Is it necessary to identify molecular defects in primary immunodeficiency disease?

Luigi D. Notarangelo, MD,[a] and Ricardo Sorensen, MD[b] *Boston, Mass, and New Orleans, La*

The identification of the molecular bases of more than 130 primary immunodeficiency diseases has prompted the use of mutation analysis in the diagnostic approach to these patients. Here we discuss the importance of and the limitations associated with molecular diagnosis of these disorders and emphasize the need that mutation analysis be accompanied by appropriate evidence that the identified genetic defect has pathologic consequences on RNA/protein expression and function.
(J Allergy Clin Immunol 2008;122:1069-73.)

Key words: *Primary immunodeficiency, mutation analysis, gene, prenatal diagnosis, carrier detection*

Abbreviations used
CVID: Common variable immunodeficiency
FHL: Familial hemophagocytic lymphohistiocytosis
GINA: Genetic Information Nondiscriminatory Act
HCT: Hematopoietic cell transplantation
HIPAA: Health Insurance Portability and Accountability Act
JAK: Janus kinase
PID: Primary immunodeficiency disease
SCID: Severe combined immunodeficiency
STAT: Signal transducer and activator of transcription
WAS: Wiskott-Aldrich syndrome
WASP: Wiskott-Aldrich syndrome protein
XHIGM: X-linked hyper-IgM syndrome
XLP: X-linked lymphoproliferative syndrome

The dramatic explosion of knowledge on the molecular bases of inherited disease that has resulted from the Human Genome Project has led to substantial improvement in understanding the mechanisms and gene defects that underlie primary immunodeficiency diseases (PIDs). The importance of identifying molecular defects in various forms of PIDs as a research tool is unquestionable, as exemplified by the observation that the same immunologic and clinical phenotype can result from distinct gene defects and that, on the other hand, different mutations in the same gene can lead to distinct phenotypes. Mutation analysis has gained relevance also as a diagnostic tool. In this article we will discuss the clinical benefits that might derive from mutation analysis of PID genes and will also discuss the possible challenges and limitations of this approach.

MOLECULAR ANALYSIS OF PID: THE CASE PRO *(LUIGI D. NOTARANGELO)*

The most recent classification of PIDs reports more than 130 different disorders.[1] In most cases, the disease-causing gene or genes have been identified. Characterization of the molecular defect has important implications to better understand the pathophysiology of the disease. In addition, there are at least 5 good reasons to pursue mutation analysis in the clinical diagnostic approach to patients with PID. In particular, mutation analysis can (1) provide a definitive diagnosis; (2) help in genetic counseling, permitting early prenatal diagnosis and carrier identification; (3) establish a diagnosis in atypical presentations; (4) provide relevant information in selected PIDs in which there is a strong genotype-phenotype correlation so that prognostic implications can be derived; and (5) permit presymptomatic identification of individuals affected with potentially lethal forms of PIDs and hence prompt timely life-saving interventions, such as hematopoietic cell transplantation (HCT).

Traditionally, the diagnostic approach to patients with a possible PID is based on detailed personal and family history, physical examination, and supportive laboratory screening assays. Although this is often sufficient to confirm the suspicion of a PID, more sophisticated assays are required to permit a conclusive diagnosis. Recently, the use of mAbs that recognize proteins that are mutated in selected PIDs has been advocated in the diagnostic approach to PIDs by using flow cytometry or Western blotting.[2] This approach is straightforward and might permit a rapid diagnosis of certain PIDs, such as severe combined immune deficiency (SCID) caused by common γc or Janus kinase (JAK) 3 deficiency; X-linked agammaglobulinemia; X-linked immunodeficiency with hyper-IgM syndrome; Wiskott-Aldrich syndrome (WAS); X-linked lymphoproliferative disease (XLP); leukocyte adhesion deficiency; immune dysregulation–polyendocrinopathy–enteropathy–X-linked syndrome; perforin deficiency; and mendelian susceptibility to mycobacterial disease caused by defects in IL-12 receptor β1 and IFN-γ receptor 1.[3] Furthermore, functional assays have been developed that might also lead to a definitive diagnosis. For example, the dihydrorhodamine-123 reduction test is a flow cytometry–based assay that is widely used in

From [a]the Division of Immunology, Children's Hospital, Harvard Medical School, Boston, and [b]the Department of Pediatrics, Children's Hospital, New Orleans.
Disclosure of potential conflict of interest: L. D. Notarangelo has served as a member of the US Immunodeficiency Network and the Immune Disease Institute. R. Sorensen has received research support from Talecris.

