CHILDREN'S HOSPITAL

PEDIATRIC REVIEW

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Diagnostic Problems in Genetics

By **Yves Lacassie, M.D., FACMG,** *director of genetics services at Children's Hospital and professor of pediatrics and head of pediatric clinical genetics at LSU Health Sciences Center.* *This issue of Pediatric Review is intended for pediatricians, family physicians and all other interested medical professionals. For CME purposes, the author has no relevant financial relationships to disclose.*

OBJECTIVES:

At the end of this activity, the participant should be able to:

1. describe that in Genetics, phenotypic diagnoses are not enough
2. discuss the diagnostic complexities establishing the specific etiology
3. list the different problems that may make the establishment of the diagnosis difficult

INTRODUCTION

With the decrease in malnutrition, infectious disease and other environmental conditions in the last century, genetic disorders became a very important problem in public health and especially in Pediatrics. Certainly the advances in molecular genetics are dramatically changing the way we practice pediatrics in practically all specialties. However, in spite of the important advances in molecular genetics and gene therapy, genetic counseling still plays a very important role in pediatric practice. As a matter of fact, in clinical genetics, prevention through genetic counseling has been the major goal in the last 40 – 50 years. In order to provide responsible and up-to-date genetics counseling, establishing the etiological diagnosis is required. This is a big difference from many other specialties, in which we treat the manifestations, the phenotype, independently of the etiology. In some specialties, like Infectious Disease, you need to establish the specific cause or germ and its resistance to the different antibiotics. However, you still have the possibility to use a wide spectrum of antibiotics. To establish the diagnosis in genetics, you can use some of the new but still expensive tests, such as Exome sequencing or comparative genomic hybridization (aCGH) arrays, to look for mutations or deletions/duplications when you do not have a specific diagnosis. However, this diagnostic method of "testing the universe" is not what should be done. This makes the cost of medicine much more expensive. Even though the diagnosis of some genetic syndromes can be established by "pattern recognition" either by gestalt or association of main findings, we always recommend a full evaluation with the method of "diagnostic hypothesis" as recommended by the late Frank Oski.

As mentioned, the only way to provide responsible genetic counseling is establishing the etiological diagnosis. The importance of differentiating the phenotypic, pathogenic and etiological diagnoses led the author to publish a multiaxial diagnostic system in 1994. However, often, in spite of a good genetic evaluation, it is hard to establish the etiological diagnosis. In the last 50 years we have been aware of two major diagnostic problems – one is genetic heterogeneity, which means that a genetic condition may be caused by different genes. For example, even though achondroplasia is the most common and best known of the skeletal dysplasias, there are more than 250 other conditions with different patterns of inheritance, prognosis, etc., that should be considered. The other classic diagnostic problem has been clinical variability, which means that the genetic diseases or syndromes rarely present most of the characteristic features; often they present variable manifestations, even in affected members from the same family.

In 1994, the author was invited to the Latin American Congress of Human Genetics in Mexico. There he presented a classification of diagnostic problems. In this first classification, we recognized diagnostic problems due to the observer, to the patient, to the disorder and to the environment. However, in 2009, with the advances in genetic testing, we added a fifth group of problems that are due to informatics. It is very important that pediatricians are aware of these different diagnostic problems.

