile range and the * denotes outliers with values greater than 3 times the interquartile range.

Line 182 Explain what is meant by average call rate of 95.1% along with quality score cut offs.

The call rate refers to the percentage of participants for each single nucleotide polymorphism that had genotyping results of high enough quality to allow us to confidently say whether that participant had that variant or not. We have edited this line to remove the “call rate” terminology and have replaced it with a new explanation for the assignment of genotypes and what these values mean (Lines 214-219).

These lines state: “On average, we were able to confidently assign a genotype to 95.1% (range 82.6% to 98.9%) of participants for each single nucleotide polymorphism tested using our selected quality score cut-off of ≥ 0.90 . Thus, for less than 5% of participants on average, we could not determine if a specific single nucleotide polymorphism was present and so these participants were not included in the simple linear regression analysis for that specific single nucleotide polymorphism.”

Table 3

The ranges in addition to the median serum concentration should be listed.

Thank you for your recommendation. We have added the ranges to Table 3 and also corrected an error noted in the reported median for rs2461817: One variant alleles row.

Figure 3

The comparison of CYP3A7*1c to wild type shows significant difference in distribution with overlap across clinically relevant level of 90.

We have added this description of the differences in distributions to the Legend for Figure 3 to help explain this finding. (Figure 3 Legend). This line states: “The distribution of serum etonogestrel concentrations significantly differs between the groups (Mann-Whitney U Test, $p=0.003$).” (Lines 499-501)

Discussion:

Line 224 although there was a 23% reduction in levels of etonogestrel to wild type the distribution and mean in the wild type is significantly greater. Looking at figure 3 it looks like the difference in medians is 19% not 23%. This needs to be clarified why the mean is used instead of median.

This larger value is from the linear model findings, as that analysis takes into consideration the confounding factors of BMI, duration of implant use, and other genetic variants. This line is the interpretation of the β -coefficient from that analysis, which demonstrates the average effect of this genetic variant. We have added a clarification to the end of the sentence to note that this association is referring to the generalized linear model. (Lines 265-267):

“Carriers of CYP3A7*1C had, on average, 23% (35 pg/mL) lower etonogestrel concentrations than participants with the wild-type genotype based upon our generalized linear model findings.”

Lines 237 What percent of wild type from figure 3 fell below the threshold of 90 pg/ml? This needs to be stated as well. Also discuss FDA and ACOG Committee Opinion 375 related to The average blood level deviation from the brand vs generic must be in the range of 80-125%.

We have added the percent of participants with the wild-type genotype who fell below 90pg/mL (9%). We have also included the acceptable range of bioequivalence as discussed in the ACOG Committee Opinion 375 and the fact that the mean difference in serum ENG concentrations from our generalized linear modeling falls outside of this acceptable range. (Lines 282-286)

These lines state: “Among participants with the respective wild-type genotype, only 9%

(30/332) had serum etonogestrel concentrations that similarly fell below 90pg/mL. Furthermore, based on the definition of bioequivalence, the mean serum etonogestrel concentration of CYP3A7*1C carriers falls outside the acceptable range when compared to participants with the wild-type genotype (77% versus acceptable range of 80-125%) (12)."

Overall the limitations not previously mentioned were acknowledged along with suggest future studies.

Thank you for all of your helpful feedback and comments.

Reviewer #2: The authors present work that they expect to lay the foundation for pharmacogenomics studies that may eventually dramatically alter the field of women's health, given the breadth of indications for steroid hormones throughout a woman's life-span.

