Peer Review File
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Reviewer A
The authors present a review on the current state of genetic testing for birth defects and pediatric diseases. They review the literature of prenatal screening, invasive diagnostic testing, and next-generation sequencing in the NICU and illustrate the challenges of testing with two clinical vignettes.

General comments
The manuscript covers the salient topics in this field and it is thoughtful and well-written.

Comments
A. Referencing. There are many sentences throughout the manuscript that appear to be missing a reference(s). A few examples (but not all) include:

Comment 1. Advances in ultrasonographic and genetic technology, namely next generation sequencing (NGS), have improved the detection of pediatric diseases and permit earlier diagnosis [ref?].
Reply 1: Two prospective trials on the yield of WES for fetal anomalies (Lord et al., Petrovski et al.) were added.
Changes (italicized here) in the text: "…have improved the detection of *birth defects* and permit earlier *genetic* diagnosis *in the prenatal period*."

Comment 2. Given the high concentration of conditions with potential genetic etiology in the neonatal intensive care unit (NICU), neonatal ES/GS studies have demonstrated high diagnostic yields in NICU settings [refs?]
Reply 2: All the refs are included in Table 1.
Changes (italicized here) in the text: "Given the high concentration of conditions with potential genetic etiology in the NICU, *we will discuss the high diagnostic yields of* neonatal ES/GS studies in NICU settings (*see Table 1*)."

Comment 3. Although NIPT was first validated in high-risk patients, professional organizations have expanded their genetic screening recommendations to include the use of NIPT in low-risk patients due to superior test performance.
Reply 3: Modified to reflect more recent recommendation with references included.
Changes (italicized here) in the text: …to include the use of NIPT in *all* patients due to superior *accuracy compared to maternal serum screening*.

Comment 4. For some genetic conditions, curated gene panels may facilitate targeted testing when a single-gene disorder is suspected, such as Noonan syndrome or skeletal dysplasia [refs?].
Reply 4: A reference has been added.

Changes (italicized here) in the text: *When a single-gene disorder is suspected, single-gene testing or* curated gene panels may facilitate targeting testing *for genetic conditions* such as *FGFR3 for achondroplasia or RASopathies panel for* Noonan syndrome*, respectively.*

Comment 5. Several multicenter studies in different countries have demonstrated the reproducible benefit of ES for diagnosis and clinical decision making [refd?].
This is not meant to be a complete list, but rather examples where the reader would benefit from a reference. Perhaps the authors can review the manuscript to add appropriate references.
Reply 5: Three references have been added

Comment 6. I would have liked to see a few sentences on the use of other testing in the NICU, in particular the single gene tests that are not readily identified by next-generation sequencing. For example, the child with suspected Prader-Willi syndrome, Beckwith-Wiedemann testing, or congenital myotonic dystrophy (>1000 repeats) to name a few.
Reply 6: A new section discussing other testing has been added (see lines 294-305).
Changes in the text:

ES does not detect variants outside of capture regions, such as noncoding variants, and many tandem repeat elements (65,66). Thus genetic conditions such as congenital myotonic dystrophy described in the second NICU case above are not well detected by most ES analyses. Current exome analytical pipelines are also less sensitive for small structural variants such as deletions involving only one exon. GS analysis can be optimized to detect many tandem repeat and small structural variants (67). Further, methylation state is not detected by ES, so some causes of Beckwith-Wiedemann and Prader-Willi will not be able to be diagnosed by ES (68). ES is able to detect uniparental disomy and deletions which could be an alternative cause of imprinting disorders. Therefore, many centers consider targeted testing for congenital myotonic dystrophy, Prader-Willi syndrome, spinal muscular atrophy, and rapid chromosomal microarray in conjunction with ES/GS (63).

Comment 7. More information. I would have liked additional information on the GWS that diagnosed a child with congenital myotonic dystrophy. Can the provide a chromatogram showing the expansion detected? I suspect this was initially identified in the mother, with a mildly increased expansion (<100ish) and then the child was followed up with a myotonic dystrophy specific diagnostic test(?). I think I (ie the reader) just need more information – even if it is just the program that was used to detect expansions.
Reply 7: Chromatogram was not available, but information on the program to detect expansion and other details of testing results were added.
Changes (italicized here) in the text: *Data analysis and interpretation were performed by the Baylor Genetics analytics pipeline. Trinucleotide repeat calling was performed using the Illumina Manta Structural Variant Caller.* Genetic results confirmed congenital myotic dystrophy *type 1* (autosomal dominant), evident in a heterozygous

pathogenic CTG repeat expansion *of approximately 2,450 repeats* in the *3' non-coding region DMPK* inherited from the mother.

