

CORRESPONDENCE

Misdiagnosed CD19 deficiency leads to severe lung disease

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To the Editor,

We describe a new case of very rare B-cell receptor (BCR) deficiency caused by a homozygous nonsense mutation of the *CD19* gene in a 11-yr-old boy suffering from severe chronic lung disease and discuss the CD19 deficiency (CVID3, MIM#613493) from the clinical and immunologic point of view. To our knowledge, only nine patients from six unrelated families with deleterious mutations in *CD19* have been described to date worldwide (1–5). In addition, this is the first European (French) patient with *CD19* deficiency who is reported in the literature.

A 7-yr-old boy was referred to the Clinical Immunology Department of Pediatrics at the Bordeaux University Hospital for chronic obstructive pulmonary disease, recurrent respiratory tract infections as well as persistent hypogammaglobulinemia. He was born after normal pregnancy and delivery at 36 wk of gestational age in a closed community where consanguineous marriages are frequent, but without known history of inbreeding. He is the first child of the family and has two younger siblings and three half-siblings (Fig. 1a). His family history was non-remarkable. From the age of 3 months, after a *Respiratory Syncytial Virus* bronchiolitis, he developed chronic wheezing, which was associated with chronic cough and recurrent infections of the upper and lower respiratory tract. More precisely, he suffered at least three new episodes of bronchitis or bronchopneumonia per year mainly due to *Haemophilus influenza* and *Streptococcus pneumoniae*. In addition, several episodes of otitis media were reported annually. At the age of 4 yr, chest X-rays already demonstrated a partial atelectasis of the left lower lobe, with thickening of the bronchial walls. The investigation for gastroesophageal reflux disease was negative. Spirometry indicated a bronchodilator-responsive obstructive pattern. Increased total IgE titer without eosinophilia was suggestive of atopy. The symptoms were attributed to unfavorable familial and living environment (smoking, household moisture and dust), although serum-specific IgE tests for common aeroallergens and skin prick assays were negative. Hypogammaglobulinemia of IgG (IgG 3.2 g/l) and IgM (0.27 g/l) was noticed for the first time when he was 5 yr old. However, it was attributed to his young age, and further immunologic investigation was not carried out. Until the age of 7, he was diagnosed to be suffering from severe childhood asthma and treated with physiotherapy, bronchodilators and antibiotics on demand.

At the admission, CT scan of the thorax confirmed a persistent incomplete atelectasis and revealed extensive bronchiectasis of the left lower lobe (Fig. 1b). Measurements of serum immunoglobulins showed a decreased IgG titer (4.4 g/l) with a selective defect in IgG1 subclass (IgG1: 1.7 g/l, IgG2: 3.4 g/l, IgG3: 0.4 g/l, IgG4: 0.002 g/l), a decreased IgM titer (0.4 g/l) and normal IgA levels. The post-vaccination serology

(correct immunization for diphtheria, tetanus, *Streptococcus pneumoniae*) and the *Varicella-Zoster* virus serology (history of previous chickenpox infection) were negative. In addition, anti-B isohemagglutinin was undetectable in group A patient's blood. Lymphocyte phenotyping by flow cytometry revealed a virtual lack of CD19+ B lymphocytes (0.06% of total blood lymphocytes), 73% CD3+ T lymphocytes and 16% CD16+/CD56+ natural killer (NK) cells. T lymphocyte proliferation assays following lectin and specific stimulations were normal. Antinuclear antibodies (ANA) were negative. Genetic analysis did not show any mutation neither in the *BTK* gene nor in the coding sequences of *BAFF-R*, *ICOS*, *TACI*, *IGLL1*, *IGHM*, *BLNK*, *XIAP* and *DCLRE1C* genes. The diagnosis of B-cell lymphocytopenia was suggested, and intravenous polyvalent immunoglobulin substitution therapy was started to prevent infections. Furthermore, left lower lobectomy was performed.

