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CORRESPONDENCE

Response to "Noninvasive prenatal screening at low fetal fraction: comparing whole-genome sequencing and single-nucleotide polymorphism methods"

This correspondence addresses the issues raised by Ryan and Martin in an accompanying letter to the editor regarding our published manuscript entitled "Noninvasive Prenatal Screening [NIPS] at Low Fetal Fraction: Comparing Whole-Genome Sequencing and Single-Nucleotide Polymorphism Methods".[1] The aim of our study was to compare the performance and clinical consequences of the two main methods of NIPS on hundreds of thousands of pregnancies at low fetal fraction. The scale of such a comparison required a simulation approach to be statistically compelling, logistically tractable, and properly controlled. We carefully modeled the whole genome sequencing (WGS) and single nucleotide polymorphism (SNP) methods impartially, accurately, and transparently: all code used for the paper is publicly available, all sources are patents or peer-reviewed publications, and no Counsyl NIPS data was used in the study. Ryan and Martin assert that our analysis is "invalidated by two significant methodological flaws", which can be distilled as follows: an overly favorable implementation of the WGS method and a misrepresentation of the SNP method. Here, we demonstrate why those criticisms are invalid.

We applied comparable scrutiny to both methods. In particular, we carefully confirmed the validity of our claims by varying relevant signal and noise parameters for both methods over many orders of magnitude (see Supplemental Figures S1, S2, S4–6). Ryan and Martin assert that our analysis of the WGS method "did not incorporate any sources of variance other than random sampling of the number of reads". Figure S2 directly refutes their claim. Indeed, the singular aim of the figure was to explore the general impact of extra variance—from any source—on WGS sensitivity. The figure demonstrates that the WGS method retains higher analytical sensitivity than the SNP method at variance levels that are actually far in excess of what was experimentally observed even in the infancy of WGS-based NIPS.[2] In addition, to further account for high-variance genomic regions in the WGS method that are typically removed during standard data analysis in a clinical setting,[3] we also removed 10% of each chromosome in our simulations.

Ryan and Martin further argue that our simulations are incomplete because we treated SNPs independently and did not model linkage across neighboring SNPs: their argument is based on the fact that the SNP-method algorithm incorporates "crossover frequency data from the HapMap database" to generate hypotheses that link SNPs probabilistically into the haplotype blocks that are highly likely to be co-inherited.[4] However, rather than handicap the SNP method by omitting this HapMap information from our model, we actually modeled the SNP method in its best-case scenario, where no crossing over occurs at all. Our approach may seem counterintuitive, but it is based precisely on Natera's published disclosures of their implementation of the SNP method: according to Natera's SNP-method patent application (US 20140162269[5]), in the absence of crossovers—that is, with deterministic rather than probabilistic linkage information—the SNP method's equation for handling linked SNPs reduces to a sum of log-likelihoods over individual SNPs. Therefore, both to evaluate the upper bound of SNP-method performance and to simplify the model to be maximally transparent, our simulations assumed the absence of crossovers. Supporting our assertion that the SNP method is well represented by our simulations is the striking correspondence between the published SNP method no-call threshold (2.8% FF[6]) and the FF level at which our simulations found a precipitous drop in sensitivity, just below 3%.

As expected, these two very different NIPS methods have particular strengths for certain rare cases: for instance, although Ryan and Martin noted that the SNP method has shown limited proficiency with triploidy detection, the WGS method is demonstrably better suited to twin,[7] egg-donor,[8] and consanguineous pregnancies.[9] In our manuscript, however, our aim was not to evaluate the methods' respective virtues on rare cases, but rather to assess the analytical performance and clinical impact for a very common occurrence: pregnancies with low fetal fraction. The SNP method routinely no-calls low-fetal-fraction samples, and publications about the SNP method (e.g., Ryan *et al.*[6])

effectively inflate calculations of sensitivity by omitting the affected fetuses that are known to be enriched in the low-fetal-fraction patients who received no test result.[10] By contrast, our analysis importantly and robustly suggests that for low-fetal-fraction pregnancies—common among patients with high BMI, at early gestational age, and with trisomy 13 or 18—the WGS method maintains high sensitivity, thereby yielding fewer false negatives, fewer no-calls, and fewer unnecessary invasive procedures than the SNP method.

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