What You Should Know About Molecular Genetic Testing
By Carol Saunders, PhD

Advances in the field of molecular genetics have made it possible to identify many disease-related genes and test for pathological mutations. There is no question that DNA testing can enhance the quality of medical care for patients and their families. Such benefits include the pre-symptomatic identification of disease susceptibility, preventative intervention, selection of pharmacotherapy, and carrier testing for at-risk relatives. However, we are a long way from the science in movies, where a drop of blood can tell you everything you need to know about your patient's gene function. The following will explain some of the limitations of DNA testing and list some considerations.

1. Are you using DNA testing to make or rule out a diagnosis? If so, is it appropriate for this particular disease?

Some disorders are easily diagnosed or ruled out with a single DNA test. This is generally because there is a common mutation or mutations in a single gene. Testing of this type is very sensitive and relatively inexpensive. (Example: Fragile X Syndrome). However, molecular testing for many diseases should ideally wait until a firm clinical diagnosis can be made. This may be for several reasons: the test is laborious (requiring complete sequencing of a gene), the disease is associated with more than one gene, and/or the detection rate is low. Since a diagnosis cannot be ruled out based on a test that is only 20% sensitive, testing of this type is best used to confirm a clinical diagnosis. (Examples: Neurofibromatosis, Tuberous Sclerosis).

2. Are you interested in DNA testing for your patient because he/she is unaffected and has a family history?

To achieve maximum sensitivity, the affected family member must be tested first to assure the mutation is detectable.

Not all parts of a gene are analyzed, and mutations sometimes lie in untested regions where we would not expect them. Once a mutation is known, the at-risk family members can be tested for that single mutation, rather than analyzing the whole gene in each person. This is much less expensive, and you can be sure a negative test result is not a false negative!

3.) Is your patient's ethnic background a factor?

The sensitivity of a DNA test may vary tremendously among different ethnicities, and may not even be appropriate or available for some ethnic groups. This is because some mutations are very common in certain populations, but very rare in others. An example is Canavan Disease, which is more prevalent in people of Ashkenazi Jewish decent. The detection rate in this population is close to 100% and only requires testing for 2 mutations. By contrast, these same two mutations only detect 3% of Canavan mutations in non-Jewish populations. For this and other tests, it is important to know which mutations are covered by testing and what your sensitivity rate is for your patient's ethnicity!

4.) Is clinical testing available? Just because a gene has been identified does not mean there is a good clinical test out there. In this case, you may be able to interest a research lab. Be aware that research labs require a signed consent form and may or may not make the results available to the patient.
Diagnostic Approach to Anemia in Children
By David Zwick, MD

Clues of the cause of anemia in a child come from consideration of the circumstances (e.g. congenital, acquired, dietary history, family history, associated syndromes, evidence of hemorrhage, and manifestations, etc), CBC and reticulocyte count results and smear morphology. Signs or symptoms of hemolysis (jaundice, hematuria, splenomegaly), blood loss (hematochesia, orthostatic hypotension), and myelosuppression (drug exposure, recent viral infection, etc.) might provide additional clues as to the etiology and aid directing further testing.

The smear findings can be very helpful and provide indication whether the anemia is hemolytic and occasionally point to a specific cause such as membrane defects (spherocytes, acanthocytes, elliptocytes), hemoglobinopathies (sickle cells, target cells, microcytes), microangiopathic (Hemolytic Uremic Syndrome, D.I.C.), nutritional deficiencies (microcytosis and hypochromia, macrocytosis and hypersegmented polys), enzymopathies (bite cells, burr cells, etc), and underlying chronic disease-related anemias (liver disease-target cells, renal disease-burr cells).

The reticulocyte count provides indication of the appropriateness of marrow response, and, when low, indicates an ineffective marrow response or a very acute loss of blood occurring prior to marrow response. Previous newsletters discussed the usefulness of the reticulocyte and immature reticulocyte index and highlighted their importance in understanding physiologic response. (See November 2002)

The following algorithm for evaluation of anemia in neonates comes from Hematology of Infancy and Childhood by Nathan and Oski; both are useful for guiding the diagnostic evaluation that recognize differing presentation and risk factors dependent upon age.

**Diagnostic consideration in Non-Neonates based on RBC size (10 physiologic basis)**

- **Microcytic**: (Impaired Hgb production in setting of appropriate driving stimuli)
  - Iron deficiency
  - Thalassemia
  - Lead poisoning
  - Sideroblastic anemia- dimorphic microcytic/ normocytic rbc's
  - Anemia of Chronic disease

- **Normocytic**: (Normal drive and production with shortened RBC survival)
  - Immune hemolytic anemia (AIHA)
  - Hemoglobinopathies (SSD)
  - RBC membrane abnormalities (HS)
  - RBC enzymopathies (G6PD)
  - INADEQUATE RETICULOCYTE RESPONSE: (loss of driving stimuli or loss of or impaired stem cell proliferation)
    - Renal insufficiency
    - Anemia of Chronic Diseases
    - Endocrine disorders e.g. hypothyroid and hypopituitarism
    - marrow replacement and marrow failures diseases

- **Macrocytic**: (Dysregulated and dysplastic precursor proliferation)
  - Megaloblastic: nutritional insufficiencies (B12 and Folate)
  - Non-megaloblastic: Liver disease, hypothyroidism, some hemolytic anemias, myelodysplasia, Fanconi's anemia.

Figure 2-9. Diagnostic approach to anemia in the newborn based on reticulocyte count. Indicates a peripheral blood smear with no specific diagnostic abnormalities.

Charles Barnes, PhD; Linda Cooley, MD; Uttam Garg, PhD; Marilyn Hamilton, MD, PhD; Joan Knoll, PhD; Carol Saunders, PhD; Lei Shao, MD; Rangaraj Selvarangan, PhD; Eugenio Taboada, MD; M. J. Willard, MD, Fellow; David Zwick, MD