

Take a Leap of Faith: Implement Routine Genetic Testing in Your Office



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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

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Learning objectives:

1. To gain a better understanding of common genetic testing techniques and their respective strengths and limitations.
2. To become familiar with currently available commercial genetic testing panels and learn by first-hand examples how genetic testing may lead to a correct diagnosis and subsequently appropriate treatment.
3. To recognize rationales for performing genetic testing in patients with presumed and/or diagnosed primary immunodeficiency disorders (PIDs) and immune dysregulation disorders.
4. To learn how to interpret genetic test results and what further action may be indicated based on the results.
5. To understand common barriers for obtaining genetic testing and how to overcome them.

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Genetic testing is a state-of-the-art and readily accessible diagnostic tool and is increasingly indicated in the evaluation process when relevant and possible, although incorporation of this modality into the daily practice of allergists-immunologists in both academic and nonacademic or community settings is still a challenge. Educational sessions and resources support the use

of genetic testing in the diagnosis and management of primary immunodeficiency by both the American Academy of Allergy, Asthma & Immunology and the Clinical Immunology Society. Genetic testing for primary immunodeficiency has become much more convenient and affordable over the past decade; allergist-immunologists in private practice are now able to offer patients high-quality and comprehensive genetic testing panels to help diagnose or characterize underlying immune abnormalities among patients with recurrent infections, and even patients with allergic disorder and noninfectious complications. Although genetic testing has not been a commonplace consideration in day-to-day practice for many nonacademic specialists, a shift toward adopting this into our standard toolkit should be taking place. Most of the commercial genetic testing is aiming for a panel of genes ranging anywhere from just a few to several hundred, so the specialist can feel comfortable clearly interpreting the data. As the panels are analyzing data from next-generation sequencing and deletion/duplication assays, this evaluation may need to be repeated when panels expand and include new relevant genes. Ultimately, for undiagnosed cases, whole-exome and whole-genome sequencing can be the next step; however, involvement of genetic counselors may be needed

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Abbreviations used

CGD- chronic granulomatous disease
CTLA-4- cytotoxic T-lymphocyte-associated protein 4
CVID- common variable immunodeficiency
NGS- next-generation sequencing
PID- primary immunodeficiency disorder
SCID- severe combined immunodeficiency
VUS- variant of uncertain significance
WES- whole-exome sequencing
WHIM- warts-hypogammaglobulinemia-infections-myelokathexis

to interpret the data. The value of genetic testing is that it may bring the clinician closer to an accurate diagnosis; therefore, we can keep treating our patients more accurately and effectively, which may result in less frequent follow-ups for unresolved or recurrent problems. In addition, we can then provide patients and their families with important information about the root cause of their disease state, risks to other family members, and offer genetic counseling services. Genetic testing results may also aid in recognizing when a referral to expert colleagues for more advanced and specialized treatments is indicated. © 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:1676-87)

Key words: Sanger sequencing; Whole genome sequencing; Granulomatous lymphocytic interstitial lung disease; T-cell receptor excision circles

INTRODUCTION

Genetic testing, or the sequencing of human DNA to discover anomalies or mutations that may be pathogenic, has rapidly become a vitally important tool in the clinical armamentarium of practicing physicians and scientists over the past half-century.¹

Genetic abnormalities presenting with allergic and/or immunologic disease are increasingly recognized. Almost 30 years ago, the first use of genetic testing in primary immunodeficiency disorders (PIDs) was in 1993 with the identification of pathogenic mutations in the *BTK* gene in X-linked agammaglobulinemia. Soon afterwards, specific genetic mutations in other X-linked PIDs were characterized, namely in X-linked severe combined immunodeficiency (SCID) and Wiskott-Aldrich syndrome. The rapid evolution in genetic testing techniques within the past 3 decades now allows us to make precise diagnoses, based not only on clinical and immunologic laboratory phenotype but also on genotype, provide the most appropriate genetic counseling, and eventually targeted treatment recommendations.²⁻⁵

In many PIDs, the diagnostic delay between clinical symptoms and disease diagnosis is years to decades. For example, the diagnostic delay in common variable immunodeficiency (CVID) of up to 61 years in adults and about 5 years in the pediatric population has cast a shadow over these patients' lives.^{6,7} Recent advances in genetic testing have identified 68 genes linked to CVID with variable frequency in 3 different regions of the world. Of interest is a subset of CVID in which regulatory T cells are key players in the disease process, such as cytotoxic T-lymphocyte-associated

protein 4 (CTLA-4) deficiency. As targeted therapy, CTLA-4-Ig can be used to offset the unbalanced immune response. Genetic testing among family members of cases with CTLA-4-deficient CVID can bring to light other relatives and expedite their journey for definitive diagnostic and treatment strategies. In other forms of PIDs, incomplete clinical features or low awareness can contribute to diagnostic delay. An example of this is WHIM (warts-hypogammaglobulinemia-infections-myelokathexis) syndrome, which may display a few of the symptoms in early childhood and often get fully diagnosed later in childhood or adulthood.⁸ WHIM syndrome is an autosomal-dominant immunodeficiency that results from excessive CXCR4 signaling (cytopenia, predominantly neutropenia as a consequence of retention of neutrophils in the bone marrow [myelokathexis]). Originally, patients with WHIM syndrome received definitive diagnosis with bone marrow biopsy for myelokathexis, beyond suggestive clinical features. With the advances of availability of genetic testing, WHIM syndrome is now increasingly identified by genetic panel testing for neutropenia and may even be identified at the asymptomatic stage with low T-cell receptor excision circles during the mandatory SCID newborn screening process. These are example how genetic testing can increase accessibility and shorten delays in diagnosis, opening up treatment options and lowering cost of care. Retrospective reviews of the literature have demonstrated that health care consumption is reduced when a diagnosis of PID is made early in life.⁹⁻¹¹

