

Evaluating Pharmacogenetic Tests

A Case Example

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Work in human genetics is revealing genes that affect the safe and effective use of pharmaceuticals. Primary care physicians are already receiving advertisements for tests for these variations that emphasize the value of learning about their patients' susceptibility to adverse reactions in order to avoid them. We propose a strategy for the critical evaluation of such offers to ensure the optimal use of these tests.

Arch Pediatr Adolesc Med. 2004;158:276-279

We live in an era of the promise of individualized medicine, in which we are told that genetic tests will tell us which medications we should use for our patients and which we should avoid. Concurrently, much concern has been expressed about how to ensure the appropriate use of genetic tests. Recently, one of us received an advertisement from Athena Diagnostics, Inc, Worcester, Mass, which led us to ask what pediatricians need to think about regarding genetic testing of children in relation to medication use. Using this advertisement as a case study, we have devised a framework to evaluate when and for whom genetic testing for medication response would be valuable.

The specific advertisement was for an aminoglycoside hypersensitivity test, intended to "identify hypersensitive patients before they are placed at risk of hearing loss." The rate of an ototoxic reaction, in general, with aminoglycosides is not known exactly, but in a meta-analysis of 8 studies in children, the overall frequency of ototoxic reactions from this class of drugs was 3.5% (9/254).¹ The promise of avoiding antibiotic-induced deafness is clearly a laudable goal, but evaluating a promise that a genetic test can prevent hearing loss caused by an aminoglycoside-induced ototoxicity requires consideration of the genetic features of the mutation itself, the test's

characteristics, and the way these drugs are used in this population.

THE MUTATION

The test detects a mitochondrial mutation, A1555G, in the 12S ribosomal RNA gene. The mitochondrion is an organelle with its own genome. The mutation affects one of the RNA species required for mitochondrial protein synthesis. Complicating our understanding of the influence of this mutation on cellular biology is the fact that each cell carries hundreds of mitochondria, and thousands of copies of the mitochondrial genome per cell.² An individual may be homoplasmic mutant, normal (all mitochondrial genomes carry the mutation or are normal), or heteroplasmic (various percentages of the mitochondrial genomes are normal or mutant). The role of heteroplasmy in the penetrance of this mutation is unclear. Tests for this genetic mutation are available from at least 7 other laboratories in addition to Athena Diagnostics, Inc.³ One study suggests the A1555G allele is found in approximately 0.09% of the population.⁴

In general, mitochondrial mutations account for less than 1% of all non-syndromic deafness.⁵ The A1555G mutation increases the risk of ototoxicity from aminoglycosides. In the United States, the A1555G mutation accounts for 15% of aminoglycoside-induced deafness.⁵ The rate is even higher in other populations in which aminoglycosides are used more fre-

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quently.⁶ In individuals who are susceptible to this adverse effect of aminoglycosides, hearing loss may occur immediately or may only be evident over time.⁷ Even so, not all persons who have this mutation develop hearing loss when exposed to these drugs.⁷ Nuclear genes have been proposed that may modify the penetrance of hearing loss due to the *A1555G* mutation. The first is as yet unidentified but is localized to chromosome 8.⁸ More recently, 2 other nuclear genes have been proposed as possible modifiers of the hearing loss phenotype based on their function in yeast. These are *MTO1* (chromosome 6)⁹ and *GTPBP3* (chromosome 19)¹⁰ that encode mitochondrial transfer RNA modification proteins. Noise exposure seems to play a role as well.¹¹ Hearing loss has been documented to occur because of the *A1555G* mutation without exposure to aminoglycosides. In 1 study, deafness was shown to occur with 50% penetrance by the age of 30 years and with 88% penetrance by the age of 65 years.¹² Even in these families, the expression of the phenotype varies, with the age of onset of hearing loss varying from infancy to adulthood.⁵

