Review Article

CHALLENGING BELIEFS: ONLY A GENETIC DIAGNOSIS OF PRIMARY IMMUNODEFICIENCY DISORDERS WILL SUFFICE FOR SOME PATIENTS, EVEN IN A RESOURCE-POOR SETTING

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ABSTRACT

Genetic testing has become important in the repertoire for the diagnosis of primary immunodeficiencies. More than 350 genetic disorders have been molecularly characterised since the first description of primary immunodeficiencies. Some authorities regard the first description of Bruton agammaglobulinaemia in 1952 as the foundation of this field, whereas others refer to the first description of neutropaenia in 1922 as its origin. Genetic testing for primary immunodeficiencies is important in order to make a definitive diagnosis, to guide definitive treatment, to arrive at a prognosis, to make prenatal genetic diagnoses, for family planning and to evaluate the risk to other family members. Primary immunodeficiencies often present in various ways, with overlapping symptoms. In resource-poor countries, primary immunodeficiency diagnoses and classifications are often based on the availability of a few basic tests with which only a probable diagnosis can be provided. To a large extent, this influences both management and outcome. In this case series, we describe eight patients in whom next-generation sequencing has proved to be of value in making a definitive diagnosis. Such diagnosis has helped practitioners to guide directed therapy, counsel family members appropriately, enable prenatal testing, predict prognosis and explain the symptoms of family members, including carrier mothers. The series also emphasises the value of and the necessity for genetic testing in resource-limited settings.

BACKGROUND

Primary immunodeficiencies (PIDs) are hereditary conditions caused by more than 350 known single pathogenic-sequence variants (mutations) that have been described, and this number continues to increase.[1] They are a heterogenous group of disorders that range in incidence from 1 : 300 to 1 : 100 000. PIDs often present as recurrent infections causing major morbidity, mainly because of missing or dysfunctional immune components. A subset of PIDs is fatal within the first two years of life when it is not recognised and treated promptly and efficiently.[2,3] These disorders need not necessarily present in childhood; indeed, patients often present only in adulthood or are misdiagnosed in childhood and present to adult physicians as recurrent infections, end-organ damage (bronchiectasis) and/or immune dysregulation with autoimmune manifestations, granulomatous infiltration or malignancies.[4–11] Patients often suffer considerable morbidity and impaired quality of life (QoL). There is often a lag of five to ten years before the condition is diagnosed, and this contributes to their morbidity.

Quantitative and qualitative immunology testing remains the mainstay of PID diagnosis, but such testing can be time-consuming and can, at best, result in a probable diagnosis.[12] Life-saving therapies may be delayed or not offered due to a lack of a definitive diagnosis. Genetic testing in routine diagnostic laboratories has always been the prerogative of developed nations, but it has recently become available in South Africa, a largely resource-poor environment. With increasing requests

and improved technology, this technology has become more affordable. Many of these PIDs demonstrate an overlap in clinical presentation (phenotype) and genetic confirmation is increasingly required in order to make a definitive diagnosis. This will enable correct classification of the PID and therefore appropriate management and care,[13–15] and we believe this is true even in resource-poor countries.

Genetic testing of PIDs has become a necessary and invaluable adjunct in the diagnostic armamentarium of these disorders, for various reasons:

- making a definitive diagnosis;
- guidance regarding definitive treatment;
- prognosis;
- prenatal diagnosis;
- family planning, and
- risk to other family members.

A genetically delineated immunodeficiency makes possible informed family planning, such as pre-implantation genetic diagnosis (PGD) and prenatal testing for affected or at-risk families.[16]

Next-generation sequencing (NGS) of a subset of genes known to be causative of PIDs has been shown to identify mutations successfully in previously described genes.[13-15]

A laboratory in South Africa has started a PID genetic panel comprising known genes associated with PIDs and using a targeted NGS molecular approach to PIDs. Genes associated with the following phenotypes are included in the panel:

- severe combined immunodeficiency syndrome (SCID)
- hyper IgM syndrome
- hyper IgE syndrome
- immune dysregulation
- common variable immunodeficiency (CVID)
- lymphoproliferative syndromes, including autoimmune lymphoproliferative syndrome
- chronic granulomatous disease (CGD)
- major histocompatibility complex (MHC) class I and MHC class II deficiencies
- anhidrotic ectodermal dysplasia with immunodeficiency
- autoimmunity with lymphoproliferation
- antibody deficiencies
- T regulatory cell defects
- familial hemophagocytic lymphohistiocytosis (FHL), including FHL with hypopigmentation
- chemokine signalling defects
- innate immunity defects
- complement deficiencies
- isotype deficiencies
- thymic defects with congenital abnormalities.

