Investigating for suspected immune deficiency in children

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Abstract

The International Union of Immunological Societies now recognises over 400 single gene inborn errors of immunity, making the investigation of suspected immunodeficiency in children increasingly complex. The use of immunomodulatory therapies, including biologics, may also cause both transient and permanent secondary immunodeficiency. This review

outlines a practical approach to guide paediatric doctors and allied health professionals investigating children with suspected immune deficiency, starting with a detailed clinical assessment of history and examination, as well as baseline investigations to exclude secondary immunodeficiency and screen for organ-specific complications for immunodeficiency. This should be followed as appropriate by a basic assessment of humoral, cell-mediated, phagocytic, and innate immunity, the results of which would instruct the necessity of confirmatory testing using functional and protein expression assays, as well as genetic testing. The authors also recommend an approach for screening for immunodeficiency in asymptomatic neonates, children with a strong family history of primary immunodeficiency, or those diagnosed with genetic syndromes associated with immunodeficiency.

Keywords

primary immunodeficiency disorders, investigation, paediatrics, screening, immunology, whole exome sequencing

Introduction

As the International Union of Immunological Societies (IUIS) now recognises more than 430 distinct single gene inborn errors of immunity (IEI) the practice of clinical immunology is both more interesting and more complex than ever before. IEI may give rise to a wide breadth of clinical phenotypes, ranging from the more familiar histories of recurrent or severe infection, through malignancy, allergy, autoinflammation, and autoimmunity, while immunodeficiency can equally form part of the phenotype of broader genetic syndromes that may present with developmental delay, dysmorphic appearance, or poor growth (1). The introduction of newborn

screening for severe combined immunodeficiency has broadened the clinical presentation of IEI further, as screened patients are diagnosed at birth before exposure to infection (2).

While individual IEIs may be rare, some, such as selective IgA deficiency that occurs at a frequency of 1:500, are relatively common. Taken together as a whole primary immune deficiency disorders (PID), which exclude selective IgA deficiency, may be as frequent as 1:2000 (1). Investigating for immunodeficiency is valuable in that it may yield a unifying diagnosis, guide management, and enable genetic counselling of the family (1). This review outlines a practical approach to investigating children with suspected immune deficiency.

When to Suspect Immune Deficiency

As the list of IEIs increases, it has become increasingly difficult to decide which patients will benefit from investigation to exclude PID. Clinical symptomatology provides a useful, if not comprehensive, framework with which to decide when and how to investigate for PID (3).

a) Asymptomatic children

While the investigation of suspected PID is often triggered by a history of infection, asymptomatic patients may require immunological investigation . The clearest example of this is newborn screening (NBS) for severe combined immune deficiency (SCID). Identifiable at birth, immediately life-threatening, and eminently treatable, the arguments in favour routine screening of newborns for SCID have been recognised, and with the development of a T-cell receptor excision circle (TREC) assay utilising dried blood spot analysis this became possible at scale (2). The TREC assay allows a quantitative analysis of the by-products from the recombination required to generate T cell receptor (TCR) diversity (2). Confirmatory testing of infants with low or absent TRECs with flow cytometric lymphocyte subsets (FC-LSS)

examining naive and memory T, B and NK cells and MHC class II expression, permits the early diagnosis and genetic confirmation of SCID, avoiding the administration of potentially life-threatening Bacille-Calmette-Guerin (BCG) or live rotavirus vaccination in these patients, and expediting curative haematopoietic stem cell transplantation or gene therapy in the first few months of life.

Newborn screening programs using TRECs have been in place across the United States since 2018, and in an increasing number of other nations (2). Some programs have included kappa-deleting recombination excision circle (KREC) alongside TREC testing in an effort to identify primary immunodeficiency characterised by congenital B cell lymphopenia, though this is not in widespread use.(2). NBS for SCID was introduced in the UK as an evaluation project in 2021, with plans in place to expand the project nationwide, assuming utility is confirmed. NBS inevitably identifies newborns with incidental T-cell lymphopenia who do not have SCID, either as an idiopathic finding, or as part of a genetic syndrome. These children will also require immunology investigation, led by a paediatric immunologist (Figure 1).

