Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Molecular Diagnosis of Monogenic Diabetes

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Slide 1:
Hello, my name is Daniela del Gaudio. I am the Associate Director of the University of Chicago Genetic Services Laboratory and Assistant Professor in the Department of Human Genetics at the University of Chicago. Welcome to this Pearl of Laboratory Medicine on "Molecular Diagnosis of Monogenic Diabetes."

Slide 2:
Diabetes Mellitus is an etiologically heterogeneous disorder. The most common forms, type 1 and type 2 diabetes, are multifactorial with variation in a number of genes together with nongenetic factors leading to disease. However, 1% to 2% of all cases result from mutations in a single gene which are therefore amenable to diagnostic testing. Monogenic diabetes can be inherited, although the majority of the cases results from a *de novo* mutation. In children, almost all monogenic diabetes results from mutations in genes that regulate beta-cell function, although diabetes can rarely occur from mutations resulting in very severe insulin resistance.

The main phenotypes suggestive of an underlying monogenic cause include:

- Neonatal diabetes mellitus (NDM)
- Maturity-onset diabetes of the young (MODY)
- Rare diabetes-associated syndromes

Slide 3:
Neonatal diabetes mellitus is defined by diabetes diagnosed within the first 6 months of life. It is a relatively rare disorder that affects approximately 1:215,000 to 1:260,000 live births. Clinical manifestations at the time of diagnosis include intrauterine growth retardation (IUGR); hyperglycemia, glycosuria, osmotic polyuria, severe dehydration, and failure to thrive. Neonatal diabetes can be either permanent requiring lifelong treatment or transient in which case the diabetes may spontaneously remit, but will often relapse, usually during adolescence. The majority of mutations causing permanent neonatal diabetes are *de novo* in origin. Although the majority of cases of neonatal diabetes involve isolated diabetes, many of the known monogenic causes are characterized by a variety of syndromic features.

Slide 4:
The diagnosis of Permanent Neonatal Diabetes Mellitus (PNDM) is based on evidence of persistent hyperglycemia (plasma glucose concentration >150-200 mg/dL) in infants younger than age six months. Other typical laboratory findings of diabetes mellitus including glycosuria, ketonuria, or hyperketonemia may also be present. Imaging of the pancreas with ultrasound or CT is used to determine its presence and size. With the advances in the last two decades in molecular genetic methodology, it has been possible to define the underlying gene or genes in most clinically recognized subgroups of monogenic diabetes, although making a molecular diagnosis is complicated by the complex genetic heterogeneity of these conditions.

Slide 5:
Increased attention to the primary genetic nature of early-onset diabetes has resulted in an expanding list of casual genes as well as an expansion of phenotypic characteristics in syndromic forms. This slide summarizes the complex genetic heterogeneity of monogenic forms of neonatal diabetes with over 20 genes known to be involved in this disorder. The transient form can be caused by the same mutations that cause the permanent type. Most frequently, however, the transient form is due to various dosage imbalances associated with chromosome 6q24. Nearly half of all cases of permanent neonatal diabetes are due to activating mutations in the *KCNJ11* and *ABCC8* genes which encode the subunits of the beta cell ATP-sensitive potassium channel. This channel plays a crucial role in insulin secretion and thus, glucose homeostasis.

In addition, mutations in the insulin gene (*INS*) are a relatively frequent explanation of neonatal diabetes. A characteristic feature of *ABCC8*, *KCNJ11,* and *INS* mutations is that they usually occur *de novo* and act dominantly. None of the parents are affected, but the patients can pass the mutation on to their descendants. There are forms of neonatal diabetes that are inherited recessively and these are due to mutations in *GCK, IPF1,* and *INS*. There are also cases of syndromic forms of diabetes where features other than diabetes dominate the clinical picture. In such cases, a careful clinical work-up is essential for selecting the most appropriate genes to screen.

Slide 6:
The most prevalent monogenic diabetes phenotype, accounting for approximately 1% of all causes of diabetes, is maturity onset diabetes of the young, or MODY. MODY is characterized by dominant inheritance of early-onset non-autoimmune diabetes that occurs in adolescence and young adulthood. However, a residual insulin secretion may be still maintained for some years after diagnosis and exogenous insulin is generally not required at the time of diagnosis. These patients are typically misdiagnosed as Type 2 Diabetes (T2D); however, two or more consecutive generations of diabetes and the absence of metabolic features (such as obesity or features of insulin resistance) are more suggestive of MODY. MODY is a heterogeneous group of disorders caused by mutations in genes important to beta cell development, function and regulation, glucose sensing, and in the insulin gene itself.

Slide 7:
Mutations in at least 11 different genes can cause MODY. Mutations in the *HNF1A* and *HNF4A* genes, which encode for transcription factors important to pancreatic development and beta cell function, and in *GCK*, the glucokinase gene, are the most common cause of MODY although the mutation prevalence

may vary across populations. The list of genes causing MODY phenotype keeps growing with some recent reports describing the identification of mutations in *ABCC8* and *KCNJ11* in MODY patients, suggesting that mutations in these genes can be associated with a large spectrum of diabetes phenotypes and can be not totally penetrant.