Other international laboratories also offer a wide selection of NGS testing with panels specifically targeted at PIDs. Genetic counselling is always provided when these tests are performed. Such counselling is imperative to equip the patients and their families with the necessary information about their conditions and sequelae.

Here we report the findings from a local genetic PID panel of testing from eight patients with PIDs in whom the definitive diagnosis was unknown or not confirmed.

CASE REPORTS

Patient 1 was a seven-year-old, HIV-negative male who presented with bronchiectasis. An immunological laboratory work-up demonstrated panhypogammaglobulinaemia, normal quantitative B cells (therefore excluding Bruton's X-linked agammaglobulinaemia), decreased class-switched memory B cells and impaired functional humoral immunity with suboptimal vaccine-induced antibody responses.

A working diagnosis of CVID was made, but because of the severity of his clinical presentation with end-organ damage, a more severe PID was considered. A family history of another male sibling of the same parents who died from fulminant liver failure after an acute febrile illness led to the suspicion of an X-linked hereditary disorder.

Patient 1 was tested on the PID genetics panel. A pathogenic sequence variant in exon 2 of the *SH2D1A* gene was detected in a hemizygous state (*SH2D1A*: c.192G>A; p.(Trp64*)). This confirmed the diagnosis of X-linked lymphoproliferative disorder (XLP). The mother of this patient had undergone targeted testing for the specific sequence variant performed and was found to carry the same pathogenic variant in a heterozygous state, therefore confirming her X-linked carrier status. The Epstein–Barr virus (EBV) serology was negative and the EBV viral load was also negative. The child is currently being treated with immunoglobulin replacement therapy and is continuously being screened for EBV seroconversion and monitored for the development of B-cell lymphoma.

In this patient, characterisation of the gene defect led to the diagnosis of XLP. This has had important implications both for his medical care and for risk counselling for the family and first-degree relatives. The patient has access to careful clinical monitoring and has had a work-up for haematopoietic stem-cell transplantation (HSCT), which is currently the only curative therapy for this condition.[17–19]

Patient 2 was an 18-month-old boy who presented with severe failure to thrive, recurrent hospital admissions and four episodes of *Pneumocystis jirovecii* pneumonia. He had absent T-cell receptor excision circles (TRECs) on PCR, decreased T and NK cells and normal B cells with increased gamma/delta T cells and reduced naive CD4 T cells. His lymphocyte proliferations to mitogens were less than 10% of the normal control and a diagnosis of severe combined immunodeficiency was made.

Genetic testing with the PID panel revealed one pathogenic sequence variant (*IL2RG* c.664C>T; p.(Arg222Cys)) in a hemizygous state. This pathogenic sequence variant is predicted to cause a missense substitution in the *IL2RG* gene, which leads to the production of an abnormal *IL2RG* protein.

This change in the gene can lead to the production of a non-functional version of the common gamma chain (CD132/ IL2RG) or prevent any protein from being produced. Without the common gamma chain, important cytokine signals are not relayed to the nucleus and lymphocytes cannot develop normally. A lack of functional, mature lymphocytes then prevents the immune system from fighting off infections.