When there is a family history of an inborn error of immunity, investigation or intervention may begin pre-implantation, with embryo selection (pre-implantation genetic diagnosis) if considered appropriate. The ethical framework surrounding this is reviewed elsewhere. Genetic diagnosis is also possible *in-utero* following chorionic villus sampling or amniocentesis. Finally, post-natal testing of possibly affected individuals can be undertaken on blood spots (TRECS) or cord blood for immunophenotyping or specific pathway investigation as directed by a paediatric immunologist and reference laboratory.

Investigating underlying immune deficiency in asymptomatic children may be indicated following the diagnosis of a genetic syndrome known to be associated with immune deficiency (Box 1). In such cases, a baseline assessment of the blood count, FC-LSS, immunoglobulin levels (IgGAM), and response to vaccination is often useful, with more detailed assessments of T-cell function, thymic output, and immunedysregulation guided by an immunologist (1).

Finally, incidental laboratory abnormalities in otherwise asymptomatic children may raise questions about immunodeficiency. Incidental lymphopenia, neutropenia, or hypogammaglobulinaemia should be qualified by the age and clinical course of the patient, as wide variations in these may be seen with acute infection, as well as secondary to medication or following cardiac or other surgery (4).

When incidental laboratory abnormalities are accompanied with a history where infection has been persistent, severe, or accompanied by opportunistic infections, such findings should be analysed by FC-LSS (including naive T-cell percentages), IgGAM, and vaccine responses if appropriate for age, with further investigation guided by the broader phenotype (3).

b) Symptomatic children

Immunological investigation may be indicated in children presenting with infectious or non-infectious symptomatology.

a. Presentation with infection

PID is often first suggested by recurrent or unusual infection.

While defining the point at which recurrent infection becomes abnormal is difficult, certain forms of recurrent infection may be suggestive of specific immune deficiency and the SPUR (serious, persistent, unusual or recurrent) mnemonic is a good starting point for assessment (3).

Recurrent sinopulmonary infection, as evidenced by otitis media, pneumonia, or sinusitis, may be an indicator of humoral or combined immunodeficiency, such as X-linked agammaglobulinaemia (XLA) or hyper-IgM syndrome (HIGM), and should be investigated in the first instance by quantifying the patient's IgGAM and FC-LSS, as well as excluding HIV infection (3).

Recurrent skin infection may also be representative of PID: extensive or troublesome viral warts with human papillomavirus may indicate possible underlying T-cell immunodeficiency, such as Dedicator of Cytokinesis-8 (DOCK8) deficiency, epidermodysplasia verruciformis, or warts, hypogammaglobulinaemia, infections and myelokathexis (WHIM) syndrome; recurrent skin abscesses or furunculosis may be indicative of a phagocyte disorder such as congenital neutropenia or chronic granulomatous disease (CGD), though may also be secondary to diabetes mellitus, poor nutrition, or skin disorders such as chronic eczema (3,4). Again, exclusion of HIV infection should be a priority in investigation, alongside an assessment of nutritional status and a baseline investigation of neutrophil count, FC-LSS, and IgGAM. If CGD is suspected, a neutrophil respiratory burst by slide method (NBT: Nitroblue Tetrazolium assay) is a quick, cheap and specific diagnostic test.

Recurrent urinary tract infections (UTI) are not usually indicative of PID: in these cases it may be more fruitful to implement a search for underlying risk factors for UTI. Equally, while recurrent central nervous system (CNS) infections may be indicative of complement deficiency

(meningococcal infection), asplenia, or Toll-like receptor defects (pneumococcal infection), clinicians must also exclude open meningeal defects as a potential cause with appropriate CNS imaging (3).

Single episodes of an infection that are unusual in severity or duration, the location affected, or the causative microbiological agent may also merit immunological investigation. Omphalitis in the neonatal period, for example, is suggestive of a leukocyte adhesion disorder (LAD): a full blood count may demonstrate a significantly elevated white cell count, and the diagnosis confirmed by assessment of CD11a/b/CD18 expression (1). Unusually severe lower respiratory tract infection should also raise the suspicion of PID, including SCID, CGD, hypogammaglobulinaemia (for example due to XLA or HIGM), and Hyper-IgE syndrome (HIES), with the autosomal dominant form of the latter being associated with pneumatocoele formation (1).