The content of this article was presented by L. D. Notarangelo and R. Sorensen in a pro-con debate at the 2008 Annual Meeting of the American Academy of Allergy, Asthma & Immunology entitled "Is it necessary to identify molecular defects in primary immunodeficiency?"

Received for publication August 27, 2008; accepted for publication August 29, 2008.
Available online November 7, 2008.
Reprint requests: Ricardo Sorensen, MD, Department of Pediatrics, LSU Health Sciences Center, c/o Children's Hospital, RIC Building, Room 4229, 200 Henry Clay Ave, New Orleans, LA 70118. E-mail: Rsoren@lsuhsc.edu.
0091-6749/$34.00
© 2008 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2008.08.038

the diagnostic approach to chronic granulomatous disease, and flow cytometry can be used to detect defects of cytokine-mediated signaling, as shown by lack of signal transducer and activator of transcription (STAT) 5 phosphorylation on *in vitro* IL-2–mediated stimulation of lymphocytes from patients with SCID caused by defects of the γc/JAK3 signaling pathway or impaired STAT1 or STAT4 phosphorylation on *in vitro* stimulation of monocytes (with IFN-γ) and activated T cells (with IL-12), respectively, in patients with mendelian susceptibility to mycobacterial disease.[3] However, protein-based and functional assays have 3 major limitations: (1) they require reagents and expertise that are not routinely available, (2) they might fail to identify affected individuals who express a mutant protein, and (3) they might experience the interference of confounding factors. For example, although expression of CD40 ligand on the surface of *in vitro* activated T cells is often used to identify patients with X-linked immunodeficiency with hyper-IgM syndrome, this assay is influenced by age (CD40 ligand expression being hardly detectable in newborns and young infants),[4] by the use of immunosuppressive drugs, or by other underlying disorders that compromise T-cell activation.

Technical considerations also support the value of mutation analysis. Taking advantage of DNA stability, molecular diagnostic assays can be repeated multiple times by using the same source of material if a search for mutations in other genes is warranted after failure to identify an abnormality in the most likely gene to be affected. Matching DNA samples to single individuals is also possible, thus reducing the risks of laboratory errors. Furthermore, at least in theory, interlaboratory assessment of quality control is feasible for DNA-based assays. In contrast, protein-based and functional assays experience the perishable nature of the required biologic samples and hence most often call for a return visit of the patient if the assay has to be repeated. For the same reason, assessment of interlaboratory quality control might be problematic.

The study of the human genome has shown that in several cases the same PID phenotype can result from mutations in different genes (Table I). For example, Omenn syndrome can result from defects in *RAG1*, *RAG2*, *DCLER1C* (Artemis), *RMRP*, *IL7R*, *IL2RG*, or *ADA* defects.[5] Identification of the disease-causing mutation is of foremost importance for genetic counseling in families with PID. Before the genomic revolution, prenatal diagnosis of PID was limited to a few disorders. With the exception of adenosine deaminase and nucleoside phosphorylase deficiency, which can also be diagnosed by analyzing enzymatic activity in chorionic villi or amniotic fluid cells, prenatal diagnosis of other forms of PID was based on immunophenotypic and functional assays performed on fetal blood samples obtained as late as 20 to 22 weeks of gestation. This approach permitted the diagnosis of typical and severe cases of SCID, chronic granulomatous disease, and leukocyte adhesion deficiency. However, interpretation of the results was certainly more problematic in fetuses at risk for X-linked agammaglobulinemia or WAS or affected with leaky forms of SCID. Mapping of PID genes opened new perspectives for prenatal diagnosis based on linkage analysis by using highly polymorphic markers that flank the disease locus. This approach expanded the number of PIDs that could be diagnosed prenatally to include many that cannot be detected by means of analysis of fetal blood sampling and allowed anticipation of the diagnosis to 11 to 12 weeks of gestation (ie, the timing for chorionic villi sampling). The indirect approach to prenatal diagnosis of PIDs, based on linkage analysis, maintains its value in families with a well-defined inheritance in which the specific mutation has not yet been identified; however, it is less accurate than searching for a known specific mutation.