This pathogenic sequence variant was deemed to be clinically relevant and was also subsequently detected in his younger brother, who presented with absent TRECs, low T- and NK-cell subsets and markedly reduced lymphocyte proliferations to mitogens.[20] The diagnosis of an X-linked SCID was made for both brothers. X-linked SCID is the most common form of SCID and has been estimated to account for 46% to 70% of all SCID cases.[21–23]

The brothers received HSCT. Haematopoietic stem-cell pre-treatment regimens and post-transplant management regimens differ between the various SCIDs (e.g. XL-SCID vs *RAG1* deficiency) and therefore the correct genetic diagnosis of SCID is important for if this disorder is to be managed and cared for precisely.[24–27]

Patient 3 was a six-week-old boy who developed *Legionella pneumophilia* liver abscesses that would not respond to optimum antibiotic therapy. On full blood count he had a persistent lymphopaenia (defined as an absolute lymphocyte count below the reference range for age on multiple occasions), which is a warning sign for an underlying PID in a baby. The T- and NK-cell lymphocytes subsets were severely decreased, raising a suspicion of a T-B+NK- SCID. The following immunological findings further strengthened the diagnosis, making this the most probable diagnosis: absent recent thymic emigrants, severely reduced naive CD4 and absent T-cell receptor excision circles (TREC) performed on a polymerase chain reaction (PCR), with lymphocyte proliferations against the mitogen phytohaemagglutinin (PHA) being less than 10% of the normal control.[28]

Genetic testing was performed using the PID panel. One pathogenic sequence variant (*IL2RG* c.562C>T; p.Glnl188Ter) was detected in a hemizygous state. This confirmed a diagnosis of X-linked SCID. *IL2RG* c.562C>T; p.Glnl188Ter is a novel sequence variation and was predicted to cause a nonsense substitution in the *IL2RG* gene. This mutation was predicted to lead to the production of a non-functional version of the common gamma chain; alternatively, it might prevent any protein from being produced.[29]

No local South African match was found for the HSCT for this patient and only a partial international match was found. This significantly increased his risk of developing graft-versus-host disease post-transplant. The patient's mother applied for participation in a gene-transfer trial at St Jude's Hospital in Memphis in the United States. The trial is entitled 'A Pilot Feasibility Study of Gene Transfer for X-Linked SCID in Newly Diagnosed Infants Using a Self-Inactivating Lentiviral Vector to Transduce Autologous CD34 + Hematopoietic Cells'. The patient received gene-transfer therapy successfully; a normal copy of the *IL2RG* gene was introduced by a genetically engineered self-inactivating lentiviral vector. He is reportedly the first African child cured by means of gene-transfer therapy and will be monitored continuously until age 18.[30]

Genetic confirmation of an X-linked SCID qualified this boy for the gene-transfer therapy trial, enabling him to receive life-saving, definitive and directed treatment.[25–27,31]

Patient 5 was a newborn boy with a family history of a maternal cousin's (boy) having received HSCT for SCID with an unknown genetic mutation. Immunological testing was performed. This showed the TREC PCR to be negative, with absent recent thymic emigrants and markedly reduced T and NK cells, with normal B cells. His lymphocyte proliferation responses to the mitogen PHA was markedly reduced (less than 10% of the normal control). X-linked SCID was suspected. NGS genetic testing for the PID genes revealed a likely pathogenic novel sequence variant in IL2RG c.442C>G; p.(Leu148Val) in a hemizygous state. The mother was confirmed to be a carrier of the same pathogenic sequence variant, as was her sister, mother of the index patient with SCID. An extensive family history revealed multiple early deaths of boys in the family.

Patient 5 successfully received HSCT. Sister 3, who happened to be pregnant at the time, tested negative for the pathogenic sequence variant. Prenatal genetic testing of the foetus was deemed unnecessary, as ultrasound scans had confirmed the foetus to be female and the mother was not a carrier. Prenatal genetic testing is an option for carrier female members of this family for family-planning purposes. Such testing should be accompanied by comprehensive and careful genetic counselling.[31]

Patient 6 was a 27-year-old man who had presented throughout his life with recurrent upper-end lower respiratory tract infections, complicated by bronchiectasis. He had severe hypogammaglobulinaemia, with a total IgG of 0.22 g/L (reference range 7–9 g/L). He had normal T- and B-lymphocyte subsets, but his recent thymic emigrants and NK cells were decreased. He had an increased gamma/delta CD8 T-cell population, with decreased NK-cell cytotoxicity relative to controls used. Secondary causes of immunodeficiency and lymphoproliferative disorders were excluded. Major histocompatibility complex (MHC) class 1 expression on monocytes, and activated lymphocytes on flow cytometry, was decreased.

