



HAL
open science

Genetic screening of male patients with primary hypogammaglobulinemia can guide diagnosis and clinical management

Nicolas Vince, Gael Mouillot, Marion Malphettes, Sophie Limou, David Boutboul, Angélique Guignet, Philippe Pellet, Pierre-Antoine Gourraud, Patrice Debré, Eric Oksenhendler, et al.

► To cite this version:

Nicolas Vince, Gael Mouillot, Marion Malphettes, Sophie Limou, David Boutboul, et al.. Genetic screening of male patients with primary hypogammaglobulinemia can guide diagnosis and clinical management. *Human Immunology*, 2018, 79 (7), pp.571-577. 10.1016/j.humimm.2018.04.014 . hal-02135208

HAL Id: hal-02135208

<https://hal.science/hal-02135208>

Submitted on 21 May 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title page**

2 **Genetic screening of male patients with primary hypogammaglobulinemia can guide**
3 **diagnosis and clinical management**

4 Nicolas Vince*^{1,2,3}, Gaël Mouillot*⁴, Marion Malphettes^{1,5}, Sophie Limou^{2,3,6},
5 David Boutboul¹, Angélique Guignet¹, Véronique Bertrand⁴, Philippe Pellet⁴,
6 Pierre-Antoine Gourraud^{2,3}, Patrice Debré⁴, Eric Oksenhendler⁵, Ioannis
7 Théodorou⁴, Claire Fieschi^{1,5}; and the DEFI Study Group.

8
9 Abbreviated title:

10 **Genetic screening of hypogammaglobulinemia**

11
12 Corresponding author:
13 Nicolas Vince, PhD
14 CRTI UMR1064 - ITUN
15 CHU Nantes Hôtel Dieu
16 30 bld Jean Monnet
17 44093 Nantes Cedex 01
18 France
19 nicolas.vince@univ-nantes.fr
20 Phone: +33 2 44 76 82 71

21
22 * These authors contributed equally to this work

23
24 1. EA3963, Université Paris 7 Denis Diderot, centre Hayem, Hôpital Saint-Louis, 1 avenue
25 Claude Vellefaux 75010 PARIS France
26 2. Centre de Recherche en Transplantation et Immunologie UMR 1064, INSERM, Université
27 de Nantes, Nantes, France
28 3. Institut de Transplantation Urologie Néphrologie (ITUN), CHU Nantes, Nantes, France
29 4. Laboratoire Central d'Immunologie Cellulaire et Tissulaire, Hôpital Pitié Salpêtrière et
30 INSERM UMR-S945, Bâtiment CERVI, Paris, France
31 5. Département d'Immunologie Clinique, Hôpital Saint-Louis, AP-HP, 1 avenue Claude
32 Vellefaux 75010 PARIS France
33 6. Ecole Centrale de Nantes, Nantes, France

34
35 Word counts: abstract 199 words, text 2876 words.
36 4 tables, 3 figures, 1 supplemental figure
37 53 references

1 **Abstract**

2 The precise diagnosis of an immunodeficiency is sometimes difficult to assess, especially due
3 to the large spectrum of phenotypic variation reported among patients. Common variable
4 immunodeficiency disorders (CVID) do not have, for a large part, an identified genetic cause.
5 The identification of a causal genetic mutation is important to confirm, or in some cases correct,
6 the diagnosis. We screened >150 male patients with hypogammaglobulinemia for mutations in
7 three genes involved in pediatric X-linked primary immunoglobulin deficiency: *CD40LG*,
8 *SH2D1A* and *BTK*. The *SH2D1A* screening allowed to reclassify two individuals with an initial
9 CVID presentation as XLP after mutations identification. All these mutations were associated
10 with a lack of protein expression. In addition, 4 patients with a primary diagnosis of CVID and
11 one with a primary IgG subclass deficiency were requalified as XLA after identifying *BTK*
12 mutations. Interestingly, two out of these 5 patients carried a damaging coding *BTK* mutation
13 associated with a lower, but detectable, BTK expression in monocytes, suggesting that a
14 dysfunctional protein explains the disease phenotype in these patients. In conclusion, our results
15 advocate to include *SH2D1A* and *BTK* in newly developed targeted NGS genetic testing, to
16 contribute to providing the most appropriate medical treatment and genetic counselling.

17 **Keywords**

18 Immunodeficiency, BTK, SAP, CD40L, CVID

19

1 **Abbreviations**

2 CVID: Common variable immunodeficiency disorders

3 EBV: Epstein-Barr virus

4 HC: healthy controls

5 HIGM: X-linked hyperIgM syndrome

6 Ig: immunoglobulin

7 IgAD: IgA deficiency

8 MFI: mean fluorescent intensity

9 NGS: next generation sequencing

10 PE: Phycoerythrin

11 SAP: SLAM-associated protein

12 XLA: X-linked agammaglobulinemia

13 XLP: X-linked lymphoproliferative disease

14

1 **Introduction**

2 Common variable immunodeficiency disorders (CVID) are characterized by a defect in
3 immunoglobulin (Ig) production. Most patients present recurrent infections of the respiratory
4 and gastrointestinal tracts and, less frequently, lymphoid proliferation or autoimmune diseases
5 [1]. The prevalence of CVID has been estimated at 1/30,000 in the European population, and
6 this disorder is the second most frequent immunodeficiency of adulthood, after IgA deficiency
7 (IgAD). About 20% of patients have at least one relative affected by IgAD or CVID, suggesting
8 a genetic origin in such cases. Genetic abnormalities in 24 genes involving different immune
9 pathways have been demonstrated in less than 10% of CVID patients to date [2]. Some genetic
10 variants, such as the ones in *TNFRSF13B* [3], do not seem sufficient to cause CVID phenotype
11 while others, such as the ones in *CD19* [2,4], present a full penetrance toward Ig deficiency.

12 The clinical presentation of patients with CVID is wide and made by exclusion of other
13 possible causes [5], which can sometimes lead to a diagnosis reclassification after identification
14 of genetic mutations or occurrence of new biological evidence or symptoms. Few CVID
15 patients were reclassified to XLA (X-linked agammaglobulinemia) when a mutation in the *BTK*
16 gene was found (OMIM: *300300) [6–16]. XLA is characterized by a failure during B-cell
17 development [17,18], and is usually diagnosed before the age of 18 months, after the loss of
18 maternal antibody protection [19], most of patients present an absence of B-cells and
19 immunoglobulin. Similarly, occasional reports of mutations in *SH2D1A* were showed in CVID
20 patients [20–24]. Mutations in *SH2D1A*, encoding SAP (SLAM-associated protein; OMIM:
21 *300490), are found in patients with XLP (X-linked lymphoproliferative disease) [25,26],
22 which is characterized by an extreme susceptibility to Epstein-Barr virus (EBV) infections,
23 resulting in near-fatal infectious mononucleosis, acquired hypogammaglobulinemia, malignant
24 lymphoma or a combination of these conditions [27,28].

1 In order to identify disease-causing mutations and their potential to support a diagnosis,
2 we explored a large cohort of French hypogammaglobulinemic patients (see figure S1) showing
3 a wide range of primary diagnosis (Table 1). We therefore systematically screened this
4 population for mutations in the *SH2D1A* and *BTK* genes, as well as for mutations in *CD40LG*
5 (OMIM: *300386), a gene responsible for the hyperIgM syndrome (HIGM1) that presents
6 numerous similarities with CVID [29,30].

7

1 **Materials and methods**

2 Patients

3 Investigated patients came from the DEFI cohort, a French cohort of patients with
4 primary hypogammaglobulinemia [1,5]. All patients gave informed consent for DNA
5 sequencing, and the study was approved by the local institutional review board, in accordance
6 with the Helsinki declaration. Initial diagnoses are reported in table 1.