TABLE I. Genetic heterogeneity of primary immunodeficiencies: Multiple gene defects, one phenotype

PID phenotype	Associated gene defects
$T^-B^+NK^-$ SCID	*IL2RG*, *JAK3*
$T^-B^-NK^+$ SCID	*RAG1*, *RAG2*, Artemis
Omenn syndrome	*RAG1*, *RAG2*, Artemis, *RMRP*, *IL7R*, *IL2RG*, *ADA*
Agammaglobulinemia	*BTK*, *IGHM*, *IGLL1*, *CD79A*, *CD79B*, *BLNK*
CVID	*TNFRSF13B (TACI)*, *ICOS*, *TNFRSF13C (BAFF-R)*, *CD40L*, *CD19*, *SH2D1A*
Hyper-IgM syndrome	*CD40L*, *CD40*, *AICDA*, *UNG*
XLP	*SH2D1A*, *XIAP*
FHL	*PRF1*, *MUNC13B*, *STX11*
CGD	*CYBB*, *CYBA*, *NCF1*, *NCF2*
SCN	*ELA2*, *HAX1*, *GFI1*, *MAPBP*, *WASP*
MSMD	*IL12B*, *IL12RB1*, *IFNGR1*, *INFGR2*, *STAT1*
HSE	*UNC93B1*, *TLR3*

CGD, Chronic granulomatous disease; *SCN*, severe congenital neutropenia; *MSMD*, mendelian susceptibility to mycobacterial disease; *HSE*, herpes simplex encephalitis; *IL2RG*, IL-2 receptor gamma; *JAK*, Janus associated kinase; *RAG*, recombinase-activating gene; *RMRP*, RNA component of mitochondrial RNA processing endoribonuclease; *IL7R*, IL-7 receptor; *ADA*, adenosine deaminase; *BTK*, B-cell tyrosine kinase; *IGHM*, Ig heavy chain mu; *IGLL1*, Ig light chain lambda; *CD*, cluster of differentiation; *BLNK*, B-cell linker; *TNFRSF13B*, TNF receptor soluble factor; *TACI*, transmembrane activator and CAML interactor (calcium modulator); *ICOS*, inducible co-stimulator; *BAFF-R*, B-cell activating factor receptor; *CD40L*, CD40 ligand; *AICDA*, activatino-induced cytidine deaminase; *UNG*, uracil N-glycosylase; *XIAP*, X-linked inhibitor of apoptosis; *PRF*, perforin; *STX11*, syntaxin; *CYBB*, cytochrome B beta; *CYBA*, cytochrome B alpha; *NCF*, neutrophil cytosolic factor; *ELA*, elastase; *HAX*, HCLS1 associated protein X-1; *GF*, growth factor; *MAPBP*, mitogen-activated protein kinase–binding protein; *WASP*, Wiskott-Aldrich syndrome protein; *IL12R*, IL-12 receptor; *IFNGR*, IFN-γ receptor; *STAT*, signal transducer and activator of transcription.

Mutation analysis permits unequivocal identification of carriers of X-linked PIDs. Thus mutation analysis has obvious implications for genetic counseling and represents a major improvement compared with the lack of reliable methods for carrier detection in the past (other than a positive family history) or to the use of more cumbersome and less reliable methods based on the analysis of the randomness of X-chromosome inactivation that came into practice in the late 1980s and early 1990s.

