Clinical Chemistry

Early Detection of Fragile X Syndrome: Applications of a Novel Approach for Improved Quantitative Methylation Analysis in Venous Blood and Newborn Blood Spots

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Guest: Dr. David Godler is a senior research fellow at the Victorian Clinical Genetics Services and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia.

Bob Barrett: This is a podcast from C*linical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Fragile X Syndrome is a severe neurodevelopmental disorder which is both complex and heterogeneous in both clinical phenotype and epigenotype. It is also one of the major inherited conditions co-morbid with autistic behaviors.

A paper in the July 2014 issue of *Clinical Chemistry* described a new Methylation Specific – Quantitative Melt Analysis method that has an immediate application in Fragile X Syndrome diagnostics. The senior author of that paper, Dr David Godler, is a Senior Research Fellow at the Victorian Clinical Genetics Services and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia and he is our guest in this podcast.

So doctor, what were the most significant findings of this study?

Dr. David Godler: The most significant and unexpected finding was that our new methylation assay could detect between 92% and 100% of all full mutation males and females in new born blood spots, without picking up FMR1 premutation allyls that are associated with increased risk of latency disorder. One 3 mm blood spot was all that was require; re-validated in future studies, this could finally make possible cost-effective, ethically acceptable, new born screening program for Fragile X Syndrome.

A non significant finding was that in various blood samples from full mutation full mutation females, there was significant correlation between MS-QMA methylation output and variable cognitive impairment.

The methylation output was not significantly associated with age. Together, these highlight potential prognostic utility of our new test in Fragile X females and finally, the last finding of importance was that MS-QMA could be used to analyze methylation of CpG sites that the reference method based

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on the EpiTYPER system could not, and this was due to the fragments being of large size. So in fact our test proved to be more informative than the EpiTYPER reference system.

Bob Barrett: Considering that methylation testing for the Fragile X gene FRM1 is already provided by the standard southern blot based testing protocol and by a number of other previously developed PCR and MLPA-based Fragile X Syndrome commercial kits now used in many diagnostic laboratories, could you please tell us what drove you to develop this MS-QMA test?

Dr. David Godler: With respect to targeted testing another, in other words, for children referred for testing due to developmental delay or unknown cause, the two main issues motivated us to develop this new test.

First, we were driven from basic science perspective to shed light upon the genetics and epigenetics for fragile X related conditions. This is the research focus of my group. Second the exiting test did not work same in males versus females, you can't have a test that only works in half of our population.

Let me comeback to this, one of the first issues in terms of shedding light on the field, there used to be a better understanding among physician and diagnostic professional about what methylation of the FMR1 gene really means. Some old tests appeared to include methylation analysis but we have to ask, ask what is being analyzed? All methylation tests are not the same. The selection of relevant DNA regions and the specific set of methylation sites within those regions is crucial. The assay performance is equally important. So it's not only what size you analyze, it's also how you go about analyzing them. This is why a MS-QMA is unique.

When considering the medical diagnostic utility of any particular methylation test, it is essential to understand whether the methylation is homogeneous between all regions targeted; does it change with time in between tissues, and what should be the lower limit of detection, especially for mosaic cases, for methylated allyls.

These factors are all relevant to prognostic and diagnostic utility of Fragile X methylation testing, but are largely not included in the current testing guidelines. Since they are not in the guidelines, they are often ignored and current market tests have limited potential benefits for the patients.

On the other hand, FREE2 MS-QMA appears to overcome several of these important limitations. The second and no less important issue is that the standard southern blot based

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protocol, and other fragile X diagnostic kits that provide Methylation information, lack demonstrated prognostic utility in females that carry fragile X mutations. This is extremely important considering that phenotype is highly variable in females with approximately half having no cogitative impairment IQ greater than 70. This is due to protective effects of X-inactivation. FREE2 MS-QMA works equally well in both sexes and it overcomes this problem of X- inactivation in females.

Bob Barrett: So what is so special about the MS-QMA methylation test?

