Expanded carrier screening and the law of unintended consequences: From cystic fibrosis to fragile X

Wayne W. Grody, MD, PhD

> "In the nature of Arctic travel there was a reason why fourteen dogs should not drag one sled, and that was that one sled could not carry the food for fourteen dogs."
>
> —Jack London, *The Call of the Wild*

In medical genetics as in all other fields of human endeavor, sometimes good intentions have unintended consequences. The initial attempts at sickle cell carrier screening in the African American community resulted in miscommunication, undue anxiety, stigmatization, and resentment,[1] and there have been similar problems related to carriers identified through expanded newborn screening programs. Some individuals who have undergone *apoE* genotyping for assessment of cardiovascular disease risk were then shocked to learn that they were also at increased risk of Alzheimer disease. Even the universal and generally successful cystic fibrosis (CF) carrier screening program has provoked confusion and uncertainty among providers and patients with regard to unpredictable genotype-phenotype correlations, diversity of mutation screening panels, and wide clinical variability of the disease.

It is notable that these adverse outcomes have occurred even in large public health programs that were planned, pilot-tested, vetted, and debated by experts and professional organizations before being put into practice. The problem usually arises either from failure to exercise in practice what was outlined in the planning, or, conversely, an impetus to go beyond the planned scope in search of increased catchment, test sensitivity, or simple market share. As illustrated by Jack London's hapless Yukon gold-seekers, it is human nature to be competitive and to try to distinguish oneself by going grander, taller, faster, stronger than one's contemporaries; this is especially so in a capitalist system. In the case of CF carrier screening, this impetus has played itself out in a quest for ever-larger and ostensibly more "comprehensive" mutation testing panels, beyond the original 25 (now 23) mutations recommended by the American College of Medical Genetics.[2,3] A previous commentary in this journal by the author and colleagues[4] bemoaned the many reasons why this trend is both unseemly and unscientific and has resulted in the inclusion of *CFTR* variants of extremely rare frequency, low pathogenicity or uncertain significance, potentially imparting to screened couples either a false sense of security or unwarranted alarm.

Now in the current issue, Strom et al[5] provide direct clinical evidence that such fears have indeed come to pass in practice. These authors work in a major commercial reference laboratory which has sufficient volume of CF tests that rare alleles are seen often enough and in different contexts (carrier screening, referrals from newborn screening, and diagnostic and prenatal testing) and that genotype-phenotype inferences can be made based on real-world experience rather than back-of-the-envelope estimates based solely on allele frequencies. Such is the case for the variant L997F which is the focus of their article. Searching their database of >2500 full-gene *CFTR* sequencing cases, they identified four patients who were compound heterozygous for L997F and a classical mutation such as ΔF508. Clinical follow-up revealed that three of the patients are asymptomatic children of ages up to 5 years, whereas one has atypical CF consisting of recurrent pancreatitis and sinusitis. These results confirm not only the rarity of the L997F allele but also its very low penetrance, essentially negligible if classic CF is taken as the at-risk phenotype, which is the one that population carrier screening was instituted to identify. The three asymptomatic cases were referred for DNA sequencing because of elevated serum trypsinogen on newborn CF screening and the L997F allele thus discovered incidentally; but when included as a deliberate target in carrier screening, referrals will ensue for a less innocuous test—prenatal diagnosis—and, disturbingly, the authors report that they have already received two of those.

The CF mutation screening panel that engendered these procedures is likely not the only one to include L997F, nor is L997F the only allele of questionable clinical significance included in other screening panels, including some that are Food and Drug Administration-approved (D1270N, D1152H, and L206W come readily to mind). But is this situation unique to CF carrier screening? Unfortunately not, as there are a number of other recessive disorders currently being screened in which reach similarly exceeds grasp. Indeed, the advent of highly parallel sequencing and microarray technologies has essentially forced the issue by making it technically easy to multiplex gene tests of unrelated biology and ethnicity together in virtually limitless number and for less total cost than that previously required for testing of one or two individual genes. One company now offers couple- and in vitro fertilization-based screening for a motley collection of more than 100 Mendelian disorders and a highly variable proportion of associated mutations, some providing carrier pick-up rates in the range of 1%. Soon we will be moving into the realm of whole-exome sequencing, initially for diagnosis but the temptation will be there for carrier screening as well, to theoretically identify couples at risk for any of the 13,000 known single-gene disorders and thereby reaching the asymptote of the "more is better" philosophy.