In addition to decreasing health care disparities (ie, proximity to clinical immunology experts in major academic centers, transportation, cost, intravenous access, etc), accessible genetic testing aids in identification of the molecular factors associated with specific diseases, from which targeted therapies have emerged. Prime examples are treatments for hereditary angioedema, Janus kinase (JAK) inhibitors, CTLA-4-Ig, Phosphoinositide 3-kinase (PI3K) inhibitor leniolisib, and other therapies targeting underlying defects.¹² This highlights the importance for the practicing allergist-immunologists to work closely with academic experts and actively prescreen their patient population for underlying genetic mutations. This effort from the community physicians will empower academic centers to focus on variant resolutions and create treatment strategies in collaboration with the primary local specialist.

TYPES OF GENETIC TESTING

Genetic testing methods include cytogenetic testing, which detects abnormalities in chromosome structure, and molecular testing, which detects abnormalities in DNA sequence (Table I).

Fluorescent *in situ* hybridization is an example of cytogenetic testing and is helpful in identifying chromosomal abnormalities such as insertions, deletions, translocations, and amplifications. Fluorescent *in situ* hybridization was commonly used in the diagnosis of full or partial DiGeorge syndrome (22q11 del). Chromosomal microarray analysis, also known as array comparative genomic hybridization, may be preferable because it provides greater sensitivity than conventional cytogenetics. In addition, chromosomal microarray analysis enables the detection of gains and/or losses of chromosomal material that are submicroscopic, can be done on uncultured cell samples, which may be more readily collected, and yields timely results.¹¹

Single-gene Sanger sequencing is a method developed by Frederick Sanger and colleagues in the 1970s that is based on

TABLE I. Methods of genetic testing

Testing method	Description	Strengths	Weaknesses
Karyotype	Cytogenetic test Microscope evaluation using standard staining procedures to pair and order chromosomes	Prenatal screening Can detect chromosomal translocations, chromosomal inversions, or ring chromosomes	Does not detect DNA gene mutations
FISH	Cytogenetic test Microscope evaluation of a specific chromosomal region using a fluorescent molecular probe	Cytogenetics in cancer diagnosis Can detect balanced chromosome abnormalities	Only detects deletions or duplications of regions specifically targeted by the probe used Very small deletions can be missed
Microarray	Cytogenetic test Microscope evaluation of genetic material using a gene chip that produces light by complementary base pairing	First-line test for suspected chromosomal deletions or duplications Similar to karyotyping but at much higher resolution	Does not diagnose balance translocations May not detect mosaicism
Sanger sequencing	Molecular genetic test Targeted sequencing using individual primer pairs in separate amplification and sequencing reactions	Criterion standard for diagnosing small DNA changes Highly accurate Detects point mutations, small deletions/ duplications, and intron mutations	Cost Labor-intensive
NGS	Molecular genetic test Simultaneous sequencing of preselected genes on a large scale	Efficient Cost-effective Detects mosaic mutations	Will not identify mutations outside of the designed panel
WES	Simultaneous sequencing of all exons, or coding sequences, within a genome	Can identify novel gene mutations Efficient Can be reanalyzed in the future	Cost High probability of uncertain results
Whole-genome sequencing	Simultaneous sequencing of all coding and noncoding DNA of a genome	Screens all genetic material Detects noncoding mutations that can be missed by WES Can identify novel gene mutations Can be reanalyzed in the future	Cost Time-consuming High probability of uncertain results

FISH, Fluorescence in situ hybridization.

TABLE II. Examples of genes included in PID genetic testing panels

Example of genetic panel	Examples of specific diagnoses	Clinical phenotypes	Examples of genes included in panel	Treatments
Cellular or combined defects	SCID	• Bacterial, viral, and fungal infections	RAG1	Immunoglobulin replacement
	DiGeorge syndrome	• T/B/NK lymphopenia	IL2RG	Antibiotic prophylaxis
	Wiskott-Aldrich	• Hypogammaglobulinemia	IL7R	Antifungal prophylaxis
	Ataxia telangiectasia	• Thymic defects with congenital abnormalities	JAK3	Enzyme replacement
		• Impaired T-cell function	ADA	Stem cell transplant
		• Immunodeficiency with congenital thrombocytopenia	CD3E	Thymic transplant
		• Ataxia	DCLRE1C	Gene therapy
			NHEJ1	
			PNP	
			ZAP70	
			CD3D	
			RAG2	
			CD40LG	
			LRBA	
			ZAP70	
			CD27	
			STK4	
			DOCK8	
			ICOS	
			RFXANK	
			CIITA	
			ITK	
			MAGT1	
			MALT1	
			TTC7A	
			WAS	
Predominantly antibody deficiencies	XLA	• Bacterial and viral infections	BTK	Immunoglobulin replacement
	AR Agammaglobulinemia	• Hypogammaglobulinemia	LRBA	Antibiotic prophylaxis
	CVID	• Granulomas	PIK3CD	Stem cell transplant
	HIGM	• Autoimmunity	CTLA4	Gene therapy
			NFKB2	
			PIK3R1	
			CD19	
			ICOS	
			IL21R	
			PLCG2	
			PRKCD	
			NFKB1	
			CR2	
			CD40LG	
			AICDA	
			CD40	
			PIK3CD	
			PIK3R1	
			UNG	

