The X-linked lymphoproliferative syndrome: molecular and cellular basis of the disease

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CHAPTER 4

Alterations of the X-linked lymphoproliferative disease gene SAP / SH2D1A in the Common Variable Immunodeficiency syndrome.

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Chapter 4

**SUMMARY**

X-linked lymphoproliferative (XLP) disease is a primary immunodeficiency caused by a defect in the *SAP* (or *SH2D1A*) gene. At least three major manifestations characterize its clinical presentation: fatal infectious mononucleosis (FIM), lymphomas and immunoglobulin deficiencies. Common Variable Immunodeficiency (CVID) is a syndrome characterized by immunoglobulin deficiency leading to susceptibility to infection. In some CVID patients a defective *btk* or *CD40-L* gene has been found, but the majority of cases remain without a clearly identified etiology.

Here, two unrelated families in which male individuals were affected by CVID were examined for a defect in the XLP gene. In one family previously reported in the literature as showing progressive immunoglobulin deficiencies, three brothers presented with recurrent respiratory infections, while female family members showed only elevated serum IgA levels. A grandson of one of the brothers died because of a severe aspergillus infection secondary to progressive immunoglobulin deficiency, FIM, aplastic anemia and a B cell lymphoma. In the second family, two brothers had B-lymphocytopenia and immunoglobulin deficiencies. The X-linked agammaglobulinemia (XLA) syndrome was excluded genetically and they were classified as CVID patients. Occurrence of FIM in a male cousin of the brothers led to the XLP diagnosis. Since the *SH2D1A* gene was found altered in both families, our findings indicate that XLP must be considered when more than one male CVID patient in the same family is encountered, and *SH2D1A* analyzed in all male CVID patients. Moreover, these data link defects in the *SH2D1A* gene to abnormal B-lymphocyte development and dysgammaglobulinemia in female members of XLP families.
INTRODUCTION

X-linked lymphoproliferative (XLP) disease is characterized by extreme complications of Epstein Barr virus (EBV) infection.\(^1\)\(^-\)\(^5\) Its identification was first reported by David Purtilo more than 25 years ago.\(^1\) XLP has three major phenotypes: fulminant infectious mononucleosis (FIM) (50%), B-cell lymphomas (20%), or dysgammaglobulinemia (30%).\(^6\)\(^-\)\(^5\) Aplastic anemia, vasculitis and pulmonary lymphomatoid granulomatosis are also often associated with the syndrome.

The gene responsible for the disease has been cloned and named SAP (for SLAM Associated Protein) or \(SH2D1A\).\(^8\)\(^-\)\(^10\) The human and mouse \(SH2D1A\) gene consist of four exons and three introns spanning approximately 25kb.\(^8\)\(^,\)\(^11\) In the mouse, \(SH2D1A\) is highly expressed in thymocytes and peripheral T cells with a prevalent expression on Th1 cells.\(^11\) While \(SH2D1A\) is also expressed by NK cells,\(^12\)\(^,\)\(^13\) its presence in B-lymphocytes is unclear.

The \(SH2D1A\) protein consists of an 128 amino acids comprising an SH2 domain and a 24 amino acid tail.\(^8\)\(^,\)\(^10\) The \(SH2D1A\) protein has been shown to bind a family of surface immune receptors, the SLAM family, which belong to the immunoglobulin-family of receptors.\(^10\)\(^,\)\(^14\)\(^,\)\(^13\) SLAM (CD150), 2B4 (CD244), CD84 and Ly-9 are the molecules that bind \(SH2D1A\).\(^10\)\(^,\)\(^13\)\(^-\)\(^15\) A \(SH2D1A\)-like molecule named EAT-2 interacts with the same SLAM-family members as \(SH2D1A\) in non-T hematopoietic cells (M Morra et al., manuscript submitted).\(^3\)\(^,\)\(^5\)