Infection with opportunistic pathogens should also raise suspicion of immunodeficiency. *Pneumocystis jirovecii* pneumonia is associated with T-cell lymphopenia or failure of co-stimulaton (CD40L), and pseudomonal infection may be indicative of neutropenia, while both *Burkholderia* sp. sepsis and staphylococcal abscesses are associated with chronic granulomatous disease (CGD). Invasive aspergillosis may be seen in chronic neutropenia or CGD, while nocardiosis may be seen in CGD, hypogammaglobulinaemia, CVID, HIES, and other forms of PID. Systemic non-tuberculous mycobacterial infection should also indicate a need for immunlogical investigation, being seen in patients with SCID, CGD, and Mendelian Susceptibility to Mycobacterial Disease (MSMD) (3).

Sepsis often occurs in apparently immunocompetent hosts, though severe or recurrent pneumococcal sepsis should lead to an effort to exclude asplenia through ultrasonography and a blood film searching for the presence of Howell-Jolly bodies. Recurrent pneumococcal sepsis may also be indicative of a defect in Toll-like receptors or their associated signalling pathways, such as IRAK-4 deficiency, or MyD88 deficiency. Such patients may additionally fail to mount normal inflammatory responses to invasive bacterial infection, with absence of fever, and limited neutrophil leucocytosis or C-reactive protein rise. Once the presence of a functioning spleen has been confirmed, these rarer PIDs can be excluded using a combination of functional or genetic testing. Recurrent meningococcal sepsis may be seen in patients with complement deficiency, particularly terminal complement components, which should be investigated by quantifying serum C3 and C4, with additional testing for complement function via CH50 and AP50 analysis (3).

b. Non-infectious presentation

PID may present with clinical features other than infection (Table 1), including symptoms and signs in the skin, gut, hair, umbilical cord, haematological indices, central nervous system, evidence of multiple allergies, autoimmunity or autoinflammation. For the latter, unusually severe or early-onset presentations may indicate a need for immunological investigation directed towards identifying immunedysregulation, which may ultimately require genetic testing (1). Faltering growth is important and plotting the child's growth is an important part of the initial assessment.

How to Investigate Suspected Immune Deficiency

a) Clinical assessment and first-line investigation

a. Clinical assessment

The initial assessment of a child with suspected immunodeficiency should include a detailed history to outline the clinical course, with a focus on identifying opportunistic, serious invasive (requiring hospitalisation or intensive care), or recurrent infection. Microbiological results of infection or colonisation are helpful. Risk factors for primary immunodeficiency, such as parental consanguinity or a family history of PID, autoimmunity, or malignancy should be noted. Vaccination records should be reviewed, with attention to whether the patient has received the BCG or other live vaccines. Clinical examination should highlight appropriateness of growth, abnormalities of hair, skin, nails, and teeth, organomegaly and lymphadenopathy, presence of tonsillar tissue, active infection, and evidence of end-organ damage such as bronchiectasis.

Children should have a baseline examination with a full blood count, assessing neutrophil count to exclude congenital or acquired neutropenia, platelet count and mean platelet volume to exclude microthrombocytopenia, lymphocyte count to detect lymphopenia, and any autoimmune cytopenia, while a concomitant blood film may be useful to exclude hyposplenism. Where hyposplenism is suspected, an ultrasound is indicated to assess splenic anatomy. Liver and renal function testing at this stage provides a useful baseline of co-morbidity secondary to potential PID, while exclusion of secondary immunodeficiency should be undertaken through HIV testing and a nutritional assessment.

b. Humoral immunity

Immunoglobulins (IgG, IgA and IgM) should be measured together with serum albumin: if albumin is low, gut or urinary loss of protein, including immunoglobulins, should be considered. Absent or extremely low levels of IgG with normal serum albumin may indicate B cell (such as X-linked agammaglobulinaemia, XLA), or combined B and T cell disorders, and should be followed up by FC-LSS to quantify the patient's CD19, CD3/4/8 and CD16/56 counts, preferably with naive and memory T-cell assessment (3).

