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Mutations of the X-linked lymphoproliferative disease gene *SH2D1A* mimicking common variable immunodeficiency

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Abstract Common variable immunodeficiency (CVID) and X-linked lymphoproliferative (XLP) disease are two immunodeficiencies that may share a similar immunological phenotype making differential diagnosis difficult. We report two patients initially diagnosed as affected with CVID who, using molecular analysis, have been subsequently found to be affected with XLP disease. Distinguishing between these two diseases is essential since they have different prognosis, treatment and genetic counselling. *Conclusion:* current techniques, such as genetic analysis of the *SH2D1A* gene and expression of signalling lymphocyte activation molecule-associated protein, allow a definite diagnosis of X-linked lymphoproliferative disease.

Keywords Common variable immunodeficiency · *SH2D1A* gene · X-linked lymphoproliferative disease

Abbreviations *CVID* common variable immunodeficiency · *EBV* Epstein-Barr virus · *HIGM* hyper IgM syndrome · *SAP* signalling lymphocyte activation molecule-associated protein · *XLA* X-linked agammaglobulinaemia · *XLP* X-linked lymphoproliferative

Introduction

Common variable immunodeficiency (CVID), the most common primary immunodeficiency, is characterized by low levels of at least two of the three major immunoglobulin isotypes, abnormal specific antibody production and variable abnormalities of T- and B-cell compartments. Patients present with recurrent infections, auto-immune phenomena and gastrointestinal disease [1].

CVID is a highly heterogeneous disease and its genetic basis remains poorly defined. Moreover, a variety of other well-known primary immunodeficiencies may present as CVID. Indeed, in a few patients mutations of genes responsible for X-linked agammaglobulinaemia (XLA) [17], hyper IgM syndrome (HIGM) [2,11] or X-linked lymphoproliferative (XLP) disease [7, 9,14] have been found indicating that a definite diagnosis of CVID requires the exclusion of these molecularly well defined immunodeficiencies.

XLP is a rare genetic disorder caused by mutations of the *SH2D1A* gene [12] and is associated with a variety of clinical manifestations occurring following primary Epstein-Barr (EBV) virus infection including fulminant hepatitis, malignant lymphoma and hypogammaglobulinaemia [8]. The XLP gene codes for a protein known as signalling lymphocyte activation molecule (SLAM)-associated protein (SAP) which in turn has been proposed to function as a regulator of the 2B4 signal transduction pathway. The 2B4 receptor, is expressed on natural killer cells, cytotoxic T-cells and can bind to SAP or SHIP-2 in a mutually exclusive way; binding to SAP mediates an activating whereas binding to SHIP-2 an inhibitory signal. It has been recently shown that XLP patients, who do not express SAP, have a defect of 2B4 receptor-mediated natural killer cell cytotoxicity, due to the binding of 2B4 to SHIP-2, and preliminary data suggest that in XLP patients, 2B4 may also negatively regulate T-cell-mediated responses. Therefore, it is possible that specific cytotoxic T-cell responses against EBV + B-cells may also be impaired by a molecular mechanism similar to that responsible for natural killer cell dysfunction. Altogether, the altered function of 2B4 may account for a general inability of different cytolytic effector cells to control EBV infection [10].

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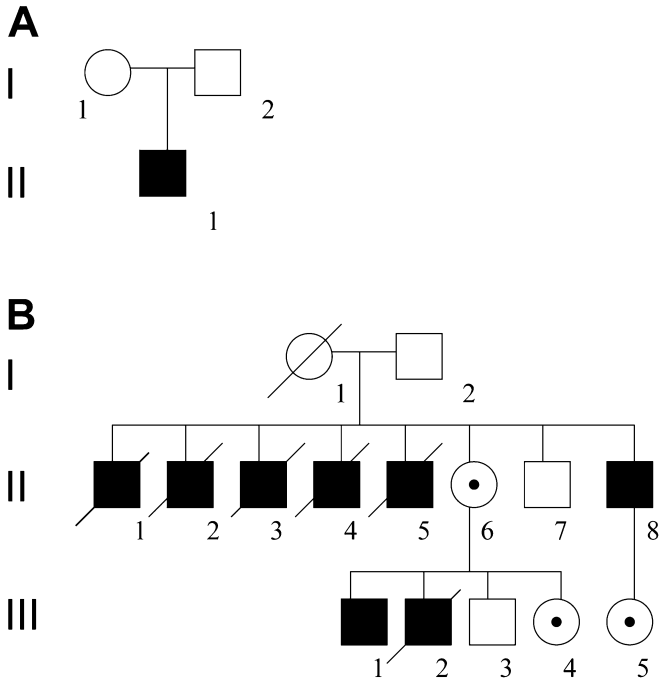