Among the different XLP phenotypes, FIM is the only one clearly linked to EBV infection. However, immunoglobulin deficiencies and non-Hodgkin’s B cell lymphomas have been observed in XLP patients who were sero- and/or PCR - negative for EBV.\(^17\)\(^,\)\(^18\) The immunoglobulin deficiency and chronic respiratory infections associated with XLP clinically resemble CVID.\(^19\)\(^,\)\(^20\) CVID is a primary immunodeficiency syndrome characterized by decreased, often fluctuating serum
immunoglobulins and clinical features of recurrent bacterial infections.\textsuperscript{19,20} Some patients develop atypical inflammatory gastro-intestinal diseases and autoimmune diseases, including autoimmune hemolytic anemia, thrombocytopenia, rheumatoid arthritis, and pernicious anemia. Patients with CVID also have an increased incidence of cancer, particularly lymphoma.\textsuperscript{20}

Our working hypothesis is that a subset of male CVID patients without a clearly defined etiology may have alterations in the $SH2D1A$ gene. In the past, the X-linked agammaglobulinemia (XLA), and X-linked and autosomal hyper-IgM syndrome (X-HIM and HIM) disease genes have been found mutated in some CVID patients, whilst the majority of patients remain without a clearly identified etiology.\textsuperscript{19,21} Here, 10 males of two families where members had been previously diagnosed with CVID were found to have alterations in the XLP gene $SH2D1A$. Our results indicate that mutations in the $SH2D1A$ gene must be studied in all male CVID patients.

**MATERIALS AND METHODS**

**Detection of mutations in the $SH2D1A$ gene**

**Genomic PCR**

Peripheral blood lymphocytes from families were collected in EDTA-containing test tubes. When lymphoblasts were available, they were grown in RPMI 1640 supplemented with 10\% fetal bovine serum under standard culture conditions. DNA was isolated using standard techniques.\textsuperscript{22} Coding sequences, 5' regulatory region (300 nucleotides from the transcription initiation site), and intronic splice-site sequences were amplified by the polymerase chain reaction (GeneAmp/XL PCR kit, Perkin Elmer, Branchburg, NJ). From each family, at least 2 affected members, 2 carriers, and 2 normal members were analyzed for mutations in the $SH2D1A$ gene. PCR were performed in 50 µl with a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA), using the following conditions: 94°C (3min); 94°C (1min), 60°C (1.30min), 72°C (1min) for 35 cycles; 72°C (10min); 4°C ($\infty$). Primer combinations: exon 1 F5'- GCC CTA CGT AGT GGG TCC ACA TAC CAA CAG -
SAP/SH2D1A mutations in CVID patients

3'; exon 1 R5'- GCA GGA GGC CCA GGG AAT GAA ATC CCC AGC -3'; exon 2 F5'- GGA AAC TGT GGT TGG GCA GAT ACA ATA TGG -3'; exon 2 R5'- GGC TAA ACA GGA CTG GGA CCA AAA TTC TC -3'; exon 3 F5'- GCT CCT CTT GCA GGG AAA TTC AGC CAA CC -3'; exon 3 R5'- GCT ACC TCT CAT TTG ACT TGC TGG CTA CAT C -3'; exon 4 F5'- GAC AGG GAC CTA GGC TCA GGC ATA AAC TGA C -3'; exon 4 R5'- ATG TAC AAA AGT CCA TTG CAG CTT TGA C -3'. Genomic DNA from the Raji human cell line (ATCC, Manassas, VA) was used as a positive control, while distilled water as negative control. PCR products were visualized on a 1.5% agarose gel and subjected either to direct sequencing procedure or sub-cloning followed by sequencing (samples from females donors).

For direct nucleotide sequencing, PCR products were purified using Microcon-PCR centrifugal filters (Amicon-Millipore, Danvers, MA) and sequenced with appropriate end-labeled primers. For sub-cloning, 1μL of the PCR product was ligated in a TA-cloning vector (TA cloning kit, Invitrogen, Carlsbad, CA). After insertion, the vector was transformed in INV-α bacteria (Invitrogen) and selection was performed through blue-white screening on LB plates containing Ampicillin (50 mg/ml) and X-Gal (40 μL of a 100mg/mL solution each plate). White colonies were grown over-night in LB-Amp liquid media. Plasmidic DNA was isolated using a Miniprep kit (Qiagen, Valencia, CA). For each exon at least 10 colonies were subjected to sequencing. Sequencing data were analyzed using the programs EditSeq and MegAlign (Dnastar software, Madison, WI).