Severely low IgG levels in combination with a raised or normal level of IgM may be indicative of HIGM syndrome, most often caused by deficiency of CD40-ligand on CD4-positive T-cells leading to failure of class-switching of antibody production from IgM to IgG (1). Low levels of IgG occur naturally around 6 months of age as maternally-acquired antibody levels wane. This may persist throughout infancy as Transient Hypogammaglobulinaemia of Infancy (THI), with varying susceptibility to sinopulmonary infection. THI is self-limiting, with antibody levels that improve spontaneously over the subsequent 6-24 months. The development of persistently low or falling levels of IgG, in combination with low IgA or IgM, may indicate alternative diagnoses. Under the age of 4 years, the differential includes multiple forms of combined immunodeficiency, while for children over 4 years of age it may be consistent with common variable immunedeficiency (CVID). For most early-onset hypogammaglobulinaemia, additional testing should be guided by an immunologist (3).

Assessing the patient's response to vaccination, particularly to tetanus (tetanus antibody levels) or pneumococcal vaccination (serotype-specific pneumococcal vaccination), provides an indication of T-dependent B cell function. T-independent antibody responses to encapsulated polysaccharides may be tested in older children (>5 years of age). Responses to vaccination may be deficient in CVID, though they are normal in THI. Specific antibody deficiency in the

face of normal IgG/A/M levels is described when there is a failure to respond to encapsulated polysaccharide vaccination, and may be associated with recurrent sinopulmonary infection.

Flow cytometric extended B cell memory phenotyping quantifies B cells at their various stages of development and activation, and certain abnormalities, such as raised levels of transitional B cells, may be indicative of impaired B-cell maturation in keeping with PID. A strong suspicion of CVID in young children may indicate a need for genetic testing to rule out monogenic mimics of CVID, as CVID is more common in adolescence and adulthood (3).

Isolated absent or low levels of IgA are associated with varying susceptibility to autoimmunity and sinopulmonary and gastrointestinal infections, but is asymptomatic in the majority of patients (3). Serum IgE testing is used to suggest HIES: the more severe autosomal dominant form (Signal Transducer and Activator of Transcription Factor 3/STAT3 loss-of-function mutations) may present as invasive infection complicated by pneumatocoele formation, while the milder, autosomal recessive forms (DOCK8 deficiency, Tyk2 deficiency) present with multiple allergies, severe eczema, and skin infections. IgE levels in these conditions are usually more than ten times greater than the age-related upper limit of normal (1).

Investigation of humoral immunity should include testing of the complement system, particularly in cases of recurrent meningococcal sepsis. This includes quantification of C3 and C4 as well as functional testing of the classical and alternative complement pathways, most often done through CH50 and AP50 testing respectively. Testing of other individual complement components, such as C1q and components of the C5b6789 membrane attack complex, are guided by clinical presentation and results of initial complement pathway testing (1). Levels of mannose-binding lectin (MBL), a lectin protein involved in opsonisation of foreign antigen, may be measured. While often asymptomatic, patients with MBL deficiency

may experience an excess of infections during the first few years of life, and may benefit from short courses of prophylactic antimicrobials (3). MBL testing in isolation is rarely helpful in our experience and absent MBL in a patient with a significant infection history should not limit further appropriate investigation.

IgG1-4 subclass testing is sometimes used to exclude IgG subclass deficiency in patients with recurrent sinopulmonary infection, though testing for functional antibody responses usually has greater clinical relevance (3).

c. Cell-mediated immunity

Reference should be made to the patient's newborn screening TRECs result, when available, as well as FC-LSS. This quantifies B cell (CD19), T-helper (CD4), T-cytotoxic (CD8) and natural killer (CD16/56) cells, with a view to identifying deficiencies in single or multiple lymphocyte lineages that may indicate primary or secondary immunodeficiency. Transient deficiencies may occur in the context of acute illness or autoimmunity, though if persistent may indicate a need to exclude PID (3). In children, FC-LSS should include an analysis of the proportions of naive and memory lymphocytes as a means to quantify thymic T-cell output, and to exclude maternal engraftment of T-cells that might conceal the presence of SCID on basic lymphocyte subset analysis. Naive T-cell proportions are highest in infancy, and wane over the years of childhood into adolescence and adulthood (1). Quantification of TRECs and recent thymic emigrants are further means of assessing thymic T-cell output and diversity (1).