7 Genes

8 The *CD40LG* gene, located in Xq26.3, encodes for the CD40 ligand. After PCR
9 amplification of the five exons and their flanking intronic regions, the PCR products were
10 directly sequenced in 150 male patients. We identified 3 SNPs (rs11575982, rs3092923 and
11 rs148594123) with a frequency equivalent to the matching 1000 Genomes population
12 (<http://www.internationalgenome.org/1000-genomes-browsers/>) [31,32].

13 The *SH2D1A* gene is located in Xq25 and encodes for SAP. After PCR amplification of
14 the four exons and their flanking intronic regions, we directly sequenced the PCR products in
15 a total of 230 male subjects. We identified 2 SNPs (rs200198093 and rs72610640) with a
16 frequency equivalent to the matching 1000 Genomes population.

17 The *BTK* gene is located in Xq22.1. After PCR amplification of the 19 exons and their
18 flanking intronic regions, we directly sequenced the PCR products in a total of 181 male
19 patients from the DEFI cohort.

20 Molecular analysis

21 Genomic DNA was extracted from buffy coats. All coding exons and intronic flanking
22 regions of *CD40LG*, *SH2D1A* and *BTK* were amplified (primers available upon request) and
23 sequenced with the Big Dye Terminator kit (Applied Biosystems) on an ABI3730 DNA
24 sequencer.

1 Total RNA was extracted from PHA T-cell blast and EBV-transformed B-cells with
2 TRIzol (Invitrogen). The mRNA was reverse-transcribed with oligo-dT primers and
3 SuperScriptIII reverse transcriptase (Invitrogen), to obtain cDNA.

4 Cloning of the amplified cDNA

5 The PCR products obtained after RNA extraction and cDNA amplification were
6 inserted into the TOPO® TA cloning vector (Invitrogen) and sequenced.

7 Flow cytometry analysis of BTK expression

8 Intracellular BTK staining was performed on thawed PBMC with the Fix and Perm®
9 kit (Caltag), used according to the manufacturer's instructions. PBMC were first stained with
10 FITC-conjugated anti-CD14 (IgG2a; Immunotech), PerCP-Cyanine5.5-conjugated anti-CD45
11 (IgG1; BD Biosciences) and APC-conjugated anti-CD19 (IgG1; BD Biosciences) for 15
12 minutes at room temperature. The cells were fixed, washed and permeabilized and Fc receptors
13 were blocked by incubation with human serum for 15 minutes at room temperature. PBMC
14 were stained by incubation with Phycoerythrin (PE)-labeled anti-BTK antibody (IgG2a; BD
15 Biosciences) or with an irrelevant Ab (CD10, IgG2a; BD Biosciences) for 15 minutes at room
16 temperature. The stained cells were analyzed with a BD FACS Canto™ flow cytometer (BD
17 Biosciences). The results are expressed by the difference (Δ) of monocytes (CD45+CD14+)
18 mean fluorescence intensity (MFI) between anti-BTK and irrelevant Ab. Fifteen male healthy
19 controls (HC) presented a median Δ MFI of 107 [79-173].

20 Flow cytometry analysis of CD40L expression

21 PHA T-cell blasts were stained by incubation with PerCP-conjugated anti-CD3 (SK7,
22 BD Biosciences) and PE-conjugated anti-CD40L (555700, BD Biosciences) antibodies for 30

1 minutes at 4°C. The stained cells were analyzed in a FACSCalibur flow cytometer (BD
2 Biosciences).

3 SAP Western blotting

4 Lysates of $5 \cdot 10^6$ PHA T-cell blasts were separated by SDS-PAGE and immunoblotted
5 with a polyclonal rabbit anti-SAP antibody (2778, Cell Signaling Technology) and a polyclonal
6 goat anti-actin antibody (1616, Santa Cruz).

7

1 **Results**

2 *CD40LG* mutation screening

3 Four patients with a primary HIGM diagnosis were included in our cohort for genetic
4 screening. Of those, only 3 presented a mutation in *CD40LG*: 228_231delAAGA, 409+1G>A
5 and 410-2A>G (Figure 1A). These mutations were associated with an absence of CD40L cell
6 surface detection on activated T-cell (see table 2). The fourth patient with an HIGM diagnosis
7 may carry a mutation in another HIGM-causing gene (e.g. *AICDA*, *CD40*) [33,34] and as such
8 should be screened for it. We did not identify any *CD40LG* mutation in any other patient of the
9 cohort.

10 *SH2D1A* mutation screening

11 No patient included in our cohort were initially diagnosed with XLP. After identifying
12 a first patient initially diagnosed with CVID but carrying a *SH2D1A* mutation (patient S1) in
13 the first 150 patients, we expanded our *SH2D1A* screening to 80 additional individuals and
14 identified a second CVID patient with a *SH2D1A* mutation (patient S2, table 3 and figure 1B).

15 Patient S1 was mutated in intron 2: 201+3A>G. We identified and sequenced two
16 splicing mRNA variants in activated T-cells by RT-PCR: a first variant where exon 2 were
17 spliced out (cDNA fragment of 323bp) and a second variant where exon 2 and a portion of exon
18 3 were spliced out (cDNA fragment of 268bp). We did not detect SAP protein expression in
19 activated T-cells protein extracts using immunoblot technique (Figure 2). Patient S2 carried a
20 E67G coding mutation in exon 2 that was associated with an absence of SAP protein on the
21 immunoblot performed with activated T-cells protein extract (Figure 2).

22 The clinical presentation of of these 2 patients was as followed. Patient S1 first diagnosis was
23 pediatric CVID at eight years of age after detection of hypogammaglobulinemia during
24 bacterial pneumonia. He also suffered from severe infectious mononucleosis at six years of age.

1 The patient was treated with gammaglobulins, administered intramuscularly for the first 10
2 years, and then intravenously. Patient S1 had 4% circulating B-cells including 87% naive
3 (CD27-IgM+), 3% switched memory (CD27+IgM-) and 7% CD27+IgM+ B-cells (Table 3).
4 He presented a family history suggestive of X-linked disease, indeed, his older brother died
5 with a refractory digestive EBV-driven B large cell lymphoma in the course of an otherwise
6 unremarkable CVID, without previous severe EBV primo-infection. Patient S2 was diagnosed
7 with CVID at the age of 40 based on the detection of profound hypogammaglobulinemia after
8 severe bacterial pneumonia without familial history of X-linked disease. He denied suffering
9 severe EBV primary infection, despite chronic positive plasma circulating EBV viral load. The
10 patient had 4% circulating B-cells including 19% naive (CD27-IgM+), 57% switched memory
11 (CD27+IgM-) and 15% CD27+IgM+ B-cells (Table 3).

12 *BTK* mutation screening

13 In our cohort, 7% of patients presented with an initial diagnosis of XLA (Table 1). As
14 our genetic screening revealed, *BTK* mutations was found only in patients presenting a very
15 low B-cell count (<1%, see table 4), we included 31 additional hypogammaglobulinemic
16 patients with B-cell counts below 1%. We detected a *BTK* mutation in 17 out of 20 patients
17 with a primary XLA diagnosis (B1-B17, table 4, Figure 1C). Despite a clinical/immunological
18 phenotype consistent with the diagnosis of XLA, we could not confirm genetically the diagnosis
19 in 3 patients (B18-B20, table 4). These 3 patients may carry a mutation in another gene causing
20 agammaglobulinemia (e.g. *BLNK*, *PI3KR1*) [35,36] and as such should be screened for it.
21 Strikingly, we found a *BTK* mutation in 5 patients (B21-B25) with a primary diagnosis of CVID
22 or IgG subclass deficiency (Table 4, boxes in Figure 1C). Overall, we described 20 different
23 mutations in 22 patients, including 5 novel mutations while the others were previously reported
24 in the *BTK* database (<https://databases.lovd.nl/shared/genes/BTK>) [37].