Reverse transcriptase-PCR

Total RNA was isolated from peripheral blood lymphocytes of patients, carriers, and healthy persons by TRIzol Reagent (BRL, Gaithersburg, MA). One microgram total RNA was reverse-transcribed using a one step RT-PCR system (Access RT-PCR kit, Invitrogen). The following primers combination was used: F5'-GCC TGG CTG CAG TAG CAG CGG CAT CTC CC -3'; R5'- ATG TAC AAA AGT CCA TTG CAG CTT TGA C -3'. The annealing temperature for both primer pairs was 60°C.

Measurement of serum immunoglobulin levels.
Immunoglobulin isotype G, A and M were determined using standard laboratory procedures. Part of the data reported in Table I were obtained from the manuscript of Buckley et al. Measured values were considered normal, above or below average relatively to the standards of the laboratory where the measurement was done.

Patients studies were done in accordance with the Helsinki protocol.

RESULTS

Family 1. Patient C.L. (Figure 1A) was born in 1996. After the first 6 months of life, the patient was examined for recurrent infections of the upper and lower respiratory tracts (bronchitis, pneumonia, and otitis media) and of the gastrointestinal tract. Determination of serum immunoglobulin levels indicated only elevated IgA at 6 months and slightly low IgM at 14 months (Table 1). Because of the family history of immunodeficiency, he did not receive live vaccines. At the age of 17 months, serum concentrations of IgG and IgA declined, and he had almost no detectable antibody titer against tetanus and diphtheria toxoids despite repeated immunizations. B- and T-cell numbers were normal. Lymphocyte proliferation tests at the age of 18 months indicated normal responses to phytohemagglutinin and concanavalin A, with a low response to pokeweed mitogen. At the age of 19 months, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase liver enzyme levels were elevated. Abdominal ultrasonography results were normal, and findings were negative for hepatitis A, B, and C, cytomegalovirus, and human immunodeficiency virus. Two months later, the patient was admitted to the hospital because of fever, pneumonia, a diffuse morbilliform rash, and an enlarged liver. Absolute neutropenia and thrombocytopenia then developed, and the patient was found to be EBV positive by PCR testing of his blood and cerebrospinal fluid. He was treated with acyclovir, granulocyte transfusions, and intravenous immunoglobulin (IVIG), but he died 5 weeks after admission because of an overwhelming *Aspergillus* infection secondary to aplastic anemia. Autopsy
Figure 1. Pedigree of the two CVID families investigated.
Genetic trees of family 1 and family 2 are shown in panels A and B, respectively. Patients are labeled with numbers and letters as indicated in the text. An arrow indicates patients analyzed for the presence of SH2D1A gene mutations. (box) Males; (circle) females; (line through) deceased; (filled) affected male; (dotted) patient carrier; (open) unaffected subjects.
Table I.

Immunoglobulin levels in members of family #1 and #2A.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>IgG (mg/dL)</th>
<th>IgA (mg/dL)</th>
<th>IgM (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.L.</td>
<td>06/12</td>
<td>391</td>
<td>74†</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>12/12</td>
<td>779</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>15/12</td>
<td>373</td>
<td>0†</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>16/12</td>
<td>119↓</td>
<td>8↓</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>17/12</td>
<td>248</td>
<td>11↓</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>18/12</td>
<td>264</td>
<td>10↓</td>
<td>97</td>
</tr>
<tr>
<td>C.G.</td>
<td>65/12</td>
<td>760</td>
<td>690†</td>
<td>114†</td>
</tr>
<tr>
<td></td>
<td>81/12</td>
<td>410↓</td>
<td>67↓</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>95/12</td>
<td>340↓</td>
<td>59↓</td>
<td>12↓</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>265†</td>
<td>53†</td>
<td>12†</td>
</tr>
<tr>
<td>C.E.</td>
<td>144/12</td>
<td>55↓</td>
<td>0↓</td>
<td>10↓</td>
</tr>
<tr>
<td>C.F.</td>
<td>119/12</td>
<td>423↓</td>
<td>253</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>139/12</td>
<td>363↓</td>
<td>167</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>144/12</td>
<td>290↓</td>
<td>117</td>
<td>18↓</td>
</tr>
<tr>
<td></td>
<td>154/12</td>
<td>223↓</td>
<td>122</td>
<td>25↓</td>
</tr>
<tr>
<td>C.B.</td>
<td>39</td>
<td>1263</td>
<td>896†</td>
<td>33↓</td>
</tr>
<tr>
<td></td>
<td>(mother)</td>
<td>41</td>
<td>1200</td>
<td>1274†</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>950</td>
<td>1000†</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>900</td>
<td>1000†</td>
<td>39</td>
</tr>
<tr>
<td>C.A.</td>
<td>43</td>
<td>690</td>
<td>450</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>(father)</td>
<td>45</td>
<td>900</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>870</td>
<td>340</td>
<td>64</td>
</tr>
<tr>
<td>A.C.</td>
<td>3</td>
<td>120↓</td>
<td>3↓</td>
<td>5↓</td>
</tr>
<tr>
<td>A.B.</td>
<td>010/12</td>
<td>90↓</td>
<td>0↓</td>
<td>4↓</td>
</tr>
</tbody>
</table>