T-cell function is assessed using proliferation assays: T-cell activation is induced in vitro using a range of targeted mitogens or antigens. Phytohaemagglutinin (PHA) stimulation cross-links

carbohydrates on the cell surface to activate T-cells irrespective of T-cell receptor function, while CD3 and recall antigen proliferation assays (such as the candida proliferation assay) activate T cells by direct stimulation of the CD3 receptor. Failure of T-cell proliferation following PHA or CD3 stimulation may indicate primary or secondary immunodeficiency, with further functional immunophenotyping indicated as below.

Response to PHA stimulation but failure to respond to CD3 stimulation may indicate actin cyto-skeleton abnormalities such as those seen in Wiskott-Aldrich Syndrome (WAS) or DOCK8 deficiency, or a T-cell receptor deficiency (1). Failure of T-cell proliferation in response to antigen stimulation may indicate the absence of prior exposure to the antigen, or an inability to mount an appropriate immune response, as seen in chronic mucocutaneous candidiasis syndromes (1). In addition to proliferation assays, cytokine production following mitogen stimulation may also be used: impaired IL-17 production following T-cell stimulation is consistent with HIES among other diseases, requiring genetic testing which may be followed by confirmatory functional assessments.(1). Ionomycin stimulation testing may be used in the investigation of suspected Calcium release-activating calcium (CRAC) channelopathies, such as ORAI-1 or MAGT-1 deficiency (1). All proliferation assays may fail in patients receiving immunomodulatory therapy such as steroids or biological therapies, and whole blood assays if the patient is lymphopenic (3).

d. Phagocyte disorders

Nitroblue tetrazolium (NBT) testing is the first-line investigation for suspected chronic granulomatous disease (CGD), with flow cytometry-based dihydrorhodamine (DHR) testing reserved for cases with a high index of suspicion despite normal NBT testing or where there is insufficient expertise in NBT analysis. Abnormalities in either will require confirmatory CGD

protein expression by fluorescence-activated cell-sorting (FACS) or immunoblot and genetic testing (3).

e. Innate immunity

Natural killer (NK) cells are quantified using FC-LSS. Functional assessment of NK cells can be undertaken by granule release assay testing, abnormalities of which may be indicative of primary haemophagocytic lymphohistiocytosis (HLH) syndromes (Familial Haemophagocytic Lymphohistiocytosis types 3-5/FLH3-5, Chediak Higashi, or Griscelli syndrome), and a need for subsequent genetic testing (1). Cytokine and interferon profiling is performed often on a research basis, may be useful in the investigation of suspected MSMD, while anti-cytokine antibodies are used in the investigation of suspected Autoimmune Polyendocrinopathy Candidiasis and Ectodermal Dysplasia (APECED) syndrome. Functional assessments, such as testing of the interleukin-12 and interferon-gamma pathway for MSMD, can then be performed to outline whether abnormalities of cytokine profiling or the presence of anti-cytokine antibodies interrupts normal cytokine signalling in vitro (1). Defects in innate immunity can be examined using functional assays of loss of CD62-ligand expression (L-selectin shedding) in the investigation of patients with suspected Toll-like receptor defects (such as IRAK4 or MyD88 deficiency) (1).

b) Confirmatory testing

Confirmatory testing of suspected PID is performed in 2 ways: functional analyses of specific immunological pathways and protein expression, and genetic testing (3).

a. Functional and protein expression testing

Functional testing of immunological pathways is a directed method of confirming specific abnormalities, offering a targeted solution that can be related to clinical phenotype and guide subsequent genetic testing (3). Investigation into the expression of specific surface and intracellular proteins also sheds light on potential immunological defects: this is done using flow cytometry or by Western Blot (3). These assays are largely restricted to specialised laboratories, with many new assays being developed. Functional and protein expression testing is easier to define in loss-of-function mutations, with gain-of-function results being harder to interpret (3).