Fig. 1 Pedigree of the two suspected CVID families investigated. Genetic trees of family 1 and family 2 are shown in panels **A** and **B**. Family members are labelled with numbers as indicated in the text. Males are indicated by *squares*, females by *circles*. Deceased members are indicated by a *diagonal line*, affected males by *solid squares*, carriers by *circles with central spot* and unaffected by *open symbols*. Probands are indicated by *arrows*

As the prognosis for XLP is much worse than for CVID syndrome in general, the differential diagnosis between CVID and XLP has important practical implications. In addition, establishing a correct differential diagnosis is essential to provide accurate genetic counselling. We describe two boys who were initially diagnosed with CVID, but who were subsequently found to carry mutations of the *SH2D1A* gene. These observations suggest that mutations of the *SH2D1A* gene must be sought in males with a presumed diagnosis of CVID.

Case reports

Case 1 is a 6-year-old boy. His clinical history has previously been reported [14]. Briefly, he was born in 1994 by normal delivery of non consanguineous parents (Fig. 1A, II-1) and came to our attention in July 2000 because of a history of recurrent respiratory infections. The immunological work-up showed a pattern (Table 1) compatible with a diagnosis of CVID and thus immunoglobulin substitution therapy was started. The review of the patient's clinical and laboratory data revealed that in February 1998 he was admitted to a local hospital because of fever, and discharged with a diagnosis of infectious mononucleosis. On that occasion, serum immunoglobulin levels were within the normal range (Table 1). Since hospital discharge, the patient started to present recurrent upper and lower respiratory tract infections; for this reason, he came to our observation on July 2000. The clinical history and the immunological data suggested XLP disease to be the most likely diagnosis. For this purpose, the *SH2D1A* gene was sequenced and a definitive diagnosis of XLP disease was made based on the detection of a single nucleotide substitution, G to T, at position 462 resulting in an arginine to a lysine substitution at residue 55. This mutation has been found in the patient's mother (Fig. 1A, I-1) and in none of the 100 normal X chromosomes studied, strongly suggesting that the mutation is disease-causing.

Case 2 is a male patient born in 1980 by normal delivery of non consanguineous parents (Fig. 1B, III-1). Since infancy he presented recurrent respiratory tract infections and an immunological work-up performed at a local hospital at the age of 3 years showed a pattern compatible with a presumptive diagnosis of CVID. Despite appropriate intravenous immunoglobulin substitution therapy (400 mg/kg/21 days) his clinical course was characterized by recurrent sino-pulmonary infections leading to bronchiectasis and chronic obstructive lung disease which required lobectomy at the age of 14 years. In 1999, he was enrolled in a prospective survey study of patients with chronic chest symptoms. On this occasion, a review of the patient's clinical and laboratory data and the acceptance by the parents to disclose documentation on the clinical history of the patient and of the other family members, allowed the physician to strongly suspect an XLP

Table 1 Immunological features of the two patients. (*ND* not done, *UN* undetected)

Parameter	Case 1		Case 2		
	February 1998	June 2000	January 1981	October 1983	December 1999
CD3 (%)	ND	58	ND	65	71
CD4 (%)	ND	31	ND	ND	33
CD8 (%)	ND	21	ND	ND	36
CD19 (%)	ND	21	ND	10	22
IgG (g/l)	8.62	0.55	4.60	0.90	6.93[b]
IgA (g/l)	0.31	0.05	0.26	0.19	0.05
IgM (g/l)	2.43	0.25	0.58	0.17	0.22
EBV-DNA	ND	Positive	ND	ND	Positive
EBV serology	Positive	Negative	Negative[a]	Negative[a]	ND
Cytomegalovirus serology	ND	Negative	Negative	Negative	ND
Antibody to tetanus toxoid	ND	UN	ND	ND	ND
Antibody to diphtheria toxoid	ND	UN	ND	ND	ND
Antibody to hepatitis B virus surface antigen	ND	UN	ND	ND	ND
Antibody to polio	ND	< 1:4	ND	ND	ND

[a]Evaluated by the Paul-Bunnel test
[b]On intravenous immunoglobulin

disease. In fact, it transpired that the patient had been hospitalized at the age of 1 year because of an episode of acute "idiopatic" hepatitis and on that occasion serum immunoglobulin levels were within the normal range. Furthermore, the patient's younger brother had died at the age of 3 years due to intestinal lymphoma (Fig. 1B, III-2). Moreover, of the seven maternal uncles of the patient, five had died early in childhood of severe infections and one of the two still alive is affected with a non-Hodgkin lymphoma (Fig. 1B, II-8), (Fig. 1B, II-7).