† Value above normal for age.
↓ Value below normal for age.
For normal range, see Methods.

showed disseminated aspergillosis (involving lungs, kidney, esophagus, large and small intestines, pericardium, and diaphragm); acute and organizing
bronchopneumonia; hypocellular bone marrow; lymphodepletion of thoracic and abdominal lymph nodes (necrosis with rare B cells and moderate number of T cells) positive for the EBV antigens LMP and EBNA-2); severe thymic atrophy with no evidence of thymopoiesis; acute and organizing splenic infarcts; centrilobular liver congestion, cholestasis, and peritoneal serous effusion; and large B-cell lymphoma involving peripancreatic lymph nodes only (LMP and EBNA-2 positive).

C.L.'s family is a well-studied sibship, previously reported by Buckley and Sidbury24 (Figure 1A), affected by a variety of progressive immunoglobulin abnormalities in male and female members. C.L.'s grandfather (C.G.), together with his 2 brothers C.E. and C.F., were first seen at Duke University Medical Center in 1963 because all 3 were affected with frequent respiratory infections that were particularly severe in C.E. C.G. had only a late onset of mild infections. When he was first seen at the age of 6.5 years, C.G.'s findings were reported to be normal, but by the time he was 8.5 years of age, splenomegaly and a low lymphocyte count were noted. Immunologic studies over a period of 4 years showed a progressive deficiency of all 3 immunoglobulin isotypes (Table 1). He had a normal number of B cells initially, but this declined with time. He was treated with IVIG, but a B-cell lymphoma of the small intestine developed. This was successfully treated, but he had chronic severe diarrhea and died of bacterial pneumonia at the age of 38 years. The oldest brother, C.E., was first seen at the age of 14 years. He was affected by severe repeated respiratory infections, and examination of his serum revealed marked deficiencies of IgA, IgM, and IgG. C.E. failed to respond to blood group A and blood group B substances and to diphtheria, tetanus, and polio vaccines. He had 4 episodes of acute pneumonia before the age of 11 and died at 15 years because of acute respiratory failure. Another brother, C.F., remained well until late childhood. After the age of 10 years, he had repeated episodes of acute pneumonia. Analysis of serum immunoglobulin (Table 1) showed progressive reduction of his IgM and IgG levels over a 4-year period beginning in 1963. He died at the age of 25 years from acute pulmonary infiltrates and carcinomatous meningitis. The mother of the 3 boys (C.B.) had no history of severe infections. Her immunologic study findings (Table
1) demonstrated marked polyclonal IgA hyperglobulinemia, selective unresponsiveness to blood group B substance injections, and poor responses to immunization with diphtheria and polio vaccines. Two maternal aunts (C.C. and C.D.) also had IgA hyperglobulinemia and low isohemagglutinin titers. The father of the 3 boys (C.A.) was healthy and had normal levels of serum immunoglobulins (Table 1). There was no history of conditions similar to those of the boys on either side of the family.