Dr. David Godler: The MS-QMA targets methylation of a region that we have named fragile X-related epigenetic element 2, which we found to be clinically more informative in both sexes. The methylation of FMR1 Cpg island. The standard region targeted by Southern blot and previously developed fragile X diagnostic kits.

MS-QMA also has one of the lowest, if the lowest limit of quantitative detection of methylated fragile X allyl which is only 2%. This is important for detection of mosaic individuals that carry low abundance, methylated fragile X allyls that may be missed otherwise.

Bob Barrett: Doctor, in your earlier work published in *Clinical Chemistry* in 2012, you've used Sequenom's EpiTYPER system to analyze the free II region. Could you comment on utility of the EpiTYPER system based analysis in fragile X and why do you decided to switch to theMS-QMA?

Dr. David Godler: This is very good question. Our earlier work using the EpiTYPER system strongly suggests that FMR1 methylation is not homogeneous entity. We found that methylation can vary significantly in space and time between CpG biomarkers located in close proximity to each other, and between tissues of special and full mutation females.

So the clinical utility of a methylation test largely depends on CpG sites it targets. In 2009, we used Sequenom's EpiTYPER system to characterize epigenetic status of the single based fair resolution or the FMR1 CpG island. The standard region targeted as well as the proximal regions. Another region was discovered on FMR1, exon 1, intron 1 boundary expanding downstream into FMR1, intron 1, this was the FREE2 region.

Methylation of FREE2 sites located within a FMR1, intron 1 were significantly associated with the levels of FMR1 protein product and severity of cognitive impairment in full mutation males and females. These did not change significantly with the agent between tissues. Some of these works was published in 2012 in our earlier *Clinical Chemistry*

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manuscript. We suggested that FMR1 promoter is much larger than previously thought.

It also became apparent that FREE2 biomarkers might have a unique role in providing prognostic information. But the main limitation was that most Fragile X research and diagnostic laboratories did not have access to the EpiTYPER system used to discover FREE2. So we developed inMS-QMA primarily to make it more accessible to other laboratories. The special thing about MS-QMA and the main reason why we developed it, is that its main requirements in terms of investment and training and resource is at minimal comparison.

This access to a real-time PCR machine can also be used to perform high resolution melt analysis. This is present in most research and diagnostic labs today and no specialized requirements for extensive training or reagents. The MS-QMA uses standard high resolutions melt reagents with total cost of about one third of the reagents required for the EpiTYPER based reference method.

Other attractive features are the turnaround time of only two hours post conversion; very high throughput and minimum DNA quality and quantity requirements; as well as fully automated analysis.

Bob Barrett: Well! Finally doctor let's look ahead in your view what does the future hold forMS-QMA?

Dr. David Godler: A number of cross-sectional and longitudinal validation studies in males and the female are now underway to reproduce and go beyond our 2014 *Clinical Chemistry* paper. Their focus is on prognostic utility of MS-QMA for cognitive and behavioral impairments that can be potentially improved through early intervention.

Data from one of these validation studies on an independent cohort will be presented at the 14th International Fragile –X Conference, Orange County, California, on 18 July this year as part of the molecular studies and screening session. The data collected so far strongly suggests thatMS-QMA analysis of FREE2 biomarkers has a potential to be market disruptive as a next generation in diagnostics of fragile X, ASD and fragile X-related conditions with important applications for both targeted testing and newborn screening.

Our institute has solid active position directed towards the use of FREE2 and other associated regions as well as of the broader patent application covering theMS-QMA technology beyond fragile X. In partnership with Bio-Link Australia, especially Biotechnology Business Development

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Consultancy, we're pursuing various strategies including licensing for global commercialization of the technology.

Bob Barrett: Dr. David Godler is a senior research fellow at the Victorian Clinical Genetics Services and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia, and he has been our guest in this podcast from *Clinical Chemistry* on Fragile X Syndrome diagnostics.

I am Bob Barrett. Thanks for listening!.

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