1 We observed a phenotypic variability in patients carrying *BTK* mutations despite
2 showing all less than 1% B-cells; indeed, while a majority of patients presented their first
3 symptoms at early age in infancy some were described at later age (up to 18 years old for B22,
4 see table 4). In addition, of all the *BTK* mutated patients only 3 had an IgG level above 4g/L
5 and all with a primary diagnosis of CVID or IgG subclass deficiency (B21, B22 and B25; see
6 table 4). We hypothesized that this variability could be explained by the impact of each
7 mutation on the *BTK* protein function and expression. Considering the absence or very low
8 levels of B-cells in XLA patients, we carried out intracellular FACS assay to measure *BTK*
9 expression in monocytes. We measured the delta between *BTK* mean fluorescent intensity
10 (MFI) and the irrelevant antibody MFI (i.e. Δ MFI) in 15 healthy controls and observed a median
11 Δ MFI of 107 [79-173] (Figure 3A). We then obtained data for patients harboring *BTK* mutations
12 (17 different mutations out of 21, see table 4 and Figure 3). As expected, *BTK* protein was
13 undetectable in all 13 patients' monocytes with a primary diagnosis of XLA, as illustrated with
14 patient B14 on Figure 3A. When considering the 3 patients with a primary diagnosis of XLA
15 but without identified *BTK* mutation, we detected a lower Δ MFI in 2 patients (B18 and B19,
16 Figure 3B) and a Δ MFI in the normal range for the B20 patient (Figure 3B).

17 Finally, we measured *BTK* expression in 4 out of 5 patients carrying a *BTK* mutation
18 but without a primary diagnosis of XLA, as we could not measure *BTK* expression for patient
19 B24. However, this patient carried the same M630V mutation as for the XLA B5 patient, so we
20 can only extrapolate that he might also present an undetectable *BTK* expression (Table 4). *BTK*
21 expression was undetectable in patients B22 and B25 monocytes, who were primarily
22 diagnosed with CVID and IgG subclass deficiency, respectively (Table 4, Figure 3C). Patients
23 B21 and B23 showed a decreased but detectable *BTK* expression in monocytes (Figure 3C), in
24 normal range for B21 (Δ MFI=80) and just below for B23 (Δ MFI=69). R28H mutation is
25 described in multiple studies as responsible for the XLA phenotype (Table 4), moreover, it is

1 reported as pathogenic in the ClinVar database
2 (<https://www.ncbi.nlm.nih.gov/clinvar/variation/11348/>). These suggest strongly that R28H is
3 responsible of patient B21 phenotype. G164D coding mutation is predicted by the CADD
4 bioinformatics tool [38] to be damaging for the protein function (score: 29.1). This mutation is
5 not described in populations databases such as the 1000 Genomes project [31]. These data are
6 not sufficient to describe G164D as responsible of the disease, additional family testing would
7 permit to settle this down. Altogether, these results suggest an alternative pathophysiological
8 mechanism to the absence of detectable BTK protein: lower level of BTK may indicate a
9 dysfunction of the BTK signaling pathway that could consequently alter the development of B-
10 cells and promote hypogammaglobulinemia.

11

1 **Discussion**

2 We sequenced three genes (*CD40LG*, *SH2D1A*, *BTK*) located on the X chromosome in
3 male patients with primary humoral deficiencies from the French national DEFI cohort (Figure
4 S1). Deleterious mutations in these genes are responsible of profound defects in
5 immunoglobulin production, are usually diagnosed in children and associated with specific
6 phenotypes. We intentionally did not restrict patients' selection to any particular clinical or
7 biological phenotypes as we intended to identify mutations outside of a commonly recognized
8 clinical presentation. We demonstrated that the systematic screening of these genes is useful to
9 confirm and/or reclassify immune defects diagnosis in adults with humoral primary
10 immunodeficiency.

11 *CD40LG* genetic screening of 150 male patients did not reveal any mutation outside of
12 the classical clinical presentation of HIGM, indicating that the *CD40LG* mutations are
13 responsible for a very specific clinical and immunological phenotype that cannot be confused
14 with CVID or any other defects. One patient with a HIGM phenotype did not exhibit any
15 mutation in *CD40LG*; to further characterize this patient's disease, additional genetic analyses
16 should be performed for autosomal recessive HIGM genes such as *AICDA*, *CD40* or *UNG*
17 [33,34].

18 Here, we characterized 2 patients primarily diagnosed as CVID but presenting a
19 *SH2D1A* mutation with an absence of SAP detection by immunoblot. XLP main feature is an
20 extreme susceptibility to EBV infection, and one of these 2 patients presented a severe
21 infectious mononucleosis at six years of age. However, the second one claimed he did not suffer
22 any severe EBV primary infection during his childhood and had no familial history of X-linked
23 disease. This indicates that, despite the low percentage of diagnosed patients (2 out of 145 if
24 we consider only CVID), the *SH2D1A* mutation knowledge is important in all male patients
25 diagnosed with CVID, whether or not they have a medical or familial history consistent with

1 severe EBV infection. Indeed, the XLP diagnosis significantly modifies the therapeutic and
2 follow-up options of the patients.

3 The systematic sequencing of *BTK* in 181 male patients confirmed the suspected XLA
4 diagnosis in 17/20 patients. Moreover, we identified 5 patients with a primary diagnosis of
5 CVID or IgG subclass deficiency carrying a mutation in the *BTK* gene. This misdiagnosis could
6 have several explanations: persistence of low levels but detectable IgG, IgA or IgM; insufficient
7 genealogic investigation to detect familial X-linked disease; onset of disease at adulthood;
8 absence of serious pulmonary complications. It emphasizes that all male patients with a
9 diagnosis of primary humoral immunodeficiency and with very low levels of circulating B-cells
10 (<1%), regardless of age at diagnosis, residual Ig concentration or familial history, should be
11 screened for *BTK* defects, either by FACS detection in monocytes or by direct sequencing of
12 the coding region.

13 The 3 patients referred to us with a diagnosis of XLA and in whom we could not identify
14 *BTK* mutations are of great interest. B18 and B19 patients do display clinical features consistent
15 with XLA, including an absence of circulating B-cells. *BTK* expression in monocytes, despite
16 being detectable, was lower in these patients compared to controls. B20 also shows an absence
17 of circulating B-cells but with a normal level of *BTK* expression and a family history of
18 autosomal recessive disease. Notwithstanding no mutations were found in these individuals,
19 including after screening the 5' and 3'UTR regulatory regions; at this stage, we cannot exclude
20 the possibility that the weak or undetectable *BTK* expression may be the result of a mutation in
21 another *BTK* regulatory region not covered by the sequencing method we implemented.
22 Alternatively, other intracellular proteins in the B-cell lineage may be absent or defective in
23 these patients, accounting for the weak translation of *BTK* and the low level of *BTK* activity in
24 B-cell precursors. These patients may carry a mutation in genes responsible of autosomal
25 recessive agammaglobulinemia, and especially B20 considering his family history. Beyond

1 these previously identified genes, an agnostic screening using whole-exome or whole-genome
2 next-generation sequencing (NGS) might also reveal novel disease-causing genes.

3 With the rising of NGS technologies, the possibility of genetic screening has increased.
4 Our results advocate to include *SH2D1A* and *BTK* genes in newly developed targeted NGS
5 panel genetic testing for all primary immunodeficiency diagnosis including CVID or related
6 hypogammaglobulinemia. This is supported by the fact that such NGS panel are already in
7 place, they include *SH2D1A* and *BTK* but also other genes responsible of HIGM, XLP or XLA
8 diseases (e.g. *CD40*, *PIK3R1*, etc.). This will further help characterize monogenic forms of
9 CVID, which still count for less than 10% of patients [2], from polygenic forms of CVID. To
10 achieve deciphering the pathophysiology and help improve treatments of these complex forms
11 of CVID, and in addition with NGS, efforts should be combined and integrated from different
12 omics approaches such as epigenomics, proteomics and metabolomics [39,40].