**Family 2.** A 2.5-year-old boy (A.C.) (Figure 1B, 2-A) was brought to the Rambam Medical Center (Haifa, Israel) in 1988 after several episodes of pneumonia and *Escherichia coli* sepsis starting when he was 1 year old. His B-lymphocyte count was very low (1%-2%), as were his serum IgG and IgM levels, and serum IgA was undetectable (Table 1). A presumptive diagnosis of XLA was made, and he was started on IVIG therapy. B cell levels rose to 7% to 8% over years. He had no major medical problems until the present; he is now 12 years of age. In 1993, his brother (A.B.) (Figure 1B, 2-A) was born and was found to have a normal number of B cells. Nevertheless at age 10 months, pneumonia and hypogammaglobulinemia developed (Table 1). IVIG treatment was begun, and, like his brother, he is now doing well. A mutation in *btk* was ruled out (courtesy of Dr M. E. Conley), and, thus, CVIID was diagnosed.

In 1999, their cousin B.C. (age 2 years) (Figure 1B, 2-B) was admitted to the hospital with clinical signs and symptoms compatible with FIM. He had marked hepatosplenomegaly and rapid deterioration of liver function. Anti-viral caspid antigen (VCA) IgM was positive, and liver biopsy showed typical features of FIM. He was treated with high-dose methylprednisolone and VP-16, but, unfortunately, he died before bone marrow transplantation could be performed. Family history (Figure 1B, 2-B) revealed that 2 other brothers died of FIM at approximately the same age range. In one of them, EBV was detected by biopsy of the liver. His 2 sisters were healthy.
Analysis of the SH2D1A gene in the 2 families

Family 1. Clinical and autopsy findings of C.L. led us to consider the possibility that he and his ancestors could have had XLP. Genomic DNA of C.G. and C.L. was extracted from B lymphocytes immortalized with EBV virus. DNA sequencing results (Figure 2A) indicated the presence of a single nucleotide substitution, C→T, in position 462 of the SH2D1A gene. This substitution alters the triplet CGA, which codes for amino acid R55, into the stop codon TGA. Because of the premature stop signal, only a 54-amino acids hypothetical SH2D1A protein (R55X) could be generated (Figure 2C). This abnormal protein has previously been described in other patients with XLP.25

Family 2. The clinical presentation of B.C. (November 1999), compatible with XLP, prompted us to examine his DNA and that of his cousins (A.C. and A.B.) for mutations in the XLP gene. Genomic DNA was extracted from peripheral blood lymphocytes of members of the 2 families (2-A and 2-B) to be tested for alterations in the SH2D1A gene. DNA sequencing (Figure 2B) indicated that the male family members B.B., B.C., A.B., and A.C. had an 8-base pair (bp) deletion located in the third exon (nucleotides 548 to 555). This alteration in the SH2D1A gene was previously unreported. Curiously, the sequence deleted in these patients (GCATTCTCA) is repeated twice in the third exon, and this deletion is situated adjacent to an internal splice acceptor site located in the third exon. This low-frequency splice acceptor site generates a physiologically shorter form of the SH2D1A protein, named SAPΔ55, which is found in all healthy persons. Because of a shift in the reading frame, this deletion leads to a premature stop codon (at a position corresponding to residue 100). This premature stop codon generates a short, altered SH2D1A protein of 99 amino acids (Y100X) (Figure 2C). The same SH2D1A gene microdeletion was also found in the 2 mothers (B.A. and A.A.), identifying them as genetic carriers.
Another brother, an asymptomatic 11-month-old (B.D.) (Figure 1B, 2-B) tested positive for the same SH2D1A gene deletion. In December 1999 the patient underwent bone marrow transplantation (BMT) from a completely matched donor (a 9-year-old sister). No complications occurred during or after BMT.