Slide 8:

So why is it important to correctly diagnose monogenic diabetes? Defining the genetic etiology can guide the most appropriate treatment, help to predict the clinical course of the patient as well as explain other associated clinical features. In addition, making a diagnosis will have implications for other family members, often correcting the diagnosis and treatment for other diabetic family members, as well as allowing appropriate genetic counseling.

Slide 9:

Activating mutations of the ATP-sensitive potassium channel genes, *KCNJ11* and *ABCC8*, are the most common causes of Neonatal Diabetes Mellitus (NDM). This channel plays a central role in glucose-stimulated insulin secretion from pancreatic beta cells. With the ingestion of food, the glucose concentration rises and enters the beta cell by way of the GLUT2 glucose transporter. Once inside the cell, glucose is metabolized, leading to changes in the intracellular concentration of adenine nucleotides that inhibit the KATP channel and thus cause channel closure. Channel closure leads to membrane depolarization, which subsequently activates voltage-dependent calcium channels, leading in turn to an increase in intracellular Ca^{2+}which triggers insulin secretion. Activating mutations in the *KCNJ11* and *ABCC8* genes cause the channel to remain open in the face of an increased ratio of ATP thereby limiting insulin secretion.

The most striking clinical implication of molecular diagnosis of a KATP channel mutation is the radical change from insulin injection to an oral sulfonylurea drug to treat diabetes. Sulfonylurea drugs specifically bind the SUR1 receptor encoded by the *ABCC8* gene, close the channels by an ATP-independent mechanism and directly stimulate insulin secretion. So patients with NDM due to activating mutations in *ABCC8* and *KCNJ11* can be successfully switched from insulin injections to oral medications. The response of patients with PNDM with mutant *ABCC8* or *KCNJ11* to sulfonylureas illustrates the power of genetics to identify patients who may benefit from a treatment.

Slide 10:

The activating *KCNJ11* mutations are associated to a wide clinical spectrum ranging from isolated diabetes or, for the more deleterious ones, diabetes in association with a range of neurodevelopmental and neuromotor disabilities. Genotype-phenotype studies correlate *KCNJ11* mutations with the extent of reduction in K_{ATP} channel ATP sensitivity. The location of the mutation seems to predict the severity of the disease, with mutations in residues that lie within the putative ATP-binding site or are located at the interfaces between channel subunits being associated with isolated diabetes mellitus and mutations occurring at codons for amino acid residues that lie at some distance from the ATP-binding site being associated with additional neurologic features. For NDM caused by *ABCC8* mutations, genotype-phenotype correlations are less distinct.

Slide 11:
Although most patients with *KCNJ11* mutations have isolated diabetes, some patients have multisystem disease, termed DEND syndrome that stands for developmental delay, epilepsy and neonatal diabetes. These patients may be unresponsive to sulfonylurea treatment and may need to remain on insulin treatment. Functional studies comparing mutations that cause either isolated PNDM or DEND syndrome to wild type Kir6.2 protein found that all mutations reduced Kir6.2 channel inhibition by ATP, leading to increased resting whole cell K_{ATP} currents. Mutations associated with more severe disease were associated with increased ATP sensitivity compared to those associated with isolated PNDM, suggesting that a larger effect on Kir6.2 function is required to cause neurological outcomes. These genotype-phenotype correlations can aid in predicting clinical course and outcomes in patients.

Slide 12:
As I mentioned before, defining the genetic etiology, in some cases, has the greatest impact on improving treatment for patients. Nearly half of all cases of PNDM are due to activating mutations in *KCNJ11* and *ABCC8*, and their diabetes respond to oral sulfonylurea instead of injected insulin. *HNF1A* and *HNF4A* mutations cause MODY that is often particularly sensitive to low-dose sulfonylurea therapy. Recent evidence of PNDM cases with *GCK* mutation also suggest a possible treatment of these patients with sulfonylurea. And ultimately, patients with mild fasting hyperglycemia caused by heterozygous inactivating *GCK* mutations do not require any pharmacological treatment.

Slide 13:
Current diagnostic testing has been using Sanger sequencing as the gold standard to detect base substitutions and small indels. However, given the long list of genes now known to cause congenital diabetes, it has become increasingly time consuming, labor intensive, and expensive to sequence all possible genes using traditional methods. Furthermore, the choice of genes to be tested using this approach relies on the availability of reliable and comprehensive phenotypic information, although such features may be subclinical. In this regard, next-generation sequencing technology provides the potential for simultaneous analysis of all the known disease genes in a single assay at a similar cost to testing a few genes by Sanger sequencing, and this approach is now becoming the standard for screening multiple genes simultaneously for genetically heterogeneous conditions, including monogenic diabetes.

Slide 14:
In conclusion, monogenic diabetes is genetically heterogeneous with over 30 genes known to cause neonatal/MODY and other syndromic forms of diabetes. A molecular diagnosis of monogenic diabetes is important since it defines the diagnostic subtype, determines the most appropriate treatment, and informs the sibling recurrence risk or risk of diabetes in offspring. Thus, genetic testing should be pursued in all patients meeting a clinical diagnosis of monogenic diabetes. Moreover, such patients should be followed longitudinally through registries to facilitate the understanding of the unique features and best treatment of each genetic cause of monogenic forms of diabetes.

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Molecular Diagnosis of Monogenic Diabetes." I am Daniela del Gaudio.