13 In conclusion, our systematic genetic screening in a large cohort of adults with primary
14 humoral immunodeficiency highlights several key features of CVID and
15 hypogammaglobulinemia genetics: (1) There is a good correlation between *CD40LG* genotype
16 and phenotype; (2) a *SH2D1A* mutation can occur in a patient with atypical clinical features;
17 (3) *BTK* genotype do not always correlates with cellular phenotype (*BTK* expression in
18 monocytes), immunological phenotype (Ig concentrations at the time of immunodeficiency
19 diagnosis) and clinical phenotype. A knowledge of the genetic status of each patient is therefore
20 crucial for a firm diagnosis, as it leads to the most appropriate medical treatment and genetic
21 counselling.

22

1 **Acknowledgements**

2 Authors thank Jean-Claude Brouet and Jean-Christophe Bories for helpful discussions.

3 This work was supported by grants from Programme Hospitalier de Recherche Clinique 2005
4 (E.O., P.D.), GIS maladies rares (E.O.), ANR MRAR-06 (C.F.), Fondation pour la recherche
5 Médicale (CF). N.V. was supported by La Ligue Nationale Contre le Cancer. D.B. was
6 supported by the ARC.

7 Conflicts of interest: none

8

1 **References**

- 2 [1] E. Oksenhendler, L. Gérard, C. Fieschi, M. Malphettes, G. Mouillot, R. Jaussaud, J.
3 Viallard, M. Gardembas, L. Galicier, N. Schleinitz, F. Suarez, P. Soulas-Sprauel, E. Hachulla,
4 A. Jaccard, A. Gardeur, I. Théodorou, C. Rabian, P. Debré, DEFI Study Group, Infections in
5 252 Patients with Common Variable Immunodeficiency, *Clin. Infect. Dis.* 46 (2008) 1547–
6 1554. doi:10.1086/587669.
- 7 [2] D.J.A. Bogaert, M. Dullaers, B.N. Lambrecht, K.Y. Vermaelen, E. De Baere, F.
8 Haerynck, Genes associated with common variable immunodeficiency: one diagnosis to rule
9 them all?, *J. Med. Genet.* 53 (2016) 575–590. doi:10.1136/jmedgenet-2015-103690.
- 10 [3] U. Salzer, C. Bacchelli, S. Buckridge, Q. Pan-Hammarström, S. Jennings, V. Lougaris,
11 A. Bergbreiter, T. Hagen, J. Birmelin, A. Plebani, A.D.B. Webster, H.-H. Peter, D. Suez, H.
12 Chapel, A. McLean-Tooke, G.P. Spickett, S. Anover-Sombke, H.D. Ochs, S. Urschel, B.H.
13 Belohradsky, S. Ugrinovic, D.S. Kumararatne, T.C. Lawrence, A.M. Holm, J.L. Franco, I.
14 Schulze, P. Schneider, E.M. Gertz, A.A. Schäffer, L. Hammarström, A.J. Thrasher, H.B.
15 Gaspar, B. Grimbacher, Relevance of biallelic versus monoallelic TNFRSF13B mutations in
16 distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody
17 deficiency syndromes, *Blood.* 113 (2009) 1967–1976. doi:10.1182/blood-2008-02-141937.
- 18 [4] N. Vince, D. Boutboul, G. Mouillot, N. Just, M. Peralta, J.-L. Casanova, M.E. Conley,
19 J.-C. Bories, E. Oksenhendler, M. Malphettes, C. Fieschi, DEFI Study Group, Defects in the
20 CD19 complex predispose to glomerulonephritis, as well as IgG1 subclass deficiency, *J.*
21 *Allergy Clin. Immunol.* 127 (2011) 538-541.e1–5. doi:10.1016/j.jaci.2010.10.019.
- 22 [5] F.A. Bonilla, I. Barlan, H. Chapel, B.T. Costa-Carvalho, C. Cunningham-Rundles,
23 M.T. de la Morena, F.J. Espinosa-Rosales, L. Hammarström, S. Nonoyama, I. Quinti, J.M.
24 Routes, M.L.K. Tang, K. Warnatz, International Consensus Document (ICON): Common
25 Variable Immunodeficiency Disorders, *J. Allergy Clin. Immunol. Pract.* 4 (2016) 38–59.

1 doi:10.1016/j.jaip.2015.07.025.

2 [6] H.B. Gaspar, M. Ferrando, I. Caragol, M. Hernandez, J.M. Bertran, X. De Gracia, T.
3 Lester, C. Kinnon, E. Ashton, T. Espanol, Kinase mutant Btk results in atypical X-linked
4 agammaglobulinaemia phenotype, *Clin Exp Immunol.* 120 (2000) 346–50.

5 [7] S. Hashimoto, T. Miyawaki, T. Futatani, H. Kanegane, K. Usui, T. Nukiwa, S.
6 Namiuchi, M. Matsushita, T. Yamadori, M. Suemura, T. Kishimoto, S. Tsukada, Atypical X-
7 linked agammaglobulinemia diagnosed in three adults, *Intern Med.* 38 (1999) 722–5.

8 [8] A. Jones, L. Bradley, L. Alterman, M. Tarlow, R. Thompson, C. Kinnon, G. Morgan,
9 X linked agammaglobulinaemia with a “leaky” phenotype, *Arch Child.* 74 (1996) 548–9.

10 [9] H. Kanegane, S. Tsukada, T. Iwata, T. Futatani, K. Nomura, J. Yamamoto, T.
11 Yoshida, K. Agematsu, A. Komiyama, T. Miyawaki, Detection of Bruton’s tyrosine kinase
12 mutations in hypogammaglobulinaemic males registered as common variable
13 immunodeficiency (CVID) in the Japanese Immunodeficiency Registry, *Clin Exp Immunol.*
14 120 (2000) 512–7.

15 [10] S.J. Kornfeld, R.N. Haire, S.J. Strong, H. Tang, S.S. Sung, S.M. Fu, G.W. Litman, A
16 novel mutation (Cys145-->Stop) in Bruton’s tyrosine kinase is associated with newly
17 diagnosed X-linked agammaglobulinemia in a 51-year-old male, *Mol Med.* 2 (1996) 619–23.

18 [11] D.M. Stewart, L. Tian, D.L. Nelson, A case of X-linked agammaglobulinemia
19 diagnosed in adulthood, *Clin Immunol.* 99 (2001) 94–9.

20 [12] M.E. Conley, D.M. Farmer, A.K. Dobbs, V. Howard, Y. Aiba, S.A. Shurtleff, T.
21 Kurosaki, A minimally hypomorphic mutation in Btk resulting in reduced B cell numbers but
22 no clinical disease, *Clin Exp Immunol.* 152 (2008) 39–44.

23 [13] K. Morwood, H. Bourne, M. Gold, D. Gillis, E.M. Benson, Phenotypic variability:
24 clinical presentation between the 6th year and the 60th year in a family with X-linked
25 agammaglobulinemia, *J Allergy Clin Immunol.* 113 (2004) 783–5.