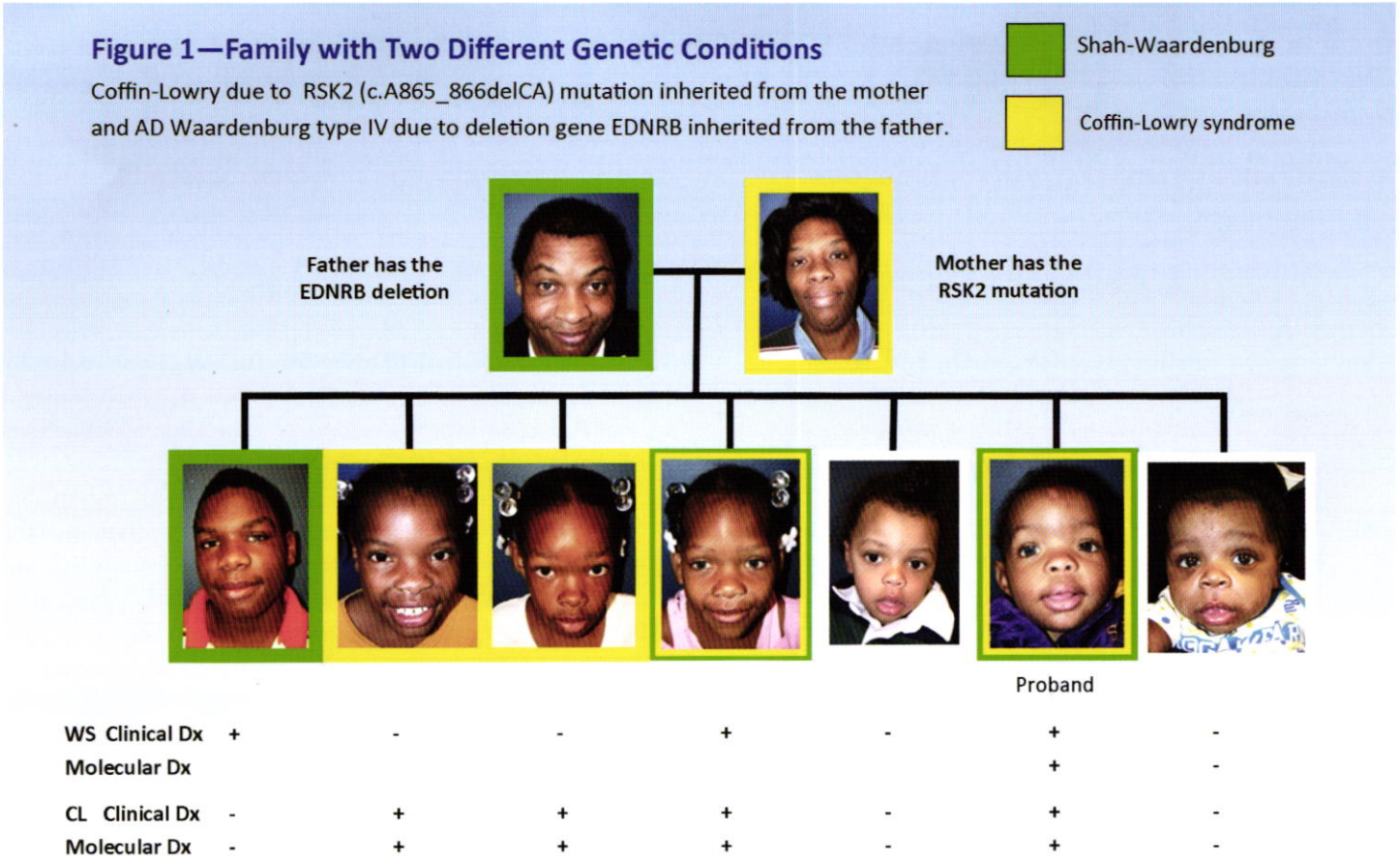
The goal of this review is dual: on one hand, to make the pediatrician aware of the importance of determining the etiology; and on the other, to be aware of the existence of different problems that may make determining the diagnosis difficult. Also, it will help to demonstrate the importance of sending proper information to the geneticist when requesting a consult. Our team, as with many other geneticists, is often faced with families that have no idea why they were referred. Proper information can reduce the cost of the evaluation, avoiding repetition of some testing.

DIAGNOSTIC PROBLEMS

The first kind of diagnostic problem is due to the observers. As physicians we often make errors because of incomplete evaluations. We often see patients that haven't had a full

Figure 1—Family with Two Different Genetic Conditions

Coffin-Lowry due to RSK2 (c.A865_866delCA) mutation inherited from the mother and AD Waardenburg type IV due to deletion gene EDNRB inherited from the father.



WS Clinical Dx	+	-	-	+	-	+	-
Molecular Dx						+	-
CL Clinical Dx	-	+	+	+	-	+	-
Molecular Dx	-	+	+	+	-	+	-

examination. The evaluation of extremities or private areas, like genitalia, may often provide important diagnostic clues. Another common problem is subjectivity. For example, low set ears are often reported; however, more frequently we detect that these are dysplastic or are posteriorly rotated. Certainly, the capacity of the observer is directly related to his knowledge and experience. We still see bias in the diagnoses or frequent stereotypes repeatedly raised by some physicians.

The second group of diagnostic problems is due to the patients or the family. Occasionally, we have patients who provide incomplete or misleading information. We need to be aware about false paternity, how to evaluate familial dysmorphism and how families usually try to justify different dysmorphic features in the patient. Another occasional problem is the coincidence of two or more genetic disorders in the same patient. One of the papers published this year shows the coincidence of two different disorders in some members of the family *(see Fig. 1)*. In this group of diagnostic problems we also include patients, usually newborns who died or stillborns. That is a unique opportunity to evaluate the patient and to try to establish the diagnosis.

