patients 1, 3.1, 5, 6.1, and 6.2 were examined by two different anti-XIAP mAbs. Using clone 48 antibody, patients 1, 2.1, 3.1, 3.2, 6.1, and 6.2 showed reduced XIAP expression, whereas XIAP was normally expressed in the lymphocytes of patients 4 and 5. In contrast to clone 48, clone 2F1 antibody showed reduced XIAP expression in patient 5. The effects of heterozygous *XIAP* mutations were studied in the lymphocytes of the patients' mothers by anti-XIAP mAb clone 48. The mothers of patients 1, 3.1, and 3.2 showed a bimodal pattern of XIAP protein (Fig. 2). The mothers of patients 2.1, 6.1, and 6.2 did not show a clear mosaic pattern, but all of these patients had reduced XIAP expression levels. Similarly to patients 4 and 5, the mothers of patients 4 and 5 demonstrated a normal XIAP expression pattern.

XIAP Expression in Lymphocytes from the Patients by Western Blot

Western blot analysis was used to evaluate the expression level of XIAP to determine the impact of patient *XIAP* mutations on protein expression and to compare this to the flow cytometric analysis. PBMCs from patients 3.1, 5.1, and 6.2 were available for Western blotting. All of these patients showed a reduction in XIAP protein expression (Fig. 3), fitting with the results obtained by flow cytometric analysis.

iNKT Cell Counts in the Patients

SAP-deficient patients had reduced numbers of NKT cells that expressed an invariantly rearranged T-cell receptor (TCR) consisting of TCRVα24 and TCRVβ11 chains [21,22]. The rare subset of iNKT cells was originally reported to be reduced in XIAP-deficient patients as well [7] but seemed to be present in normal numbers in a later study involving a larger patient cohort [23]. We analyzed the iNKT cell frequencies in 100,000 $CD3^+$ T cells in our XIAP-deficient patients and compared these with healthy controls (Fig. 4). The average frequency of iNKT cells within the $CD3^+$ T cell compartment of our XIAP patients was significantly reduced by twofold when compared with healthy

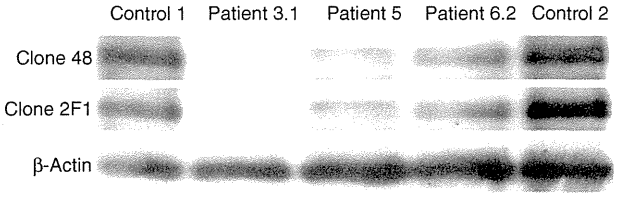

Fig. 3 XIAP expression in lymphocytes from the patients by Western blot. Analysis of XIAP expression in PBMC generated from patients with XIAP deficiency and normal controls using the antibody clone 48 (*upper panel*), the antibody clone 2 F1 (*middle panel*), and the β-actin antibody as an internal control (*lower panel*)

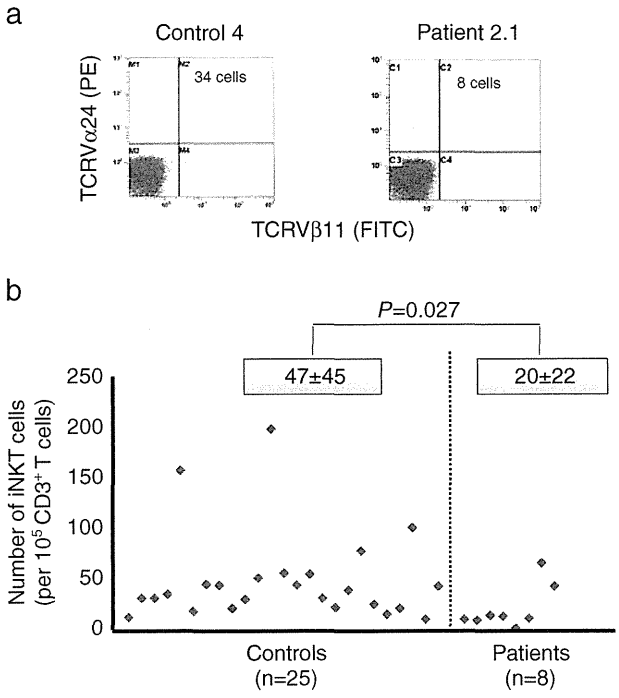

Fig. 4 iNKT cell counts in the patients and healthy controls. **a** Representative flow cytometric analysis of iNKT cells in $CD3^+$ lymphocytes from one XIAP-deficient patient and one healthy control. **b** Comparison of the number of iNKT cells in 100,000 $CD3^+$ lymphocytes between XIAP-deficient patients and control individuals. Statistical significance between patients and controls was determined with the Student's *t*-test (*p*-value=0.027)

controls (20 vs. 47 per 10^5 $CD3^+$ T cells). Therefore, we concluded that the number of iNKT cells was reduced in our patients with XIAP deficiency.

Functional Analysis of CTL Lines Established from the Patients

To test whether our XIAP-deficient patients have similar defects in $CD8^+$ T cell cytotoxicity as described in other subtypes of familial HLH [20,38], we generated $CD8^+$ alloantigen-specific CTL from patients 1, 2.1, 3.1, and three healthy controls (Fig. 5). The cytotoxic activity of the CTL of these patients was similar to that of the healthy controls, indicating that XIAP patients clearly differ from other familial HLH patients in this aspect of the disease.

Discussion

XIAP deficiency is a rare but severe and life-threatening inherited immune deficiency [12,13]. Early diagnosis and life-saving treatment such as hematopoietic stem cell transplantation is especially important. The causative gene for

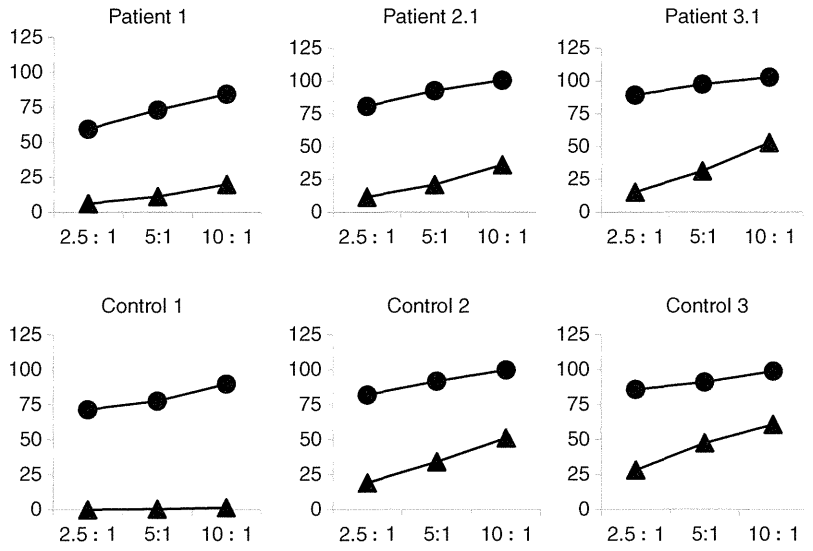

Fig. 5 Cytotoxicity of alloantigen-specific $CD8^+$ T cell lines. $CD8^+$ T cell lines were generated from PBMC of patients with XIAP deficiency and healthy controls by stimulation with allogeneic LCL (KI-LCL). Their cytotoxity was determined against allogeneic KI-LCL (*circles*) and against allogeneic TA-LCL (*triangles*), which does not share alloantigens with KI-LCL

XIAP deficiency was identified to be *XIAP/BIRC4*, and 25 mutations in the *XIAP* gene have been previously reported [7,12–14]. In the present study, we described four novel mutations (W217CfsX27, E349del, deletion of exons 1 and 2 and N341YfsX7) in the *XIAP* genes as well as previously described patients with R381X and R238X mutations [13,14]. The mother of patients 6.1 and 6.2 had no mutation in the *XIAP* gene. Because this is an X-linked inheritance, the failure to identify the same mutation in the mother suggests that the mother had a germline mosaicism for the mutation. Such mosaicism has not yet been described in XIAP deficiency, but it has been reported in Duchenne muscular dystrophy, X-linked severe combined immunodeficiency, X-linked agammaglobulinemia, and many other inherited diseases [24–26]. HLH is common in XIAP-deficient patients, and it is often recurrent [13,14]. In our study, six patients had HLH and five patients presented with recurrent HLH. Therefore, XIAP deficiency should be suspected in certain boys with HLH, especially in those with family history or recurrent HLH. The reason why XIAP deficiency increases susceptibility to HLH remains unclear. Murine studies have also failed to disclose a mechanism for the development of HLH [27]. Interestingly, Xiap-deficient mice possess normal lymphocyte apoptosis induced by a variety of means [28]. Three of our patients presented with EBV-associated HLH. EBV infection has been reported to be a trigger of the first HLH episode in patients with XIAP deficiency [13]. The excess of lymphocyte apoptosis in XIAP deficiency might account for the abnormal immune response to EBV [28]. Splenomegaly is not frequently observed in XLP type 1 or SAP deficiency but might be a common clinical feature in XIAP deficiency [12,13] as four (50%) of eight Japanese patients developed splenomegaly. Pachlopnik Schmid et al. [13] reported that recurrent splenomegaly occurring in the absence of systemic HLH was often associated with fever and cytopenia. XIAP-deficient patients are at risk for chronic colitis, which is possibly a more frequent cause of mortality than HLH [13]. Our study included two patients who developed colitis, and one of the patients died of colitis at 4 years of age. Although we did not have enough clinical information or samples from that patient because of his early death, his symptoms suggest that he had a XIAP deficiency complicated with colitis because he was the maternal uncle of patient 2.1. The other patient was 2 years old and also suffered from chronic hemorrhagic colitis.