(continued)

TABLE II. (Continued)

Example of genetic panel	Examples of specific diagnoses	Clinical phenotypes	Examples of genes included in panel	Treatments
Phagocytic defects	CGD LAD Chediak-Higashi Congenital neutropenias	• Skin infections/abscesses • Abscesses of internal organs • Bacterial, viral, and fungal infections • Granulomas • Oculocutaneous albinism • Hemophagocytosis • Hepatosplenomegaly • Neutropenia	ELANE SLC37A4 HAX1 TAZ CXCR4 G6PC VPS13B WAS G6PC3 CYBB CYBA NCF2 CYBC1 NCF4 FERMT3 SLC35C1 ITGB2	Cytokine replacement Antibiotic prophylaxis Immunoglobulin replacement G-CSF Stem cell transplant Gene therapy
Complement deficiencies	Complement component deficiency C1 esterase inhibitor deficiency Paroxysmal nocturnal hemoglobinuria	• Bacterial infections (predominantly encapsulated bacteria) • Neisserial infections • Angioedema • Hemolysis • Glomerulonephritis • Autoimmunity	C1QA C1QB C1QC C1S C2 C3 C5 C6 C7 C8A C8B C9 CD46 CD55 CD59 CFB CFD CFH CFI CFP SERPING1 THBD	Hyperimmunization Antibiotic prophylaxis C1 esterase inhibitor replacement Biologic agents Stem cell transplant Gene therapy
Well-defined disorders with syndromic features	Chronic mucocutaneous candidiasis HIES WHIM syndrome Netherton syndrome	• Bacterial and fungal infections • Skin infections/abscesses • Lung abscesses • Elevated IgE • Atopic dermatitis • Hypogammaglobulinemia • Warts • Hair shaft abnormalities	STAT3 DOCK8 ERBIN IL6R IL6ST TYK2 CARD11 CXCR4	Antibiotic prophylaxis Antifungal prophylaxis Hyperimmunization Stem cell transplant Gene therapy

			SPINK5 AIRE BCL10 CARD9 IL12B IL12RB1 IL17F IL17RA IL17RC RORC STAT1 STAT3	
Other disorders of immune dysregulation	ALPS HLH EBV-associated lymphoproliferative disease	• Lymphoproliferative disease • Bacterial, viral, and fungal infections • Hepatosplenomegaly • Cytopenias • Vasculitis	FAS CASP10 FASLG PRF1 UNC13D STXBP2 STX11 RAB27A LYST SLC7A7 SH2D1A CD27 ITK	Antibiotic prophylaxis Antifungal prophylaxis Hyperimmunization Immunosuppressants Biologic agents Stem cell transplant Gene therapy
Autoinflammatory syndromes	Periodic fever syndromes	• Fevers • Mucosal ulcers • Rash • Joint inflammation • Uveitis	ADA2 ASAH1 ELANE LPIN2 MEFV MVK NLRC4 NLRP12 NLRP3 POMP PSMA3 PSMB4 PSMB8 PSMG2 PSTPIP1 SCO2 TNFRSF1A TRNT1	Corticosteroids Other immunosuppressants Biologic agents Stem cell transplant Gene therapy

ALPS, Autoimmune lymphoproliferative syndrome; *CVID*, common variable immunodeficiency; *HIES*, hyper-IgE syndrome; *HIGM*, hyper-IgM syndrome; *HLH*, hereditary hemophagocytic lymphohistiocytosis; *LAD*, leukocyte adhesion defects; *LRBA*, LPS-responsive and beige-like anchor protein; *NK*, natural killer; *STAT1*, signal transducer and activator of transcription 1; *WAS*, Wiskott-Aldrich syndrome; *XLA*, X-linked agammaglobulinemia.

selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during *in vitro* DNA replication. It is targeted sequencing that uses oligonucleotide primers to seek out specific regions of DNA. This technique is highly accurate and can detect point mutations, small deletions/duplications, and intron mutations. It is considered a criterion standard of genetic sequencing.^{12–14}

Most currently available Sanger sequencing is used to sequence specific genes or single nucleotide variants; however, it is unable to detect mosaic alleles below a threshold of 15% to 20% and can miss a significant proportion of low-level mosaic mutations. The utility of single-gene Sanger sequencing is high when screening for familial variants, and/or the candidate gene can be narrowed easily by clinical phenotype and immunologic readout. An example is a scenario where family members with recurrent infections show X-linked inheritance of clinical phenotype, laboratory features are notable for absence of immunoglobulins and B cells, and abnormal btk protein expression in monocytes: hence, targeted sequencing of the *BTK* gene is pursued. In addition, single-gene Sanger sequencing is of high importance for PIDs in which a genetic variant may occur in the deep intronic or untranslated regions, such as chronic granulomatous disease (CYBB c.253-1879A>G, with the variant hiding >1000 nucleotides in the intron, away from the coding regions).^{14–16}

