Commentary

You Can Build It . . . But Will They Come?

The Potential “Expansion” of Testing Methodologies for Fragile X Syndrome

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Many years ago, when I was a clinical molecular genetics fellow, our laboratory received a hand written request for “DNA *leakage* studies for Fragile X.” Of course we knew exactly what the clinician meant, since DNA *linkage* analysis was the molecular test of record at the time as the initial reports of the causative mutation underlying the disorder were just beginning to appear in the literature.^{1–4} However, we also understood the challenges that many practitioners were facing as they tried to grasp (both conceptually as well as linguistically) the role that molecular diagnostics was beginning to play in the contemporary practice of medicine. Nevertheless, we still had a good laugh.

Well, much has changed since then. From our understanding of the molecular genetics and pathobiology of Fragile X syndrome (FXS) and its related disorders (fragile X associated tremor/ataxia syndrome and premature ovarian failure), to the development of technical standards and guidelines for FXS testing⁵ (http://www.acmg.net/Pages/ACMG_Activities/stds-2002/fx.htm, accessed March 9, 2009) and the evolution of methodological approaches for the detection and classification of *FMR1* alleles,^{1–4,6–13} the study of FXS and *FMR1* related disorders provides an excellent example of the development of molecular diagnostics and, over a 20-year period, instructive case studies for the successful integration, adoption, and reimbursement of new and novel molecular tests.

In this issue of the *Journal of Molecular Diagnostics*, Iwahashi and colleagues,¹⁴ in a brief technical report, describe an enhancement to an antibody-based enzyme-linked immunosorbent (ELISA) assay for the detection of the *FMR1* protein (FMRP) in peripheral blood. The authors demonstrate that with the generation of an affinity-purified chicken anti-human FMRP peptide-specific capture antibody and a murine anti-FMRP detection antibody, they could develop a highly sensitive and specific sandwich ELISA that was suitable for the detection and quantitation

of FMRP. Of note is the ability of this assay to accurately and reliably quantitate FMRP levels across the entire range of CGG expansion and to use non-transformed peripheral blood lymphocytes for the analyses. The authors conclude that the “FMRP ELISA is potentially a powerful tool in expanding our understanding the relationship between FMRP levels and the various *FMR1*-associated clinical phenotypes.”

This manuscript is instructive for two reasons. First, through the development of a simple and robust immunochemical assay, the authors have provided a relatively straightforward, inexpensive, scalable, and potentially automatable assay to study the role of variable FMRP expression in Fragile X syndrome and the *FMR1* related disorders. This is accomplished by the author’s recognition of the inherent utility in generating anti-avian antibodies when studying a protein (FMRP) that maintains strong phylogenetic conservation across mammalian species. Clearly the authors understood and appreciated the challenges engendered by the original concepts of immunological self tolerance,^{15–16} and developed a practical work around. This was a critical insight. By using the chicken as the host for the production of the primary antibody reagent coupled with specific targeting of the carboxyl terminal domain of FMRP, they were able to generate an antibody with a high enough affinity to meet the performance and sensitivity requirements of the ELISA. Second, and more germane to the title of this commentary, this technical report also provides an excellent opportunity to discuss the challenges associated with the demonstration of the clinical utility of technologies with potentially novel applications. While the authors clearly make no direct claims that, outside of a research setting, this test currently has any clinical applicability, it provides a very nice model for highlighting the processes that are involved in the evo-

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lution and transition of robust clinical research applications to tests with defined clinical utility.

In vitro diagnostic manufacturers and molecular diagnostics companies frequently talk in terms of systems placed and reagents sold. Conversely, molecular pathologists and laboratory diagnosticians commonly discuss test adoption patterns and reimbursement. These are not mutually exclusive concepts. The maturation and acceptance of a novel test or application reflects the relative weight and contribution from all of these variables. To some extent this process can be modeled, as has been recently done for *HLA-B*5701* screening.¹⁷ However, while this approach is instructive for highlighting the variables and concurrent processes that contribute to the successful adoption of a molecular diagnostic test into clinical practice, the ultimate checkpoint with regard to the integration of testing into routine clinical care remains the demonstration of clinical utility.

Establishing clinical utility is usually incremental in nature.¹⁷ For diagnostic tests, the demonstration of robust analytical, and more importantly, clinical performance claims can collectively drive the development of practice guidelines, consensus statements, and test adoption. This evidence-based medicine approach also drives the valuation of the test itself, ultimately resulting in reimbursement. This is the model routinely used by third party payers, like BlueCross BlueShield and others, to determine whether new or novel technologies meet the standard for improving health outcomes (<http://www.bcbs.com/blueresources/tec/tec-criteria.html>, accessed March 9, 2009).

With specific regard to FXS, the evolution of testing from DNA linkage to Southern blot to PCR for diagnostic testing, carrier testing and eventually newborn screening is the direct result of technological and methodological enhancements that that happen to sync very nicely with our increased breadth and depth of understanding of the disorder. Although these tests (Southern blot and PCR) have different strengths and weaknesses and can be used in either a mutually exclusive or complementary manner, their dual existence continues to underscore their validity and reflects their wide-spread acceptance, adoption, and clinical utility.

So where does protein-based testing for a disorder whose primary clinical phenotype is manifested by changes within the central nervous system and ovaries fall on the spectrum of clinical utility? Are the most relevant clinical samples accessible or available to test? Is there incremental clinical benefit derived by knowing the expression level of FMRP in peripheral blood or would knowledge of FMRP levels affect changes in treatment or current standard of care or support a letter of medical necessity for testing? Clearly, these issues remain unresolved and must wait, as the authors allude to in the final sentence of the discussion, for the design and completion of further large scale studies "to recognize the value of the measurement and how FMRP influences the multitude of phenotypes associated with the *FMR1* gene."

In summary, FMRP testing, when viewed from the perspective of current clinical utility, remains a clinical research application whose place on the continuum of FXS testing has yet to be determined. However, when one views disease specific molecular diagnostic testing as a

series of continuous enhancements and improvements rather than as a set of specific applications one can begin to appreciate the potential for novel applications that have yet to be fully realized. You see once on a time we all did "DNA *leakage* studies for Fragile X" and as anachronistic as this may seem today, at *that* time it was the best test for a yet to be molecularly characterized genetic disorder.

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