In contrast to SAP deficiency, lymphoma has never been reported in XIAP deficiency, including our patients. Some studies indicate that the XIAP protein is a potential target for the treatment of cancer based on the anti-apoptotic function of XIAP [29]. Therefore, the absence of XIAP may protect patients from cancer, explaining why XIAP-deficient patients do not develop lymphoma. We generated a clinical summary to compare XIAP-deficient patients with the previous reports (Table II). Although our study included a relatively small number of patients, our results appear to be consistent with previous large studies [12,13] and confirm the clinical characteristics of XIAP deficiency.

Flow cytometry can be used for the rapid screening of several primary immunodeficiencies including XLP [30]. XIAP protein has been found to be expressed in various human tissues, including all hematopoietic cells [7,10]. Marsh et al. [16] described that XIAP was readily detectable in normal granulocytes, monocytes, and all lymphocyte subsets. Moreover, patients with *XIAP* mutations had decreased or absent expression of XIAP protein by flow cytometry [14,16]. We investigated XIAP expression in lymphocytes from eight patients by flow cytometry as previously described [16,17]. As demonstrated by Marsh et al. [16], clone 48 antibody provided brighter staining compared

Table II Comparison of patients with XIAP deficiency

	Marsh R et al. [12]	Pachlopnik Schmid J et al. [13]	Our study
Number of patients	10	30	9
HLH	9 (90%)	22/29 (76%)	6/9 (67%)
Recurrent HLH	6 (60%)	11/18 (61%))	5/6 (83%)
EBV-associated HLH	3 (30%)	16/19 (84%)	4/6 (67%)
Splenomegaly	9 (90%)	19/21 (90%)	4/8 (50%)
Hypogammaglobulinema	2 (20%)	8/24 (33%)	2/8 (25%)
Lymphoma	0	0	0
Colitis	0	5 (17%)	2 (22%)

to clone 2F1 antibody. In patients 5, 6.1, and 6.2, XIAP protein expression was normal when using clone 48 antibody but decreased when using clone 2F1 antibody. Western blot analysis showed XIAP expression in patients 3.1, ,5 and 6.2, and using clone 48 antibody, we found a discrepancy between flow cytometry and Western blot. Flow cytometric diagnosis may thus result in false positive results, and the gene sequencing of *XIAP* should be performed even when the patient shows normal XIAP expression levels.

All of the mothers examined in this study except for one were carriers of *XIAP* mutations. Analysis of XIAP expression in the mothers of patients 1, 3.1, and 3.2 revealed a bimodal expression pattern of XIAP in lymphocytes with cellular skewing towards expression of the wild-type XIAP allele as previously demonstrated [16]. However, the mother of patients 2.1, 6.1, and 6.2 demonstrated a normal expression pattern, possibly resulting from an extremely skewed pattern of X chromosome inactivation as shown in XIAP deficiency and other primary immunodeficiencies, and de novo mutations in *XIAP* are also observed [16,31]. The mother of patients 6.1 and 6.2 might have a germline mosaicism for the mutation, resulting in normal XIAP protein expression.

iNKT cells represent a specialized T lymphocyte subpopulation with unique features distinct from conventional T cells [32,33]. Human iNKT cells express an invariant TCR that recognizes self and microbacterial glycosphingolipid antigens presented by the major histocompatibility complex class I-like molecule CD1d [28]. The first series of XIAP-deficient patients showed decreased iNKT cell counts similar to SAP deficiency [7]. However, Xiap-deficient mice have normal numbers of iNKT cells and did not show an abnormal response to apoptotic stimuli [34]. Marsh et al. [23] reported a cohort of XIAP-deficient patients with normal numbers of iNKT cells, indicating that XIAP-deficient patients differ from SAP-deficient patients in this respect. In our cohort, we observed significantly decreased iNKT cell numbers in XIAP-deficient patients compared to healthy controls. However, we could not identify a correlation between the number of iNKT cells and the clinical disease features. Flow cytometric evaluation of iNKT cell counts can allow for the discrimination of XLP and other primary immunodeficiency diseases because patients may have normal XIAP protein expression in their lymphocytes.

CTLs kill their targets by one of two mechanisms: granule- or receptor-mediated apoptosis [35]. A recent study showed that the main pathway of cytotoxicity mediated by alloantigen-specific human $CD4^+$ and $CD8^+$ T cells is granule exocytosis and not the FAS/FAS ligand system [18]. Granzyme B is a major effector molecule of granule-mediated killing that rapidly induces cell death after entering the cytoplasm of the target cell [36]. The enzymatic activity of granzyme B is key to its ability to induce cell death. The executioner caspase-3 has been shown to be proteolytically processed and activated by granzyme B [37]. Although XIAP possesses an inhibitory effect for caspases, it is important to study the cytotoxic activities of CTLs in XIAP deficiency. Furthermore, many studies have indicated that some subtypes of patients with familial HLH show a deficiency in their cytotoxic activities [20,38]. To further investigate the function of antigen-specific CTLs, we studied $CD8^+$ alloantigen-specific CTL analysis among three XIAP-deficient patients. XIAP-deficient patients showed a normal level of cytotoxic activity, suggesting that XIAP might not play an important role in the cytotoxic responses of $CD8^+$ T cells as was previously suggested based on the normal NK cell-mediated cytotoxicity found in XIAP-deficient patients [7,12].

In this study, we have described nine Japanese patients with XIAP deficiency with clinical characteristics similar to those of patients in Europe and USA [12,13].

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References

1. Sumegi J, Huang D, Lanyi A, Davis JD, Seemayer TA, Maeda A, et al. Correlation of mutations of the SH2D1A gene and Epstein–Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. Blood. 2000;96:3118–25.
2. Seemayer TA, Gross TG, Egeler RM, Pirruccello SJ, Davis JR, Kelly CM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. Pediatr Res. 1995;38:471–8.
3. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. Nature. 1998;395:462–9.
4. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet. 1998;20:129–35.
5. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci USA. 1998;95:13765–70.
6. Gilmour KC, Cranston T, Jones A, Davies EG, Goldblatt D, Thrasher A, et al. Diagnosis of X-linked lymphoproliferative disease by analysis of SLAM-associated protein expression. Eur J Immunol. 2000;30:1691–7.
7. Rigaud S, Fondanèche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. Nature. 2006;444:110–4.
8. Uren AG, Pakusch M, Hawkins CJ, Puls KL, Vaux DL. Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. Proc Natl Acad Sci USA. 1996;93:4974–8.
9. Liston P, Roy N, Tamai K, Lefebvre C, Baird S, Cherton-Horvat G, et al. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature. 1996;379:349–53.
10. Duckett CS, Nava VE, Gedrich RW, Clem RJ, van Dongen JL, Gilfillan MC, et al. A conserved family of cellular genes related to the baculovirus iap gene and encoding apoptosis inhibitors. EMBO J. 1996;15:2685–94.
11. Galbán S, Duckett CS. XIAP as a ubiquitin ligase in cellular signaling. Cell Death Differ. 2010;17:54–60.
12. Marsh RA, Madden L, Kitchen BJ, Mody R, McClimon B, Jordan MB, et al. XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. Blood. 2010;7:1079–82.
13. Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F, et al. Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP-deficiency) versus type 2 (XLP-2/XIAP-deficiency). Blood. 2011;117:1522–9.
14. Zhao M, Kanegane H, Ouchi K, Imamura T, Latour S, Miyawaki T. A novel XIAP mutation in a Japanese boy with recurrent pancytopenia and splenomegaly. Haematologica. 2010;95:688–9.
15. Filipovich AH, Zhang K, Snow AL, Marsh RA. X-linked lymphoproliferative syndromes: brothers or distant cousins? Blood. 2010;116:3398–408.
16. Marsh RA, Villanueva J, Zhang K, Snow AL, Su HC, Madden L, et al. A rapid flow cytometric screening test for X-linked lymphoproliferative disease due to XIAP deficiency. Cytometry B Clin Cytom. 2009;76:334–44.
17. Marsh RA, Bleesing JJ, Filipovich AH. Using flow cytometry to screen patients for X-linked lymphoproliferative disease due to SAP deficiency and XIAP deficiency. J Immunol Methods. 2010;362:1–9.
18. Yasukawa M, Ohminami H, Arai J, Kasahara Y, Ishida Y, Fujita S. Granule exocytosis, and not the fas/fas ligand system, is the main pathway of cytotoxity mediated by alloantigen-specific CD4(+) as well as CD8(+) cytotoxic T lymphocytes in humans. Blood. 2000;95:2352–5.
19. Yanai F, Ishii E, Kojima K, Hasegawa A, Azuma T, Hirose S, et al. Essential roles of perforin in antigen-specific cytotoxity mediated by human CD4+ T lymphocytes: analysis using the combination of hereditary perforin-deficient effector cells and Fas-deficient target cells. J Immunol. 2003;170:2205–13.
20. Ishii E, Ueda I, Shirakawa R, Yamamoto K, Horiuchi H, Ohga S, et al. Genetic subtypes of familial hemophagocytic lymphohistiocytosis: correlations with clinical features and cytotoxic T lymphocyte/natural killer cell functions. Blood. 2005;105:3442–8.
21. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. Nat Med. 2005;11:340–5.
22. Pasquier B, Yin L, Fondanéche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. J Exp Med. 2005;201:695–701.
23. Marsh RA, Villanueva J, Kim MO, Zhang K, Marmer D, Risma KA, et al. Patients with X-linked lymphoproliferative disease due to *BIRC4* mutation have normal invariant natural killer T-cell populations. Clin Immunol. 2009;132:116–23.
24. Puck JM, Pepper AE, Bedard PM, Laframboise R. Female germ line mosaicism as the origin of a unique IL-2 receptor gamma-chain mutation causing X-linked severe combined immunodeficienc. J Clin Invest. 1995;95:895–9.
25. O'Marcaigh A, Puck JM, Pepper AE, Santes KD, Cowan MJ. Maternal mosaicism for a novel interleukin-2 receptor gamma-chain mutation causing X-linked severe combined immunodeficiency in a Navajo kindred. J Clin Immunol. 1997;17:29–33.
26. Sakamoto M, Kanegane H, Fujii H, Tsukada S, Miyawaki T, Shinomiya N. Maternal germinal mosaicism of X-linked agammaglobulinemia. Am J Med Genet. 2001;99:234–7.
27. Harlin H, Reffey SB, Duckett CS, Lindsten T, Thompson CB. Characterization of XIAP-deficient mice. Mol Cell Biol. 2001;21:3604–8.
28. Latour S. Natural killer T cells and X-linked lymphoproliferative syndrome. Curr Opin Allergy Clin Immunol. 2007;7:510–4.
29. Schimmer AD, Dalili S, Batey RA, Riedl SJ. Targeting XIAP for the treatment of malignancy. Cell Death Differ. 2006;13:179–88.
30. Oliveira JB, Notarangelo LD, Fleisher TA. Applications of flow cytometry for the study of primary immune deficiencies. Curr Opin Allergy Clin Immunol. 2008;8:499–509.
31. Kanegane H, Futatani T, Wang Y, Nomura K, Shinozaki K, Matsukura H, et al. Clinical and mutational characteristics of X-linked agammaglobulinemia and its carrier identified by flow cytometric assessment combined with genetic analysis. J Allergy Clin Immunol. 2001;108:1012–20.
32. Godfrey DI, Berzins SP. Control points in NKT-cell development. Nat Rev Immunol. 2007;7:505–18.
33. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. Annu Rev Immunol. 2007;25:297–336.
34. Bauler LD, Duckett CS, O'Riordan MX. XIAP regulates cytosol-specific immunity to *Listeria* infection. PLoS Pathog. 2008;4:e1000142.
35. Hersperger AR, Makedonas G, Betts MR. Flow cytometric detection of perforin upregulation in human CD8 T cells. Cytometry A. 2008;73:1050–7.
36. Motyka B, Korbutt G, Pinkoski MJ, Heibein JA, Caputo A, Hobman M, et al. Mannose 6-phosphate/insulin-like growth