Major suggestions for improving the presentation of this work are:

1. identifying the potential clinical relevance of this work in settings which are not routinely genotyping patients. Perhaps having a genetic predisposition to lower hormone levels should be included on the differential diagnosis of reasons why women may experience an undesired pregnancy despite reporting perfect adherence to a contraceptive pill or ring? Currently, most clinicians assume such women are just over-reporting their medication adherence, which may adversely affect clinician-patient relationships

Thank you for this suggestion. We have added these clinically relevant implications to our Discussion section with the following lines:

"As more genetic data becomes available, clinicians may need to consider adding genetic predisposition to increased steroid hormone metabolism in their differential diagnosis for unintended pregnancies in women reporting perfect adherence to hormonal contraceptive methods. The assumption that these unintended pregnancies are solely due to medication adherence issues may adversely affect clinician-patient relationships for women with genetic factors that result in lower steroid hormone levels." (Lines 368-373)

2. clarifying in the abstract the hormone level that has been identified as the threshold for implant effectiveness and whether any subjects were found to have levels below this threshold (eg the data presented on lines 236-238 perhaps belongs in the abstract)

Thank you for this suggestion. We have added this information into the abstract in Lines 53-55 which now state: "Women with this variant may potentially have increased metabolism of all steroid hormones, as 27.8% (5/18) of CYP3A7*1C carriers had serum etonogestrel concentrations that fell below the threshold for consistent ovulatory suppression (<90pg/mL)."

More minor issues:

1. Line 36- has an extra "and"

We have deleted the extra "and" from Line 36. This sentence now states:

"We enrolled healthy, reproductive-aged, women using etonogestrel implants for 12-36 months without concomitant use of hepatic enzyme inducers or inhibitors."

2. Line 41-"most" is not the best word for 41%

This text which was included in the demographic information has been removed per Reviewer #1's recommendation. (Lines 42-43)

3. Line 90-missing word?

Thank you for identifying this error. We have added the missing word: "proteins" to line 99.

Reviewer #3: The primary objective of this manuscript was to identify genetic variants that influence steady-state ENG concentrations among ENG implant users, 12-36 months after implant insertion. I found this to be a very novel and important study and had only a couple of minor comments.

1) Results: I was confused by Lines 208-9 on page 12: "Both genetic variants remained significant with equivocal β -coefficients when self-reported participant race/ethnicity was factored into the regression model." What do you mean by the word "equivocal" here? You say that the variants remained significant; do you mean that their β -coefficients in the expanded model were similar to the β -coefficients in the prior model? Since you don't show the β -coefficients for the variants in the expanded model, and I thought that "equivocal" was synonymous with "uncertain", I couldn't figure out what Lines 208-9 were saying. It would be helpful if you expanded Table 2 to show the β -coefficients for both the final and final expanded models, so that we could easily compare them, and/or added the β -coefficients for the expanded model to the text.

We apologize for the confusion regarding these lines. We have replaced "equivocal" with "minimal change" to designate that the β -coefficients for the variants did not change in the expanded model. We have also added the β -coefficients from the expanded model into Table 2 to demonstrate the minimal differences in these values. (Lines 246-248). These lines now read:

"All genetic variants had minimal change in their β -coefficients when self-reported participant race and ethnicity were factored into the linear model (Table 2)."

2) Results, Lines 209-2011: I think it would be also helpful to add the word "decreased" in front of the words "serum ENG concentrations" so that the reader doesn't have to think too much about the β -coefficients to determine directionality of the association. Not all readers of this journal will be familiar with β -coefficients and how to interpret them. On a related note, I think you should also specify directionality of the associations with the variations, duration of implant use, and BMI in the previous sentences as you currently have to look at the β -coefficients in Table 2 to find that information.

Upon rerunning our statistical analysis based on the Statistical Editor's and Reviewer #1's feedback, this finding was not reproduced and was therefore removed (Lines 248-251). We have added a line to the Results to specify the directionality of the associations in our final model (Lines 243-246).

These lines now state: "Only one variable was associated with increased serum etonogestrel concentrations (NR1I2[PXR] rs2461817), whereas CYP3A7*1C carrier status, PGR rs537681 carrier status, longer duration of implant use, and higher BMI were all associated with decreased serum etonogestrel concentrations."