- 1 [14] J.G. Noordzij, S. de Bruin-Versteeg, N.G. Hartwig, C.M. Weemaes, E.J. Gerritsen, E.
2 Bernatowska, S. van Lierde, R. de Groot, J.J. van Dongen, XLA patients with BTK splice-site
3 mutations produce low levels of wild-type BTK transcripts, *J Clin Immunol.* 22 (2002) 306–
4 18.
- 5 [15] D.C. Saffran, O. Parolini, M.E. Fitch-Hilgenberg, D.J. Rawlings, D.E. Afar, O.N.
6 Witte, M.E. Conley, Brief report: a point mutation in the SH2 domain of Bruton's tyrosine
7 kinase in atypical X-linked agammaglobulinemia, *N Engl J Med.* 330 (1994) 1488–91.
- 8 [16] J.R. Sigmon, E. Kasasbeh, G. Krishnaswamy, X-linked agammaglobulinemia
9 diagnosed late in life: case report and review of the literature, *Clin Mol Allergy.* 6 (2008) 5.
- 10 [17] S. Tsukada, D.C. Saffran, D.J. Rawlings, O. Parolini, R.C. Allen, I. Klisak, R.S.
11 Sparkes, H. Kubagawa, T. Mohandas, S. Quan, et al., Deficient expression of a B cell
12 cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia, *Cell.* 72 (1993) 279–
13 90.
- 14 [18] D. Vetrie, I. Vorechovsky, P. Sideras, J. Holland, A. Davies, F. Flinter, L.
15 Hammarstrom, C. Kinnon, R. Levinsky, M. Bobrow, et al., The gene involved in X-linked
16 agammaglobulinaemia is a member of the src family of protein-tyrosine kinases, *Nature.* 361
17 (1993) 226–33.
- 18 [19] J.A. Winkelstein, M.C. Marino, H.M. Lederman, S.M. Jones, K. Sullivan, A.W.
19 Burks, M.E. Conley, C. Cunningham-Rundles, H.D. Ochs, X-linked agammaglobulinemia:
20 report on a United States registry of 201 patients, *Med. Baltim.* 85 (2006) 193–202.
- 21 [20] A. Aghamohammadi, H. Kanegane, M. Moein, A. Farhoudi, Z. Pourpak, M.
22 Movahedi, M. Gharagozlou, A.A. Zargar, T. Miyawaki, Identification of an SH2D1A
23 mutation in a hypogammaglobulinemic male patient with a diagnosis of common variable
24 immunodeficiency, *Int J Hematol.* 78 (2003) 45–7.
- 25 [21] D. Eastwood, K.C. Gilmour, K. Nistala, C. Meaney, H. Chapel, Z. Sherrell, A.D.

1 Webster, E.G. Davies, A. Jones, H.B. Gaspar, Prevalence of SAP gene defects in male
2 patients diagnosed with common variable immunodeficiency, *Clin Exp Immunol.* 137 (2004)
3 584–8.

4 [22] M. Morra, O. Silander, S. Calpe, M. Choi, H. Oettgen, L. Myers, A. Etzioni, R.
5 Buckley, C. Terhorst, Alterations of the X-linked lymphoproliferative disease gene SH2D1A
6 in common variable immunodeficiency syndrome, *Blood.* 98 (2001) 1321–5.

7 [23] K. Nistala, K.C. Gilmour, T. Cranston, E.G. Davies, D. Goldblatt, H.B. Gaspar, A.M.
8 Jones, X-linked lymphoproliferative disease: three atypical cases, *Clin Exp Immunol.* 126
9 (2001) 126–30.

10 [24] A. Soresina, V. Lougaris, S. Giliani, F. Cardinale, L. Armenio, M. Cattalini, L.D.
11 Notarangelo, A. Plebani, Mutations of the X-linked lymphoproliferative disease gene
12 SH2D1A mimicking common variable immunodeficiency, *Eur J Pediatr.* 161 (2002) 656–9.

13 [25] A.J. Coffey, R.A. Brooksbank, O. Brandau, T. Oohashi, G.R. Howell, J.M. Bye, A.P.
14 Cahn, J. Durham, P. Heath, P. Wray, R. Pavitt, J. Wilkinson, M. Leversha, E. Huckle, C.J.
15 Shaw-Smith, A. Dunham, S. Rhodes, V. Schuster, G. Porta, L. Yin, P. Serafini, B. Sylla, M.
16 Zollo, B. Franco, A. Bolino, M. Seri, A. Lanyi, J.R. Davis, D. Webster, A. Harris, G. Lenoir,
17 G. de St Basile, A. Jones, B.H. Behloradsky, H. Achatz, J. Murken, R. Fassler, J. Sumegi, G.
18 Romeo, M. Vaudin, M.T. Ross, A. Meindl, D.R. Bentley, Host response to EBV infection in
19 X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding
20 gene, *Nat Genet.* 20 (1998) 129–35.

21 [26] J. Sayos, C. Wu, M. Morra, N. Wang, X. Zhang, D. Allen, S. van Schaik, L.
22 Notarangelo, R. Geha, M.G. Roncarolo, H. Oettgen, J.E. De Vries, G. Aversa, C. Terhorst,
23 The X-linked lymphoproliferative-disease gene product SAP regulates signals induced
24 through the co-receptor SLAM, *Nature.* 395 (1998) 462–9.

25 [27] D.T. Purtilo, D. DeFlorio Jr., L.M. Hutt, J. Bhawan, J.P. Yang, R. Otto, W. Edwards,

1 Variable phenotypic expression of an X-linked recessive lymphoproliferative syndrome, *N*
2 *Engl J Med.* 297 (1977) 1077–80.

3 [28] T.A. Seemayer, T.G. Gross, R.M. Egeler, S.J. Pirruccello, J.R. Davis, C.M. Kelly, M.
4 Okano, A. Lanyi, J. Sumegi, X-linked lymphoproliferative disease: twenty-five years after the
5 discovery, *Pediatr Res.* 38 (1995) 471–8.

6 [29] A. Etzioni, H.D. Ochs, The hyper IgM syndrome--an evolving story, *Pediatr Res.* 56
7 (2004) 519–25.

8 [30] R.C. Allen, R.J. Armitage, M.E. Conley, H. Rosenblatt, N.A. Jenkins, N.G. Copeland,
9 M.A. Bedell, S. Edelhoff, C.M. Disteché, D.K. Simoneaux, et al., CD40 ligand gene defects
10 responsible for X-linked hyper-IgM syndrome, *Science.* 259 (1993) 990–3.

11 [31] 1000 Genomes Project Consortium, A. Auton, L.D. Brooks, R.M. Durbin, E.P.
12 Garrison, H.M. Kang, J.O. Korbel, J.L. Marchini, S. McCarthy, G.A. McVean, G.R.
13 Abecasis, A global reference for human genetic variation, *Nature.* 526 (2015) 68–74.
14 doi:10.1038/nature15393.

15 [32] S. Limou, A.M. Taverner, C.A. Winkler, Ferret: a user-friendly Java tool to extract
16 data from the 1000 Genomes Project, *Bioinformatics.* 32 (2016) 2224–2226.
17 doi:10.1093/bioinformatics/btw147.

18 [33] K. Imai, G. Slupphaug, W.I. Lee, P. Revy, S. Nonoyama, N. Catalan, L. Yel, M.
19 Forveille, B. Kavli, H.E. Krokan, H.D. Ochs, A. Fischer, A. Durandy, Human uracil-DNA
20 glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch
21 recombination, *Nat Immunol.* 4 (2003) 1023–8.

22 [34] P. Revy, T. Muto, Y. Levy, F. Geissmann, A. Plebani, O. Sanal, N. Catalan, M.
23 Forveille, R. Dufourcq-Labelouse, A. Gennery, I. Tezcan, F. Ersoy, H. Kayserili, A.G.
24 Ugazio, N. Brousse, M. Muramatsu, L.D. Notarangelo, K. Kinoshita, T. Honjo, A. Fischer, A.
25 Durandy, Activation-induced cytidine deaminase (AID) deficiency causes the autosomal

1 recessive form of the Hyper-IgM syndrome (HIGM2), *Cell*. 102 (2000) 565–75.

2 [35] Y. Minegishi, J. Rohrer, E. Coustan-Smith, H.M. Lederman, R. Pappu, D. Campana,
3 A.C. Chan, M.E. Conley, An essential role for BLNK in human B cell development, *Science*.
4 286 (1999) 1954–1957.