One particular disorder has recently become a popular target for population "carrier screening," seemingly in the absence of any pilot studies or professional recommendations, and, to this author at least, illustrates the law of unintended consequences only too well. Fragile X syndrome is considered the most common inherited cause of mental retardation, with an aggregate carrier frequency for the *FMR1* premutation allele in the

From the Divisions of Medical Genetics and Molecular Pathology, Departments of Pathology & Laboratory Medicine, Pediatrics, and Human Genetics, UCLA School of Medicine, Los Angeles, California.

Wayne W. Grody, MD, PhD, Divisions of Medical Genetics and Molecular Pathology, Departments of Pathology & Laboratory Medicine, Pediatrics, and Human Genetics, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90095. E-mail: wgrody@mednet.ucla.edu.

Disclosure: The author declares no conflict of interest.

DOI: 10.1097/GIM.0b013e31823c49a2

US population recently ascertained at 1 in 178.[6] Given this frequency and the risk of the premutation CGG repeat to expand into the full mutation range in the subsequent generation, it has long been accepted practice to offer premutation carrier testing in the prenatal setting when there is a family history of developmental delay, learning disabilities, autism, or any of the other neurologic or physical manifestations suggestive of the disorder.

Over the past few years, however, many laboratories have noticed a several-fold increase in *FMR1* test requests being ordered on pregnant women. It seems that this is the result of a shift in screening protocol, from offering the test only to those women with a suggestive family history, to offering it to women of reproductive age with no family history. Yet it can be argued that fragile X syndrome and the molecular biology of the *FMR1* gene are significantly more complex than the other single-gene screening targets discussed here. In particular, it is concerning that the carrier state being screened for—the CGG premutation allele—is itself disease causing, unlike the heterozygous carrier mutations screened in autosomal recessive diseases such as CF. This refers, of course, to the still poorly understood, late-onset syndromes of fragile X-associated premature ovarian insufficiency in women and fragile X-associated tremor and ataxia syndrome in men (and maybe women) who carry premutation alleles.

Consider the experience thus far with this expanded *FMR1* premutation screening in all pregnant women, which is not unexpected. The majority of these women test negative and go on with their pregnancies unbothered. But given the relatively high premutation population frequency and the large numbers of women now being screened, an appreciable number show up positive as carriers. The majority of these positive alleles are in the low-premutation range (<65 CGG repeats), which is known to carry a low risk of expansion into the full-mutation range in the next generation.[7] Yet all of these women will be offered prenatal testing, and most will accept. Because of the low risk of expansion, nearly half of their fetuses will be found to carry the same premutation allele as the mother, or perhaps one slightly expanded although still in the premutation range. The mother is then reassured that her child will not have fragile X syndrome. But what of the late-onset effects of the premutation? If these mothers are counseled that their child has a premutation with risk of potentially devastating effects in adulthood, then we have essentially performed predictive/presymptomatic genetic testing on a child for an adult-onset disease for which there is no known prevention or treatment (except perhaps for harvesting and freezing oocytes from young women before the onset of premature ovarian insufficiency). Such predictive testing in children for a disorder which has no effective intervention to be initiated during childhood has long been recognized in the genetics community as unethical.