STATISTICAL EDITOR COMMENTS:

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

line 47: An R^2 for the model may be statistically significant, but may not have any clinically useful predictive ability

We included the R^2 in the abstract to demonstrate that our best fit model still only explains a small amount of the variability in serum etonogestrel concentrations.

lines 166-169: This statistical analysis would apply had the hypothesis been limited to one comparison, but there were 120-19, or 101 SNPs tested in 14 genes. Therefore using an inference threshold of $< .05$ is too high and likely to include apparent associations that were spurious, due to multiple hypothesis testing. Apart from the multiple SNPs, simply testing 14 candidate genes would have required an inference threshold of .0035. Therefore both the NR1H2(PXR) and CYP3A7*1C do not achieve the needed threshold.

Thank you for this comment. We have included a Bonferroni corrected p-value and an explanation for the multiple hypothesis testing that was performed as you described (Lines 187-190).

These lines state: “Given the multiple hypothesis testing performed using simple linear regression, we determined a Bonferroni corrected p-value based on the number of simple linear regression analyses performed. We used this corrected p-value to determine overall significance for the variables included in our final generalized linear model.”

We agree that the variants do not achieve statistical significance based on the corrected p-value and have included explanations as such. (Lines 248-251)

These lines state: “To determine overall statistical significance, we used a Bonferroni correction to account for the 100 statistical tests performed: corrected p-value cut-off of 0.0005. Only duration of implant use ($p=0.000058$) and BMI ($p=0.0000007$) remained statistically significant based on this conservative threshold.”

On the other hand, months of implant use and BMI do appear to have significant negative associations with etonogestrel.

Thank you for your assistance with this clarification and we have highlighted these persistent significant negative associations as outlined above.

From the figures, it appears that serum ENG levels have significant skewing to higher values. Perhaps a transformation of the data (?log) would result in a more normal distribution and better overall model fitting.

We decided to utilize a generalized linear model to account for the non-normal distribution of serum ENG concentrations. This has some benefits over a log transformation as discussed by Changyong et al (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4120293/>). We feel that the generalized linear model provides a better overall model without some of the concerns with log transformation.

The description of the generalized linear model and our backward step-wise approach are contained in Lines 178-190.

“Given that serum etonogestrel concentrations do not follow a normal distribution (9), we used a generalized linear model, which allows for multivariable linear regression analysis

regardless of the distribution of the dependent variable (24). In this generalized linear model, we included all genetic variants found to be significantly associated with serum etonogestrel concentrations in simple linear modeling, and also included the variables of months of implant use and BMI based on our prior findings (Lazorwitz et al, work presented at the 2018 North American Forum on Family Planning). We utilized a backward-stepwise approach to create our final linear model where all variables were entered into the model initially. We then sequentially removed variables without significant associations ($p < 0.05$) until we obtained a model with the minimal Akaike's Information Criterion value (25). Given the multiple hypothesis testing performed using simple linear regression, we determined a Bonferroni corrected p-value based on the number of simple linear regression analyses performed. We used this corrected p-value to determine overall significance for the variables included in our final generalized linear model."

EDITOR COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. In addition to the comments from the reviewers above, you are being sent a notated PDF that contains the Editor's specific comments. Please review and consider the comments in this file prior to submitting your revised manuscript. These comments should be included in your point-by-point response cover letter.

The notated PDF is uploaded to this submission's record in Editorial Manager. If you cannot locate the file, contact Randi Zung and she will send it by email - rzung@greenjournal.org.

- In general, we avoid the word "impact" as it implies a force or blow. Quirky green journal thing. Could you substitute "association of" or "Influence of"?

We have substituted "Influence of" in place of "Impact of" – Line 1

We have also removed all other instances of "impact" throughout the manuscript and replaced these instances with suitable alternatives.