Next-generation sequencing (NGS) sequences DNA fragments but in a large-scale parallel technique, simultaneously sequencing millions of fragments. Most NGS platforms are targeting protein coding regions (exons), whereas some can provide the capacity to analyze the entire intronic sequence of a target gene from a genomic DNA sample rather than just the coding exons and conserved splice sites with increase in cost and decrease in feasibility as a screening tool in the community. Panel testing is a compromise of NGS platform when sequencing is broad but analysis is limited to targeted or known immune genes (panel testing, most commonly used in practice). This is a highly valuable approach for patients with CVID phenotype, antibody deficiency syndromes, or combined immunodeficiencies. NGS is recognized as a cost-effective and efficient testing tool that has an additional benefit over Sanger sequencing in that it can detect mosaic mutations, such as may be seen in incontinentia pigmenti, and more frequently detects large deletions, duplications, or inversions than does Sanger sequencing.

It is widely accepted that NGS may be less sensitive than Sanger sequencing, and sequence variants identified by NGS may need to be validated by Sanger sequencing. There may be instances in which the accuracy of a specific variant is in doubt; Sanger sequencing is the easiest method to resolve these uncertainties. In addition, Sanger sequencing can serve as a “patch” to more closely inspect regions that are poorly covered by NGS. These regions are often rich in guanine-cytosine content and may lead to poor capture, amplification, or other idiosyncrasies.^{13–17}

Whole-exome sequencing (WES) efficiently and selectively sequences all exons of a genome. Exons represent about 1% to 2.5% of a human genome. By focusing on these coding regions, cost and time can be reduced, and is most commonly done by hybridization with oligonucleotide probes to capture targeted DNA fragments, which are sequenced by NGS and compared with whole-exome computer algorithms. WES can determine inheritance patterns when performed with parental samples or may detect unrelated genes and lead to the discovery of novel immunophenotypes.¹⁸

Whole-genome sequencing assesses the whole genome and can detect noncoding mutations such as intronic mutations and

splice site mutations, as well as deletions and duplications that can be missed by WES. Whole-genome sequencing is costly, time-consuming, and complex; therefore, clinical utilization is limited, but it has the potential to detect novel mutations.¹⁹

UTILITY OF GENETIC TESTING IN PID Screening

Genetic testing can be done to make a prenatal diagnosis in families with a well-defined genetic PID. Sequencing, or chromosomal microarray analysis when clinically applicable, can be done on embryos before *in vitro* fertilization implantation, or by amniocentesis or chorionic villous sampling postconception. Prenatal diagnosis of a genetic PID is advantageous for counseling regarding prognosis and preparedness for postdelivery treatment plans.

Diagnosis

As clinical immunologists and allergists, we know that certain phenotypic presentations and infection histories have long served as necessary clues in forming a differential diagnosis and subsequently deciding which immunology laboratory and functional testing is indicated. We are currently practicing in an exciting age in which several improved genetic assays, novel pathogenic gene mutations, and new clinical PID phenotypes are being described each year. Therefore, it should now be considered appropriate practice to routinely assess when further genetic evaluation should be offered. This could be considered for patients with immune abnormalities and history of recurrent infections, especially those with noninfectious complications also, which require immune modulation. In addition, patients with refractory hives, severe atopic dermatitis, or recurrent fevers may qualify for genetic approach. In selected cases, it makes sense to take a targeted sequencing approach, restricted only to specific genes or to specific regions of interest. With expanding clinical spectrum of PID with known genes and the continued discovery of novel genes, it is now preferable to pursue broad-panel testing for PIDs. Most of the commercially available genetic testing is aiming for a panel of immune genes ranging anywhere from just a few to several hundred where the specialist could feel comfortable to clearly interpret the data. Because the panels are analyzing data from NGS and deletion/duplication assays, this evaluation step may need to be repeated when panels expand and include new relevant genes.

As previously mentioned, one should be mindful about the limitation of the WES approach. Targeted genetic studies have recently suggested that disease-causing mutations in the deep intronic or untranslated regions may be more common than previously thought. A good example of this is the relatively recent detection of incontinentia pigmenti and ectodermal dysplasia with immunodeficiency caused by a deep intronic variant of *IKBKG*, a late diagnosis of phenotypically mild chronic granulomatous disease (CGD) due to a deep intronic mutation (c.253-1879A>G) activating a cryptic acceptor splice site, or even in X-linked SCID with novel 3' untranslated region variant.^{20,21}

We currently do not know the full impact of mutations in the deep intronic and untranslated regions, but it is anticipated that many more disease-causing mutations have yet to be discovered outside of the exons, and flanking intronic regions, and may also be located outside the intronic regions covered by WES.