Genetic testing with the PID panel detected one pathogenic sequence variant in exon 7 of the *TAP1* gene, c.1744C>T and one variant of uncertain clinical significance (VOUS); *TAP1* c.2117G>A; p.Gly706Asp was detected in exon 10 in the TAP1 gene. Some patients with class I MHC deficiency (bare lymphocyte syndrome type I, MIM #604571) have defects in *TAP1* (transporter, ATP-binding cassette, major histocompatibility complex 1). Patients may have chronic bacterial infections, often beginning in the first decade of life, which are restricted to the

respiratory tract and extend from the upper to the lower airway. Bronchiectasis, emphysema, panbronchiolitis and bronchial obstruction have been described.

These mutations were judged to be the most likely cause of the patient's immune defect, as a result of clinical and laboratory functional correlation. The genetic diagnosis was a significant finding in this patient; HSCTs are not indicated in these patients and immunosuppression should be avoided. It also answered many questions for the family and explained why he had been so unwell for most of his life.[32–34]

Patients 7 and 8 were a boy, two years and eight months of age, and his 25-year-old mother. The boy presented with recurrent upper and lower respiratory infections and an episode of *Staphylococcus aureus* pneumonia. The *in vitro* flow cytometric dihydrorhodamine 123 (DHR) assay identified an abnormal neutrophil respiratory burst of only 3% after Phorbol myristate acetate (PMA) stimulation. The mother had a history of always being sick as a child and suffered various upper respiratory infections and a couple of episodes of pneumonia. She was chronically unwell as an adult and was admitted for six weeks for a *Salmonella* septic arthritis when patient 7 was 18 months old. Anti-SSA antibodies (associated with autoimmune connective-tissue diseases such as systemic lupus erythematosis (SLE), and Sjögrens' disease) were detected in the mother, confirming the induction of autoimmunity. The *in vitro* flow cytometric dihydrorhodamine 123 (DHR) assay identified a dual neutrophil population; one population with a good neutrophil respiratory burst and one neutrophil population without it. After PMA stimulation, a 40% residual respiratory burst was noted. A provisional diagnosis of X-linked chronic granulomatous disease (XL-CGD) was made, with the mother suspected as being a carrier.

Sanger sequencing of the *CYBB* gene detected a sequence variant: *CYBB* c.1613G>A; p.Gly538Glu in exon 13, in a hemizygous state in patient 7. This detected novel sequence variant was predicted to be disease-causing, supported by *in silico* analysis using PolyPhen-2, SIFT, Mutation Taster and Align GVGD. *In silico* analysis of the mutation revealed partial, sub-optimal function of CYBB protein, but not complete absence. This explained the 3% residual oxidative burst observed on flow cytometry (as opposed to a complete absence of the oxidative burst usually observed in X-linked CGD) and in this way explained why he presented at a slightly later age. The sequence variant was subsequently detected in a heterozygous state in his mother, patient 8, confirming her carrier status.

Approximately 50% of X-linked carriers have reported to have recurrent mouth lesions in the form of gingivitis or stomatitis. They have a high risk of developing autoimmune disorders, mainly SLE. The skewing of X-chromosome inactivation (lyonization) has been reported to confer a mild clinical phenotype in female carriers. Most of these female carriers have less than 10% normal neutrophils, although clinical symptoms have also been described in female carriers with more than 10% residual oxidative burst.

The genetic testing confirmed the diagnosis in the patient but also provided prognostic information. Even though HSCT is the definitive treatment, expertise in South Africa in transplanting these children is limited. If this child is unable to receive HSCT in an overseas unit, he should be treated with prophylactic antibiotics and antifungals and have proactive aggressive treatment when infections do occur. If a different pathogenic sequence variant had been detected, he may have presented much earlier with a much slimmer chance of survival. Genetic diagnostic information is therefore key in predicting morbidity in XL-CGD. Genetic testing of the mother also explained her symptoms and would allow for informed family planning to occur for a possible future pregnancy.[24,35–40]

DISCUSSION

These clinical cases suggest that NGS PID panels (available in South Africa and abroad) should be seen as a necessity in detecting specific genetic defects in patients with PIDs and should be promoted as standard of care to facilitate better access to definitive therapy.