Reviewer B

Comment 1: This commentary is an ambitious attempt to integrate the multiple sources genetic information (prenatal, carrier testing, carrier testing, family history, diagnostic tests that include sequencing) all of which pose a major challenge for pediatricians. Importantly, the authors fail to mention newborn screening. Although the latter is mostly focused on a discrete set of rare metabolic disorders, there are similar challenges in phenotype diversity and the need for confirmatory studies. Newborn screening presents a different paradigm in which neither the patient (baby) nor parents are consented or provided (prior to testing) detailed information about the disorders (in nearly all cases). There is no question that prenatal screening should involve provision of information prior to testing. The dilemma is how much information can realistically be provided given the diversity of conditions, variable expressivity, need for confirmatory tests, etc.

Reply 1: We added discussions on newborn screening in two sections (lines 254-264, 308-317).

Changes (italicized here) in the text:

(Line 254) Genetic testing can be an important component of care provided in a NICU. *All newborns receive screening by dried blood spot for a discrete list of early-onset disorders (varies by state), with DNA testing as primary- or second-tier confirmatory testing for the following conditions: cystic fibrosis, hemoglobinopathies, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, severe combined immunodeficiency (SCID), and more recently, SMN1-related spinal muscular atrophy (SMA). Expanding to universal genomic newborn screening is under investigation on the feasibility, clinical utility, public health benefits vs. costs, and ethical, legal, and social implications. Genomic newborn screening will not be discussed in this review as it is currently not used in the clinical setting, but has been addressed by others including a systemic review by Downie et al.*

(Line 308) As the landscape of prenatal and neonatal genetic testing evolves, we will continue to encounter new challenges that require thoughtful strategies to maximize benefits and minimize harms while centering the patient's goals and values. *For example, concerns over newborn screening dried blood spot storage and utilization and possible universal genomic newborn screening are related to an insufficient informed consent process in current newborn screening programs, in contrast to consent obtained for genetic testing performed for a suspected genetic condition (69,70).* For the latter, three major challenge areas are: 1) patient understanding of testing, 2) detection of secondary findings, incidental findings, and reinterpretations, and 3) impact on clinical management.

Comment 2: The authors also should consider the different modes of inheritance. For most of the rare disorders identified by ES, the mode of inheritance is de novo autosomal dominant and it is generally not possible or practical to counsel prior to the identification of specific clinical findings. Conversely, although aneuploidy is also de novo in origin, it is possible to counsel prior to provide NIPS. Monogenic inherited disease is the major burden but how to pre-test counsel for broad-based expanded panels is problematic. The latter problem is exasperated by the fact that carrier screening is usually provided as a prenatal test (but would optimally be provided prior to conception).
Reply 2: We added discussion on modes of inheritance (see lines 318-333).
Changes (italicized here) in the text:

The decision to pursue genetic screening or testing should occur after a comprehensive discussion about the options and with informed consent, especially given the rapid advances in technology that have added complexity and nuance to prenatal and neonatal genetics. In the prenatal setting, consultation with a genetic counselor or comprehensive educational resources should be offered to all patients as a standard component of prenatal care (28,71,72). *If a genetic diagnosis is suspected and confirmed, the genetic counselor can provide information on recurrence risk for future family planning. Most pediatric rare diseases are due to pathogenic de novo genetic variants in autosomal dominant disorders or de novo aneuploidy, both with extremely low risk of recurrence (73). In cases of autosomal recessive disorders where each unaffected parent is found to be a carrier, recurrence risk is higher and so decisions affecting future pregnancies such as preimplantation genetic testing may be considered. While these scenarios have long been a part of the genetic counselor's experience in single gene disorders, the landscape has changed significantly with the expansion of ES/GS (74).*

The paper would be improved by addressing these broader issues.

Some specific points.

Comment 3. 1. Introduction Line 60. ACOG and ACMG guidelines should reflect most recent guidance documents. ACMG (2022) does not limit NIPS to high risk populations and monogenic disease carrier screening is advocated for all (Gregg, et al 2021).
Reply 3. Modified to reflect more recent recommendation with references included. References, including the most recent guidelines pertaining to aneuploidy NIPT (2022) by ACMG, have also been added (see lines 58-60 and 107-110).
Changes (italicized here) in the text:
(Line 58) Recommendations for genetic testing by the American College of Obstetrics and Gynecology (ACOG) and the American College of Medical Genetics (ACMG) provide screening and diagnostic guidance in the prenatal and newborn period.
(Line 107) Although NIPT was first validated in high-risk patients, professional organizations have expanded their genetic screening recommendations to include the use of NIPT in *all* patients due to superior *accuracy compared to maternal serum screening*.

Comment 4. Lines 72-3. GS includes intron (non-coding region) sequencing rather than "expanded coverage of genes".
Reply 4: This was revised as suggested (see lines 72-74).
Changes (italicized here) in the text: GS generally provides information obtained from ES but with expanded coverage of genes, *inclusion of noncoding regions such as introns,* and increased sensitivity for structural variants.