factor II receptor is a death receptor for granzyme B during cytotoxic T cell-induced apoptosis. Cell. 2000;103:491–500.
37. Martin SJ, Amarante-Mendes GP, Shi L, Chuang TH, Casiano CA, O'Brien GA, et al. The cytotoxic cell protease granzyme B initiates apoptosis in a cell-free system by proteolytic processing and activation of the ICE/CED-3 family protease, CPP32, via a novel two-step mechanism. EMBO J. 1996;15:2407–16.
38. zur Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. Am J Hum Genet. 2009;85:482–92.

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CASE REPORT

GATA-2 anomaly and clinical phenotype of a sporadic case of lymphedema, dendritic cell, monocyte, B- and NK-cell (DCML) deficiency, and myelodysplasia

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Abstract A Japanese patient presented with lymphedema, severe *Varicella zoster*, and *Salmonella* infection, recurrent respiratory infections, panniculitis, monocytopenia, B- and NK-cell lymphopenia, and myelodysplasia. The phenotype was a mixture of the monocytopenia and mycobacterial infection (MonoMAC) and Emberger syndromes. Sequencing of the *GATA-2* cDNA revealed the heterozygous missense mutation 1187 G>A. This mutation resulted in the amino acid mutation Arg396Gln in the zinc fingers-2 domain, which is predicted to cause significant structural change and prevent a critical interaction with DNA. Functional analysis of the patient's *GATA-2* mutation is required to understand the relationship between these distinctive syndromes.

Keywords Emberger syndrome · MonoMAC · Monocytopenia · B- and NK-cell lymphopenia · Immunodeficiency · Myelodysplasia

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Recent studies have characterized a novel primary immunodeficiency known as monocytopenia and mycobacterial infection (MonoMAC), also known as dendritic cell, monocyte, B and NK lymphoid (DCML) deficiency. This form of immunodeficiency occurs either as an autosomal dominant form or sporadically. It is primarily characterized by persistent and profound peripheral monocytopenia, diagnostic B- and NK-cell lymphocytopenia, and variable T cell lymphocytopenia, along with increased susceptibility to mycobacterium or papilloma virus infections [1, 2, 13]. Moreover, most patients with MonoMAC eventually develop acute myelogenous leukemia (AML) following myelodysplastic syndrome (MDS). Another rare disorder called Emberger syndrome (MIM614038) is characterized by congenital deafness and primary lymphedema of the lateral lower limb; typically, onset occurs in childhood and is associated with a predisposition to MDS or AML in addition to other minor anomalies such as hypotelorism and long tapering fingers. It is also a sporadic or familial disorder [8]. Familial MDS/AML without other hematopoietic defects has also been reported [6]. Surprisingly, it was reported recently that these three distinctive syndromes are all caused by *GATA-2*

mutations, which suggests that these syndromes are different phenotypes caused by the same genetic alteration [5–7, 9]. Here, we report the case of a patient with a *GATA-2* mutation bearing the characteristic features of MonoMAC/Emberger syndrome.

Case report

The patient was the second child of non-consanguineous parents. Neither the parents nor the elder brother had a history of increased susceptibility to infection. The medical history of the patient included BCG vaccination 3 months after birth without any side effects and a severe *Varicella zoster* infection at 2 years of age. After that, she suffered repeated upper and lower respiratory tract infections that required antibiotics. At 4 years of age, the patient's peripheral blood showed mild neutropenia and profound monocytopenia ($0–20\times10^6$/L), and mild hypocellularity but no dysplasia was observed in the bone marrow. At 8 years of age, she experienced a prolonged *Salmonella* enterocolitis infection. Lymphedema in the left leg first appeared at 13 years of age. She subsequently developed recurrent panniculitis. Recently, the patient (now 19 years old) was admitted to hospital with fever (with no apparent cause) and panniculitis (Fig. 1a). She had mild hypotelorism and lymphedema, with warts on her left leg (Fig. 1b). Her mental ability was appropriate for her age. An immunodeficiency was first suspected after the severe *Varicella zoster* and *Salmonella* infections during early childhood. The most recent recurrent episode of fever supported this suspicion.

Peripheral blood analysis revealed a white blood cell count of 1.5×10^9/L with 45% neutrophils, 54 % lymphocytes, and 1 % monocytes, a hemoglobin level of 11.0 g/dl, and a platelet count of 146×10^9/L. Flow cytometric analysis of the peripheral blood also revealed a deficiency in dendritic cells (lineage$^-$/DR$^+$/CD123$^+$ or CD11c$^+$ cells, 0%), B cells (CD19$^+$ cells, 0.7%), and NK cells (CD3$^-$/CD56$^+$ cells, 0.5%), and profound monocytopenia (CD14$^+$ cells, 0.2%). Lymphocytes comprised 97% T cells (CD4/8 ratio, 0.54), 33% of which were TCR γδ$^+$ T cells. Immunological analyses revealed IgG, IgA, IgM, and IgE levels of 711, 65, 131,