Examples like these have not always been the trend, and there are numerous instances in the past in which the genetics community, and the College in particular, have voluntarily stepped back from potentially lucrative screening targets after careful evidence-based assessment. In the same year that our recommendations for universal CF carrier screening were issued, another consensus statement in this journal argued strongly against doing the same for factor V-Leiden on the grounds that its penetrance is so low that the risk of prophylactic intervention (oral anticoagulant administration) causing a major bleed far exceeds the risk of a thromboembolic event caused by the mutation itself.[8] Similar conclusions have been reached regarding population screening for hereditary hemochromatosis (*HFE* gene) mutations, despite the availability of a rather benign prophylactic therapy (phlebotomy). In the face of a shockingly high carrier frequency of particular *BRCA1* and *BRCA2* mutations in the Ashkenazi-Jewish population, it was nevertheless judged inappropriate to offer general mutation screening in this group in the absence of personal or family history of breast/ovarian cancer, again because of questions of penetrance in an unselected population and the risk of unintended consequences as a result of screening, which in this case includes unnecessary and irreversible prophylactic surgery. And irrespective of much commercial hype and a significant "scare factor," the College has not endorsed routine pharmacogenetic screening for patients prescribed warfarin for thrombotic problems.[9] All of these are examples where our better natures prevailed and we chose not to proceed with population-based screening (i.e., in the absence of other indications), despite superficially appealing medical and economic inclinations to do so. Meanwhile, a challenging unsettled example is the case of spinal muscular atrophy, where an American College of Medical Genetics recommendation for general prenatal carrier screening exists[10] but uptake has been slow due to the technical difficulty of the DNA test, uncertainties about genotype-phenotype prediction, and pushback by the obstetricians.[11]

In light of the current disarray in the field, the American College of Medical Genetics has appointed a committee to develop general guidelines and criteria for determining if and when a particular allele target is suitable for inclusion in a population carrier screening panel. Of course, we cannot police every last rare disease or mutation, and such decisions must ultimately be left to the discretion of the laboratory director. Moreover, it is possible that in time the incorporation of whole-exome sequencing may render many such decisions moot. But given the inability of ordering physicians and patients to meaningfully filter variant screening results, we are the ones entrusted with assuring that what is tested and reported is of clinical import and appropriately actionable. Indeed, that is the unique value that medical geneticists bring to the table, and to shirk that responsibility virtually guarantees that we will have to contend with unintended and very unpleasant consequences.

REFERENCES

1. Rutkow IM, Lipton JM. Some negative aspects of state health departments' policies related to screening for sickle cell anemia. *Am J Public Health* 1974;64:217–221.
2. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ; Subcommittee on Cystic Fibrosis Screening, Accreditation of Genetic Services Committee. American College of Medical Genetics. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* 2001;3:149–154.
3. Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med* 2004;6:387–391.
4. Grody WW, Cutting GR, Watson MS. The cystic fibrosis mutation "arms race": when less is more. *Genet Med* 2007;9:739–744.
5. Strom CM, Redman JB, Peng M. The dangers of including nonclassical cystic fibrosis variants in population-based screening panels: p.L997F, further genotype/phenotype correlation data. *Genet Med* 2011;13:1042–1044.
6. Hantash FM, Goos DM, Crossley B, et al. FMR1 premutation carrier frequency in patients undergoing routine population-based carrier screening: insights into the prevalence of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and fragile X-associated primary ovarian insufficiency in the United States. *Genet Med* 2011;13:39–45.
7. Nolin SL, Lewis FA, 3rd, Ye LL, et al. Familial transmission of the FMR1 CGG repeat. *Am J Hum Genet* 1996;59:1252–1261.
8. Grody WW, Griffin JH, Taylor AK, Korf BR, Heit JA; ACMG Factor V. Leiden Working Group. American College of Medical Genetics consensus statement on factor V Leiden mutation testing. *Genet Med* 2001;3:139–148.
9. Flockhart DA, O'Kane D, Williams MS, et al; ACMG Working Group on Pharmacogenetic Testing of CYP2C9, VKORC1 Alleles for Warfarin Use. Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. *Genet Med* 2008;10:139–150.
10. Prior TW; ACMG Professional Practice and Guidelines Committee. Carrier screening for spinal muscular atrophy. *Genet Med* 2008;10:840–842.
11. ACOG Committee on Genetics. ACOG committee opinion No. 432: spinal muscular atrophy. *Obstet Gynecol* 2009;113:1194–1196.