Comment 5. Lines 82-84. See above. Provide references.
Reply 5: Recent references to ACOG (2020) and ACMG (2021, 2022) guidelines have been added (see lines 83-88).
Changes (italicized here) in the text: Current ACOG, Society for Maternal-Fetal Medicine (SMFM), and ACMG guidelines recommend all pregnant individuals be offered genetic screening *in the form of carrier screening, serum screening, and/or cell-free DNA, and prenatal* diagnostic testing regardless of risk. Previously, risk factors such as maternal age, *presence of abnormality on fetal ultrasound, ethnicity,* and family history were considered when recommending genetic *screening* and testing.

Comment 6. Line 85. Or ultrasound abnormality.
Reply 6: Revised as suggested by the reviewer (see line 87).
Changes (italicized here) in the text: Previously, risk factors such as maternal age, *presence of abnormality on fetal ultrasound, ethnicity*, and family history were considered when recommending genetic screening and testing.

Comment 7. Line 95. Delete (or define) "penta" screening.
Reply 7: Revised as suggested by the reviewer (see line 96-100).
Changes (italicized here) in the text: Screening tests include first *and/or second trimester* maternal serum analytes (pregnancy-associated plasma protein [PAPP-A], beta human chorionic gonadotropin [hCG], alpha fetoprotein [AFP], estriol, inhibin A), first-trimester nuchal translucency ultrasound, and non-invasive prenatal testing of cell-free fetal DNA.

Comment 8. Line 102. Trisomy 21, 18, 13 and detection of sex chromosome abnormalities.
Reply 8: This was revised as suggested (see lines 112-115).
Changes (italicized here) in the text: All tests assess the risk of fetal Trisomy 21 (T21; Down syndrome), Trisomy 18 (T18; Edwards syndrome), Trisomy 13 (T13; Patau syndrome), and sex chromosome *abnormalities* such as 45,X (Turner syndrome) and 47,XXY (Klinefelter syndrome).

Comment 9. Line 111. Better to reference a more recent meta-analysis and use their overall rates for NIPS detection and false-positive rates. E.g., Demko et al J Clin Med. 2022.
Reply 9: We have updated the detection rates and included a reference to Rose et al., 2022 (see line 115-117).

Changes (italicized here) in the text: The *detection rate* of NIPT is *98.8%* for *T21, 98.83% for T18, and 92.85% for T13;* the specificity is >99% for all three trisomies.

Comment 10. Beginning line 204. The two cases do not have a prenatal component and therefore it would be better place these under the sub-heading of "Advances of diagnostic testing in newborns" instead of being separate sections.
Reply 10: The cases have been moved directly under "Advances of diagnostic testing in newborns" (now starting at line 187) as suggested by the reviewer.

Comment 11. Line 211. "Non-intervenable" is not quite accurate since lung transplant was a possible intervention.
Reply 11: This was revised as suggested (see line 192).
Changes (italicized here) in the text: Earlier identification of *serious* conditions.

Comment 12. Line 214. Perhaps "labored or difficulty breathing"?
Reply 12: This was revised as suggested (see line 195).
Changes (italicized here) in the text: At birth, the newborn required routine resuscitation in the delivery room, but due to his *labored* work of breathing and oxygen requirement, he was brought to the NICU on 1 L/min nasal cannula with FiO_2 of 100%.

Comment 13. Line 223. What is meant by the "Neonatal Respiratory Distress Panel" A test? A group of experts?
Reply 13: This was revised to reflect the commercial test (see lines 204-206)
Changes (italicized here) in the text: *Surfactant NGS panel that included ABCA3, FOXF1, NKX2-1, SFTPB, and SFTPC was sent (Fulgent Genetics, Temple City, CA).*

Comment 14. Line 257. "A number"?
Reply 14: This was edited as recommended (see line 242)
Changes (italicized here) in the text: *A* number of genetic conditions were suspected.

Comment 15. Line 293-295. The sentence is misleading. NIPS has fewer positive results and therefore there is less post-test counseling and testing. However, this does not necessarily support the concern that there is increased misinterpretation of those results that are screen-positive.
Reply 15: We agree with the reviewer's comment and removed the sentence regarding "decrease in referrals for genetic counseling and frequency of diagnostic testing" since neither are about the patient's interpretation of screen positive results.

Comment 16. Line 364. Pediatricians are already at the forefront of precision of precision medicine; at least as it relates to the children whose parents can now receive information about their children's lifetime health risks. A practical reality is that pediatricians need to have access to genetics services and understand contemporary testing.

Reply 16: We appreciate the thoughtful suggestion by the reviewer and made revisions (see lines 438-440).
Changes (italicized here) in the text: With these goals in mind we *can continue to improve equitable access to* precision medicine in maternal and pediatric health.