- Slightly longer precis than it should be. its efficacy in women with this genotype, not the genotype itself, isn't it? Could you make the precis less specific? The precis is used in the TOC to draw readers in to paper--for the average clinician, this precis is likely not appealing as the reference to the gene name at the beginning may make them flip past it. Could you say something more general like "Some genetic variants such as those influencing cytochrome P450 function may decrease efficacy of steroid-hormone contraception"

Thank you for your suggestion. We have changed the Précis to be more general and appealing to the average clinician. The precis now states: "Some genetic variants, such as those influencing cytochrome P450 enzyme function, can affect steroid hormone drug concentrations and may decrease the efficacy of hormonal contraceptive methods." (Lines 28-31)

- Please consult the Instructions for Authors regarding the use of abbreviations, and what constitutes an acceptable abbreviation. This is not an acceptable abbreviation. Please spell the words out throughout the manuscript. On line 36, you use a virgule (/). The Journal style doesn't not use the virgule (/) except in numeric expressions. Please edit here and in all instances

We apologize for the use of unacceptable abbreviations and virgules. We have removed all instances of the abbreviation "ENG" and "SNP" and replaced these instances with

“etonogestrel” and “single nucleotide polymorphism,” respectively.

We have removed the virgule from line 37 and all other occurrences of virgules except in numeric expressions.

- how were these women selected?

Women were recruited using flyers, online advertising, and through our contraceptive clinics on the Anschutz Medical campus.

We have added this information to the Methods section (Lines 111-112):

“We recruited participants through community advertising and contraceptive clinics at the University of Colorado Anschutz Medical Campus.”

- please read instructions for authors and follow guidelines for organization of your paper. For instance, we don't use 1.0 type of headings.

We apologize for this oversight and have removed the numbering.

- did you measure height and weight or go by patient report?

We measured the height and weight for each participant. We have edited lines 119-122 to better describe this process. These lines now state: “As low body mass index (BMI) has been associated with abnormal etonogestrel metabolism, we measured a height and weight for each participant and excluded women with a measured BMI less than 18.5 kg/m².”

- why did you select 50%?

We selected 50% based on the amount of change in mean serum etonogestrel concentrations to cause the level to fall below 90pg/mL. This explanation has been added in lines 193-195:

“We selected a 50% difference as this would cause the mean serum etonogestrel concentration to fall below 90pg/mL, which is the manufacturer’s reported threshold for maintaining ovulatory suppression with the implant (13).”

- where were these women recruited from? GYN clinics? Community advertising?

Our recruitment was conducted through community advertising and directly through our contraceptive clinics on our medical campus. We have added this information in Lines 111-112 for clarification.

“We recruited participants through community advertising and contraceptive clinics on the University of Colorado Anschutz Medical Campus.”

2. Please pay special attention to the Statistical Editor's concerns.

Given the number of hypotheses you are testing (the panel of candidate genes, the histories and demographics of the women) you need to use a stricter threshold to determine significance. Unfortunately, it seems that by so doing, only BMI and duration of time with the implant will end up being significant and that the presence or absence of a mutation will not.

Thank you for your recommendation and we have included a stricter threshold for statistical significance based on a Bonferroni correction for 100 hypothesis testing procedures (99 simple linear regression and 1 generalized linear model). We have clarified throughout the manuscript that the genetic variants are not statistically significant using this stricter

threshold, but may still have some physiologic plausibility.

Specific note from Editor-in-Chief:

As it appears that there is a serum threshold of etonogestrel below which the efficacy of the implant declines.

Please include the following:

1. Of the 17 women with the mutation that is associated with lower steroid hormone levels, what % had levels below the effective level. This would need to be in the abstract.

We have added this percentage (27.8%) to the Conclusion section of the abstract. (Lines 53-55) These lines state:

“Women with this variant may potentially have increased metabolism of all steroid hormones, as 27.8% (5/18) of CYP3A7*1C carriers had serum etonogestrel concentrations that fell below the threshold for consistent ovulatory suppression (<90pg/mL).”

2. What percent of women in each group were below this level? How were BMI, time with the implant associated with this?

We have included the percentages of women in each group with ENG levels <90pg/mL in the Discussion section of the manuscript. (Lines 279-283)

“For 27.8% (5/18) of CYP3A7*1C carriers, the increased etonogestrel metabolism resulted in etonogestrel concentrations that fell below 90pg/mL, which is the manufacturer’s reported threshold for consistent ovulatory suppression with the implant (13). Among participants with the respective wild-type genotype, only 9% (30/332) had serum etonogestrel concentrations that similarly fell below 90pg/mL.”