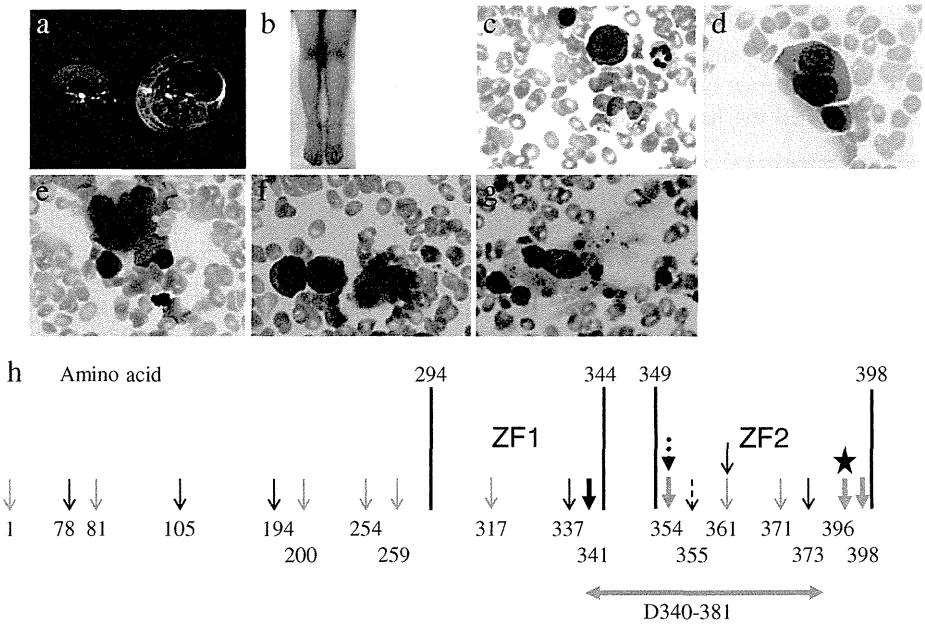

Fig. 1 Clinical and bone marrow features and *GATA-2* protein mutation sites. **a** A gadolinium-enhanced T2-weighted MRI image of the left thigh was performed when the patient developed panniculitis at 19 years of age. An increased signal was observed in the subcutaneous tissue and fascial layers. **b** After she was cured, the patient showed lymphedema in her left leg. **c–g** Bone marrow taken at the same time revealed decreased granule numbers within neutrophils and a pseudo-Pelger anomaly (**c**), binucleation (**d**), and megaloblastic changes in erythroblasts, dysplastic nuclei in megakaryocytes (**e**) and micromegakaryocytes (**f**), and hemophagocytosis (**g**). **h** Depiction of the *GATA-2* protein mutations previously identified in MonoMAC/DCML deficiency and Emberger syndrome. ZF1 and ZF2 are functional DNA-binding domains. The *star* indicates the Arg396Gln mutation identified in the present case. *Arrows* indicate previously reported mutations. These include missense, nonsense, and frameshift mutations (*short downward arrows*, respectively) and long deletions (*horizontal arrows*). *Black arrows* denote mutations associated with Emberger syndrome, *gray arrows* denote mutations associated with MonoMAC syndrome/DCML deficiency, *long horizontal arrows* indicate long deletions that have been observed in MonoMAC syndrome/DCML deficiency, *broken black arrows* denote mutations associated with familial MDS/AML, and *bold arrows* denote multiple pedigrees with the same mutation

and 5 mg/dl, respectively, and lymphocyte stimulation responses to phytohemagglutinin at the lower limits of the normal range. Antibody memory responses to infections contracted in early childhood (*Varicella* and *measles*) were maintained, and fibroblast sensitivity to radiation was normal. Flow FISH analysis of peripheral blood lymphocytes revealed normal telomere length; however, the peripheral blood contained 160 copies/μg WT1-mRNA (the upper limit of normal is 50 copies/μg RNA), and bone marrow aspirates showed hypocellularity, particularly of myeloid and lymphoid cells. Strikingly, despite monocytopenia in the peripheral blood, $CD64^+$ macrophages (accompanied by a few hemophagocytes) were observed in bone marrow specimens. Significant trilineage dysplasia was also present (Fig. 1c–g). Cytogenetic and chromosomal breakage analyses showed normal results. Meanwhile, profiles of familial peripheral blood showed a white blood cell count of 5.6×10^9/L with 50% neutrophils, 30% lymphocytes, and 8% monocytes, a hemoglobin level of 15.1 g/dl, and a platelet count of 199×10^9/L in the father; 5.1×10^9/L with 51 % neutrophils, 36% lymphocytes, and 9% monocytes, 10.7 g/dl, and 225×10^9/L in the mother; and 6.6×10^9/L with 41% neutrophils, 45% lymphocytes, and 10% monocytes, 15.4 g/dl, and 208×10^9/L in the brother. Flow cytometric analysis of peripheral blood samples taken from these family members showed a normal frequency of B cells ($CD19^+$ cells) and NK cells ($CD3^-/CD56^+$ cells) (the father 11 and 8%, the mother 10 and 12%, and the brother 9 and 15%, respectively). Taken together, these findings suggested that the patient might have sporadic MonoMAC/Emberger syndrome.

Sequencing of *GATA-2* cDNA revealed a 1187 G>A heterozygous missense mutation. This mutation resulted in an Arg396Gln substitution in the zinc finger-2 domain, which is predicted to cause significant structural changes that prevent critical interactions with DNA (Fig. 1h). Furthermore, sequencing of cDNA from her healthy familial members revealed no mutations, including 1187 G>A in *GATA-2* gene. Ultimately, the patient was diagnosed with MonoMAC/Emberger syndrome with a de novo *GATA-2* mutation.

Discussion

GATA-2 plays a critical role in both hematopoietic stem cell development and the maintenance of normal adult stem cell homeostasis [10]. It is likely that the significant protein structural alterations caused by mutations in *GATA-2* result in loss-of-function or have a dominant-negative effect on the DNA-binding ability of wild-type *GATA-2* [9] It seems reasonable to suggest that the loss of hematopoiesis-indispensable transcription factor activity results in impaired hematopoietic-cell differentiation and hematopoietic stem cell exhaustion; this in turn may promote the development of related diseases such as MDS and AML. Additional genetic alterations may also be required.

The patient's phenotype included hypotelorism, primary lymphedema (which had an onset during childhood before the recurrent episodes of panniculitis), peripheral monocytopenia, B- and NK-cell lymphocytopenia, neutropenia since early childhood, and myelodysplasia. The Arg396Glu mutation in *GATA-2* identified in this patient was not detected in 150 healthy individuals [7]. Taken together, these factors confirmed the diagnosis of MonoMAC/Emberger syndrome with a de novo *GATA-2* mutation; however, the *GATA-2* mutations alone cannot explain the phenotypic diversity between these three syndromes (MonoMAC, Emberger syndrome, and familial MDS/AML) and the presented patient. Interestingly, she developed neither BCG dissemination nor severe lymphadenitis after her BCG

Table 1 Summary of the clinical features of MonoMAC, Emberger syndrome, and the present case

	MonoMAC/DCML deficiency	Emberger syndrome	Present case
DCML[a] deficiency	+	+/–	+
MDS/AML	+	+	+
Lymphedema	ND	+	+
Deafness	ND	+/–	–
Hypotelorism	ND	+/–	+
Long slender fingers	ND	+/–	–
Mycobacterial infection	+	ND	–
Fungal infection	+/–	+/–	–
Papillomaviral infection/warts	+	+	+
Severe *varicella* and/or *Salmonella* infection	+/–	ND	+
Pulmonary alveolar proteinosis	+/–	ND	–
Panniculitis/erythema nodusa	+/–	+/–	+

ND not described, *MDS* myelodysplastic, *AML* acute myelogenous syndrome, + most cases, +/– some cases

[a]Dendritic cell, monocyte, B and NK lymphoid syndrome

vaccination 3 months after birth. This indicates normal functioning of tissue macrophages, because protective immunity to mycobacteria is dependent upon the interleukin (IL)-12/IL-23-interferon (IFN)-γ axis, possibly mediated by intracellular killing of phagocytes following the production of IFN-γ by CD4 T lymphocytes in response to IL-12/IL-23 secreted by infected macrophages [3]. Patients with MonoMAC/DCML deficiency show very low numbers of circulating monocytes and no detectable myeloid or plasmacytoid dendritic cells in the peripheral blood, but relatively normal numbers of Langerhans cells and tissue macrophages accompanied by prominent hemophagocytosis in the bone marrow [1, 2]. This supports the idea that tissue and marrow macrophages, in addition to Langerhans cells, may be maintained by a distinct precursor from circulating monocytes or dendritic cells [1].

Mansour et al. [8] reported that the age of onset of MDS/AML in Emberger syndrome is 9–14 (median 11) years of age. This appears to be earlier than that of MDS/AML in MonoMAC syndrome (7–52 years, median 32 years) [2]. Moreover, the level of WT1-mRNA in the peripheral blood increases significantly as MDS progresses and is a strong predictor of rapid AML transformation in adult patients with de novo MDS [11]. The level of WT1-mRNA in the peripheral blood of the current patient was as high as that in patients that show worse survival than those with a low level WT1 mRNA (10^2–10^4 vs. $<10^2$ copies/μg) [12]. However, it is unclear whether phenotypic variation and increased WT1 mRNA level are related to hematological disease progression. In any case, neutropenic patients who suffer recurrent infections and/or MDS are likely to need a transplant in the near future. Therefore, for such cases, we perform hematopoietic stem cell transplantation with a reduced intensity conditioning regimen before the disease has progressed [4]. Table 1 summarizes the clinical features of MonoMAC, Emberger syndrome, and the present case.

Our observations suggest that children with recurrent or prolonged common infections that respond to antibiotics and recover well may suffer from unknown primary immunodeficiencies. Although the relationship between *GATA-2* and lymphedema or deafness requires further investigation, tissue-specific lesions such as lymphedema provide important clues to primary immunodeficiencies that also affect non-hematopoietic cells.

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Conflict of Interest Statement The authors declare no competing financial interests.