5 [36] M.E. Conley, A.K. Dobbs, A.M. Quintana, A. Bosompem, Y.-D. Wang, E. Coustan-
6 Smith, A.M. Smith, E.E. Perez, P.J. Murray, Agammaglobulinemia and absent B lineage cells
7 in a patient lacking the p85 α subunit of PI3K, *J. Exp. Med.* 209 (2012) 463–470.
8 doi:10.1084/jem.20112533.

9 [37] J. Valiaho, C.I. Smith, M. Vihinen, BTKbase: the mutation database for X-linked
10 agammaglobulinemia, *Hum Mutat.* 27 (2006) 1209–17.

11 [38] M. Kircher, D.M. Witten, P. Jain, B.J. O’Roak, G.M. Cooper, J. Shendure, A general
12 framework for estimating the relative pathogenicity of human genetic variants, *Nat. Genet.* 46
13 (2014) 310–315. doi:10.1038/ng.2892.

14 [39] R. Ameratunga, K. Lehnert, S.-T. Woon, D. Gillis, V.L. Bryant, C.A. Slade, R. Steele,
15 Review: Diagnosing Common Variable Immunodeficiency Disorder in the Era of Genome
16 Sequencing, *Clin. Rev. Allergy Immunol.* (2017). doi:10.1007/s12016-017-8645-0.

17 [40] A.-K. Kienzler, C.E. Hargreaves, S.Y. Patel, The role of genomics in common
18 variable immunodeficiency disorders, *Clin. Exp. Immunol.* 188 (2017) 326–332.
19 doi:10.1111/cei.12947.

20 [41] J.P. DiSanto, J.Y. Bonnefoy, J.F. Gauchat, A. Fischer, G. de Saint Basile, CD40
21 ligand mutations in x-linked immunodeficiency with hyper-IgM, *Nature*. 361 (1993) 541–3.

22 [42] Y. Tabata, J. Villanueva, S.M. Lee, K. Zhang, H. Kanegane, T. Miyawaki, J. Sumegi,
23 A.H. Filipovich, Rapid detection of intracellular SH2D1A protein in cytotoxic lymphocytes
24 from patients with X-linked lymphoproliferative disease and their family members, *Blood*.
25 105 (2005) 3066–71.

- 1 [43] B. Pasquier, L. Yin, M.C. Fondaneche, F. Relouzat, C. Bloch-Queyrat, N. Lambert, A.
2 Fischer, G. de Saint-Basile, S. Latour, Defective NKT cell development in mice and humans
3 lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product, *J Exp*
4 *Med.* 201 (2005) 695–701.
- 5 [44] M.E. Conley, D. Mathias, J. Treadaway, Y. Minegishi, J. Rohrer, Mutations in *btk* in
6 patients with presumed X-linked agammaglobulinemia, *Am J Hum Genet.* 62 (1998) 1034–
7 43.
- 8 [45] E. Holinski-Feder, M. Weiss, O. Brandau, K.B. Jedele, B. Nore, C.M. Bäckesjö, M.
9 Vihinen, S.R. Hubbard, B.H. Belohradsky, C.I. Smith, A. Meindl, Mutation screening of the
10 *BTK* gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations
11 without correlation to clinical course, *Pediatrics.* 101 (1998) 276–284.
- 12 [46] J. Väliäho, I. Faisal, C. Ortutay, C.I.E. Smith, M. Vihinen, Characterization of all
13 possible single-nucleotide change caused amino acid substitutions in the kinase domain of
14 Bruton tyrosine kinase, *Hum. Mutat.* 36 (2015) 638–647. doi:10.1002/humu.22791.
- 15 [47] I. Vorechovsky, M. Vihinen, G. de Saint Basile, S. Honsova, L. Hammarstrom, S.
16 Muller, L. Nilsson, A. Fischer, C.I. Smith, DNA-based mutation analysis of Bruton’s tyrosine
17 kinase gene in patients with X-linked agammaglobulinaemia, *Hum Mol Genet.* 4 (1995) 51–8.
- 18 [48] H.B. Gaspar, L.A. Bradley, F. Katz, R.C. Lovering, C.M. Roifman, G. Morgan, R.J.
19 Levinsky, C. Kinnon, Mutation analysis in Bruton’s tyrosine kinase, the X-linked
20 agammaglobulinaemia gene, including identification of an insertional hotspot, *Hum Mol*
21 *Genet.* 4 (1995) 755–7.
- 22 [49] R.A. Brooimans, A.J. van den Berg, G.T. Rijkers, L.A. Sanders, J.K. van Amstel,
23 M.G. Tilanus, M.J. Grubben, B.J. Zegers, Identification of novel Bruton’s tyrosine kinase
24 mutations in 10 unrelated subjects with X linked agammaglobulinaemia, *J. Med. Genet.* 34
25 (1997) 484–488.

- 1 [50] Y. Ohta, R.N. Haire, R.T. Litman, S.M. Fu, R.P. Nelson, J. Kratz, S.J. Kornfeld, M. de
2 la Morena, R.A. Good, G.W. Litman, Genomic organization and structure of Bruton
3 agammaglobulinemia tyrosine kinase: localization of mutations associated with varied clinical
4 presentations and course in X chromosome-linked agammaglobulinemia, Proc. Natl. Acad.
5 Sci. U. S. A. 91 (1994) 9062–9066.
- 6 [51] B. Duriez, P. Duquesnoy, F. Dastot, P. Bougneres, S. Amselem, M. Goossens, An
7 exon-skipping mutation in the btk gene of a patient with X-linked agammaglobulinemia and
8 isolated growth hormone deficiency, FEBS Lett. 346 (1994) 165–70.
- 9 [52] M. de Weers, R.G. Mensink, M.E. Kraakman, R.K. Schuurman, R.W. Hendriks,
10 Mutation analysis of the Bruton's tyrosine kinase gene in X-linked agammaglobulinemia:
11 identification of a mutation which affects the same codon as is altered in immunodeficient xid
12 mice, Hum Mol Genet. 3 (1994) 161–6.
- 13 [53] M. Velickovic, M.L. Prasad, S.A. Weston, E.M. Benson, Identification of the bruton
14 tyrosine kinase (BTK) gene mutations in 20 Australian families with X-linked
15 agammaglobulinemia (XLA), Hum Mutat. 23 (2004) 398–9.
- 16

1 **Tables**

2 **Table 1:** Diagnosis provided by the clinical investigators before the genetic screening of *CD40LG*, *SH2D1A* and *BTK*.

	<i>CD40LG</i> <i>n=150</i>	<i>SH2D1A</i> <i>n=230</i>	<i>BTK</i> <i>n=181</i>
<i>HIGM</i>	4	4	4
<i>XLP</i>	0	0	0
<i>XLA</i>	11	14	20
<i>CVID</i>	109	145	120
<i>IgAD</i>	0	3	0
<i>IgG-SCl</i>	9	15	13
<i>Good syndrome</i>	4	9	8
<i>Other</i>	13	40	16

3

4 *HIGM*: X-linked hyperIgM syndrome. *XLP*: X-linked lymphoproliferative disease. *XLA*: X-linked agammaglobulinemia. *CVID*: Common
5 variable immunodeficiency disorders. *IgAD*: IgA deficiency. *IgG-SCl*: IgG subclass deficiency.