| 2A. | wt       | ATACATACCCAGTGTC | wt       | ATACATACCCAGTGTC |
|     | CL       | ATACATACTGAGTGTC  | CH       | ATACATACTGAGTGTC  |
|     | CG       | ATACATACTGAGTGTC  | CH       | ATACATACCCAGTGTC  |

| 2B. | wt       | TCAGCATTTTCAGAAGCC | wt       | TCAGCATTTTCAGAAGCC |
|     | AB       | TCAG------AAGCC    | AA       | TCAG------AAGCC    |
|     | AC       | TCAG------AAGCC    | AA       | TCAGCATTTTCAGAAGCC |
|     | BB       | TCAG------AAGCC    | BA       | TCAG------AAGCC    |
|     | BC       | TCAG------AAGCC    | BA       | TCAGCATTTTCAGAAGCC |
|     | BD       | TCAG------AAGCC    |          |                    |

| 2C. | SH2D1A   | MDAVA VYHGKISRETGEKL LLLATGLDGSYLLRDSSESPGVYCLC |
|     | F 1      | MDAVA VYHGKISRETGEKL LLLATGLDGSYLLRDSSESPGVYCLC |
|     | F 2      | MDAVA VYHGKISRETGEKL LLLATGLDGSYLLRDSSESPGVYCLC |

| SH2D1A | VLYHGYIYT YRVSQT ETGSWSAETAPVHKRYFRK LKNI LSAFQ |
| F 1    | VLYHGYIYT Y* |
| F 2    | VLYHGYIYT YRVSQT ETGSWSAETAPVHKRYFRK LKNI LSEAR |

| SH2D1A | KPDQGIVIPLQYPVEKKSSARSTQGTTGIREDPDVCLKAP |
| F 1    |                                            |
| F 2    | SRHCNTSAVSS*                              |
Figure 2. Alterations in the SH2D1A gene and protein.

2A. Alignment of the wild-type SH2D1A complementary DNA (cDNA) sequence with the nucleotide sequences obtained from patients C.L. and C.G. (left) and C.H. sequences (right). Panels depict the gene segment of interest. Nucleotide differences are indicated in gray. A single nucleotide substitution was detected at position 462 of the SH2D1A cDNA coding region of patients C.L. and C.G. (C462T) (left). After subcloning, approximately half the exon 2 PCR products of C.H. (mother of C.L. and daughter of C.G.) contained the C462T nucleotide substitution (right panel).

2B. The SH2D1A cDNA nucleotide sequence was aligned with the sequences obtained from patients A.C., A.B., B.B., B.C., and B.D. (left) and A.A. and B.A. sequences (right). Panels depict the gene segment of interest. Nucleotide differences are indicated in gray. An 8-nucleotide deletion was detected between positions 548 and 555 of the SH2D1A cDNA coding region of patients A.C., A.B., B.B., B.C., and B.D. (left). After subcloning, approximately half the exon 3 PCR products of A.A. (mother of A.B. and A.C.) and B.A. (mother of B.B., B.C., and B.D.) contained the 8-nucleotide deletion detected in their sons (right).

2C. Comparison of the 2 mutant protein sequences with wild-type SH2D1A. The single nucleotide C462T substitution detected in family 1 resulted in a change of the triplet CGA that coded for R55 to the stop codon triplet TGA. This generated a shorter SH2D1A protein of 54 amino acids (R55X) (indicated in the figure as F1). The 8-nucleotide deletion in the third exon of family 2 resulted in a change of the protein reading frame, generating a premature ending signal at a position corresponding to Y100 in SH2D1A. The shorter SH2D1A protein of 99 amino acids (Y100X) is indicated in the figure as F2. The gray area indicates the identity of residues among wild-type SH2D1A and the 2 mutant proteins. Asterisks mark the premature stop codon signals.

DISCUSSION

CVID is a heterogeneous syndrome both clinically and immunologically. A precise clinical and laboratory definition of the disease has been difficult because of the heterogeneity in phenotypes. In a large study of 248 patients with CVID, 40% had impaired T-cell proliferation to mitogens. Based on B-lymphocyte responses to plate-bound -IgM, patients with CVID were divided into 4 subgroups. Numerous studies have attempted to establish diagnostic criteria for the disease and to determine molecular etiologies. Recently, guidelines for the evaluation of CVID have been published. A much stricter definition of the disease must now include
the genetic exclusion of mutations in *btk*, *CD40-L*, *AID*, and *SH2D1A* genes.19,21,27