Phagocytic functional assays include DHR testing for suspected CGD, functional assessment of phagocytosis, and testing of the expression of the CD11a/b/CD18 integrins when investigating suspected LAD (3). Specific gene expression studies can be performed depending on the results of initial immunophenotyping. For example, Bruton's Tyrosine Kinase (BTK) expression can be analysed in the work-up of suspected XLA with monocyte functional assessment for those with novel mutations, and WAS protein expression in the work-up of suspected X-linked thrombocytopenia or WAS. Signaling lymphocytic activating molecule-associated protein (SAP), X-linked inhibitor of apoptosis protein (XIAP) or perforin expression may be tested during investigation for suspected primary HLH or lymphoproliferative disease, in conjuction with CD107a induction after PHA or CD3 stimulation for FLH3-5 (1). Suspected HIGM syndrome can be investigated by testing expression of CD40-ligand in the first instance, and DOCK8 expression can be assessed in the investigation of suspected autosomal recessive HIES (1). STAT phosphorylation pathways may be analysed following stimulation, allowing confirmation of the pathogenicity of genetic variants in, for example, the STAT1 or STAT3 genes. Common gamma chain abnormalities in suspected X-linked SCID can be assessed by

FACS, coupled with confirmatory STAT5 phosphorylation assessment, which may also be used to confirm common JAK3 and IL7ra SCID (1).

b. Genetic testing

Genetic testing for PID is indicated to confirm underlying genetic abnormalities consistent with those demonstrated on functional or protein expression testing, or to exclude known PID in patients without proven specific functional immunological defects (3). Genetic testing may be targeted around a specific diagnosis where there is a high index of suspicion, such as sequencing of the BTK gene in boys with agammaglobulinaemia to confirm XLA. The choice of genomic over functional assessment should be based on both the sensitivity of the functional assay for a confirmed genetic defect and the need for clinical expediency.

XLA is preferentially assessed by genetics in boys with no B-cells, agammaglobulinaemia and no tonsillar tissue, because protein expression alone, although timely, will miss 10-15% of those affected who have functional mutations with residual protein expression. In suspected primary HLH, Perforin, MUNC, Syntaxin, STXB2, XIAP and SAP abnormalities can all be reliably excluded by functional and protein expression assays in under 24 hours, whereas at present most genetic screening takes a minimum of 2 weeks. Increasingly, in the non-acute setting, whole exome (WES) or even whole genome sequencing (WGS) are the approach of choice in investigating suspected PID, particularly when the presentation and immunophenotyping are not sufficiently specific to suggest a single diagnosis or when an assay is not yet available for this. Furthermore, in undertaking WES for PID, the initial approach targets known PID genes in an effort to establish a genetic diagnosis – if results are equivocal and the index of suspicion very high for PID, a more agnostic approach can be taken via WGS with a trio (both parents plus affected child) and may identify novel genetic causes of PID.

However, as WES and WGS generate novel variants of unknown significance, protein and functional data are required to classify these variants (Table 2).

Limitations

Ultimately, individual PIDs remain rare, so the clinician must strike a balance between requesting fruitless investigations and investigating PID thoroughly. Many of the tests described are limited to highly specialised laboratories, while the nature of certain functional tests means that false positives are sometimes inevitable, and require adequate sampling procedures, transport, and submission of an accompanying control sample. Positive results may need to be repeated, which is both an inconvenience to the patient and their family and an added expense to the service-provider. Negative results, including from genetic testing, may conceal unknown PIDs that are not yet fully understood or characterised, and limited by our understanding of epigenetics and non-coding space disorders, so that clinicians investigating potential PID should be guided by the clinical course of the patient regardless of the results of immunological investigation. It is likely that a number of PIDs, such as CVID, are complex genetic traits that at present we do not have the knowledge to investigate at a molecular level (3).