Complete results

Gene	Variant	Zygosity	Variant classification
AIRE	c.967 979del (p.Leu323Serfs*51)	Homozygous	PATHOGENIC
IRF7	c.908A>G (p.Asp303Gly)	Heterozygous	Uncertain significance
POLE	c.5480C>T (p.Ser1827Leu)	Heterozygous	Uncertain significance
STXBP2	c.1001C>T (p.Pro334Leu)	Heterozygous	Uncertain significance
The following genes were evaluated for sequence changes and exonic deletions/duplications: ACD, ACP5, ACTB, ADA, ADA2, ADAM17, ADAR, AICDA, AIRE, AK2, AP3B1, ATM, B2M, BCL10, BLNK, BLOC1S6, BTK, CARD11, CARD14, CARD9, CASP10, CASP8, CD247, CD27, CD3D, CD3E, CD3G, CD40LG, CD79A, CD79B, CD8A, CEBPE, CHD7, CIITA, CLPB, COPA, CORO1A*, CR2, CSF3R, CTC1, CTLA4, CTPS1, CTSC, CXCR4, CYBA, CYBB, DCLRE1B, DCLRE1C, DKC1, DNMT3B, DOCK2, DOCK8, ELANE, EPG5, FADD, FAS, FASLG, FERMT3, FOXP1, FOXP3, FPR1, G6PC3, GATA2, GFI1, HAX1, ICOS, IFIH1, IFNGR1, IFNGR2, IGLL1, IKBKB, IL10, IL10RA, IL10RB, IL12B, IL12RB1, IL17F, IL17RA, IL17RC, IL1RN, IL21, IL21R, IL2RA, IL2RG, IL36RN, IL7R, IRAK4, IRF7, IRF8, ISG15, ITCH, ITGB2, ITK, JAGN1, JAK3, LAMTOR2, LCK, LIG4, LPIN2, LRBA, LYST, MAGT1, MALT1, MAP3K14, MEFV, MOGS, MYD88, NBN, NCF2, NCF4, NFAT5, NFKB2, NFKBIA, NHEJ1, NHP2, NLRC4, NLRP12, NLRP3, NOD2, NOP10, ORAI1, PARN, PGM3, PIK3CD, PIK3R1, PLCG2, PMM2, PNP, POLE, PRF1, PRKCD, PRKDC, PSMB8, PSTPIP1, PTPRC, RAB27A, RAC2, RAG1, RAG2, RBCK1, RFX5, RFXANK, RFXAP, RHOH, RMRP, RNASEH2A, RNASEH2B, RNASEH2C, RORC, RTEL1, SAMHD1, SEMA3E, SH2D1A, SH3BP2, SLC29A3, SLC35C1, SLC37A4, SLC7A7, SMARCA1, SP110, SPINK5, STAT1, STAT2, STAT3, STAT5B, STIM1, STK4, STX11, STXBP2, TAP1, TAP2, TAPBP, TAZ, TBX1, TCN2, TERC, TERT, TICAM1, TINF2, TLR3, TMC6, TMC8, TMEM173, TNFRSF13B, TNFRSF13C, TNFRSF1A, TNFRSF4, TNFSF12, TPP2, TRAF3, TRAF3IP2, TREX1, TRNT1, TTC7A, TYK2, UNC13D, UNC93B1, UNG, VPS13B, VPS45, WAS, WIPF1, XIAP, ZAP70, ZBTB24 The following genes were evaluated for sequence changes only: CSF2RA Results are negative unless otherwise indicated			

Benign and likely benign variants are not included in this report but are available upon request. An asterisk (*) indicates that this gene has a limitation. Please see the limitations section for details.

FIGURE 1. Positive genetic test result for homozygous AIRE mutation.

Prognosis

Characterizing a specific gene defect in PID can guide prognosis and treatment in some cases. An example of a PID with variable phenotypes and hence respectively variable treatment recommendations is Wiskott-Aldrich syndrome. Identification of a nonsense mutation leading to absence of protein expression predicts a severe clinical course and significant risk for autoimmunity and malignancy. However, mutations that allow for small amounts of Wiskott-Aldrich syndrome protein production more likely exhibit an X-linked thrombocytopenia phenotype, warranting different treatment.^{22,23} Similarly, prognosis of a patient with CGD is determined by the type of pathogenic mutation that can be identified by sequencing. X-linked CGD tends to be associated with lower residual oxidative capacity and therefore has a more severe phenotype than autosomal-recessive CGD genotypes.²⁴ Genotyping can also identify PIDs more amenable to hematopoietic cell transplantation, guide pretransplant conditioning regimens, and identify PIDs associated with radiosensitivity, such as Artemis, Ligase4, Cernunnos, and Nijmegen breakage syndrome.^{24,25}

HOW TO “TAKE A LEAP OF FAITH”: KNOWING WHEN AND HOW TO PROCEED WITH GENE TESTING IN YOUR PATIENTS

Many practitioners believe that genetic testing is readily accessible only to geneticists and/or academicians. It is also not uncommon that genetic evaluation is only considered for those with signs of immunodeficiency, although monogenic allergic diseases and atopic features of idiopathic environmental illnesses are on the rise. As clinical immunologists and allergists we have

expertise in both common and rare diseases, and as such are in a unique position to provide specialized medical care to our patients. It is abundantly clear with SCID newborn screening that early diagnosis is lifesaving. Therefore, we encourage all practicing allergist-immunologists to apply a genetic screening approach in our everyday management of a subset of patients with allergic and immunologic diseases in the community, particularly for those who do not readily have access to academic centers.