And Lines 298-302:

“ In support of the latter, only 11.7% (31/266) of carriers for rs537681 had serum etonogestrel concentrations that fell below 90pg/mL (7.3% in participants with the respective wild-type genotype) and the difference in mean serum etonogestrel concentrations (19.9%) falls within the acceptable range for bioequivalence (12).”

BMI and duration of implant use maintain their significant negative associations with serum etonogestrel concentrations when controlling for the genetic variants in the final model. Though they remain statistically significant, the majority of women will not fall below 90pg/mL solely from these two factors. The average decrease in etonogestrel levels between 12 and 36 months is 37pg/mL based upon our model and each 1kg/m² increase in BMI is associated with a 3.1pg/mL decrease in etonogestrel level. We discussed these associations in-depth in our manuscript currently under review (discussed in the cover letter) and currently cited as a presentation in Lines 183-184 while we await acceptance. We will update this citation as soon as it is available.

While we are very interested in personalized medicine as a topic, our assessment of the results of your paper with the proper statistical analysis is that the genetic mutation presence is not going to be a very good predictor of lack of efficacy. If so, this could by itself be a very important thing to report and a decision about acceptance or rejection of your paper does not hinge on whether or not they are left being significant but these analysis are required for us to proceed with the paper further.

We greatly appreciate your consideration of our manuscript and have incorporated the

statistical analysis feedback into the manuscript. We agree that the genetic variants are no longer statistically significant, and so have focused on the physiologic plausibility behind these variants to support future investigations.

3. 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, as well as subsequent author queries. 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:

1. OPT-IN: Yes, please publish my response letter and subsequent email correspondence related to author queries.
2. OPT-OUT: No, please do not publish my response letter and subsequent email correspondence related to author queries.

OPT-IN

4. As of December 17, 2018, Obstetrics & Gynecology has implemented an “electronic Copyright Transfer Agreement” (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on “Revise Submission.” Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Any author agreement forms previously submitted will be superseded by the eCTA. During the resubmission process, you are welcome to remove these PDFs from EM. However, if you prefer, we can remove them for you after submission.

We will remove the author agreement forms from the submission and complete the eCTAs.

5. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript’s lead author. The statement is as follows: “The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.” *The manuscript’s guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission in Editorial Manager.

The lead author has included this statement in the cover letter as requested.

6. 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), 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, 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 and gynecology data definitions at <https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize>. 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 26 typed, double-spaced pages (6,500 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.

The Short title is provided on Page 1, Line 19

“Short Title: Genetic variants and etonogestrel concentrations”

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 limits for different article types are as follows: Original Research articles, 300 words. Please provide a word count.

The word counts are provided in Line 27: “Word counts: Abstract = 275, Manuscript = 3839”

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.

We have removed all virgule symbols from the manuscript except where used for data or measurements.

14. 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.

15. Figures

Figure 1: The n values in the first exclusion box total 32. Are items not mutually exclusive?

We apologize for this oversight. We have edited the figure to appropriately show n=32 for the first exclusion box and the total screened n=418.

Figures 2-4: May be resubmitted as-is.

We have added a Figure 5 for the additional genetic variant included in our revised statistical analysis.

16. Authors whose manuscripts have been accepted for publication have the option to pay an article processing charge and publish open access. With this choice, articles are made freely available online immediately upon publication. An information sheet is available at <http://links.lww.com/LWW-ES/A48>. The cost for publishing an article as open access can be found at <http://edmgr.ovid.com/acd/accounts/ifauth.htm>.

Please note that if your article is accepted, you will receive an email from the editorial office asking you to choose a publication route (traditional or open access). Please keep an eye out for that future email and be sure to respond to it promptly.

17. If you choose to revise your manuscript, please submit your revision via Editorial Manager for Obstetrics & Gynecology at <http://ong.editorialmanager.com>. It is essential that your cover letter list point-by-point the changes made in response to each criticism. Also, please save and submit your manuscript in a word processing format such as Microsoft Word.