It is becoming more and more evident that certain mutations in PID genes can lead to atypical clinical and immunologic presentations. For example, although null mutations in *RAG1* or *RAG2* genes cause $T^-B^-NK^+$ SCID, hypomorphic mutations in the same genes can associate with Omenn syndrome, leaky SCID, or combined immunodeficiency with granulomas.[6] Thus investigation into the molecular bases of human diseases has disclosed an unanticipated level of both phenotypic (Table II) and genotypic heterogeneity, making mutation analysis inevitable to solve differential diagnosis of complex presentations.

In some forms of PID, a genotype-phenotype correlation that can have prognostic and therapeutic implications has emerged. One such example is represented by mutations in the Wiskott-Aldrich syndrome protein gene *(WASP)*. Mutations in *WASP*, which are predicted to abrogate protein expression, are typically associated with a severe clinical form of the disease. In contrast, missense mutations in exons 1 and 2 typically cause isolated

TABLE II. Phenotypic heterogeneity of primary immunodeficiencies: One gene, multiple phenotypes

Mutated genes	Associated phenotypes
RAG1, *RAG2*, Artemis	SCID, OS, leaky SCID
LIG4	SCID, LIG4 syndrome
WASP	WAS, XLT, XLN
SH2D1A	XLP, CVID
RMRP	CHH, SCID, OS
ELA2	SCN, cyclic neutropenia

RAG, Recombinase-activating gene; *LIG4*, ligase 4; *WASP*, Wiskott-Aldrich syndrome protein; *RMRP*, RNAse mitochondrial ribonucleoprotein processing enzyme; *ELA*, elastase; *OS*, Omenn syndrome; *LIG4*, DNA ligase 4; *XLT*, X-linked thrombocytopenia; *XLN*, X-linked neutropenia; *CHH*, cartilage hair hypoplasia; *SCN*, severe congenital neutropenia.

X-linked thrombocytopenia, a milder form of the disease.[7] HCT is the only curative approach to WAS; however, the risks associated with this procedure would make identification of candidates for HCT highly desirable. Before identification of the *WASP* mutation, patients were referred to HCT when they had experienced severe clinical problems (life-threatening infections, significant autoimmunity, and malignancies). The overall results of HCT in patients with WAS reflected inclusion of patients with advanced disease and complications. After identification of the *WASP* gene and demonstration of a relatively solid genotype-phenotype correlation, several groups have started to refer to patients undergoing HCT with severe mutations and lack of WASP protein expression early in life, before severe complications might develop. At the same time, recognition that missense mutations in exons 1 and 2 are associated with X-linked thrombocytopenia has offered the basis for a prospective longitudinal study of patients affected with this disease, about whom little information is currently available.

Presymptomatic identification of genetically affected individuals is of foremost importance also in other forms of PID, such as XLP and familial hemophagocytic lymphohistiocytosis (FHL). These disorders are genetically heterogeneous but share a high mortality rate early in life. In the past, the diagnosis of XLP and FHL was mostly based on a constellation of typical and severe clinical and laboratory abnormalities after infection with Epstein-Barr virus (in XLP) or with a variety of infectious agents (in FHL). Attempts to use HCT in this setting were often unsuccessful, and the high mortality rate, even after HCT, has been attributed to either pre-existing organ damage, abnormal immune activation, or both.[8] Identification of one of the multiple gene defects that can cause XLP and FHL and characterization of the specific disease-causing mutation in affected families might permit presymptomatic identification of genetically affected individuals, thus prompting an immediate search for a suitable donor for HCT. This strategy, along with improvements in the procedures used to favor donor stem cell engraftment and in monitoring and early treatment of infections, has already resulted in dramatic changes in the success rate of HCT for XLP and FHL,[8] thus emphasizing again the value of mutation analysis.