Conclusion

With the list of known single gene PIDs ever growing, confirming or excluding PID has become more complex, and the tools to help the clinician achieve this have grown in number and complexity. Initial assessments should be guided by a detailed clinical history and

examination, and incorporate basic testing of full blood count, renal and liver function, HIV serology, and nutritional status. Humoral immunity is assessed in the first instance by checking immunoglobulin levels, C3 and C4 levels, quantifying B cell number on FC-LSS. Cell-mediated immunity is investigated through memory and naive FC-LSS and T-cell proliferative assays, and suspicion of phagocytic abnormalities investigated with an NBT test or LAD markers as appropriate. Vaccine responses are used to assess both B and T cell function. Further investigation should be guided by the results of initial immunophenotyping, and may include functional testing of specific immunological pathways, analysis of protein expression, and genetic testing, which may be targeted towards sequencing of specific genes, or adopt a wider WES/WGS approach. In cases with multiple clinical indicators of underlying PID, genetic testing may be indicated despite normal immunophenotyping.

Practice points

- Target investigations depending on clinical phenotype, then broaden investigations depending on the results.
- Basic assessments are essential: full blood count, vaccination responses, excluding other risk factors for infection such as HIV, anatomical defects, nutritional deficiencies, or bacterial colonisation.
- Ask for help: this is a new and growing field and there are plenty of experts who are ready to help.
- Genetics may be the only way to reliably exclude known PID in some cases.

Boxes

Syndromic immunodeficiency
DiGeorge syndrome
CHARGE syndrome
Kabuki syndrome
Wiedemann-Steiner syndrome
Trisomy 21
Ataxia-telangiectasia
Immunodeficiency-centromeric instability-facial anomalies syndrome
Nijmegen Breakage Syndrome
Schimke's Immuno-osseous dysplasia
Cartilage-hair hypoplasia
Multiple intetstinal atresia with combined immunodeficiemncy/TTC7a deficiency

Box 1. Genetic syndromes often associated with immunodeficiency

Tables

Clinical presentation		PID
Gastrointestinal	Failure to thrive	SCID, CGD, XLA, syndromic immunodeficiency
	Inflammatory bowel disease	IPEX, LRBA deficiency, CTLA4 deficiency
Haematology	Microthrombocytopenia	Wiskott-Aldrich syndrome, WIP
	Autoimmune cytopenia	ALPS, APDS, CTLA4 deficiency, LRBA deficiency
Neurology	Ataxia	Ataxia-telangiectasia
	Developmental delay	Nijmegen-Breakage syndrome
Skin	Eczema	Hyper-IgE syndrome
		Wiskott-Aldrich Syndrome
		Omenn syndrome (SCID)
	Hypopigmentation	Chediak Higashi syndrome
		Griscelli syndrome
		Hermansky-Pudlak syndrome
Other	Delayed cord separation	Leukocyte adhesion disorders

Table 1. Non-infectious presentations of primary immunodeficiency disorders. (PID: primary immunodeficiency disorders)

Classification of genetic variants

Benign	1 stand-alone benign criterion OR ≥2 strong benign criteria
Likely benign	1 strong and 1 supporting benign criterion OR ≥2 supporting benign criteria
Uncertain significance	Variant does not fulfill criteria for pathogenic or benign OR evidence for benign or pathogenic is conflicting
Likely pathogenic	1 very strong and 1 moderate pathogenic criteria OR 1 strong and 1-2 moderate pathogenic criteria OR 1 strong and ≥2 supporting pathogenic criteria OR ≥3 moderate pathogenic criteria OR 2 moderate and ≥2 supporting pathogenic criteria OR 1 moderate and ≥4 supporting pathogenic criteria
Pathogenic	1 very strong pathogenic criterion and ≥1 strong/≥2 moderate/1 moderate and 1 supporting/≥2 supporting pathogenic criteria OR ≥2 strong pathogenic criteria OR 1 strong and ≥3 moderate/2 moderate and ≥2 supporting/1 moderate and ≥4 supporting pathogenic criteria

Table 2. Classification of genetic variants (Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May; 17(5): 405-424).

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Figure 1. Clinical pathway for SCID newborn screening in the UK. (TRECs=T-cell receptor excision circles; SCID=severe combined immune deficiency; CMV=cytomegalovirus)