In 1 review of medical records at 2 institutions, hearing loss was attributable to *A1555G* mutations in 7 of 41 patients who had aminoglycoside-induced ototoxicity.⁷ Assuming that these data about the mutation frequency and the rate of ototoxicity can be replicated in future studies, the sensitivity of the test to detect those at risk of developing an ototoxicity (the number of *A1555G* mutations detected in patients who have an ototoxic reaction divided by the total number of people who have an ototoxic reaction) is 7 of 41 or 17%. Significantly, 83% of people who have an ototoxic reaction would not have been protected by being tested for the *A1555G* mutation. Thus, if the *A1555G* mutation test had been done before aminoglycoside exposure, 34 people still would have suffered hearing loss. Those individuals may have had a different mutation in the 12S ribosomal RNA gene, such as 961delT + C_(n),⁴ or a mutation in a gene as yet unidentified as related to aminoglycoside hypersensitivity.

Notably, 4 of the 7 *A1555G* mutation-bearing individuals had a maternal family history of aminoglycoside-related hearing loss.⁷ Thus, maternal family history of ototoxicity may provide a substantial indication of an individual child's risk for having an ototoxic reaction. Family history cannot be relied on alone, however, since the absence of a family history of hearing loss may be a false-negative report. Since relatively few people in the United States receive aminoglycoside agents, a negative family history may be because of a lack of exposure to these drugs. Furthermore, some people who experienced an aminoglycoside-induced ototoxicity in the presence of the *A1555G* mutation had a mild initial hearing loss that progressed over many years.⁷ Slowly progressing hearing loss due to an aminoglycoside-induced ototoxicity in a family member may result in a report of a negative family history.

TEST FEATURES AND TARGET POPULATION

It is not enough simply to understand the biological consequences of the genetic mutation in deciding whether

Settings in Which *A1555G* Genetic Testing May Prevent an Ototoxic Reaction

Whom to Test	Who Could Be Protected
Children or adults who have experienced an ototoxic reaction	Maternal relatives
Maternal relatives of individuals who have experienced an ototoxic reaction	The tested individual (in case aminoglycoside therapy is ever considered)
Children with chronic medical conditions such as cystic fibrosis, for whom aminoglycoside exposure is likely	The tested individual

to use a genetic test. One must also examine the practical aspects of the tests. In the test from Athena Diagnostics, Inc, DNA from 8.5 mL of whole blood is assessed by restriction fragment length polymorphism. Results are returned in 7 to 14 days. These facts are important given the way these drugs are used.

Aminoglycosides are among the most frequently used antibiotics in pediatric inpatient care for infections involving gram-negative organisms. Clinical situations that warrant aminoglycoside usage may include prophylaxis and treatment of sepsis and pneumonia for neonatal and pediatric populations, kidney and urinary tract infections in children of all ages, and serious pulmonary infections for patients with cystic fibrosis. Even so, the overwhelming majority of children never receive these drugs during their lifetimes, and it is usually impossible to predict beforehand which children are going to need them. When a child presents with signs of the sort of serious bacterial infections that warrant the use of aminoglycoside therapy, the typical practice is to start antibiotic therapy while awaiting cultures for confirmation. Waiting 1 to 2 weeks to receive the genetic test results before starting therapy would not be in the best interest of the patient in most cases. The damage from the drug is likely to have occurred before the genetic test results are returned. Thus, it is unlikely that this test will avert the risk of ototoxicity in the acute setting. Furthermore, drawing 8.5 mL of whole blood, the amount requested by Athena Diagnostics, Inc, from an infant is infeasible. While testing in some settings could be accomplished with a smaller volume of blood, and completed in several hours, access to this technology is not widely available. The time frame of hours may still be too long to wait when considering antibiotic therapy. By contrast, obtaining a maternal family history of aminoglycoside-induced ototoxicity, while somewhat less sensitive, could be accomplished promptly and direct the practitioner to select another form of therapy if available.