From: [REDACTED]
To: [Randi Zung](#)
Subject: RE: Your Revised Manuscript 18-2235R1
Date: Friday, January 18, 2019 2:36:04 PM
Attachments: [Green Journal - Transparency Declaration.pdf](#)
[18-2235R1_ms \(1-16-19v2\) with edits.docx](#)
[STROBE_checklist_v4_combined_for_Green_journal.doc](#)

Dear Ms. Zung,

Thank you so much for coordinating the changes to the manuscript. Please find attached the manuscript with our edits in track changes, the STROBE checklist, and the signed transparency declaration statement. Our responses to the Editors' queries are below:

1. General: The Editor has made edits to the manuscript using track changes. Please review them to make sure they are correct.

Thank you for your edits and comments. We agree with all the edits made by the Editor.

2. Please provide a completed STROBE checklist. The checklist is available at <http://ong.editorialmanager.com>.

A completed STROBE checklist is attached along with the revised manuscript.

3. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained." *The manuscript's guarantor.

Please provide a signed version of this statement.

A signed version of this statement is attached along with the revised manuscript.

4. Electronic Copyright Transfer Agreement: All co-authors will need to complete our electronic Copyright Transfer Agreement, which was sent to them through Editorial Manager.

I will contact the co-authors and ensure the eCTA's are completed.

5. Line 54-55: Please be sure this is stated in the body of your paper. Statements and data that appear in the Abstract must also appear in the body text for consistency.

We have added a line to the *Results* section containing this specific variant allele frequency information.

Lines 267-269: "PGR rs537681 was the most common variant allele included in the final model (found in 84.9% of participants), followed by NR1I2(PXR) rs2461817 in 69.4% of participants and CYP3A7*1C in only 5.1% of participants."

6. Line 57 and elsewhere: Please express this p-value and all the p-values in your paper to no more than three decimal places.

We have replaced the p-values in Line 57 with the appropriate scientific notation to ensure no more than three decimal places: “ $p=7.0 \times 10^{-7}$ ” and “ $p=5.8 \times 10^{-5}$ ”

We have also replaced the values in the following locations:

Line 263: “ 5.0×10^{-4} ”

Line 264: “ 5.8×10^{-5} ” and “ $p=7.0 \times 10^{-7}$ ”

7. Line 159: Please put in parenthesis what dbSNP is. I assume it's a database of SNP results. Can you explain if it's a website, what the URL is, etc.

We have added what dbSNP refers to and how it can be accessed in parentheses in lines 160-162: “(The Database for Short Genetic Variations managed by the National Center for Biotechnology Information, catalog available online at <http://www.ncbi.nlm.nih.gov/projects/SNP/>)”

8. Line 192: Can you provide a full reference for this? Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references

We unfortunately do not have a full reference for our work as that manuscript is still under review. However, we feel that published data already cited in this paper support the inclusion of these two variables in our analysis. The work by Le et al (2001) demonstrate a very slight negative trend in serum etonogestrel concentrations with longer use of the implant, thereby supporting inclusion of duration of use as a variable. As discussed in lines 132-134, McNicholas et al (2017) and Morell et al (2016) found opposing associations between BMI and serum etonogestrel concentrations (a positive association in one and negative association in the other), which supports inclusion of BMI in our modeling as a potential confounder. We apologize for not having a citation for the work originally cited, but feel this change addresses this concern.

Line 195: “...prior published associations (9, 17, 20).”

9. Line 247: In order to break up this long string of gene names, which can be difficult to read (I had to look hard for the period here) would you consider writing this sentence as: “We excluded CYP3A4rs5578340 from the multivariable analysis as it had a prevalence of 0.3% (1/350).”

Thank you for this suggestion and we have made the change you recommended. We agree that this makes the sentence easier to read:

Lines 251-253: “We excluded CYP3A4 rs55785340 from the multivariable analysis as it had a prevalence of 0.3% (1/350).”

10. Line 252-255: The highlighted information about duration of use and BMI is not mentioned in your discussion at all. Since these were contributors to the variation in protein levels, don't you think they should be?