Sequence analysis of the patient's *SH2D1A* gene showed the presence of a single nucleotide substitution, A to C, at position 263 resulting in a glutamine to proline substitution at residue 88, allowing us to make a definite diagnosis of XLP disease. The same mutation was present in the patient's sister, demonstrating that she is a carrier of the disease gene and in the maternal uncle with non-Hodgkin lymphoma (II-8), whereas no mutation was observed in the patient's younger EBV-seronegative healthy brother (III-3).

Discussion

The two cases described here presented symptoms in early childhood and fulfilled the diagnostic criteria of CVID. In fact, at present, the diagnosis of CVID is based only on a clinical history of infections and/or auto-immune manifestations associated with hypogammaglobulinaemia in the presence of normal number of circulating B-cells.

Within the last decade, progress in molecular biology has allowed identification of the genes responsible for several forms of hypogammaglobulinaemia. These include XLA which is due to mutations of *BTK* gene [16], four forms of HIGM due to mutations of *CD40L*, *AID, CD40*, and *IKKγ* genes respectively [2, 5, 6,11], and *XLP* which is due to mutations of the *SH2D1A* gene [12].

Since atypical cases of these immunodeficiencies may present with an immunological and clinical phenopype resembling CVID, a stricter definition of CVID should now be based also on genetic exclusion of mutations of these genes. However, the presence in both of our patients of normal number of circulating B-cells as well as of low serum IgM levels, and normal IgG levels early in the disease, made the diagnosis of XLA or of HIGM syndromes less likely than that of XLP disease, which on the contrary was strongly supported by a positive EBV infection which preceded the occurrence of hypogammaglobulinaemia and, in addition, in case 2, by a positive family history of early deaths or lymphoma. Sequence analysis of the *SH2D1A* gene in both cases led to a definitive diagnosis of XLP disease.

Characterization of the gene defect has many practical implications. First, it changes the genetic risk in the family from what observed in a multifactorial disorder (as CVID) into that of a monogenic, X-linked disease (as XLP disease). Second, it raises the need for careful monitoring of the clinical and immunological status of the patient himself and allows a search for a potential stem cell donor to be initiated, as haematopoetic stem cell transplantation is at present the only successful curative therapy [4,18]. It is well known that XLP disease has a worse prognosis as compared to CVID, with over 70% of affected males dying within 10 years of age (most often due to fulminant hepatitis, EBV-related lymphoma, or haemophagocytic lymphohistiocytosis) [13]. Among the 30% of the XLP disease patients who become hypogammaglobulinaemic upon EBV infection, the survival rate is 55%, far better than that observed in those who present with fulminant hepatitis (4% survival rate), or a lymphoproliferative disorder (35%) [13]. The poor outcome of XLP disease contrasts with a survival rate of 65.5%, 20 years after diagnosis, in a large survey of 248 CVID patients [1]. Nevertheless, it is also recognized that in spite of the better long-term outcome, the XLP disease patients who become hypogammaglobulinaemic (as the cases reported here) remain at high risk for lymphomas [13]. As a consequence, it is crucial to perform a definite diagnosis of XLP disease in all male patients with a presumed diagnosis of CVID and a positive history of EBV infection. This can be achieved by sequence analysis of the *SH2D1A* gene. However, mutation analysis in individuals with typical XLP disease presentation and family histories, has only detected abnormalities in about 60% of patients. Thus, genetic analysis alone cannot confirm a diagnosis of XLP disease. Recently, a SAP expression assay has been developed that seems to be a more sensitive diagnostic indicator for XLP disease, since lack of expression of SAP protein has been found in some XLP disease patients with no mutations in the *SH2D1A* coding region [3]. Furthermore, it has been shown, following the characterization of the gene defect, that some genotypically affected XLP disease males may develop clinical symptoms even in the absence of primary EBV infection [15]. Thus, what was considered a hallmark of the disease (development of symptoms after EBV infection) is no longer true. We therefore suggest that XLP disease should be suspected in all males who develop clinical signs of XLP regardless of their EBV serology and in certain boys previously diagnosed as affected with CVID and recommend that patients are investigated both by genetic analysis of *SH2D1A* and by expression of SAP protein.

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