6

1 **Table 2:** *CD40LG* mutations and clinical features of the patients

2

3

<i>Patient ID</i>	<i>Age at first symptoms (years)</i>	<i>B-cells (%)</i>	<i>IgG (g/L)</i>	<i>IgA (g/L)</i>	<i>IgM (g/L)</i>	<i>Family history</i>	<i>Opportunistic infections</i>	<i>Initial diagnosis</i>	<i>Mutation</i>	<i>Previously described</i>	<i>FACS expression</i>
<i>C1</i>	4	9	1.3	0	7.5	no	yes	HIGM	228-231delAA GA	no	none
<i>C2</i>	0	7	3.3	0.4	1.2	no	yes	HIGM	409+1G> A	yes [41]	none
<i>C3</i>	1	7	0.1	0	0.9	no	yes	HIGM	410-2A>G	no	none

1 **Table 3:** *SH2D1A* mutations and clinical features of the patients

2

<i>Patient ID</i>	<i>Age at first symptoms (years)</i>	<i>B-cells (%)</i>	<i>IgG (g/L)</i>	<i>IgA (g/L)</i>	<i>IgM (g/L)</i>	<i>Family history</i>	<i>Initial diagnosis</i>	<i>Mutation</i>	<i>Previously described</i>	<i>WB expression</i>
<i>S1</i>	3	4	1.8	0.4	0.4	yes	CVID	201+3A>G	yes [42]	none
<i>S2</i>	6	4	1.2	0	0.2	no	CVID	E67G	yes [43]	none

3 See figure 2 for Western blot.

4

1 **Table 4:** *BTK* mutations and clinical features of the patients

<i>Patient ID</i>	<i>Age at first symptoms (years)</i>	<i>B-cells (%)</i>	<i>IgG (g/L)</i>	<i>IgA (g/L)</i>	<i>IgM (g/L)</i>	<i>Family history</i>	<i>Bronchiectasis</i>	<i>Initial diagnosis</i>	<i>Mutation</i>	<i>Previously described *</i>	<i>FACS expression **</i>
<i>B1</i>	0	0	0.5	0.3	0.6	yes	yes	XLA	T117P	yes [44,45]	ND
<i>B2</i>	1	0	NA	0.1	0.1	no	no	XLA	R332L,H333L	no	none
<i>B3</i>	0	0	0	0	0	no	no	XLA	C502R	yes [46]	none
<i>B4</i>	3	0.1	0	0	0	no	no	XLA	K577N	yes [46]	none
<i>B5</i>	1	0	ND	0	0	yes	no	XLA	M630V	yes [46]	none
<i>B6</i>	0	0	1.2	0	0	yes	yes	XLA	F644L	yes (unpublished***)	ND
<i>B7</i>	0	0	0.7	0	0.4	yes	yes	XLA	R525X	yes [47]	ND
<i>B8</i>	2	0	2.0	0	0.4	no	yes	XLA	100delG	no	none
<i>B9</i>	0	0	0.8	0.2	0.3	yes	yes	XLA	1953delT	yes [47]	none

<i>B10</i>	4	0	ND	0.1	0	no	yes	XLA	g.2275_13114del10838insCC C	no	none
<i>B11</i>	4	0	ND	0	0.1	yes	no	XLA	174_175in sT	yes [45]	none
<i>B12</i>	1	0	3.7	0.1	0.1	no	yes	XLA	215_216in sA	yes [48]	ND
<i>B13</i>	1	0	1.5	0	0	yes	no	XLA	1349+5G >A	yes [49]	none
<i>B14</i>	0	0	ND	0	0	yes	no	XLA	1566+1G >T	yes [45]	none
<i>B15</i>	0	0	0.8	0	0	no	no	XLA	1567- 12delTTT G	yes [50]	none
<i>B16</i>	0	0	0	0	0	yes	yes	XLA	1750+5G >A	yes [51]	none
<i>B17</i>	0	0.1	0	0	0.2	no	yes	XLA	1750+5G >A	yes [51]	none
<i>B18</i>	22	0	0	0	0.1	yes (XL)	yes	XLA	No mutation	-	low
<i>B19</i>	14	0.4	0	0	0.1	yes	no	XLA	No mutation	-	low

<i>B20</i>	0	0	1	0	0	yes (AR)	yes	XLA	No mutation	-	yes
<i>B21</i>	5	0.3	4.4	1.6	0.4	no	no	CVID	R28H	yes [50,52]	yes
<i>B22</i>	18	0.1	4.4	1.8	0.1	no	no	CVID	F114V	no	none
<i>B23</i>	15	0.1	2.1	0	0.3	no	yes	CVID	G164D	no	low
<i>B24</i>	0	0	0	0	0	yes	yes	CVID	M630V	yes [46]	ND
<i>B25</i>	0	0.1	4.2	1.2	0.4	no	yes	IgG s/class	1350-2A>G	yes [53]	none

1

2 *Only the oldest description is cited, see the btkbase for a complete list: <https://databases.lovd.nl/shared/genes/BTK>.

3 **See figure 3 for FACS expression.

4 ***See in btkbase.

5 Patients B1 to B17 had clinical and biological features consistent with XLA and an identified BTK mutation. Patients B16 and B17, as well as,
6 B5 and B24 do not belong to the same family. Patients B18 to B20 had an initial diagnosis of XLA based on phenotypic data, no mutation in
7 *BTK* were found. Newly described mutations are in bold. CVID or IgG subclass deficiency patients' mutations are boxed. Patients B21 to B25
8 had a primary diagnosis of humoral deficiency, 4 CVID and 1 IgG subclass deficiency. XL: X-linked; AR: autosomic recessive.

9

1 **Figure Legends**

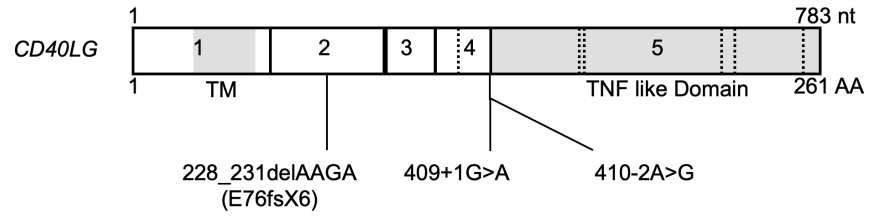
2 **Figure 1:** (A) Schematic diagram of the *CD40LG* coding region, with 5 coding exons, 783
3 nucleotides (nt) and 261 amino acids (AA). The 3 patients' mutations are represented. TM:
4 transmembrane domain. Dotted lines are the trimer interface. (B) Schematic diagram of the
5 coding region of *SH2D1A*, with 4 exons, 387 nt and 128 AA. SH2: Src homology 2 domain.
6 The 2 patients' mutations are represented. (C) Schematic diagram of the coding region of *BTK*,
7 with 18 coding exons, 1980 nt and 659 AA. Newly described mutations are in bold. CVID or
8 IgG subclass deficiency patients' mutations are boxed. PH: pleckstrin homology. ZF: zinc
9 finger. SH3: Src homology 3 domain. SH2: Src homology 2 domain. TK: tyrosine kinase.

10 **Figure 2:** Immunoblot of SAP in PHA T-cell blasts protein extraction. Actin is represented as
11 a control of protein expression. HC: healthy control.

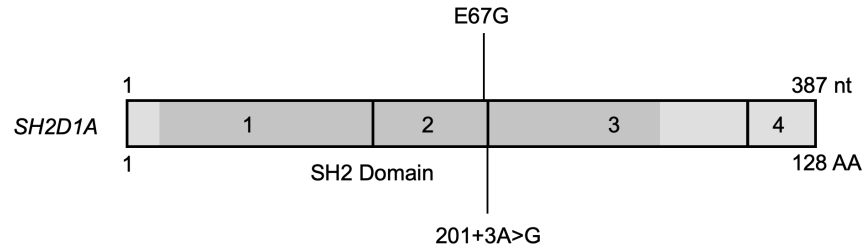
12 **Figure 3:** BTK expression in gated monocytes (CD45+, CD14+). (A) healthy control (HC) and
13 a representative XLA patient (B14, absence of BTK detection). We measured the delta between
14 BTK mean fluorescent intensity (MFI) and the irrelevant antibody MFI (i.e. Δ MFI), the 15 HC
15 showed a median of Δ MFI = 107 [79-173]. (B) Patients with XLA as primary diagnosis but
16 without identified *BTK* mutations (WT: wild type). B18 and B19 patients showed reduced BTK
17 expression but not B20. (C) Patients with CVID or IgG subclass deficiency as primary diagnosis
18 but with *BTK* mutations. B21 and B23 patients showed reduced BTK expression; B20 show no
19 BTK expression. Ab: Antibody.