PIDs often present in diverse ways, with overlapping symptoms and syndromes. Genetic testing for patients with an atypical presentation of an immunodeficiency will allow clinicians to make a definitive diagnosis, better inform patients with respect to the expected prognosis and will also promote directed therapy. A subset of conditions qualify for and need definitive treatment (including HSCT) for a patient's survival. If these conditions are not identified timeously, QoL and survival will be shortened, with significant morbidity. Genetic testing is often the only way to distinguish a humoral immunodeficiency, which is treated mainly with immunoglobulin replacement therapy, from a combined immunodeficiency that will need HSCT.

The case study of patient 1 with XLP (classified as an immune dysregulation syndrome by the International Union of Immunological Societies' classification of inborn errors of immunity[41]) clearly demonstrated this scenario. In patient 3, a definitive genetic diagnosis was life-saving and allowed him to be accepted as part of a clinical trial to receive gene-transfer therapy. This option would never have been possible without a genetic diagnosis.

The identification of a specific mutation can help to predict the clinical course of the disease and is of prognostic value. Chronic granulomatous disease (CGD) can have a variable phenotype: from patients presenting in the first year of life with fulminant life-threatening infections to patients remaining undiagnosed until adulthood and suffering from mild abscesses. In CGD, the phagocyte respiratory burst is markedly reduced to absent. This can be demonstrated by a functional test performed by flow cytometry that is currently the primary diagnostic tool for this disorder. The oxidative burst test cannot, however, distinguish between the X-linked and the autosomal recessive variants. The X-linked variants have a more severe course, whereas the autosomal recessive variants often present later and have a milder phenotype. The X-linked variants may also present with variable clinical profiles that have variable outcomes depending

on the underlying genetic defect. This was clearly demonstrated in patient 7.

Prognostic counselling for an affected family can lead to the more effective use of preventive measures, including pre-implantation gender selection and plans for specific treatments post-delivery to reduce the risk of infant morbidity and mortality. Genetic testing is available via PGD or prenatal diagnosis, using chorionic villus sampling (CVS) or amniocentesis for established pregnancies.[42]

Prenatal genetic testing is not available for families that do not have a proven genetic abnormality.[42,31]

One limitation of NGS using a specific panel of genes is that diagnosis can be made only if pathogenic changes are detected in the subset of genes. Whole exome sequencing (WES) and whole genome sequencing (WGS), as it becomes more readily available, may replace targeted testing for the diagnosis of unknown PIDs in the future, as more genes are identified as causative of PIDs. Genetic testing, no matter the methodology, is predicted to become a standard diagnostic procedure for PIDs as more functional studies are conducted on the resultant proteins.[31,43–45]

For the reasons outlined, we believe that this service should become a routine of care in resource-limited settings and for all population groups. The importance and frequency of PIDs in all populations now require governments and health providers to provide this standard of care to everyone who may require it.

The need for geneticist (clinical and laboratory) and bio-informatics training would be an important component of this care as developing nations realise the frequency of genetic disorders in the population.

CONCLUSION

A targeted NGS panel of testing for 99 genes, found to be causative for many PIDs, has proven value in order to:

- make a definitive diagnosis;
- guide directed therapy;
- counsel family members appropriately;
- enable PGD and prenatal testing;
- predict prognosis, and
- explain symptoms of family members, including carrier mothers.

These case studies have demonstrated that the PID genetic panel used in our laboratory made accurate genetic diagnoses and influenced the course of many patients' lives. With the advent of improved technology and increased demand, these assays will become more affordable and more widely available. This service should become a routine of care in resource-limited settings and for all population groups. The importance and frequency of PIDs in all populations now requires governments and health providers to provide this standard of care to everyone who may require it.

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Some of the authors are employed at Ampath Laboratories.

