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Translating cell-free fetal DNA technology: Structural lessons from non-invasive RhD blood typing.

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New techniques employing the detection and analysis of cell-free fetal DNA in maternal serum have raised the possibility of non-invasive prenatal screening and diagnosis. One of the first available applications of cffDNA testing, Rhesus D blood typing, has had significantly differential uptake profiles between the United States and European nations. RhD testing using cffDNA is becoming widely used in countries like the United Kingdom but has generated little interest in the United States. This difference can be explained by wider social and regulatory considerations in these countries that can have significant impact on the successful translation of new biomedical technologies. We suggest that technology developers should consider the legitimate needs of the medical realm when developing and introducing new tests.

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Competing interests

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The projected market for non-invasive prenatal genetic screening and diagnosis using cell-free fetal DNA has expanded rapidly in recent years. CffDNA consists of fragments of fetal DNA floating freely in the maternal bloodstream as early as five weeks into pregnancy. Over the past decade, United States (US)-based biotechnology companies have broadened interest in cffDNA testing by commercializing several tests, including those for fetal Rhesus D (RhD) blood type, sex, and most recently, trisomy 13, 18, and 21 (Down syndrome) and monosomy X. CffDNA technology is now entering an expanding \$1.3 billion prenatal testing market in the US [1], driven by several companies, including Sequenom, Verinata Health and Ariosa Diagnostics. The growing interest in these technologies stems from the distinguishing features of cffDNA testing: the absence of procedure-related risks of miscarriage and the increased sensitivity and specificity over current non-invasive methods [2].

Rhesus D (RhD) blood typing targets blood type incompatibility between a fetus and the pregnant woman by identifying an RhD-positive fetus when the mother is RhD-negative. The RhD blood protein appears on the red blood cells of a majority of the population (RhD-positive), while a minority of individuals lack the RhD antigen and therefore have an RhD-negative phenotype. When an RhD-negative woman is pregnant with an RhD-positive fetus, the health of the fetus or newborn may suffer if alloimmunization, or maternal sensitization to the RhD antigens and consequent production of antibodies, occurs [3]. In 2001, polymerase chain reaction (PCR) was used to target cffDNA in maternal serum and successfully determine fetal RhD blood type [4]. It was subsequently suggested that cffDNA technology might provide an inexpensive, safe screening method for patients at risk of RhD incompatibility, namely pregnant RhD-negative women. Research and development efforts were subsequently expended on developing an affordable and accurate test for fetal RhD status.

Although these technical efforts were successful, cffDNA screening for RhD blood type has been met with significantly different uptake across countries. The International Blood Group Reference Laboratory in the United Kingdom (UK) has been offering cffDNA testing for RhD blood type since 2001, and use of the test has expanded rapidly in Europe [5], leading to suggestions that it would enjoy similar success in the US [6]. But an analysis conducted in 2011 suggested that, due to structural factors, replacement of a widely available prophylactic with cffDNA testing to triage for fetal blood type would result in a 20-fold increase in Rh alloimmunization among pregnant RhD-negative women in the US [7]. Based on this analysis, it was concluded that cffDNA testing would only add costs without improving outcomes.

The fact that other aspects of the standard of prenatal care in the two countries are very similar suggests that there are larger external forces influencing the translation of this technology. Here, we explore these larger forces and suggest that, in order to be successful, the technology development process should consider not only the capabilities of novel technologies, but also take into account the larger social and regulatory arena in which the technology is destined to be introduced. Such a broad strategic view would allow new technology to target legitimate medical needs with a greater chance of success.

RhD Blood Type Testing

Over the past several decades, there have been major advances in the management of maternal-fetal RhD incompatibility, which have led to greatly improved fetal outcomes and lower morbidity and mortality among newborns [8]. The risk of Rh disease in a pregnancy, by virtue of a pregnant women with an RhD-negative blood type having an RhD-positive partner, varies with the genetic diversity of a population. In the United States, Caucasians have the highest prevalence of RhD-negative phenotypes, which occur in approximately 15 to 17% of the population [9]. Alloimmunization can occur when RhD-positive fetal cells cross into the maternal circulation, leading to an antibody response in the mother. These specific antibodies can then cross back over the placenta and ultimately cause hemolysis of fetal red blood cells. The spectrum of severity of RhD disease is wide, ranging from mild anemia in the infant at birth, to miscarriage or stillbirth. Moreover, because maternal antibodies persist following a sensitizing event, the manifestation of RhD disease is typically more severe with increasing gravidity.

Since 1966, prophylactic Rh immune globulin (RhIG, brand name: RhoGAM) has been shown to effectively prevent maternal sensitization to the Rh factor and thus Rh disease in the fetus or newborn. Initial screening can be conducted by screening the father for RhD status – only if the father is Rh positive is the fetus at risk – further reducing the relevant patient population for fetal RhD testing. However, without knowledge of the RhD status of the fetus, the standard treatment regime entails the indiscriminate administration of RhIG to all RhD-negative women with RhD positive partners, even those whose fetuses may also be RhD-negative [10]. Diagnostic testing for fetal RhD status via chorionic villus sampling or amniocentesis is possible but rarely performed unless the procedures are otherwise indicated, due to the invasiveness and potential for miscarriage associated with these tests. A non-invasive blood test to establish the RhD status of the fetus would allow care-providers to limit RhIG administration to those who have a genuine medical need.