Lastly, it is important to consider that advances in technology are making mutation analysis faster and cheaper every day. Use of DNA chips that permit screening for a large series of mutations in a wide group of genes is overcoming the potential difficulty associated with genetic heterogeneity for many forms of PID. For example, Lebet et al[9] have recently reported that using a newly developed custom resequencing microarray, they were able to screen simultaneously for mutations in the SCID-causing *IL2RG*, *IL7R*, *JAK3*, and *DCLRE1C* genes and were able to detect all of 47 point mutations in a known sample. It can be anticipated that this approach will enter into practice for other forms of genetically heterogeneous PIDs, thus decreasing both the costs and the turnaround time that can currently limit the usefulness of mutation analysis.

Because of all of the above considerations, the guidelines to diagnosis of PID that have been developed jointly by the European Society for Immune Deficiencies and the Pan-American Group for Immune Deficiencies acknowledge mutation analysis as a key step to attaining definitive diagnosis of molecularly defined PIDs.[10]

CONCERNS ABOUT MOLECULAR DIAGNOSIS: THE CASE CON *(RICARDO SORENSEN)*

Although there are arguments that make a case for the molecular diagnosis of PIDs, it is also important to consider a significant number of limitations to this methodology's practicality and usefulness.

At the present time, the availability and cost of molecular tests are a problem. Most analyses are done in research laboratories at the researcher's and not at the patient's convenience. Commercial laboratories are few and face the problem of patented genes.[11] Gene patents typically cover the clinical applications of mutation analysis, the methods to study these genes, and treatment. Companies holding gene patents, including patents on several genes involved in PID, charge initial licensing fees and royalties to other laboratories wanting to run genetic tests. This fact has slowed the development of affordable and easily available commercial tests.

The cost of molecular testing is compounded by the fact that mutations in different genes can all lead to the same phenotype (Table I). If no mutations are found in the gene the clinician chose to investigate, the costs and logistics problems will further increase because of the need for studying other candidate genes.

As mentioned above, progress in technology is likely to reduce cost and improve the availability of testing, but it will also introduce new problems in the interpretation of results. The search for mutations will inevitably unravel many polymorphisms and genetic variants that are unlikely to be clinically relevant. Determining the disease-causing role of nucleotide changes might be particularly challenging. Laboratories offering these tests will be unlikely to provide expert advice on the significance of the abnormalities detected. Inevitably, interpretation of gene sequences will be left to physicians who will not be prepared to deal with the complexity of genetics, and this might cause significant harm to patients given diagnoses of such perceived abnormalities.

The need to be aware of the intricacies of the assessment of the risks associated with specific mutations, even if in some patients these mutations are clearly associated with abnormal immune function causing a clinical immunodeficiency, is further exemplified by conditions in which the mutation is present throughout life but the clinical manifestations either improve with age or appear later in life.

It has been observed that patients with mutations of *IRAK4*, a kinase involved in signaling through Toll-like receptors, outgrow their need for Toll-like receptor–mediated signaling. This *IRAK4* kinase helps protect young children from specific pathogens but is

dispensable in adults who no longer are susceptible to life-threatening infections.[12]

Several clinical observations point out that the clinical expression of a mutation is often influenced by factors outside the realm of conventional genetics. Common variable immunodeficiency (CVID) can be caused by mutations in one of many different genes. However, clinically, patients with CVID can present at any age.[13] In other instances, the same mutation can result in very different PID phenotypes, even in the same family. In a large kinship of patients with XLP followed in Louisiana, 2 patients died at an early age of a fatal infectious mononucleosis, a third patient had B-cell lymphomas twice, and the proband presented with a phenotype of CVID after having mononucleosis at 3 years of age. All these phenotypes plus aplastic anemia and vasculitis have been reported in patients with XLP.[14] The fact that they can present within the same family further emphasizes that other factors do regulate normal and abnormal gene expression, that the disease phenotype is the result of the interaction of many genes (not just the mutated one) and environmental factors, or both.