Genetic testing for PIDs has become much more convenient and affordable over the past decade; allergist-immunologists in all practice settings are now able to offer patients high-quality and comprehensive gene testing panels to help diagnose or characterize PID. Although genetic testing has not been a commonplace consideration in day-to-day practice for many nonacademic physicians, a shift toward adopting this into our standard toolkit should be taking place.

One commonly cited reason for not routinely initiating genetic testing is that many physicians have practiced their entire careers without practical access to gene testing yet have successfully cared for patients with PID. We want to encourage all practicing clinical immunologists to learn the importance of genetic characterization of patients with PID, because doing so may advance our ability to care for our patients with PID. In addition, we will correctly diagnose more patients with PID who may otherwise be missed when B- and T-cell populations and function are normal on initial screening laboratory evaluations. Recognizing in which patients genetic testing is indicated is the first step.

Factors that may be helpful in determining who should have genetic testing performed include those individuals with a strong

Summary

**Positive result. Homozygous pathogenic variant identified in AIRE.
Variants of Uncertain Significance identified in IRF7, POLE, and STXBP2.**

Clinical Summary

- A homozygous, pathogenic variant, c.967_979del (p.Leu323Serfs*51), was identified in AIRE.
 - The AIRE gene is associated with autosomal-recessive and autosomal-dominant autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED) (MedGen UID: 39125).
 - This result is consistent with a predisposition to, or diagnosis of, APECED.
 - APECED is typically characterized by adrenocortical insufficiency, hypoparathyroidism, and chronic mucocutaneous candidiasis. Other common features include premature ovarian insufficiency, pernicious anemia, vitiligo, alopecia, enamel hypoplasia, and keratitis. APECED has variable presentation and severity. Heterozygous individuals typically have later onset, milder phenotype, and reduced penetrance than those who are homozygous or compound heterozygous (PMID: 26141571, 26084028).
 - It is likely that one pathogenic variant was inherited from each parent. When 2 parents each carry a pathogenic variant, their chance of having an affected child is 25%. Any children of this individual would inherit a pathogenic variant. The chance of having a child affected with APECED depends on the carrier state of this individual's partner. Other relatives may also be carriers. Testing for this variant is available.

FIGURE 2. APECED genetic test report. *APECED*, Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy.

family history of recurrent infections and/or inflammatory disease especially exhibiting a genetic pattern of inheritance (ie, autosomal-dominant, X-linked, autosomal-recessive). One example of this is a family history of early infant death in males due to recurrent respiratory infections and *Pneumocystis pneumonia*. Additional considerations are a family history or personal history of unusual infections such as encephalitis due to herpes simplex virus, sepsis due to atypical organisms such as salmonella or tuberculosis, or recurrent or invasive infections with common organisms. Patients with frequent, recurrent bacterial, viral, and/or fungal infections, or those with severe or unusual infections, warrant PID laboratory evaluation as well as genetic testing. Patients with granulomatous disease, autoimmunity, immune dysregulation, or lymphoproliferative diseases with invasive or recurrent infection history would also benefit from genetic testing. Patients with syndrome features (ie, dysmorphic facial features, microcephaly, atopic dermatitis and/or ectodermal dysplasia, conical teeth, conjunctival telangiectasia, oculocutaneous albinism, bony disorders, congenital heart disease) should also be evaluated by genetic testing, particularly when they also experience recurrent or severe infections. Finally, severe inflammatory disease targeting specific organs such as the lungs, gastrointestinal tract, or bone marrow is another indication that genetic testing is warranted.

Most of the major laboratories offering genetic testing now offer genetic testing panels specifically designed to evaluate PIDs. Depending on a patient's phenotypic and laboratory characteristics, one may order targeted panels testing for only a few genes, or very broad panels that test for several hundred genes at a time (Table II).

As we better characterize disease-gene associations in PID, we also can better tailor current and future treatments on the basis of genetic testing results. A good example of this would be the preferential use of abatacept to treat granulomatous lymphocytic interstitial lung disease in patients with CTLA-4 haploinsufficiency or LPS-responsive and beige-like anchor protein deficiency.^{26,27} In addition, emerging treatments of PI3K

inhibition are expected to be available for patients with activated PI3KD syndrome in the near future. Genetic characterization can also help us recognize risks for noninfectious complications in some of our patients with PID, and therefore prompt timely evaluation by other specialists, such as rheumatology in patients with CVID, or endocrinology in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, when clinically applicable.

When we have made an accurate diagnosis, we can treat our patients more correctly and effectively, prevent frequent follow-ups for unresolved or recurrent problems, and provide them and their families with invaluable education on their disease state and risks to other family members, and offer genetic counseling services when needed. Most clinical geneticists do not focus their testing on identifying PIDs, so it really is up to us, the clinical immunologist, to fill this gap and advocate for our patients in the way only we can. Fortunately, we are practicing during a time when we can readily offer this level of proficient care to patients, even those without access to academic centers.