References

1. Bigley V, Haniffa M, Doulatov S, Wang XN, Dickinson R, McGovernet N et al (2011) The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. J Exp Med 208:227–234
2. Calvo KR, Vinh DC, Maric I, Wang W, Noel P, Stetler-Stevenson M, Holland SM et al (2011) Myelodysplasia in autosomal dominant and sporadic monocytopenia immunodeficiency syndrome: diagnostic features and clinical implications. Haematologica 96:1221–1225
3. Carneiro-Sampaio M, Coutinho A (2007) Immunity to microbes: lessons from primary immunodeficiencies. Infect Immun 75:1545–1555
4. Cuellar-Rodriguez J, Gea-Banacloche J, Freeman AF, Hsu AP, Zerbe CS, Calvo KR et al (2011) Successful allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. Blood 118:3715–3720
5. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M et al (2011) Exome sequencing identifies *GATA-2* mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. Blood 118:2656–2658
6. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC et al (2011) Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet 43:1012–1017
7. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY et al (2011) Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood 118:2653–2655
8. Mansour S, Connell F, Steward C, Ostergaard P, Brice G, Smithson S et al (2010) Lymphoedema research consortium. Emberger syndrome-primary lymphedema with myelodysplasia: report of seven new cases. Am J Med Genet A 152A:2287–2296
9. Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ et al (2011) Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 43:929–931
10. Rodrigues NP, Boyd AS, Fugazza C, May GE, Guo Y, Tipping AJ et al (2008) *GATA-2* regulates granulocyte-macrophage progenitor cell function. Blood 112:4862–4873
11. Tamaki H, Ogawa H, Ohyashiki K, Ohyashiki JH, Iwama H, Inoue K et al (1999) The Wilms' tumor gene WT1 is a good marker for diagnosis of disease progression of myelodysplastic syndromes. Leukemia 13:393–399
12. Tamura H, Dan K, Yokose N, Iwakiri R, Ohta M, Sakamaki H et al (2010) Prognostic significance of WT1 mRNA and anti-WT1 antibody levels in peripheral blood in patients with myelodysplastic syndromes. Leuk Res 34:986–990
13. Vinh DC, Patel SY, Gl U, Anderson VL, Freeman AF, Olivier KN et al (2010) Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood 115:1519–1529

Clinical profile and genetic basis of Wiskott-Aldrich syndrome at Chandigarh, north India

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Summary

Background: **The Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency disorder characterized by thrombocytopenia with small sized platelets, eczema, and recurrent infections. There is paucity of information on WAS from the Indian subcontinent. We describe the clinical and molecular profile of 8 patients with WAS as seen in the Pediatric Immunodeficiency Clinic at the Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India.**

Methods: **A detailed analysis of the clinical profiles, investigations and outcome of the 8 children diagnosed with WAS during the period 2006- 2010 was performed. Confirmation of the genetic diagnosis was done at the Service d'Hématologie, d'Immunologie et de Cytogénétique, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France and the National Defense Medical College, Saitama, Japan.**

Results: **8 patients were diagnosed as WAS in 5 years. The ages at diagnosis ranged from 13 weeks to 9 years while the mean age of onset of the symptoms was 117 days ± 136 days. The diagnosis was established within a mean period of 31 months (ranging1-108 months) from the onset of symptoms. Recurrent infections and diarrhea were seen in 6 and 7 out of the 8 patients, respectively, while eczema was variable. Autoimmunity manifestations were observed in 2 children. Thrombocytopenia and small platelet size was the hallmark of the disease and the main clinical clue to diagnosis in our patients. Mutations in the *WASP* gene were seen in 8 children, out of which 2 were novel mutations. While one child successfully underwent bone marrow transplantation, two children are doing well on immunoglobulin replacement and cotrimoxazole prophylaxis. Out of 8 children 4 children in our cohort died - all had high WAS scores and could not be offered hematopoietic stem cell transplantation.**

Conclusion: **WAS should be suspected clinically in any male infant with persistent unexplained thrombocytopenia and especially if the platelet size is small. Clinical presentation can be very variable and it is therefore important to recognize the entire spectrum of the disease. Understanding the molecular basis has important implications for the diagnosis, treatment, and genetic counseling of patients with WAS. *(Asian Pac J Allergy Immunol 2012;30:71-8)***

Key words: *Wiskott-Aldrich Syndrome, Thrombocytopenia, North India*

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Key Messages/ Clinical Implications:

One should suspect Wiskott-Aldrich syndrome in boys who have persistent unexplained thrombo-cytopenia.

Introduction

The Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency disorder characterized by a clinical phenotype that includes thrombo-cytopenia with small platelets, eczema, and recurrent infections. The condition was first described by Wiskott in 1937[1] while the X-linked nature of the syndrome was identified by Aldrich in 1954.[2] The identification of the molecular defect in 1994 by Derry et al.[3] has broadened the clinical spectrum of the syndrome to include chronic or intermittent X-linked thrombocytopenia (XLT), a relatively mild form of WAS[4,5,6] and X-linked neutropenia caused by arrest of myelopoiesis.[7] The *Wiskott-Aldrich Syndrome Protein (WASP)* gene codes for a protein selectively expressed in the hematopoietic system and is involved in the cytoskeletal-organizing complex, its maturation, activation and transport of blood elements.[8]

The incidence of WAS is estimated to be between 1-10/million of the population.[9,10] Recent reports however, suggest that this may be an underestimate, as the phenotype of the disorder is much broader than hitherto recognized[11,12]. However, there is paucity of information on WAS from the Indian subcontinent.

The present paper describes clinical experience in managing 8 patients suffering from WAS as seen at the Pediatric Allergy Immunology Unit, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Methods

Patients and methods:

The case records of all children attending the Pediatric Immunodeficiency Clinic at the Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research, Chandigarh from January 2006 to December 2010 were reviewed. The institute serves as a tertiary care referral center for North West India. A detailed analysis of clinical profiles, investigations and outcome of the children with this syndrome was carried out. The severity of WAS-associated symptoms was expressed as a clinical score of 1-5, based on a previously published scoring system.[13,14] Skin biopsy was performed in patient with cutaneous vasculitis and subjected to histopathology and immunoflorescence studies . Serum IgG, IgM and IgA were estimated by end-point nephelometry on a semi-automated nephelometer while IgE was estimated by enzyme immunoassay.

The confirmation of the genetic diagnosis was carried out with the consent of the parents at Service d'Hématologie, d'Immunologie et de Cytogénétique, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France and at the National Defense Medical College, Saitama, Japan.

Results

During this period of 5 years, WAS was clinically suspected in 13 patients, all of Caucasian ethnicity. The diagnosis was genetically confirmed in 8 patients. No mutations could be demonstrated in the *WASP* gene in 3 children, while 2 were lost to follow-up.

The ages at diagnosis ranged from 13 weeks - 9 years, while the mean age of onset of the symptoms was 117 days $\pm$ 136 days. The diagnosis was established within a mean of 31.9 months, from the onset of symptoms ranging from1-108 months.

Clinical features

Out of the total of 8 children, 7 (87.5%) presented with loose stools with 6 (75%) having blood in them. Recurrent infections were very prominent in all except patient numbers 2 and 6 (75%). Major infections included pneumonia (75%), otitis media (37.5%), septic arthritis and osteomyelitis (37.5%), meningitis (25%), and Herpes zoster (12.5%). The details are shown in Table 1. Patients 1 and 7 suffered from many pyogenic infections before the diagnosis was established. The degree of eczema was variable and was extremely difficult to control in patient 4. Autoimmunity was observed in two children [patient 3 and patient 8]. Patient 3 had a prominent vasculitic rash on the dorsum of the hands and feet, while patient 7 had developed vasculitic skin lesions and scrotal gangrene. A detailed family history revealed the death of a maternal uncle in early infancy in 3 families.

Investigations

Thrombocytopenia was the hallmark and the main clue to diagnosis. Even though the mean platelet volume was not very low, the small platelet morphology was observed on smear by an experienced hematologist. Ig M was low in 4, normal in 3 and high in 1 child. Ig G was increased in 4 children, while Ig A and Ig E fractions were

Table 1. Clinical Features of patients with Wiskott Aldrich Syndrome Patient

Patient	State of residence	Age at onset of symptoms (days)	Age at diagnosis (months)	Infections/manifestations	Bleeding	Eczema	Auto immunity	WAS score	Family history	Treatment	Age at last follow up (years)	Approximate follow up duration (years)	Present status/comments
Patient 1	Karnataka	60	54	Diarrhoea; Staphylococcal epidermidis meningitis; pneumonia; septic arthritis; gluteal abscess; otitis media	Blood in stools	+	none	4	2nd degree consanguinity	IV Ig	8.5	4	Presently well, no major hospitalizations
Patient 2	Himachal Pradesh	45	2.5	Loose stools with blood	Epistaxis	+	none	3	Death of maternal uncles	IV Ig HSCT	4	4	Well
Patient 3	Chandigarh	15	2.5	Staphylococcal osteomyelitis of tibia; Varicella zoster over scalp; Candidaemia disseminated candidiasis with meningitis and pneumonia; loose stools with blood	Blood in stools	+	Leucocytoclastic vaculitis on dorsum of hands and feet	5	Not significant	IV Ig; Steroids for vasculitis, splenectomy done before diagnosis of WAS established	3.5	3.5	Died; pre-terminally developed intractable diarrhea and pneumonia
Patient 4	Haryana	180	12	Staphlococcal aureus otitis media;pneumonia; anemia; micronutrient deficiency	Hemetemesis	+ +	none	4	Death of maternal uncles	IVIg	3	2	Died; at home
Patient 5*	Uttar Pradesh	5	5	Pneumonia; Diarrhoea	Blood in stools	+ +	none	4	Cousin of patient 4	No treatment	-	No follow up	Died; at home
Patient 6	West Bengal	8	4.5	Loose stools with blood;	Hematuria, skin bleeds	+	none	4	Death of maternal uncles	IV Ig	4.9	4	Well
Patient 7*	Punjab	360	108	Pneumonia; septic arthritis; diarrhea	Hemoptysis, skin bleeds	+	none	4	Not significant	No treatment	9	No follow up	Died; at home
Patient 8	Haryana	270	60	Pneumonia; otitis media;anemia	Skin bleeds	-/+	Leucocytoclastic vasculitis with scrotal gangrene	5	Not significant	Steroids for vasculitis	8	3	Well

increased in all patients. Table 2 summarizes the laboratory findings in all the 8 patients.