The third group of diagnostic problems is due to the genetic disorder. This has been most prevalent and important in the medical genetic literature. As we mentioned, genetic heterogeneity exists in many conditions. Different genes, sometimes with different patterns of inheritance, may produce a similar phenotype. Furthermore, we now recognize the importance of gene-gene interaction as well as of epigenetic phenomenon. We now better understand pleiotropism, or how one gene may affect different structures, as well as the clinical variability. Abiotropism means that not all genetic disorders are congenital or present at birth – some appear at different ages. There are conditions that may simulate Mendelism, for example by environmental conditions. These phenocopies may simulate an autosomal recessive or dominant disorder. In the last 30 years we have recognized the importance of nontraditional patterns of inheritance, such as the contiguous gene syndromes, or microdeletions and recently the recognition of microduplications; the epigenetic mechanism, such as uniparental disomy or imprinting; dynamic mutations that are expansions of triplets like the CGG in Fragile X syndrome; and the importance of mitochondrial or cytoplasmic inheritance. We need to be aware of gonadal mosaicism in which normal parents may have two or more affected children with the same autosomal dominant disorder; and the presence of triallelic inheritance or maybe even a more complex disorder. In these cases it is not enough to have two recessive alleles mutated to have the disorder; sometimes you need three or maybe even more. In spite of all the recent advances, still there are many genetic disorders of unknown cause.

The fourth group of diagnostic problems is due to the environment. This idea was born more than 30 years ago. In developing and underdeveloped countries, or in regions where environmental conditions like malnutrition prevail, these may mask some genetic disorders. Children of families within a good socioeconomic level in which a child presents

failure to thrive, developmental delay, etc., the pediatrician quickly refers the infant to the neurologist, geneticist or other specialists; while, when the same patient is born in a poor environment, it was usually thought that the lack of growth or lack of development was due to malnutrition (Lacassie et al., 1980). Also, technological limitations play an important role. There are patients that have undetected abnormalities with old cytogenetic techniques. As mentioned, there are patients referred with no information, or with misinterpretation of tests, error of transcription and communication, etc. Also some rules and regulations, including time pressure, often prevent detailed, appropriate genetic evaluation. All these may play a role making the diagnosis difficult.

The fifth group is diagnostic problems due to informatics. Problems with the interpretation of results of new techniques, such as microarray, exome sequencing, etc., due to incomplete knowledge about phenotype and genotype correlations, is a common problem that we are seeing at the present time practically every day.

Recent Genetic Cases

I will mention two different problems which were the subject of two publications during 2014. As mentioned in Figure 1, we presented two generations in an African-American family that was identified after the proposita was referred for diagnostic evaluation at 4-1/2 months with a history of Hirschsprung and dysmorphic features that were typical of Waardenburg syndrome. The family evaluation revealed that the father had heterochromia iridis and hypertelorism, supporting the clinical diagnosis of Waardenburg. However, examination of the mother revealed characteristic facial and digital features of Coffin-Lowry syndrome (CLS). Molecular testing of the mother identified a novel 2-bp deletion (c.865_866delCA) in codon 289 of the RPS6KA3, leading to a frameshift and premature termination of translation 5 codons downstream. This deletion also was identified in the proposita and her three sisters with the clinical suspicion of CLS, all of whom, as carriers for this X-linked disorder, had very subtle manifestations. The molecular confirmation of Waardenburg syndrome type 4, also known as Shah-Waardenburg or WS4, was not as straightforward. After multiple sequential molecular testing, a new technique (MLPA) identified a heterozygote deletion of the entire *EDNRB* gene in the father. This deletion was also found in the proposita and the oldest child. Since the heterozygote's deletion was the only change identified in EDNRB, this family represented one of the few cases of an autosomal dominant inheritance of WS4 involving the endothelin pathway. Altogether, clinical evaluation of the family revealed one child to be positive for WS4 and two positive for CLS, while two children were positive for both diseases simultaneously, including the proposita, while another pair tested negative for either disease. This kinship is an example of the coincidence of two conditions cosegregating in one family, with variable phenotypes requiring molecular testing to confirm the clinical diagnosis (Loupe et al., 2014). The coincidence of two or more different conditions in a patient is not so exceptional.

