Improving the Accuracy of Prenatal Screening with DNA Copy-Number Analysis

TO THE EDITOR: Despite reported specificities of more than 98% for noninvasive prenatal screening with the use of cell-free DNA obtained from maternal blood,[1] the published positive predictive values for trisomies 21, 18, and 13 are 93%, 64%, and 44%, respectively.[2] The apparent discrepancy between specificity and positive predictive value is due to the low prevalence of these disorders, technical limitations of the assays, and biologic variation, such as a vanishing twin or confined placental mosaicism. Snyder and colleagues described two cases of falsely positive results on noninvasive prenatal screening for trisomy 18 that were later found to be the result of maternal microduplications of chromosome 18.[3]

We performed noninvasive prenatal screening using whole-genome shotgun sequencing, a method that involves sequencing fragments of DNA that, in the aggregate, represent almost all the genome. This approach allows us to generate a karyogram that graphically represents z scores throughout the entire genome. After reading the report by Snyder et al., we instituted a process in which for every positive result obtained on noninvasive prenatal screening, we generated and examined the karyogram for the affected chromosome. For a true positive result, sequence reads are increased throughout the entire chromosome. When a maternal microduplication is present, only the region of the chromosome that is duplicated is represented through an increased number of sequence reads. In evaluating data from a series of 31,278 pregnant women who underwent such screening, our process allowed us to identify 61 women in whom maternal microduplications occurring on chromosomes 13, 18, and 21 showed false positive results.

Until we were confident that karyograms correctly predicted maternal microduplications, we confirmed suspected microduplications by means of microarray analysis (CytoScan HD Array, Affymetrix). Subsequently, maternal microarray analysis was performed at the discretion of the ordering physician. A genetic counselor contacted the physician with the report, which included a description of the suspected maternal microduplication and an offer of confirmatory microarray analysis (at no charge for underinsured patients).

Microarray analysis showed the presence of a maternal microduplication in all the confirmatory tests that were performed. The identification of maternal microduplications as a source of false positive results improved the positive predictive values of our screening to a rate of 98% for trisomy 21, 92% for trisomy 18, and 69% for trisomy 13 (Table 1). True positive results for trisomy 21 were confirmed by means of karyotype or microarray analysis of amniocytes. True positive results for trisomies 13 and 18 were confirmed by means of distinctive ultrasonographic abnormalities or amniocyte analysis.

These analyses had no effect on the negative predictive value of the prenatal screening, since we found no ultrasonographic evidence of fetal abnormalities suggestive of trisomy or an ab-

Table 1. Identification of Maternal Microduplications as a Source of False Positive Results on Noninvasive Prenatal Screening.*

Chromosome	Positive Trisomy	Maternal Microduplication	Confirmed on Microarray Analysis	Improvement in Positive Predictive Value
	number of patients			*percentage points*
21	313	12	9	+4 (from 94% to 98%)
18	106	21	3	+20 (from 72% to 92%)
13	93	28	2	+30 (from 39% to 69%)

* The identification of maternal microduplications as a source of false positive results improved the absolute positive predictive value of prenatal screening to a rate of 98% for trisomy 21, 92% for trisomy 18, and 69% for trisomy 13.

normal prenatal diagnosis. We received no notice from any delivering physician or neonatologist of the birth of an affected infant. Because there were no known infants with trisomy 13 or 18, we calculated a negative predictive value of 100%. One baby was born with trisomy 21, which resulted in a negative predictive value of greater than 99.9%.

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1. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med 2014;370:799-808.
2. Wang JC, Sahoo T, Schonberg S, et al. Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases. Genet Med 2015;17:234-6.
3. Snyder MW, Simmons LE, Kitzman JO, et al. Copy-number variation and false positive prenatal aneuploidy screening results. N Engl J Med 2015;372:1639-45.

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TARGT Gene Therapy Platform for Correction of Anemia in End-Stage Renal Disease

TO THE EDITOR: Anemia in patients with end-stage renal disease who are undergoing hemodialysis is caused, in part, by a reduction in or absence of secretion of endogenous erythropoietin. Recombinant human erythropoietin given intravenously has been associated with an increased risk of cardiovascular complications and death, which is considered to be related, at least in part, to the high peak levels of plasma erythropoietin that are achieved after intravenous administration.[1,2] Transduced autologous restorative gene therapy (TARGT), a new autologous protein-delivery platform designed to provide sustained secretion of proteins such as recombinant human erythropoietin, uses a small autologous dermal explant or explants (see Fig. S1 in Supplementary Appendix, available with the full text of this letter at NEJM.org) in which several biopsy samples of a given patient's dermis are transduced ex vivo with the use of a helper-dependent adenovirus system that is engineered to carry a gene — in this case *EPO*, which encodes the human erythropoietin protein. The resulting delivery system is referred to as $TARGT_{EPO}$.

We performed studies of $TARGT_{EPO}$ in vitro and in vivo (in SCID [severe combined immunodeficient] mice) and measured the secretion of erythropoietin. By assessing the rate of erythropoietin secretion into the media and the amount of erythropoietin secreted, we estimated the dose of recombinant erythropoietin that would typically be needed for each patient. We then washed the $TARGT_{EPO}$ units extensively to remove residual culture media and implanted the appropriate number subcutaneously into each patient.[3] We found that erythropoietin secreted from $TARGT_{EPO}$ units had an isoform pattern similar to that of secreted endogenous erythropoietin.

After we performed the in vitro and in vivo studies to determine individual initial doses, we administered $TARGT_{EPO}$ in a phase 1–2, open-label, multicenter, single-group, dose-escalating clinical study (ClinicalTrials.gov number, NCT02117427) to treat anemia in 10 patients with end-stage renal disease who were undergoing hemodialysis. Patients were assigned to one of two dose groups — low dose (18 to 25 IU per kilogram of body weight per day, 6 patients) and higher dose (35 to 45 IU per kilogram per day, 4 patients). The primary outcomes were clinical safety, pharmacokinetic properties, and time within the target hemoglobin range (9 to 12 g per deciliter) in accordance with clinical guidelines for patients with end-stage renal disease. The study protocol is available at NEJM.org.

All patients who underwent $TARGT_{EPO}$ implantation had an initial increase in plasma levels of erythropoietin, and plasma levels of hemoglobin remained above or within the desired range for most patients (Fig. 1). The plasma levels of erythropoietin remained stable within the physiologic range. In 7 of the 10 pa-