Structural Comparisons and Implications

While the technological capability of cfDNA testing to establish RhD status with a high rate of sensitivity and specificity has been established [5], a wide variety of social factors have influenced the uptake of the test. To be useful, cfDNA testing for RhD status needs to demonstrate meaningful improvement over the standard of care for the limited population of RhD-negative pregnant women. RhIG administration is already low risk – there are limited side effects, even when the fetus and mother are blood compatible – but availability varies. While relatively inexpensive and readily available in the US, RhIG is often in short supply in Europe. Furthermore, the UK has a nationalized health care system that regulates both uptake and cost of new medical technologies while the US has a decentralized, partially-privatized system with no national standardization. Although the US Federal Food and Drug Administration (FDA) does have jurisdiction over genetic tests, it has largely declined to enforce regulation of genetic laboratory developed tests [11]. Furthermore, several states have their own prenatal screening regimes that may impact the uptake of additional prenatal technologies. Thus, there is a considerably higher barrier to entry and uncertainty of success for new technologies in the US, especially if there is an accepted standard of care.

Intellectual property regimes provide additional constraints. A single company – Sequenom – currently holds the intellectual property rights for RhD testing using cffDNA and thus controls the cost and potential applications of the test. The out-of-pocket cost for the company's RhD test is reportedly \$250 [3]. However, due to a pre-patent licensing agreement with the Institut de Biotechnologies Jacques Boy in France, RhD testing using one specific polymerase chain reaction method is permitted in Europe outside of Sequenom's licensing arrangement in the US. The reduced cost of these tests, approximately GBP 46.50, means that using cffDNA testing for RhD is considerably more cost effective in Europe, especially given the limited availability of RhIG [12]. It also means that there is very little incentive for health care providers in the US to pursue insurance coverage of RhD testing using cffDNA, even in patients for whom RhIG administration is indicated. Anecdotally, some physicians have chosen to send patient samples to the UK rather than pay for domestic testing.

Sequenom has already asserted its rights to its broadest patent, US 6,258,540, which covers a number of analytic techniques using cffDNA, against other companies wishing to enter the cffDNA technology market [13]; this patent includes a specific claim related to the detection of RhD status. This stance has the potential to severely limit the development and use of many cffDNA technologies by smaller laboratories around the United States. The defense of this patent and any patents with similar claims may have negative implications for translation of all cffDNA tests, including those for RhD status. If Sequenom successfully defends its patent, it will be in a monopoly position with regard to non-invasive prenatal RhD testing. Indeed, competing companies, Natera, Ariosa Diagnostics, and Verinata Health have already sued Sequenom, stating that its patent claims are too broad or invalid or that Sequenom is over-exerting the strength of its patent. Litigation is ongoing [13]. This financial uncertainty surrounding companies' intellectual property rights impacts product development and investment in new technologies.

Structural Factors and Translation

RhD testing is not the only example of how these structural factors can impact the uptake of new technologies. Another potential example of how differential regulation impacts implementation of new technologies is the use of cffDNA testing for fetal aneuploidy. Again, there are widely available, cost-effective testing options in both the UK and the US – maternal screening and when indicated, invasive follow-up through amniocentesis – but cffDNA offers considerable improvements in detection rates with far lower screen positive rates and may allow women to avoid invasive procedures [3]. Here, the structural features of the US and UK health systems may have a different impact than on RhD testing. The relatively unregulated environment for genetic testing in the US has allowed companies to freely begin offering aneuploidy testing using cffDNA without regulatory approval [14]. Uptake has been relatively swift, at least among academic medical centers, suggesting that a genuine need has been targeted. By contrast, the need for preapproval from individual regulatory agencies has slowed the distribution of cffDNA tests in Europe. Despite this relatively expeditious introduction in the US, the regulatory and intellectual property uncertainty surrounding cffDNA testing remains. If cffDNA testing for aneuploidy does

receive approval from European regulators, producers are likely to enjoy greater security in the long term than those operating under the uncertainty in the US.

Outlook

The demonstrated importance of considering social and regulatory structural factors in technology development and application should complement, rather than impinge on, the importance of ensuring technical precision and validity. The lack of implementation of RhD testing using cfDNA in the US as compared to the translation of this technology in Europe provide a salutary case study for technology developers in this field. The solution is not to slow commercial progress in the medical testing realm, but to encourage innovators to be aware of the genuine needs of broader stakeholder populations as well as the regulatory environment. There is room for additional input by physicians, regulators, patients, and other stakeholders to identify and target existing gaps in the care system; translation simply for the sake of translation may result in a lack of trust in the biomedical establishment and the misappropriation of resources, damaging not only individual producers but the field as a whole. The example of non-invasive RhD testing should encourage technology developers to broaden the variety of normative and practical inputs into the technology development process so that they can address real and immediate social needs.

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