The genetic mutation analysis was done at the Hôpital de Bicêtre, Le Kremlin-Bicêtre, France and at National Defense Medical College, Saitama, Japan. Out of the 8 mutations identified, 6 were point mutations and 2 frame shift mutations. Out of the point mutations, 2 novel mutations - 1 in intron 9 and another in exon 1 (patients 1 and patient 6) were noted. The 2 frame shift mutations in patients 2 and patient 7 were identical, involving the exon 10. Patient 2 was diagnosed early and managed by Hematopoietic stem cell transplant and is doing well whereas patient 7 did not take any treatment and died.

Amongst the 2 novel mutations mentioned above, patient 1 had severe life threatening infections with minimal eczema but is doing well on immunoglobulin supplementation. Patient 6 had mild infections with predominant bleeding manifestations and is also doing well on immune-globulin therapy. The details of mutations and outcome of therapy for all patients are presented in Table 1 and 3.

Discussion

Wiskott-Aldrich Syndrome is a rare primary immunodeficiency disorder with variable clinical presentations. With the advent and availability of molecular diagnosis, laboratory confirmation of the condition is now possible. However, there are hardly any data on this condition from the Indian subcontinent. A review of literature revealed occasional case reports but there is no information on molecular abnormalities in WAS from India.[15,16,17]

A perusal of the records of the Pediatric Immunodeficiency Clinic and the Pediatric Allergy Immunology Unit in the Advanced Pediatrics Centre at Post Graduate Institute of Medical Education and Research shows that till 2006, this condition had been suspected in only 2 patients. The diagnosis was presumptive and was based on the features of persistent thrombocytopenia in a boy along with other clinical features and supporting laboratory profile. Facilities for molecular diagnosis, however, were not available till very recently. Both the children were treated with prophylactic antimicrobials but did not survive for long.

In 2007, we were able to arrange for the molecular diagnosis of WAS at the Hôpital de Bicêtre, Le Kremlin-Bicêtre, France and later at National Defense Medical College, Saitama, Japan. Since then 8 children have had a confirmed diagnosis of WAS, out of 13 patients in whom the diagnosis was suspected. It was clinically suspected in 3 but no mutation was found in the *WAS* gene; 2 children were lost to follow up before the diagnosis could be genetically confirmed.

An increase in awareness could have resulted in an increasing number of children diagnosed during the period 2006-2010. It is likely that many children with WAS may have died undiagnosed and untreated prior to 2006. Considering the rarity of the condition in the general population, the number observed within a span of 3-5 years seems high. This calls for a concerted efforts to screen males with thrombocytopenia who are refractory to conventional therapy for WAS.

Table 2. Summary of the investigations

Patients	Platelet count	MPV (fL)	Ig G (G/L)	Ig A (G/L)	Ig M (G/L)	Ig E (IU/ml)
1	$38x10^9$/L	7.3	21.91 (4.9-16.1)	5.86 (0.4-2.0)	1.21 (0.5-2.0)	400 (0.3-25.0)
2	$18x10^9$/L	7	13.03 (2.1-7.7)	1.57 (0.05-0.4)	1.44 (0.15-0.7)	260 (0-6.6)
3	$72x10^9$/L	6.7	5.84 (2.1-7.7)	6.19 (0.05-0.4)	0.15 (0.15-0.7)	500 (0-6.6)
4	$50x10^9$/L	small	16.00 (3.1-13.8)	2.51 (0.3-1.2)	0.41 (0.5-2.2)	480 (0-20)
5	$142x10^9$/L	small	14.21 (2.4-8.8)	3.2 (0.1-0.5)	0.64 (0.2-1.0)	360 (0-6.6)
6	$9x10^9$/L	6.5	7.35 (2.4-8.8)	3.9 (0.1-0.5)	0.13 (0.2-1.0)	464 (0-6.6)
7	$67x10^9$/L	7.0	8.26 (5.4-16.1)	3.6 (0.7-2.5)	0.42 (0.5-1.8)	330 (3.6-81.0)
8	$33x10^9$L	7.2	6.34 (4.9-16.1)	2.42 (0.4-2.0)	1.20 (0.5-2.0)	250 (0.2-17.6)

Figures in parenthesis indicate range of age related normal values for the respective patients.MPV: mean platelet volume; (G/L):grams/litre; (IU):International units

The clinical spectrum of WAS is very broad.[12,18-20] General pediatricians, as well as those working in pediatric sub-specialties, need to be aware of this disorder. Most of our patients had initially presented to other specialties like Pediatric Gastroenterology, Pediatric Dermatology and Pediatric Hematology. The classical triad of eczema, thrombocytopenia and immune deficiency at first presentation was seen in 6 out of 8 patients. The average incidence of bleeding manifestations before diagnosis is reported to be more than 80%,[21] consisting of petechiae and ecchymoses in most cases. We found bleeding from the GI tract in the form of bloody diarrhea and or haemetemesis as the most consistent symptoms and were seen in 75% of the children. This is also probably the earliest symptom which should raise the suspicion of this disorder among the treating physicians.

Patients with WAS are more susceptible to bacterial infections as well as viral and fungal infections. Polysaccharide-coated bacteria pose a special risk because of the impaired capacity to produce antibodies of the IgG2 subclass against polysaccharide antigens. In our cohort of children, recurrent infections were the dominant features in 6 children (Table 2). Pneumonia was the commonest infection and was seen in 6 children. Otitis media, meningitis, deep seated abscess, osteomyelitis, and septic arthritis were other infections encountered. Patient 3 developed disseminated candidiasis with meningitis and also varicella zoster infection. Eczema affects 80% of WAS patients during the disease.[21] In the present cohort eczema was seen in all, though it was mild in 3 children while severe and difficult to treat in 2 children. In a multicentric series analyzed by Sullivan et al. in 1994, 40% of patients had at least one autoimmune event and 25% had several manifestations. In a series of patients analysed in a single centre study (Hospital Necker, Paris) published,[22] 72% of patients presented at least one autoimmune event and 36% had multiple manifestations. Autoimmune or inflammatory events were reported in 24% of patients by Imai et al.[11] Autoimmune hemolytic anemia, cutaneous vasculitis, including Schoenlein-Henoch syndrome, nephropathies and arthritis, together have being reported to account for more than 80% of autoimmune manifestations. Among our patients, 1 child had an unusual presentation of acute hemorrhagic edema on hands and feet which on biopsy revealed leucocytoclastic vasculitis.[23]

It may be noted that persistent unexplained thrombocytopenia in boys is the most important clinical clue to the diagnosis of WAS. MPV in patients with WAS varies and there is no absolute diagnostic range. Interpretation depends on expertise and should be made considering the clinical context. MPV was found to be between 6-7.3 fL in our subset of children.

Immunologic abnormalities observed in WAS are complex and involve both B and T cell function. Affected male infants have a normal number of circulating lymphocytes but develop lymphopenia by 6 to 8 years of age due to a loss of T lymphocytes.[24] Classical features include normal levels of IgG, moderately reduced serum IgM levels, whereas IgA and IgE are elevated. However, these

Table 3. Details of mutation analysis

Patient	WAS score	Mutation	Type of mutation	Screening/Carrier/Status
Patient 1	4	intron 9,c.931+1>A	Point mutation Intronic /Splice defect	Not done
Patient 2	3	exon 10, c.1031delC, p.Pro344Leufs*101	Deletion, frameshift mutation	Not done
Patient 3	5	exon 1, c.91G>A, p.Glu31Lys	Point mutation, missense mutation	Mother and sister carrier
Patient 4*	4	exon 1, c.37C>T, p.Arg13*	Point mutation, nonsense mutation	Mother, maternal aunt carrier
Patient 5*	4	exon 1, c.37C>T, p.Arg13*	Point mutation, nonsense mutation	Mother, maternal aunt carrier
Patient 6	4	exon 1, c.100C>T, p.Arg34*	Point mutation, nonsense mutation	Mother carrier
Patient 7	4	exon 10, c.1031delC, p.Pro344Leufs*101	Deletion, frameshift mutation	Not done
Patient 8	5	exon 2, c.257G>A, p.Arg86His	Point mutation, missense mutation	Not done

*Patient 4 and 5 are related.

changes are not constant features and mainly affect older patients. A significant number of WAS subjects present with normal or even raised IgM values, the latter being a risk factor for the development of autoimmunity. Eczema is also often associated with raised IgE. The immunoglobulin profiles in our children showed decreased IgM in 50% children while IgA and E were elevated in all.

The severity of the clinical presentation can vary depending largely on the mutation and its effect on protein expression.[18,25] Understanding the molecular basis also has important implications for the diagnosis, treatment, genetic counseling and gene therapy of patients with WAS. The *WASP* gene, which encodes for the WAS protein (WASP), has 12 exons and 5 major functional domains. *WASP* gene mutations can impair all or part of the protein's expression and function. The severity of impaired protein expression is directly correlated with the severity of the clinical phenotype.[18] In particular, missense mutations in exons 1 and 2 (which usually diminish but do not suppress protein expression) are associated with a milder phenotype, with thrombocytopenia (XLT) being the major manifestation. Conversely, nonsense mutations or mutations impairing the reading frame of the RNA messenger, disrupts the protein's aminoacid sequence, abolishes or radically reduces protein expression and tends to be associated with a severe clinical phenotype (WAS).