Cellular immunologic alterations in patients with XLP are not well understood. T and B lymphocytes undergo sustained proliferation in XLP. Extensive tissue infiltration and multi-organ failure are the primary causes of death in these patients.6 The failure to eliminate EBV-transformed B cells in XLP does not seem to be caused by a defect in the B cell.28 SH2D1A expression in B lymphocytes is probably limited only to certain subpopulations.12 Moreover, no major B-lymphocyte defects have been found in SH2D1A null mice (C. Gullo, C. Terhorst, personal communication). On the contrary, variable defects in T cells and natural killer cells of patients with XLP have been reported. SH2D1A-deficient natural killer cells are unable to lyse appropriate target cells.29-33 B-lymphocyte developmental abnormalities were detected in one member of the 2 families. Such a defect in B cells has been described in the past.34 Whether these B-lymphocyte abnormalities and abnormal immunoglobulin levels result from a SH2D1A deficiency in B cells or from abnormal T-B lymphocyte interactions among SLAM-family members is unknown at this time. The SH2D1A-interacting molecules SLAM and CD84 and the 2B4-ligand CD48 are highly expressed in B cells,35 and their expression increases after cell activation or EBV infection. In particular, SLAM has been demonstrated to play a role in B-lymphocyte proliferation and immunoglobulin synthesis after ligation by its soluble form (sSLAM).36 The complex network of interactions among SH2D1A, EAT-2, and their ligands SLAM, 2B4, CD84, and Ly-9 may account for the clinical variability of manifestations in XLP. Recent data (M.M. et al) indicate that EAT-2 is probably the SH2D1A-like molecule functional in B lymphocytes. One could predict that mutations of EAT-2 might give rise to CVID.

Decreases in serum immunoglobulin levels with time in patients C.L., C.E., C.G., and C.F. (family 1) suggest that a cumulative effect of sequential environmental factors must play a strong role in determining the expression of the SH2D1A
mutations. Because SLAM has been recently identified as another receptor for the measles virus, a role for measles virus as a potential precipitant of disease expression in SH2D1A-deficient patients can be presumed. Dysgammaglobulinemia complicated by disseminated measles has been described in the past.

Of particular interest is the fact that female members of family 1 had abnormal immunoglobulin levels. Female carriers of XLP have been reported to have abnormal antibody responses to EBV. In male patients with XLP, IgG1 and IgG3 serum levels are often low with elevated IgA and IgM classes. Therefore, in females with 1 of 2 altered SH2D1A alleles, a modest reduction in SH2D1A protein levels could result in mild laboratory alterations, such as the hyper-IgA reported in family 1. Decreased cellular levels of the SH2D1A protein could lead to immunoglobulin dysregulation through alterations in the T-B lymphocyte network. Patients with XLP who have reduced SH2D1A protein levels have been described. One patient had a critically reduced SH2D1A wild-type protein level because of a regulatory mutation in the 5' splicing acceptor site of the second exon.

Family 1 is of further interest because all 3 major phenotypes developed in C.L. in only few months, and he died before the age of 2 years. The other affected male relatives had hypogammaglobulinemia or hypogammaglobulinemia and malignant lymphoma and lived until 15 years and 38 years. This clinical variation in patients with the same SH2D1A mutation indicates that other host or environmental factors are important in determining disease expression. Environmental factors are not limited to EBV infection because XLP phenotypes may develop in its absence. Age may be a critical factor in determining disease severity hypogammaglobulinemia developed in C.L. at 17 months of age, and he succumbed of FIM and aplastic anemia at 20 months of age.

In conclusion, the work reported here indicates the presence of SH2D1A mutations in patients diagnosed with CVID. Therefore, together with \( \text{btk}, \text{CD40-L}, \) and \( \text{AID} \)
genes, we suggest that \textit{SH2D1A} must be included in the molecular diagnosis of CVID. Because of the high rate of new mutations occurring in other human X-linked immunodeficiencies, such as XLA,\textsuperscript{41} the \textit{SH2D1A} gene should be studied in all male patients with CVID. Clinically polarized XLP presentations must be considered when patients with CVID are encountered. A similar conclusion could be drawn from data published elsewhere.\textsuperscript{42} This is particularly true when more than one male member of a family is affected. Besides allowing genetic counseling, a correct diagnosis of XLP will allow for the selection of more aggressive therapy (such as BMT) because the prognosis for XLP is much worse than for CVID syndrome in general.

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\textbf{REFERENCES}