We agree that they should be mentioned and thank you for your recommendation. We had added a few sentences to the beginning of the *Discussion* to highlight these significant contributors. These lines explain how to interpret these associations and that they are not clinically significant on their own, but may contribute to significant effects in combination with other factors.

Lines 278-288: “In this large and diverse group of subdermal etonogestrel implant users, we found two patient characteristics and three genetic variants associated with serum etonogestrel concentrations. BMI and duration of implant use remained significantly associated with decreased serum etonogestrel concentrations using our conservative threshold. However, for the majority of women, the effects of increasing BMI and longer duration of implant use will not cause their serum etonogestrel concentrations to fall below the level needed for consistent ovulatory suppression (90pg/mL). For every 1kg/m² increase in BMI, the serum etonogestrel concentration will decrease on average 3pg/mL. Similarly, for every month of implant use past 12 months, the serum etonogestrel concentration will decrease on average by 1.6pg/mL. Though these decreases are not clinically significant on their own, they may contribute to clinically significant effects in combination with other factors, such as genetics.”

11. Line 287-289: I’ve highlighted “results” here to question if think that the gene variant is causative for the increased metabolism or associative? If you think causative, can you explain why? If you don’t think its causative (or could be permissive, for example) would you please change the language to more associations?

We do think there is some physiologic plausibility to this gene variant being causative for increased metabolism, but appreciate your comment and cannot declare causation with the results of this study. We have changed the language in these lines to better express the association between this gene variant and increased metabolism.

Lines 303-307: “Our findings reaffirm that the expression of fetal CYP3A7 enzymes in CYP3A7*1C carriers is associated with increased steroid hormone metabolism, specifically etonogestrel in contraceptive implant users. In further support of this metabolic association, 27.8% (5/18) of CYP3A7*1C carriers had etonogestrel concentrations that fell below 90pg/mL, which is the manufacturer’s reported threshold for consistent ovulatory suppression with the implant (13).”

Sincerely,
Aaron Lazowitz

From: Randi Zung [mailto:RZung@greenjournal.org]

Sent: Wednesday, January 16, 2019 8:05 AM

To: Lazowitz, Aaron [REDACTED]

Subject: Your Revised Manuscript 18-2235R1

Dear Dr. Lazowitz:

Your revised manuscript is being reviewed by the Editors. Before a final decision can be made, we need you to address the following queries. Please make the requested changes to the latest version of your manuscript that is attached to this email. **Please track your changes and leave the ones made by the Editorial Office.** Please also note your responses to the author queries in your email message back to me.

1. General: The Editor has made edits to the manuscript using track changes. Please review them to make sure they are correct.
2. Please provide a completed STROBE checklist. The checklist is available at <http://ong.editorialmanager.com>.

3. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained." *The manuscript's guarantor.

Please provide a signed version of this statement.

4. Electronic Copyright Transfer Agreement: All co-authors will need to complete our electronic Copyright Transfer Agreement, which was sent to them through Editorial Manager.

5. Line 54-55: Please be sure this is stated in the body of your paper. Statements and data that appear in the Abstract must also appear in the body text for consistency.

6. Line 57 and elsewhere: Please express this p-value and all the p-values in your paper to no more than three decimal places.

7. Line 159: Please put in parenthesis what dbSNP is. I assume it's a database of SNP results. Can you explain if it's a website, what the URL is, etc.

8. Line 192: Can you provide a full reference for this? Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references

9. Line 247: In order to break up this long string of gene names, which can be difficult to read (I had to look hard for the period here) would you consider writing this sentence as: "We excluded CYP3A4rs5578340 from the multivariable analysis as it had a prevalence of 0.3% (1/350)."

10. Line 252-255: The highlighted information about duration of use and BMI is not mentioned in your discussion at all. Since these were contributors to the variation in protein levels, don't you think they should be?

11. Line 287-289: I've highlighted "results" here to question if think that the gene variant is causative for the increased metabolism or associative? If you think causative, can you explain why? If you don't think its causative (or could be permissive, for example) would you please change the language to more associations?

To facilitate the review process, we would appreciate receiving a response by January 22.