For those who are unsure where to start, we would like to share the following illustrative cases from our own collective experiences as allergist-immunologists practicing outside the academic setting, which serve as examples of how genetic testing significantly impacted the diagnosis, clinical course, and current managements of some of our own patients. We encourage those not already offering genetic testing to consider using new diagnostic tools such as genetic testing; there is increasing evidence to support the use of genetic testing in PID diagnosis, and genetic testing is more accessible today than ever before. It is never too late to take the leap of faith into a new modality and keep advancing our patient care practices.

CASE 1

A 44-year-old man presented with a history of mucocutaneous candida infections since childhood, severe fatigue, and

widespread dermatophytosis refractory to multiple treatments. Genetic testing revealed a pathogenic homozygous mutation for autoimmune regulator (c.967_979del) (Figure 1). This genetic test result confirmed the diagnosis of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (also commonly referred to as autoimmune polyglandular syndrome type 1) (Figure 2). He was successfully treated with antifungal posaconazole. Furthermore, the diagnosis prompted endocrinology referral, which led to diagnosis and treatment for previously unrecognized adrenal insufficiency and hypogonadism.

CASE 2

A 9-year-old girl presented with neutropenia and recurrent respiratory infections. She was followed by a hematologist specialist for neutropenia. Immune evaluation for repeated infections revealed low IgG. Bone marrow biopsy unveiled retention of senescent neutrophils. The patient did not have any history of cutaneous warts. Targeted genetic panel for neutropenia revealed a pathogenic variant in CXCR4, resulting in a diagnosis of WHIM syndrome. This patient exhibited common features of WHIM syndrome including recurrent respiratory infections, hypogammaglobulinemia, and myelokathexis with a remote history of very limited warts that spontaneously resolved before her diagnosis was made. The family had been hesitant to receive the human papillomavirus vaccine, and after her diagnosis they agreed that she would greatly benefit. She was also started on intravenous immunoglobulin for treatment of her hypogammaglobulinemia, trimethoprim-sulfamethoxazole prophylaxis, and G-CSF for her neutropenia and is awaiting clinical trial of CXCR4 antagonist therapy.

CASE 3

A 9-year-old boy presented with recurrent oral thrush and dental abscesses. He had difficulty with behaviors, struggled with brushing his teeth, and therefore it did not occur often. The primary care physician was concerned about recurrent oral candidiasis, and he was treated with nystatin mouthwash multiple times without improvement. The patient presented to the immunology clinic and was treated with 1 course of oral fluconazole with resolution of his infection. He did not have a recurrence of infections until months later when he was admitted with an episode of acute anemia, and oral thrush. The patient was treated with high-dose steroids and antifungals during his hospitalization with resolution of his anemia and candidiasis. Based on his history, WES was performed and revealed signal transducer and activator of transcription 1 gain-of-function pathogenic mutation. The patient was offered JAK1 inhibitor treatment to prevent further episodes of autoimmune and autoinflammatory manifestations related to signal transducer and activator of transcription 1 gain-of-function. In addition, targeted genetic testing was obtained on his twin sibling, which demonstrated the same signal transducer and activator of transcription 1 gain-of-function mutation and similarly his treatment options were considered when clinically indicated.

CASE 4

Recurrent lip swelling in several family members led to an immunologic and genetic evaluation. Immune evaluation revealed a phenotype with hereditary angioedema with normal C1 inhibitor. Genetic evaluation revealed a pathogenic mutation

in exon 9 of the coagulation factor XII in all affected family members. In addition to factor XII mutations, hereditary angioedema with normal C1 inhibitor phenotype may occur from defects involving plasminogen (*PLG*), angiotensin-converting enzyme (*ACE*), kininogen-1 (*KNG1*), and myoferlin (*MYOF*) genes. The recent expansion in genetic testing for patients with hereditary angioedema with normal C1 inhibitor helps ensure appropriate diagnosis, and management can be made for this potentially life-threatening rare disease.²⁸

CASE 5

A 15-year-old male patient is followed by the immunologist for history of recurrent infections, bronchiectasis, and agammaglobulinemia, on immunoglobulin replacement therapy. In the past few years, the patient also developed type 1 diabetes, which is difficult to control with insulin therapy. The endocrinologist was concerned about an underlying cause. Genetic testing revealed a variant of uncertain significance (VUS) in the *FOXP3* gene, in the essential forkhead domain. *In silico* prediction programs found the variant damaging, and healthy individuals did not carry this variant based on large data sets. Although the *FOXP3* protein was present, there was a likelihood that it was dysfunctional. Currently, the patient is considered to have immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. No clinical assays are available for functional studies; however, a regulatory T suppressor assay was low on research grounds. Parents were eager to initiate family testing.

INTERPRETATION OF RESULTS

Genetic mutations are reported as either negative, or 1 of 5 positive variant classifications—benign, likely benign, pathogenic, likely pathogenic, or VUS—based on recommendations from the American College of Medical Genetics and Genomics.^{29,30} To interpret genetic testing results with confidence, it is important to critically assess variants of uncertain/undetermined significance, and not only be familiar with the implications of a pathogenic, likely pathogenic, benign, and likely benign mutation. A heterozygous pathogenic variant may be reported as likely pathogenic, if homozygosity is required to manifest a disease state or phenotype.

