Genotype–Phenotype Correlations of Glucose-6-Phosphate–Deficient Variants Throughout an Activity Distribution

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Guest: Dr. David Grenache is the Chief Scientific Officer at TriCore Reference Laboratories and Clinical Professor of Pathology at the University of New Mexico.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

Glucose-6 phosphate dehydrogenase (G6PD) deficiency is a genetic disorder that mostly affects red blood cells. A low activity of this enzyme can cause red cells to lyse leading to a hemolytic anemia and elevated bilirubin. The gene that codes for the enzyme is located on the X chromosome, so the disorder is more common in males than females.

Most affected males and homozygous females have very low enzyme activities while heterozygous females may have a range of activities. Several testing options are available for identification of G6PD deficiency but these do not perform equally across all genotypes and populations.

"Genotype–Phenotype Correlations of Glucose-6-Phosphate–Deficient Variants Throughout an Activity Distribution" was published in the May 2018 issue of *The Journal of Applied Laboratory Medicine*. This work examines G6PD mutations across a range of enzyme activities and seeks to establish enzyme activity cutoffs that could be implemented to rule out G6PD deficiency.

The first author is Dr. David Grenache. Dr. Grenache is the Chief Scientific Officer at TriCore Reference Laboratories in Albuquerque, New Mexico, and clinical professor of pathology at the University of New Mexico. He is our guest for today's podcast. Welcome, Dr. Grenache.

For our first question, I'd like to know, what was the reason that you performed the study? Why was it important to do?

David Grenache: Well, first, let me give you some background information to help frame my answer to those questions. So, G6PD deficiency is the most common enzyme deficiency worldwide, and it affects about 400 million people. It's a red blood cell enzyme and it functions to maintain NADPH and reduced glutathione at sufficient concentrations inside

of the red cell. And in its absence, NADPH and reduced glutathione end up being low and then red blood cells often can lyse due to oxidative damage.

The clinical manifestations of a G6PD enzyme deficiency include things like acute hemolysis, chronic hemolytic anemia, neonatal hyperbilirubinemia, but remember that some people can also be asymptomatic. G6PD deficiency is an X-linked inherited disorder that most commonly affects persons of African, Asian, Mediterranean, and Middle Eastern descent. Both homozygotes and heterozygotes can be symptomatic, but the disease is typically more severe in people who are homozygous for the deficiency.

The diagnosis of G6PD deficiency is usually made by measuring the enzyme activity inside of red blood cells. And genetic tests are available but they usually look for specific common mutations. And because there are hundreds of mutations that can lead to a deficiency, targeted screening can miss some individuals with rare enzyme variants.

On the other hand, enzymatic activity tests, they have a high diagnostic sensitivity and specificity for detecting very deficient males or homozygous females, but they have a lower sensitivity for detecting heterozygous females. And that's because random X chromosome inactivation leads to these heterozygous females having a mixture of both G6PD deficient red cells and G6PD sufficient red cells.

So, they end up showing a wide range of enzyme activities which makes the partial deficiencies difficult to detect. And that's important because I'm frequently asked if G6PD enzyme testing can be used to identify heterozygous females. So, we did the study to examine the relationship between an individual's G6PD enzyme activity, that is the phenotype, and their G6PD genotype. And by doing so, we hope to be able to identify an enzyme activity cutoff above which a G6PD deficiency would be considered unlikely for both males and females.

Randye Kaye: I see. So, how robust is that relationship between the phenotype and genotype?

David Grenache: Well, it depends on the individual sex and genotype. So, for example, hemizygous males and homozygous females that inherit a gene or genes that causes a severe deficiency will have extremely low G6PD activities.

But heterozygous females could have activities that range from deficient to sufficient. Also, different mutations in the G6PD gene lead to different deficiency phenotypes that are grouped into classes. And they're grouped in classes -- identified as group one, two, three and four. Class one and

two variants are the severe deficiencies. And class three variants have a moderate deficiency while class four variants really show mild to, often, no enzyme deficiency.

In our study, all of the males and the homozygous females with the Class 2 mutations did have very low activities. But in contrast, we had 19 females that were heterozygous for a Class 3 mutation and they had activities that range from 2.4, which is very deficient to 9.4, which is just barely below an established reference interval. And that's why using G6PD activity tests to identify heterozygous females is so challenging.

Randye Kaye: So, this study, it sounds like you already have explained some of the findings. Are there any other novel findings from this study you'd like to tell us about?

David Grenache: Sure, yeah. I think there were really probably three novel findings. First, we were able to identify G6PD activity cutoffs above which the presence of an abnormal G6PD variant was unlikely. So, for males, this cutoff was 7.85 and it was 100% sensitive, meaning that an activity above that cutoff would rule out the possibility that the male had a G6PD deficiency. A female cutoff was not as robust because, as I've already explained, females can be heterozygous. Accordingly, we found that a G6PD cutoff of 8.95 was 90% sensitive at identifying the presence of an enzyme variant.

The second novel finding is that it may be inappropriate to strictly categorize G6PD mutations into one of four classes. So, for example, our study included three males that were homozygous for a variant called, "Viangchan." That's often in the literature described as a Class 2 variant with severely deficient activity. The G6PD activity of two of those males in our study were indeed severely deficient, but one of those males had an activity that was more consistent with a Class 3, or a moderate, deficiency variant.

We think that for many Class 2 and Class 3 variants, if enough people are examined, a wide range of enzyme activities will be found. So, it may not always be appropriate to think of a particular variant as either Class 2 or Class 3.

And then lastly, we discovered a new G6PD variant that we predicted would cause severe deficiency based upon its extremely low activity. We named this variant, "G6PD Salt Lake," based on where it was discovered. We were in Salt Lake City. But unfortunately, our samples for the study were de-identified, so we couldn't correlate that new variant that we discovered with any clinical data from the patient that it come from.

Randye Kaye: So, what's next? Like what could be done next to expand on the conclusions of the study?

David Grenache: Well, it's a good question. Our study was really limited by the number of samples that we were able to include, and our inability to access the clinical information on the patients from whom those samples came from.

Our conclusions could be definitely strengthened and expanded by collecting G6PD genotype and activity data from patients who are known to have G6PD deficiencies as well as documenting their clinical phenotypes as well. Also, our cutoffs were determined from a population that ranged in age from newborns to 81 years old, and it's likely that different G6PD cutoffs would be needed for newborns if they were to be applied to neonatal screening. Performing a similar study on a population of only infants and young children would definitely be something that would be interesting to pursue.

Randye Kaye: All right, very interesting. Thank you so much for joining us today.

David Grenache: You're welcome. It's my pleasure. Thank you for having me.

Randye Kaye: That was Dr. David Grenache, the chief scientific officer at TriCore Reference Laboratories talking about genotype-phenotype correlations of glucose-6 phosphate deficient variants throughout an activity distribution from the May 2018 issue of JALM for this podcast. Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about.