The conventional genetics assumption is that 1 gene codes for 1 protein. However, new information shows that 22,000 genes code for 100,000 proteins. The emerging field of epigenetics postulates that alternative splicing or clipping out not only introns but also some exons and posttranscriptional control of gene expression determine the characteristics of the protein produced. We now understand that disease is more about how genes are regulated than about identification of gene mutations and polymorphisms. The study of epigenetics is likely to revolutionize our knowledge about diseases, including PIDs.[15,16] In the future, understanding of inheritance that is not based on changes in DNA sequencing will be required to asses the potential effect of mutations and polymorphisms that we are presently trying to diagnose.

Considering the many pitfalls of molecular diagnosis, it is important to examine the effect the detection of some mutations can have on the affected individual and his or her insurability. Genetic discrimination is prohibited by law. The Genetic Information Nondiscriminatory Act (GINA) offers legal protection to encourage genetic screening, counseling, and testing and prohibits health insurers from denying coverage or adjusting premiums based on individual predisposition to a genetic condition. Furthermore, it stops both employers and insurers from requiring applicants to submit to or to disclose genetic test information.[17]

The Health Insurance Portability and Accountability Act (HIPAA) recognizes genetic information as protected health information. HIPAA further states that genetic information in the absence of a diagnosis cannot be considered a pre-existing condition. HIPAA provides restrictions on health-related information coverage in setting premiums by group health insurers, although it does not provide such restrictions for non–group plans.

Although GINA and HIPAA do protect individuals against genetic discrimination and their insurability and cost of insurance, it needs to be remembered that molecular diagnosis of clinically healthy family members identifies risk and not disease and that the risk alters the patients' perception of health. Furthermore, GINA and HIPAA protect the health insurability of some individuals with a genetic abnormality, but access to affordable life insurance and job access are not equally protected.

The rush to identify genes involved in immune function and mutations has had a remarkable yield: more than 130 genes and innumerable mutations of those genes linked to PID. In light of this remarkable achievement, it is important to ask the following: What advantages has this success brought for the patient? Some patients now know that there is a mutation causing their immunodeficiency, and some family members know that they are at risk. However, the diagnosis of a mutation has afforded better treatment for a tiny fraction of patients. Although the exiting promise of gene therapy has had some stunning successes, it also has led to severe adverse events.[18] Furthermore, it has become clear that many larger genes are not candidates for gene therapy and that successful gene insertion does not ensure effective gene expression. This latter observation should not be a surprise in light of our emerging knowledge about the importance of epigenetics on gene expression.

The overemphasis on the identification of gene mutations and experimental gene knockout models probably has had a negative effect as well. Functional and biochemical studies of cell activation and signal transduction pathways that would possibly allow identification of novel PID-causing genes have been largely neglected in the field of PIDs. Such research is also essential to develop algorithms that could guide the approach to molecular diagnosis. In addition, it needs to be considered that some of the most frequent immunodeficiencies are most likely polygenic. Much emphasis has been placed on the mutations detected in some genes in a minority of patients with CVID. However, some of these mutations are rare and present only in geographically restricted, inbred patient populations. In the vast majority of patients with CVID, no single gene mutation has been identified. This is also true for the rather frequent IgG subclass deficiencies and in specific antibody deficiencies with normal immunoglobulins. Thus many patients with clinically relevant and frequent forms of immunodeficiency are unlikely to benefit from the progress in the detection of molecular abnormalities.

Research on potentially innovative forms of treatment also has not been adequately promoted. In clinical practice we still have to contend with variants of antibiotic and gamma globulin treatment and various form of stem cell transplantation. But for notable exceptions analyzed in the first section of this review, decisions about treatment are still based mostly on the patient's clinical presentation and not on the type of mutation. Accurate clinical and immunologic phenotyping of immunodeficiencies is still the cornerstone of diagnosis and management. Although identification of a gene defect provides a definitive diagnosis, the assessment of its phenotypic expression needs to be perfected and used to monitor the effectiveness of preventive and therapeutic strategies over a patient's lifetime.