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REFERENCE

1. Picard C, Al-Herz W, Bousfiha A, Cassanova JL, et al. Primary Immunodeficiency Diseases: An update on the classification from the International Union of Immunological societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol 2015;35(8):696–726.
2. De Pagter AP, Bredius RG, Kuijpers TW, Tramper J, et al. Overview of 15-year severe combined immunodeficiency in the Netherlands: Towards new born blood spot screening. Eur J Pediatr 2015;174(9):1183–1188.
3. Chan A, Scalchunes C, Boyle M, Puck JM. Early vs delayed diagnosis of severe combined immunodeficiency: A family perspective survey. Clin Immunol 2011;138(1):3–8.
4. Ghosh S, Thrasher AJ, Gaspar HB. Gene therapy for monogenic disorders of the bone marrow. Br J Haematol 2015;171(2):155–170.
5. Genovese P, Schiroli G, Escobar G, Di Tomaso T, et al. Targeted genome editing in human repopulating haematopoietic stem cells. Nature 2014;10(7504):235–240.
6. Hacein-Bey Abina S, Gaspar HB, Blondeau J, Caccavelli L, et al. Outcomes following gene therapy in patients with severe Wiskott-aldrich syndrome. JAMA 2015;313(15):1550–1563.
7. Pai SY, Logan BR, Griffith LM, Buckley RH, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. N Eng J Med 2014;371(5):434–446.
8. Montiel-Equihua CA, Thrasher AJ, Gaspar HB. Gene therapy for severe combined immunodeficiency due to adenosine deaminase deficiency. Curr Gene Ther 2012;12(1):57–65.
9. Henderson C, Goldbach-Mansky R. Monogenic autoinflammatory diseases: New insights into clinical aspects and pathogenesis. Curr Opin Rheumatol 2010;22(5):567–578.
10. Milman N, Andersen CB, Hansen T, Nielsen FC, et al. Favourable effects of TINF-alpha inhibitor (infliximab) on Blau syndrome in monozygotic twins with a de novo CARD15 mutation. APMIS 2006;114(12):912–919.
11. Aksentjievich I, Masters SL, Ferguson PJ, Dancey P, et al. An auto inflammatory disease with deficiency of the interleukin-1-receptorn antagonist. N Eng J Med 2009;360(23):2426–2437.
12. Abraham RS. Relevance of laboratory testing for the diagnosis of primary immunodeficiencies: A review of case-based examples of selected immunodeficiencies. Clin Mol Allergy 2011;9:6.
13. Nijman IJ, Van Montfrans JM, Hoogstraat M, Boes ML, et al. Targeted next-generation sequencing: A novel diagnostic tool for primary immunodeficiencies. J Allergy Clin Immunol 2014;133(2):529–534.
14. Moens LN, Falk-Sorqvist E, Asplund AC, Bernatowska E, et al. Diagnostics of primary immunodeficiency diseases: A sequencing capture approach. PloS One. 2014;9(12).
15. Al-Mousa H, Abouelhoda M, Monies DM, Al-Tassan N, et al. Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases. J Allergy Clin Immunol 2016;137(6):1780–1787.
16. Heimall JR, Hagin D, Hajjar J, Henrickson SE, et al. Use of genetic testing for primary immuinodeficiency patients. J Clin Immunol 2018;38:320–329.