Although not useful when a patient presents with signs of a serious bacterial infection, this test may, however, have a role in several contexts (**Table**). Testing individuals who do experience ototoxicity for the *A1555G* mutation could be valuable to prevent hearing loss in maternal relatives. The value to maternal relatives, however, would depend on the mutation status of the mother because new mutations in mitochondrial DNA are about

What This Study Adds

Biomedical literature and biotechnology companies are eager to promote the possibility of using genetic tests to improve the safety and efficacy of drugs. Physicians desire to improve the safety and efficacy of treatments for their patients and may desire to use new technology, especially when improved outcomes with little physical risk are promised. This article explores a case for aminoglycoside-induced ototoxicity susceptibility testing that is advertised to prevent hearing loss. We provide a framework for considering test features when deciding when, how, and for whom to use pharmacogenomic tests.

10-fold more frequent than in nuclear DNA.² Similarly, the presence of a maternal family history of aminoglycoside-induced ototoxicity might warrant testing. The mother could be tested to see if she carries the A1555G mutation, and if so, then the child could be tested in case aminoglycoside therapy ever needed to be considered. The test may also be useful in pediatric patient populations for whom exposure to aminoglycosides is reasonably predicted. For example, patients who have cystic fibrosis often receive repeated courses of aminoglycosides for lung infections, as do children with urologic abnormalities that predispose them to develop urinary tract infections. Testing these individuals prior to use of aminoglycoside treatment might actually fulfill the promise of preventing hearing loss. The context for counseling the families of these patients about the genetic testing is also more amenable to obtaining clear understanding and good informed consent.

One strategy that would be inappropriate is screening all individuals at birth for the A1555G mutation. The A1555G mutation contributes to ototoxicity in less than 0.6% of children exposed to aminoglycosides (17% of the 3.5% who experience this adverse effect, not 0.6% of all newborns). Although 1 to 5 per 1000 newborns are at risk for neonatal sepsis and, therefore, may be treated with aminoglycosides, newborn testing in this setting would not be accomplished in time to alter therapy. Because of the few children who receive aminoglycoside therapy during childhood, widespread newborn screening is unwarranted.

LESSONS LEARNED—A FRAMEWORK FOR THINKING ABOUT PHARMACOGENETIC TESTS

Numerous bodies have developed criteria for the clinical application of genetic tests.^{13,14} The Secretary's Advisory Committee on Genetic Testing (Department of Health and Human Services) concluded that tests need to be evaluated for their analytic validity, clinical validity, clinical utility, and social consequences.¹⁵ The Secretary's Advisory Committee on Genetic Testing also insisted that patients (or parents of pediatric patients) be provided with detailed counseling about the implications of genetic tests.

Developing a framework for analyzing claims of improved outcomes based on these recommendations will aid clinicians in assessing and appropriately applying new

genetic tests. Three broad categories of questions, which are widely applicable to thinking about pharmacogenetic tests, can help clarify the genetic issues:

The Genetics

- How effectively can family history illuminate the risk for this patient?
- What is the nature (eg, penetrance, expressivity) of the mutation?
- How frequent is the phenotype?
- What is the relationship between the marker and the phenotype in children? (The relationship between a mutation and a drug response from studies in adults may not always be applicable in the pediatric context, because of different pharmacokinetics and pharmacodynamics in children, as well as different patterns of gene expression over time.)

The Likelihood of Exposure

- How frequent is the environmental exposure (ie the medication) that elicits the phenotype?
- How predictable is the exposure?

Practical Test Characteristics

- What are the characteristics of the test? (That is, the amount of blood required, interval until the results are received.)

Asking these questions makes clear that testing for the A1555G mutation is inappropriate either in the acute setting or on a population basis.

Many resources are available for pediatricians to understand the implications of various genetics tests. Some provide curricula for self-directed learning. The following Web sites are particularly useful: Genetics in Primary Care at: http://genes-r-us.uthscsa.edu/resources/genetics/primary_care.htm (This site has a specific chapter on genetic issues in congenital hearing loss at: <http://genes-r-us.uthscsa.edu/resources/genetics/pdfs/gpc-congenhearing.pdf>.) and the National Coalition for Health Professional Education in Genetics at: <http://www.nchpeg.org/>. We recommend that pediatricians, and indeed, all physicians avail themselves of these opportunities if we are to ensure the optimal use of new genetic tests.

Accepted for publication November 3, 2003.

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