Often times VUS occur with broader sequencing methods (whole-exome or whole-genome sequencing) but may be present in the resulting reports for gene sequencing panels from individual laboratories or even targeted genetic sequencing if the variant has not been previously reported. It is of utmost importance to counsel patients and families, before performing genetic testing, that VUSs may be likely on the report and sometimes, although rarely, need further determination. The likelihood of a VUS being reported can be reduced when detailed clinical and family history is provided with the genetic testing being ordered.²⁹

A VUS is reported when a genetic change is detected, but the significance of its role in disease is not known. In other words, there has not been identification of this mutation/molecular change in others with a disease, for example, not enough people with or without disease have this change, or the variant occurs at a high frequency in the reference database. When a VUS is identified in a patient with a clinical and laboratory phenotype suggestive of a disease, it can be challenging to prove or disprove the diagnosis at that time. Attempts to resolve this should be undertaken, including performing targeted sequencing on family

members with or without the disease, and collaborating with institutions/laboratories at which functional assays can be performed. Keeping this in mind, the results may not be conclusive and as a provider continues to care for the patient and the disease evolves, repeat analysis down the line may be offered as over time more data are accumulated and genetic mutations are reclassified.

When enough individuals with a disease are found to have 1 genetic variant, or advances in computer modeling show that there is no likely impact on genetic function, a genetic mutation is reclassified.²⁸⁻³⁰ Equally important is having a broad differential and considering alternative diagnosis when a VUS is identified or the resulting mutations are found to be benign/likely benign.

In addition, when a positive result is obtained, it is essential that we recognize when a diagnosis warrants a multidisciplinary treatment team. Referral to expert colleagues in academic facilities may be indicated, particularly to initiate and manage complex therapeutics or even stem cell transplant when indicated.

BARRIERS TO PERFORMING GENETIC TESTING

The primary barrier for obtaining genetic testing in our patients is a lack of physician referral for testing. Therefore, it is imperative that we continue to educate ourselves and our colleagues on the benefits as well as the logistical steps in initiating genetic testing for our patients.³¹

Several genetic testing laboratories have websites with user-friendly dashboards in which one may select from a number of testing panels, organized by types of PIDs, such as humoral PIDs, innate PIDs, neutropenias, complement deficiencies, and so on. Once an order is placed, test kits are mailed to patients' homes, containing materials and simple instructions on saliva collection or buccal swab, which can be either self-collected by the patient, or obtained in the physician's office. In circumstances when a venipuncture is required, blood can be collected in the physician office. In addition, many of the laboratories can arrange for mobile blood draw to patients' homes, often at no additional cost. Samples are then mailed to the laboratory; results are typically released by fax and/or electronic mail in roughly 3 to 4 weeks.

Many health insurance companies will approve part or all of the testing cost when applicable diagnostic codes and supporting documentation, such as relevant office notes and/or letters of medical necessity, are provided. These can be easily attached to the initial order electronically as uploaded documents, and generally do not require additional time or review by the physician or staff once the order is placed. Fortunately, there are no time-consuming prior authorization processes required by the ordering physician or staff once the order is placed. In cases when an out-of-pocket cost exists for testing, many larger genetic testing laboratories offer discounted pricing for their testing panels, which are relatively affordable. If a patient expresses an inability to afford the discounted price, certain laboratories may be able to provide testing at lower cost, or sometimes at no cost, by way of charitable foundations, or sponsored testing programs in cases when specific clinical criteria are met.

GENETIC TESTING IN OTHER ALLERGIC AND IMMUNOLOGY DISEASES

The role of genetic testing is expanding in other facets of allergic and immunologic diseases as we increasingly recognize

the importance of genetic characterization in developing targeted and novel therapeutic modalities. For example, the primary risk factors in atopic dermatitis have been identified as mutations in the gene encoding filaggrin (chromosome 1q21.3). This discovery led to the understanding that a combination of skin barrier function as well as adaptive and innate immune dysfunction plays a role in disease pathogenesis. As more genetic loci are linked to atopic dermatitis in the future, new insights into disease pathogenesis and novel drug targets can be identified.³²

Currently, much exciting research is being done to characterize genetic defects and polymorphisms associated with other conditions such as asthma, food allergies, and drug hypersensitivity reactions; however, a detailed discussion into these topics is beyond the scope of this article.

CONCLUSIONS

Genetic testing is a state-of-the-art and readily accessible diagnostic tool that can and should be incorporated into the daily practice of allergists-immunologists including those in community or nonacademic settings. Genetic testing laboratories provide commercially available comprehensive genetic testing panels for PIDs and other disorders of immune dysregulation. Genetic testing can be efficiently ordered through user-friendly online platforms, allowing all practicing allergist-immunologists to incorporate genetic testing into our everyday practice, and readily offer comprehensive genetic testing to our patients. This modality enhances patient care, particularly when immune dysregulation or PID is suspected, or when treatments and/or further expert referral may depend on further genetic characterization.

The American Academy of Allergy, Asthma & Immunology, as well as the Clinical Immunology Society, supports the use of genetic testing in the diagnosis and management of patients with PID and have educational resources available for the practicing allergist-immunologist. Clinical immunologists have the necessary knowledge to determine which genes should be considered, the most reasonable testing modality to determine a diagnosis, and to counsel patients regarding the use of genetic testing in PID and immune dysregulation.³³

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