In our series, there were 2 patients with novel mutations. Patient 6, had a mutation in the exon 1, with a WAS score of 4 and is presently doing well with immunoglobulin replacement therapy. The other patient (patient 1) had a new mutation in intron 9, with same WAS score and is also doing well with replacement immunoglobulins. Unfortunately, we were not able to quantify the WASP protein in our patients for better phenotypic-genotypic correlation. Genotype–phenotype correlation in WAS/XLT is not absolute and progression to a severe phenotype is possible. The arginine residue at position 86 is a mutation hotspot, as it is located in the CpG island. Missense mutations in the R86 residue are more commonly associated with XLT phenotype, but in a recent paper, Albert et al. described patients with R86H mutations progressing from a WAS score 1 or 2 to 5 due to development of autoimmunity.[26] Patient 8 in our series with a mutation in exon 2, c.257G> A, p.Arg86His has also shown a similar progressive trend from WAS score of 2 to 5 on development of autoimmunity in the form of cutaneous vasculitis.

Management of patients with WAS continues to present major challenges in resource poor settings, as is evident from the fact that two of our patients died at home as the parents could not afford any form of therapy. Early diagnosis is most important for effective prophylaxis and treatment. The natural history of disease progression is less predictable, especially in the attenuated phenotypes. At present, the only curative therapy is hematopoietic stem cell transplantation (HSCT). The results are promising for patients with HLA-matched family or unrelated donors or partially matched cord blood donors but less satisfactory for other donor types.[27,28] One of our patient successfully underwent a related HLA matched bone marrow transplantation at a sister institute.[29]

Immunoglobulin therapy was given to 5 out of the 8 patients based on clinical assessment. This significantly improved the quality of life in these patients. The number of infections decreased and so did the hospitalization for serious infections. However, we do not have any double blind data to prove this contention. At present, two children are doing well on immunoglobulin replacement and cotrimoxazole prophylaxis.

Mortality continues to be high in India and 50% children died in our cohort. The patient who was given HSCT therapy is still doing well after 4 years of follow up. However, there is an urgent need to create awareness regarding proper implementation of therapy, particularly with regards to the beneficial effects of HSCT. At present there are no regional or national level registries for patients with primary immune deficiencies in India. A centre for diagnosis and research is under consideration at our institute. Transplant facilities are available in 10-12 centres in India, though predominantly for hematological malignancies. Transplants for immune deficiencies are being actively considered at several centres.

As such, efforts to promote general and educational awareness of not only WAS but all primary immune deficiency diseases has been initiated by the Indian Patients' Society for Primary Immune Deficiency (www.ipspiindia.org). In order to further the cause of these patients, this year the Indian Society for Primary Immunodeficiency Diseases has been established with an objective to create awareness amongst the medical fraternity associated with the diagnosis, management and treatment of these multidisciplinary disorders.

Conclusions

WAS, though a rare primary immunodeficiency disorder, should be suspected clinically in any male infant who presents with bloody loose stools and persistent thrombocytopenia. Mutations in the *WASP* gene result in phenotypically unique disease entities, depending on the effect of the mutation on WASP expression. Understanding the molecular basics has important implications for the diagnosis, treatment and genetic counseling of patients with WAS.

References

1. Wiskott A. Familiaereer, angeborner morbus Werlhoffi? Monatsschr Kinderheilkd 1937; 68:212-216.
2. Aldrich RA, Steinberg AG, Campbell DC. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhea. Pediatrics1954; 13:133-138.
3. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell 1994; 78:635-44.
4. Villa A, Notarangelo L, Macchi P, Mantuano E, Cavagni G, Brugnoni D, et al. X-linked thrombocytopenia and Wiskott-Aldrich syndrome are allelic diseases with mutations in the WASP gene. Nat Genet. 1995; 9(4):414-7.
5. Notarangelo LD, Mazza C, Giliani S, D'Aria C, Gandellini F, Ravelli C, et al. Missense mutations of the WASP gene cause intermittent X-linked thrombocytopenia. Blood 2002; 99:2268-9.
6. Zhu Q, Zhang M, Blaese RM, Derry JMJ, Junker A, Francke Chen SH, Ochs HD. The Wiskott-Aldrich syndrome and X-linked thrombocytopenia are caused by mutations of the same gene. Blood. 1995; 86(10):3797-804.
7. Devriendt K, Kim AS, Mathijs G, Frints SG, Schwartz M, Van Den Oord JJ, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet. 2001; 27:313-7.
8. Thrasher AJ. New insights into the biology of Wiskott-Aldrich syndrome (WAS). Hematology Am Soc Hematol Educ Program. 2009:132-8.
9. Ryser O, Morell A, Hitzig WH. Primary immunodeficiencies in Switzerland: first report of the national registry in adults and children. J Clin Immunol 1988; 8:479-85.
10. Stray-Pedersen A, Abrahamsen TG, Froland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol. 2000; 20:477-85.
11. Imai K, Nonoyama S, Ochs HD. *WASP* (Wiskott-Aldrich syndrome protein) gene mutations and phenotype. Curr Opin Allergy Clin Immunol. 2003; 3:427-36.
12. Ochs HD, Rosen FS. The Wiskott-Aldrich syndrome. In: Ochs HD, Smith CIE, Puck JM, editors. Primary Immunodeficiency Diseases: A Molecular and Genetic Approach. NewYork. Oxford University Press; 2007
13. Stiehm ER, Ochs HD, Winkelstein JA. Immunologic Disorders in Infants and Children. Fifth edition. Philadelphia: Saunders; 2004.
14. Zhu Q, Watanabe C, Liu T, Hollenbaugh D, Blaese RM, Kanner SB, et al. Wiskott-Aldrich syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression, and phenotype. Blood. 1997; 90:2680-9.
15. Gupta MC, Agarwal VK, Mittal AK, Rajvanshi VS. Wiskott-Aldrich Syndrome: A case report. JAPI. 1964; 12:513-3.
16. Mathew LG, Chandy M, Dennison D, Srivastava A, Ganapathy K, Cherian T. Successful bone marrow transplantation in an infant with Wiskott-Aldrich syndrome. Indian Pediatr.1999; 36:707-10
17. Srivastava A, Swaid HA, Kabra M, Verma IC. Management of Wiskott-Aldrich Syndrome. Indian J Pediatr.1996; 63:709-712.
18. Imai K, Morio T, Zhu Y, Jin Y, Itoh S, Kajiwara M, et al. Nonoyama S.Clinical course of patients with WASP gene mutations.Blood. 2004; 103:456-64.
19. Ochs HD, Filipovich AH, Veys P, Cowan MJ, Kapoor N. Wiskott-Aldrich syndrome: diagnosis,clinical and laboratory manifestations, and treatment. Biol Blood Marrow Transplant. 2009; 15:84-90.
20. Notarangelo LD, Miao CH, Ochs HD. Wiskott-Aldrich syndrome. Curr Opin Hematol. 2008; 15:30-36.
21. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr.1994; 125:876-85.
22. Dupuis-Girod S, Mediani J, Haddad E, Quartier P, Cavazzana-Calvo M, Le Deist F, et al. Autoimmunity in Wiskott-Aldrich syndrome: Risk factors, clinical features, and outcome in a single-center cohort of 55 patients. Pediatrics.2003; 111:622-627.
23. Chandrakasan S, Singh S, Dogra S, Delaunay J, Proust A, Minz RW. Wiskott-Aldrich syndrome presenting with early onset recurrent acute hemorrhagic edema and hyperostosis. Pediatr Blood Cancer. 2011; 56:1130-2.
24. Ochs HD, Slichter SJ, Harker LA, Von Behrens WE, Clark RA,Wedgwood RJ. The Wiskott-Aldrich syndrome: Studies of lymphocytes, granulocytes, and platelets. Blood. 1980; 55:243-52.
25. 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.
26. Albert MH, Bittner TC, Nonoyama S, Notarangelo LD, Burns S, Imai K, Espanol T, et al. Ochs HD. X-linked thrombocytopenia (XLT) due to WAS mutations: clinical characteristics, long-term outcome, and treatment options. Blood. 2010; 115:3231-8.
27. Filipovich AH, Stone JV, Tomany SC, Ireland M, Kollman C, Pelz CJ, et al. Impact of donor type on outcome of bone marrow transplantationfor Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program. Blood. 2001; 97(6):1598-603.
28. Moratto D, Giliani S, Bonfim C, Mazzolari E, Fischer A, Ochs HD, et al. Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic

cell transplantation in the period 1980- 2009: an international collaborative study. Blood. 2011; 118(6):1675-84.

29. John JM, Philip C C, Sharma K, Baldev V, Dheer G, Kakkar N, Bhatti A. Standard Bulsphan /Cyclophosphamide / ATG regimen as an option for conditioning in for a one antigen mismatch allogenic stem cell transplant with father as donor in a patient with wisskott Aldrich syndrome(WAS): A case report. Paper presented at: First International Conference on Primary Immunodeficiency Diseases;2011 March 4-6; New Delhi, India

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ORIGINAL ARTICLE

Endocrine complications in primary immunodeficiency diseases in Japan

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Summary

Background In spite of the accumulating evidence on the interaction between the immune and endocrine systems based on the recent progress in molecular genetics, there have been few epidemiological studies focused on the endocrine complications associated with primary immunodeficiency diseases (PID).

Objective To investigate the prevalence and clinical features of endocrine complications in patients with PID in a large-scale study.

Design and participants This survey was conducted on patients with PID who were alive on 1 December 2008 and those who were newly diagnosed and died between 1 December 2007 and 30 November 2008 in Japan. We investigated the prevalence and the clinical data of the endocrine complications in 923 patients with PID registered in the secondary survey.