In another paper, (Casci et al., 2014), we reported an unexpected exome sequencing result which showed de novo TRPS1 mutation in an infant with infantile scoliosis, mild developmental delay and history of consanguinity. This was very interesting as we expected an autosomal recessive disorder, however, the exome sequencing showed a de novo mutation in an autosomal dominant gene, the trichorhinophalangeal syndrome type 1 (TRPI). This was interesting as the patient didn't have any of the characteristic features of trichorhinophalangeal syndrome. This is a well-known condition in which we had the opportunity to publish the first black patient with Perthes-like disease and the trichorhinophalangeal syndrome in 1990, along with Drs. Z.R. Stearns and Dean MacEwen, who was the chairman of orthopaedics at Children's Hospital. This is a good example of finding unexpected results. This family also illustrates the importance of this new testing, as in their second pregnancy this couple had a baby with neonatal severe hyperparathyroidism (NSHPT). A mutation in the CASR gene was suspected. As the parents had been sequenced, Dr. Zambrano contacted the laboratory which quickly confirmed that both parents were heterozygous for a mutation in the CASR gene: c.206G>A (p.R96H). This allowed the team of neonatologists, endocrinologists and geneticsts (Baez et al., 2015) further support of the clinical diagnosis of NSHPT. The concomitant occurrence of several of these "diagnostic problems" in a patient is not exceptional and should be considered in our professional practice.

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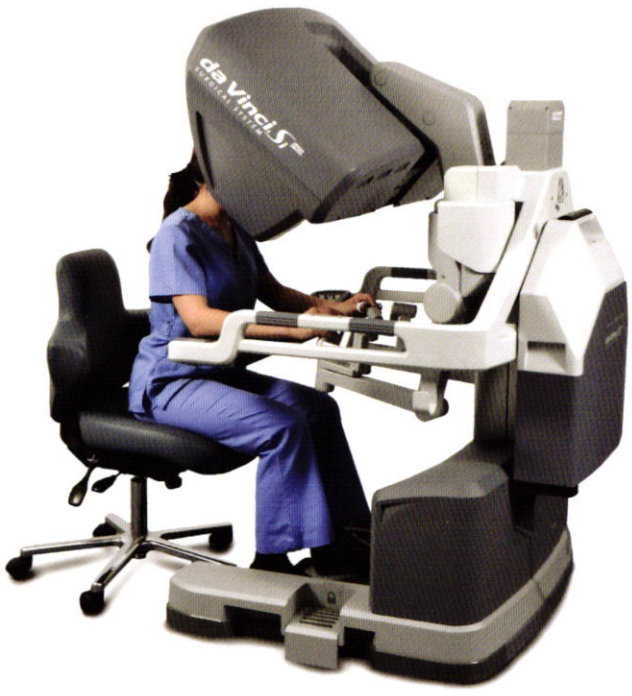

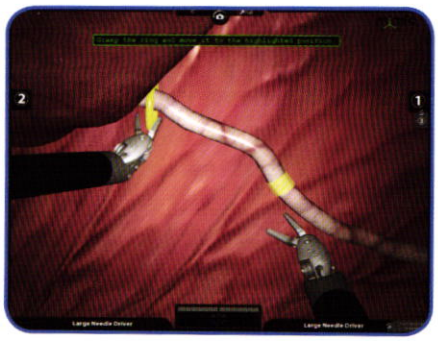

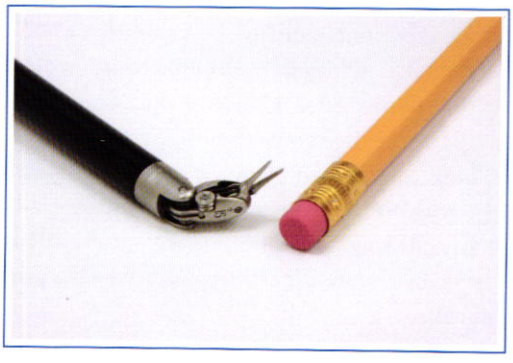

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1. Genetic disorders have become an important issue in pediatric public health due to the decrease in _____.
   a. Malnutrition
   b. Infectious disease
   c. Other environmental conditions
   d. All of the above

2. True or False, establishing etiological diagnosis isn't required in order to provide responsible and up-to-date genetics counseling
   a. True
   b. False

3. How many kinds of diagnostic problems did the author present?
   a. One
   b. Three
   c. Five
   d. Seven

4. The first kind of diagnostic problem discussed is due to _____.
   a. Physicians
   b. Patients/families
   c. Genetic disorder
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