Best,

Randi Zung

--

Randi Zung (Ms.)

Editorial Administrator | *Obstetrics & Gynecology*

American College of Obstetricians and Gynecologists

409 12th Street, SW

Washington, DC 20024-2188

T: 202-314-2341 | F: 202-479-0830

<http://www.greenjournal.org>

From: [REDACTED]
To: [Stephanie Casway](#)
Subject: RE: O&G Figure Revision: 18-2235
Date: Tuesday, January 22, 2019 9:56:26 AM

Hi Stephanie,

Thanks for making the changes and I am fine with the “progesterone receptor” change the copyeditor requested. I have no additional edits and really appreciate your assistance with this process.

Sincerely,
Aaron

From: Stephanie Casway <SCasway@greenjournal.org>
Sent: Tuesday, January 22, 2019 7:52 AM
To: Lazorwitz, Aaron [REDACTED]
Subject: RE: O&G Figure Revision: 18-2235

Good Morning Aaron,

Thank you so much for your review. Also, my apologies for the delayed reply. I wanted to run your edits by both of our copyeditors. I have made all of your requested edits, with the exception of italicizing PGR (this has also been replaced with “progesterone receptor”). This was at the request of our in-house copyeditor. The manuscript will also be reviewed by our publisher’s copyeditor, and if they make any edits we will update the art to match.

Please let me know if this is okay, or if you have any additional edits. Have a great day!

From: Lazorwitz, Aaron [REDACTED]
Sent: Thursday, January 17, 2019 11:50 AM
To: Stephanie Casway <SCasway@greenjournal.org>
Subject: RE: O&G Figure Revision: 18-2235

Hi Stephanie,

Thanks so much for the edited figures and revisions. Here are my suggested changes and responses to your queries:

18-2235 Legend:

For the Figure 4 legend, can you please italicize “NR1I2 (PXR)” in the first line and not italicize “rs2461817” in the second line? The rs numbers should not be italicized, but gene names should be. For the Figure 5 legend, similarly can you italicize “PGR” in the first and second line and not italicize “rs537681” in both lines?

18-2235 Fig 5

Similarly, can we italicize “PGR” and not italicize “rs537681” in the x axis labels?

AQ1: Note that we updated the BMI in the second exclusion box for Figure 1 (8.5 to 18.5). If this is incorrect, please let me know.

This is correct, thank you for changing this!

AQ2: We have added an x-axis label in Figure 2. If this is incorrect, please let me know.

The x-axis label is correct. Thank you for adding this.

AQ3: We have added a description of the symbols (circles and asterisks) to the legends for Figures 3–5. If this is incorrect, please let me know.

The added description of the symbols is correct for all of the legends.

Thanks so much,
Aaron

From: Stephanie Casway <SCasway@greenjournal.org>

Sent: Wednesday, January 16, 2019 11:53 AM

To: Lazorwitz, Aaron [REDACTED]

Subject: O&G Figure Revision: 18-2235

Good Afternoon Dr. Lazorwitz,

Your figures and legend have been edited, and PDFs of the figures and legend are attached for your review. Please review the figures CAREFULLY for any mistakes. In addition, please see our queries below.

AQ1: Note that we updated the BMI in the second exclusion box for Figure 1 (8.5 to 18.5). If this is incorrect, please let me know.

AQ2: We have added an x-axis label in Figure 2. If this is incorrect, please let me know.

AQ3: We have added a description of the symbols (circles and asterisks) to the legends for Figures 3–5. If this is incorrect, please let me know.

PLEASE NOTE: Any changes to the figures must be made now. Changes at later stages are expensive and time-consuming and may result in the delay of your article’s publication.

To avoid a delay, I would be grateful to receive a reply no later than Friday, 1/18. Thank you for your

help.

Best wishes,

Stephanie Casway, MA
Senior Production Editor
Obstetrics & Gynecology
American College of Obstetricians and Gynecologists
409 12th St, SW
Washington, DC 20024
Ph: (202) 314-2339
Fax: (202) 479-0830
scasway@greenjournal.org