20

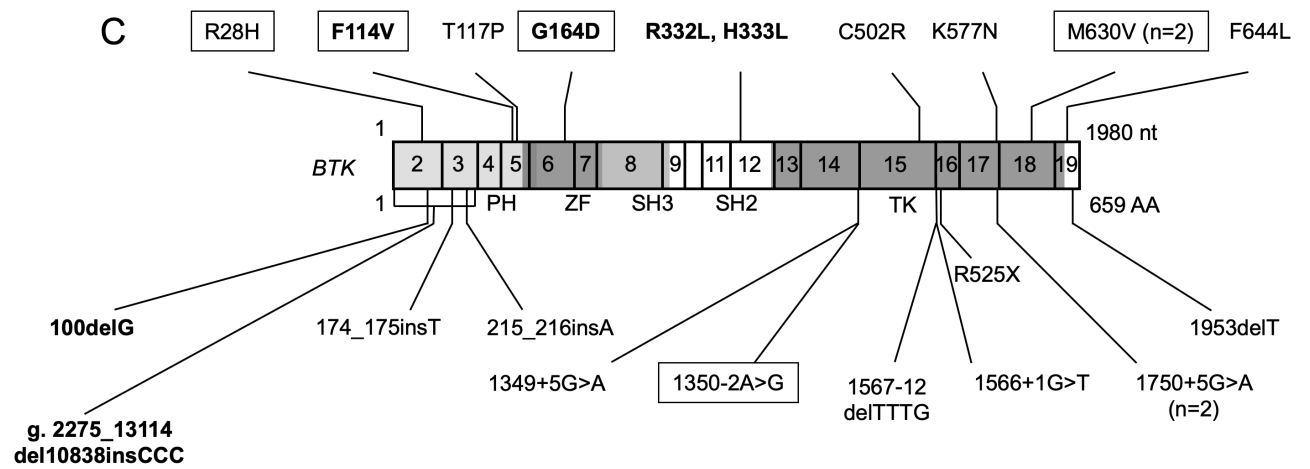
Fig1 A



B



C



1

2 Figure 1

3

4

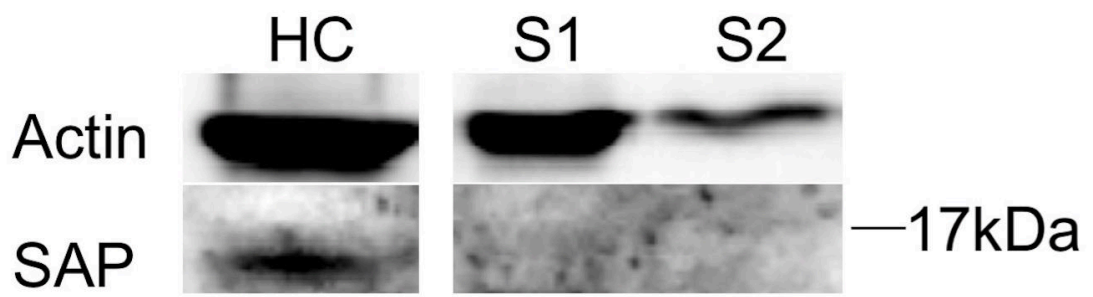
5

6

7

8

Fig2



1

2 Figure 2

3

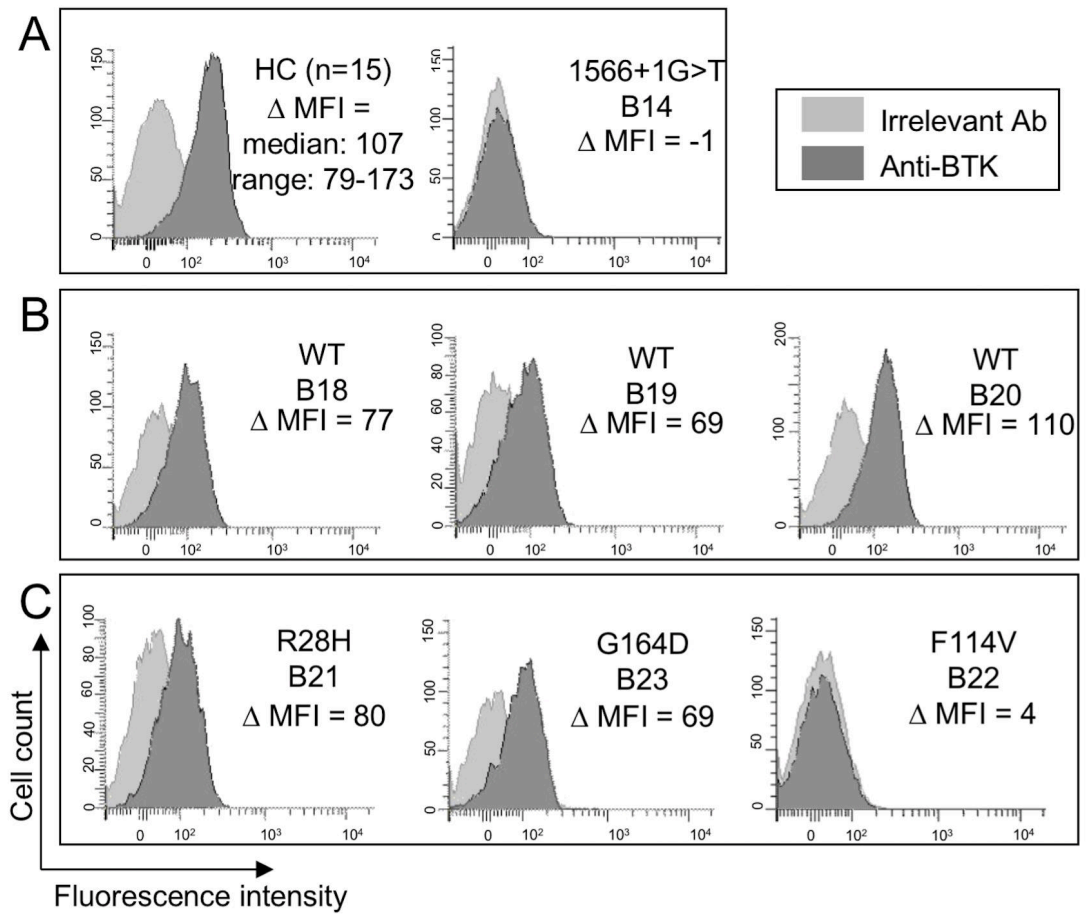
4

5

6

7

Fig3



1

2 Figure 3

Table S1: Diagnosis provided by the clinical investigators before the genetic screening of *CD40LG*, *SH2D1A* and *BTK*.

	<i>CD40LG</i> <i>n=150</i>	<i>SH2D1A</i> <i>n=230</i>	<i>BTK</i> <i>n=181</i>
<i>HIGM</i>	4	4	4
<i>XLP</i>	0	0	0
<i>XLA</i>	11	14	20
<i>CVID</i>	109	145	120
<i>IgAD</i>	0	3	0
<i>IgG-SCI</i>	9	15	13
<i>Thymoma</i>	4	9	8
<i>Other</i>	13	40	16

HIGM: X-linked hyperIgM syndrome. XLP: X-linked lymphoproliferative disease. XLA: X-linked agammaglobulinemia. CVID: Common variable immunodeficiency disorders. IgAD: IgA deficiency. IgG-SCI: IgG subclass deficiency.

FigS1