A CONSENSUS *(LUIGI D. NOTARANGELO AND RICARDO SORENSEN)*

In summary, investigation into the molecular bases of human disease has clearly increased our understanding of the pathogenesis of many forms of PID. Mutation analysis has also become an important tool in the approach to definitive diagnosis of PIDs. However, the expectation that mutation analysis will inevitably solve the differential diagnosis of complex presentations will not be met until biomarkers are defined that differentiate clinically relevant disease subtypes and help identify molecular targets for application to diagnosis and prevention. If sensitive and specific enough, these biomarkers might represent a very valuable tool in attempting an early diagnosis and might even be considered for

population screening, as proposed for newborn screening of SCID based on quantitation of T-cell receptor excision circles.

Beyond immediate clinical application, identification of molecular defects should help in the understanding of molecular pathways in health and disease. Mutation analysis performed through DNA sequencing should be supported by analysis of mRNA/cDNA and protein expression and by functional assays, if possible, to prove the disease-causing role of newly identified mutations. If these are not available or inconclusive, there are bioinformatics tools available that might help estimate the significance of the observed DNA changes.[19] Eventually, such a development should improve approaches to disease diagnosis, treatment, and prevention.

REFERENCES

1. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, et al. International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol 2007;120:776-94.
2. Kinnon C, Gaspar HB. Genetics of inherited immunodeficiency diseases and diagnostic techniques. In: Cunningham-Rundles C, editor. Manual of clinical laboratory immunology. 6th ed. Washington (DC): American Society for Microbiology; 2002. p. 810-8.
3. Oliveira JB, Notarangelo LD, Fleisher TA. Applications of flow cytometry for the study of primary immune deficiencies. Curr Opin Allergy Clin Immunol 2008. In press.
4. Brugnoni D, Airò P, Graf D, Marconi M, Molinari C, Braga D, et al. Ontogeny of CD40L expression by activated peripheral blood lymphocytes in humans [erratum published in Immunol Lett 1996;52:61]. Immunol Lett 1996;49:27-30.
5. Villa A, Marrella V, Rucci F, Notarangelo LD. Genetically determined lymphopenia and autoimmune manifestations. Curr Opin Immunol 2008;20:318-24.
6. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med 2008; 358:2030-8.
7. Jin Y, Mazza C, Christie JR, Giliani S, Fiorini M, Mella P, et al. Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. Blood 2004;104:4010-9.
8. Jordan MB, Filipovich AH. Hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis: a journey of a thousand miles begins with a single (big) step. Bone Marrow Transplant 2008 [Epub ahead of print].
9. Lebet T, Chiles R, Hsu AP, Mansfield ES, Warrington JA, Puck JM. Mutations causing severe combined immunodeficiency: detection with a custom resequencing microarray. Genet Med 2008;10:575-85.
10. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999;93:190-7.
11. Matthijs G. Patenting Genes. BMJ 2004;329:1358-60.
12. Bashyam H Outgrowing IRAK. J Exp Med 2007;204:2242.
13. Pan-Hammarström Q, Salzer U, Du L, Björkander J, Cunningham-Rundles C, Nelson DL, et al. Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. Nat Genet 2007;39:429-30.
14. Gaspar H, Sharifi R, Gilmour K, Thrasher A. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. Br J Haematol 2002;119:585-95.
15. Hatchwell E, Greally J. The potential role of epigenomic dysregulation in complex human disease. Trends Genet 2007;23:588-95.
16. Holliday R. Epigenetics: a historical overview. Epigenetics 2006;1:76-80.
17. Genetic Information Nondiscrimination Act, Public Law No. 110-233; 2008.
18. Hacein-Bey-Abina S, Garrigue A, Wang G, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in four patients after retrovirus-mediated gene therapy of SCID X1. J Clin Invest 2008;118:3132-42.
19. Fleisher TA, Notarangelo LD. What does it take to call it a pathogenic mutation? Clin Immunol 2008;128:285-6.