17. Soresina A, Lougaris V, Giliani S, Cardinale F, et al. Mutations of the X-linked lymphoproliferative disease gene SH2D1A mimicking common variable immunodeficiency. Eur J Pediatr 2002;161:656–659.
18. Morra M, Silander O, Calpe S, Choi M, et al. Alterations of the X-linked lymphoproliferative disease gene SH2D1A in common variable immunodeficiency syndrome. Blood 2001;98:1321–1325.
19. Panchal N, Booth C, Cannons JL, Schwartzberg PL. X-Linked Lymphoproliferative Disease Type 1: A clinical and molecular perspective. Front Immunol 2018;9:666.
20. https://www.ncbi.nlm.nih.gov/clinvar/variation/10027/.
21. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Ann Rev Immunol 2004;22:625–655.
22. Stephan JL, Donadieu J, Griscelli C. Severe combined immunodeficiency: A retrospective single-center study of clinical presenation and outcome in 117 patients. J Paediatr 1993;123(4):564–572.
23. Fischer A, Cavazzana-Calvo M, De Saint Basile G, De Villartay JP, et al. Naturally occurring primary deficiencies of the immune system. Ann Rev Immunal 1997;15:93–124.
24. Van den Berg JM, Van Koppen E, Åhlin A, Belohradsky BH, et al. Chronic Granulomatous Disease: The European Experience. PLoS One 2009;4(4).
25. Puck JM, Middelton L, Pepper AE. Carrier and prenatal diagnosis of X-linked severe combined immunodeficiency: Mutation detection methods and utilization. Hum Genet 1997;99:628–633.
26. Fischer A, Le Deist F, Hacein-Bey-Abina S, André-Schmutz I, et al. Severe combined immunodeficiency: A model disease for molecular immunology and therapy. Immunol Rev 2005;203:98–109.
27. Gennery AR. Recent advances in treatment of severe primary immunodeficiencies. F1000Research. 2015;1459.
28. European Society for Immunodeficiencies [Internet]. Clinical working party diagnostic criteria for PID – SCID diagnostic criteria [updated 2018 June 26]. Available from: http://esid.org/Working-Parties/Clinical -Working-Party/Recourses/Diagnostic-criteria-for-PID2.
29. https://www.ncbi.nlm.nih.gov/clinvar/variation/463379/.
30. www.stjude.org/research/clinical-trials/lvxscid-nd-hematological-disorder.html.
31. Sponzilli I, Notarangelo LD. Severe combined immunodeficiency (SCID): from molecular basis to clinical management. Acta Biomed 2011;82:5–13.
32. Furukawa H, Murata S, Yabe T, Shimbara N, et al. Splice acceptor site mutation of the transporter associated with antigen processing-1 gene in human bare lymphocyte syndrome. J Clin Invest 1999;103(5):755–758.
33. De la Salle H, Hanau D, Fricker D, Urlacher A, et al. Homozygous human TAP peptide transporter mutation in HLA class I deficiency. Science 1994;5169(265):237–241.
34. Elliott T. TAP2 – Transporter associated with antigen processing. Advances Immunol 1997;65:47–109.
35. Holland SM. Chronic Granulomatous Disease. Hematol Oncol Clin North Am 2013;27(1):89–99.
36. Khuns DB, Alvord WG, Heller T, Feld JJ, et al. Residual NADPH oxidase and survival in chronic granulomatous disease. N Eng J Med 2010;363(27):2600–2610.
37. Anderson-Cohen M, Holland SM, Kuhns DB, Fleisher TA, et al. Severe phenotype of chronic granulomatous disease presenting in a female with a de novo mutation in gp91-phox and a nonfamilial, extremely skewed X chromosome inactivation. Clin Immunol 2003;109:308–317.
38. Roos D, Weening RS, De Boer M, Meerhof LJ. Heterogeneity in chronic granulomatous disease. In progress in immunodeficiency research and therapy. 2 edition. Edited by: Vossen J, Griscelli C, Amsterdam: Elsevier. 1986:139–146.
39. Rosen-Wolf A, Soldan W, Heyne K, Bickhardt J, Roeslei GM. Increased susceptibility of a carrier of X-linked chronic granulomatous disease (CGD) to Aspergillus fumigatus infection associated with age-related skewing of lyonization. Ann Hematol 2001;80:113–115.
40. Winkelstein JA, Marino MC, Johnston RB, Boyle J, et al. Chronic Granulomatous disease – Report on a National registry of 368 patients. Medicine (Baltimore). 2000;79(3):155–169.
41. Picard C, Gaspar HB, Al-Herz W. International union of immunological societies: 2017 Primary Immunodeficiency Diseases Committee report on inborn errors of immunity. J Clin Immunol. 2018;38:96–128.
42. Lee W, Huang J, Yeh K, Cheng P, et al. The effects of prenatal genetic analysis on foetuses born to carrier mothers with primary immunodeficiency diseases. Ann Med 2016;(48):103–110.
43. Stray-Pedersen A, Orange S, Lupski JR. Primary immunodeficiency diseases – genomic approaches delineate heterogeneous Medelian disorders. J Allergy Clin Immunol 2017;139(1):232–245.
44. Van Schouwenburg PA, Davenport EE, Kienzler A, Marwah I, et al. Application of whole genome and RNA sequencing to investigate the genomic landscape of common variable immunodeficiency disorders. Clin Immunol 2015;160:301–314.
45. Yang Y, Muzny DM, Reid JG, Bainbridge MN, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Eng J Med 2013;369(16):1502–1511.