Results Among 923 PID patients, 49 (5·3%) had endocrine disorders. The prevalence of the endocrine diseases was much higher in patients with PID than in the general population in the young age group, even after excluding patients with immune dysregulation.

Conclusions Endocrine disorders are important complications of PID. Analysis of the endocrine manifestations in patients with PID in a large-scale study may provide further insights into the relationship between the immune and endocrine systems.

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Introduction

A wide variety of clinical complications have been described in primary immunodeficiency diseases (PID).[1,2] PID have been reported to be associated with an increased risk of cancer, in particular non-Hodgkin lymphoma,[2] and the contribution of immune dysfunction in PID to cancer risk is receiving much attention. It is also well known that patients with PID often have complications such as autoimmune and allergic disorders.[1,3] Recently, the interaction between the immune and endocrine systems has been getting increasing attention.[4,5] However, there have so far been no reports focusing on the endocrine complications associated with PID in a large-scale survey.

Many endocrine disorders in patients with PID are thought to be due to the development of the autoimmunity, which is closely related to the pathophysiology of PID.[6] However, it is not known how the immunological and molecular defects in individual PID contribute to the development of various autoimmune endocrine disorders. In addition, the genetic defects in some PID can lead to these complications directly or indirectly via nonimmunological mechanisms.[6]

We analysed the endocrine complications in PID from the information obtained from the nationwide PID survey in Japan conducted in 2008. This is the first large-scale survey focusing on the endocrine complications in PID.

Materials and methods

This survey was performed according to the nationwide epidemiological survey manual of patients with intractable diseases (2nd edition 2006, Ministry of Health, Labour and Welfare of Japan) as described previously.[7] PID classification was based on the criteria of the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee in 2007.[8] The survey was conducted on patients with PID who were alive on 1 December 2008 and those who were newly diagnosed and died between 1 December 2007 and 30 November 2008 in Japan. The initial survey covered 1224 paediatric departments and 1670 internal medicine departments, which were randomly selected according to the number of beds among the 2291 paediatric departments and 8026 internal medicine departments in Japan. Primary questionnaires regarding the number of patients and the disease names based on the PID classification

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were sent to the selected hospitals. The initial survey was conducted to investigate the prevalence of the respective PID. The secondary survey was performed to study the detailed clinical features of individual patients with PID. Secondary questionnaires regarding age, gender, clinical manifestations and complications other than those related to haematopoietic stem cell transplantation of individual patients with PID were sent to the respondents who answered that they observed at least one PID patient with characteristics listed in the primary questionnaires. The details of the methods of the questionnaire investigation, the response rates and the breakdown of the number of patients in both paediatric and internal medicine departments were described elsewhere.[9] The questionnaires were designed to elucidate the clinical characteristics including the manifestations and laboratory data of the patients. In this study, all endocrine manifestations in patients with PID were included as complications of PID, even if they were well known major symptoms of PID.

Results

Detailed clinical information was available from 923 (secondary survey) out of 1240 patients with PID (initial survey).[9] Among the 923 patients with PID, 49 (5·3%) had endocrine disorders. As shown in Table 1, more than two thirds of the patients with PID were <20 years old and the prevalence of endocrine diseases was much higher in the young population of patients with PID than that in the general young population,[7,10–14] even after excluding patients with immune dysregulation (PID category IV). As expected, hypoparathyroidism was the most common endocrine disorder, because it is very frequently observed in patients with DiGeorge syndrome. Endocrine manifestations were also common in patients with diseases of immune dysregulation, such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Although the number of patients with defects in innate immunity was small, endocrine complications seemed to be more common than expected. Interestingly, endocrine disorders were not observed in patients with complement deficiencies. In addition, Graves' disease and Addison's disease were not observed in any of the patients with PID in this study.

Type 1 diabetes mellitus (T1D) was observed in six patients with PID (Tables 1 and 2) including four with type 1A (autoimmune) and two with type 1B (autoantibody-negative, idiopathic). Type 1A diabetes mellitus occurred frequently in patients with IPEX or IPEX-like syndrome (two of six patients, 33·3%) (Table 1). One patient of unknown aetiology in PID category IV showed type 1A diabetes and Hashimoto's thyroiditis along with recurrent viral infections (Tables 1, 2 and S1). In the cases of type 1A diabetes mellitus, anti-glutamic acid decarboxylase (GAD) autoantibodies and anti-insulin autoantibodies (IAA) were positive in all patients and in two of four patients, respectively (Table 2). The patients with IPEX and IPEX-like syndrome had a history of diabetic ketoacidosis with poor glycaemic control, and they developed T1D at a younger age than the other patients with PID. The first case of warts, hypogammaglobulinaemia, infections, and myelokathexis (WHIM) syndrome with T1D and hypothyroidism was included (Tables 2 and S2).[15] With regard to type 1B diabetes mellitus, the patient with hypogammaglobulinaemia of unknown aetiology had diabetic ketoacidosis (Table 2). On the other hand, type 2 diabetes mellitus (T2D) was observed in two patients with PID (Table 1).

Hashimoto's thyroiditis was observed in five patients with PID (Tables 1 and S1). The onset was very early in the patient with IPEX syndrome (at birth). All patients had at least 1 autoantibody among the anti-thyroid peroxidase (TPO), anti-thyroglobulin (Tg) and thyroid stimulating hormone receptor autoantibodies (TRAb).

Nonautoimmune hypothyroidism was reported in seven patients with PID (Tables 1 and S2). Anti-thyroid autoantibodies were all negative when measured. Among these, three patients with X-linked agammaglobulinaemia (XLA), IgG subclass deficiency or WHIM syndrome had primary (congenital) hypothyroidism detected by newborn mass screening. Hypothyroidism in the other four patients with normal TSH levels was considered to be due to central hypothyroidism, a disorder of the pituitary, hypothalamus or hypothalamic-pituitary portal circulation. Two patients with severe combined immunodeficiency (SCID) developed hypothyroidism before they received haematopoietic stem cell transplantation.

Growth hormone deficiency (GHD) was observed in six patients with PID (Tables 1 and S3), whose heights at the diagnosis of GHD ranged from −11·3 SD to −2·5 SD. Five patients were treated with growth hormone. One patient with SCID received cord blood transplantation when she was 20 months old, without conditioning chemotherapy or radiation.

Hypogonadism was observed in three patients with PID (Tables 1 and S4). Among them, two had hypergonadotrophic (primary) hypogonadism, whereas the other had hypogonadotrophic (central) hypogonadism. None of the patients received haematopoietic stem cell transplantation.

One common variable immunodeficiency disease (CVID) patient had isolated ACTH deficiency (Table 1). The other endocrine complications included hypophosphataemia, pseudohypoaldosteronism, adrenal crisis, hypoglycaemia and hypophosphataemic rickets as shown in Table 1.

Discussion

This is the first nationwide survey focusing on the endocrine complications of PID. Among these, hypoparathyroidism was the most common, observed in patients with DiGeorge syndrome and APECED.[16,17] In APECED, the calcium-sensing receptor has been reported to be the autoantigen responsible for hypoparathyroidism.[18] Although it has been reported that 79% of patients with APECED have hypocalcaemia due to hypoparathyroidism,[17] only 1 (25%) among four patients with APECED developed hypoparathyroidism in this study, which might be one of the clinical characteristics of patients with APECED in Japan.

The prevalence (33·3%) of T1D in patients with IPEX syndrome in this study seemed to be lower than that (>70%) of the previous reports.[19,20] The low prevalence of T1D might be due to

Table 1. Endocrine complications in PID patients

PID category	Hypopara-thyroidism	Diabetes mellitus			Thyroid disease		GHD	Hypogonadism	Isolated ACTH deficiency	Others	The number of PID patients			
		T1D		T2D	Autoimmune hypothyroidism (Hashimoto's thyroiditis)	Non-autoimmune hypothyroidism					*n*	0–19 years	Total	Percent in total
		1A	1B											
I. Combined T and B cell immunodeficiencies											**4**	**67**	**75**	**5·3**
RAG1 deficiency						1					1	6	6	16·7
CD4 deficiency					1						1	2	2	50·0
Undetermined											2	10	10	20·0
T-B-SCID						1	1				2	4	4	50·0
II. Predominantly antibody deficiencies											**13**	**231**	**378**	**3·4**
X-linked agammaglobulinaemia						1				2*	3	93	138	2·2
Common variable immunodeficiency disorders			1		1††		1		1	2†	6	29	93	6·5
IgG subclass deficiency						2					2	45	50	4·0
Undetermined			1				1**	1**			2	9	9	22·2
III. Other well-defined immunodeficiency syndromes											**20**	**126**	**165**	**12·1**
Hyper-IgE syndrome							1	1		1‡	3	31	46	6·5
DiGeorge syndrome	14										14	29	32	43·8
Ataxia telangiectasia				1							1	8	13	7·7
Chronic mucocutaneous candidiasis					1††						1	9	13	7·7
ICF syndrome								1			1	0	1	100·0
IV. Diseases of immune dysregulation											**6**	**31**	**38**	**15·8**
IPEX syndrome		2			1					1§	4	5	6	66·7
APECED	1										1	3	4	25·0
Undetermined		1**			1**						1	2	2	50·0
V. Congenital defects of phagocyte number, function or both											**3**	**106**	**153**	**2·0**
Chronic granulomatous disease						1	1				2	54	87	2·3