Four years later, a re-evaluation of the flow cytometry immunophenotypic analysis using double anti-CD19 and anti-CD20 monoclonal antibody staining revealed the lack of CD19+ membrane expression, whereas the frequency of CD20+ B lymphocytes was normal (12.80%). These findings suggested an isolated CD19 defect, rather than global B-cell lymphocytopenia. Moreover, extensive B-cell immunophenotyping of CD20+ cells demonstrated an excess of naive B cells and a slight excess in circulating plasmablasts. Memory B-cell subsets were found to be significantly decreased (Table 1) (6). Sequencing of the coding and intron–exon boundaries regions of the *CD19* gene revealed a homozygous G>T mutation on the splicing acceptor site of the intron 5 (IVS5-1G>T) leading to a truncated protein, which is not expressed at the B-cell surface explaining the absence of CD19+ B lymphocytes in immunophenotyping (2). Moreover, the parents were found to be heterozygous for the same mutation (Fig. 1a,c). Finally, definitive diagnosis of CD19 deficiency was established at age of 11 yr. At time of this writing, and despite regular IV immunoglobulin replacement therapy, this teenager suffers from treatment-dependant chronic bronchopathy and impaired pulmonary function reflecting obstructive disease (FEV1 66% of normal value and Tiffeneau index 63%). However, the compliance for continuous physiotherapy, inhalation treatments and antibiotic prophylaxis is poor.

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary antibody deficiency and is a generic clinical term used to describe several variants that are characterized by marked hypogammaglobulinemia of at least two isotypes (IgG and IgA and/or IgM), impaired functional antibody responses, normal or low counts of B cells, and recurrent bacterial infections, mainly of the respiratory tract. The vast majority of CVID cases are sporadic (80–90%), and although clinical phenotype and immunologic tests lead to

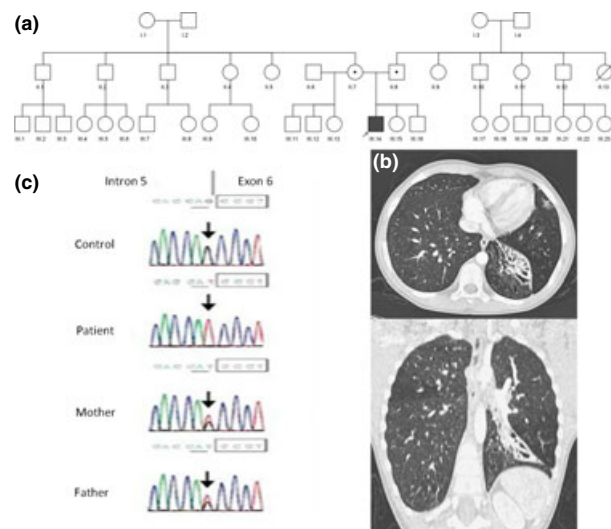


Figure 1 (a) Family pedigree, black box indicates the patient (b) Axial and coronal chest CT, before the administration of immunoglobulin replacement therapy, demonstrate extensive left lower lobe bronchiectasis and partial atelectasis (c) CD19 gene sequencing. The analysis demonstrates a homozygous mutation of the splicing acceptor site on intron 5 for the patient and the same mutation at the heterozygous state for both parents.

diagnosis, causal genetic defects are detected in <10% of the patients (7). During the last decade, CVID phenotype has been associated with distinct and rare mutations in genes encoding immune receptors involved in B-cell activation and maturation, such as ICOS (inducible costimulator), TACI (transmembrane activator and calcium-modulating cyclophilin ligand interactor), CD19, BAFF-R (B-cell activating factor), CD81, CD20 and CD21 (7).

We identified the tenth case worldwide (seventh family, first patient of European descent) of CD19 deficiency due to an intronic mutation of *CD19* gene (IVS5-1G>T) (Table S1). This mutation leads to the skipping of exon 6 and coupling of exons 5–7 encoding a truncated protein with four altered amino acids and a premature stop codon at position 320. As a consequence, the mutant protein lacks the entire intracytoplasmic region and is not expressed on the membrane of B lymphocytes (2).

CD19 protein is a transmembrane protein of the B lymphocytes that is expressed on the B-cell surface from the early stage of differentiation contributing to antigen specific B-cell activation. Along with CD21, CD81 and the CD225, it forms a transmembrane complex which enhances the signal induced by the ligation of an antigen on the BCR, to decrease the threshold for antigen receptor-dependent stimulation (8). Although CD19 deficiency does not impair B-cell differentiation *per se*, it seems to diminish the antigenic response of B cells due to impaired signal transduction leading to ineffective humoral responses (7).

The same causal mutation has been described only once, in a 8-yr-old Japanese boy by Kanegane et al. (2). Our patient was found to be homozygous for IVS5-1G>T mutation, but the

Table 1 Percentages of peripheral blood B cell subpopulations

Tube	Phenotype	Subpopulation	% of total B cells (CD20+)	
			Patient	Control
1	CD27– IgD+	Naive	91	75
1	CD27+ IgD+	Non-switched memory	2	10
1	CD27+ IgD–	Switched memory	1	6
1	CD27– IgD–	CD27– memory	6	5
2	CD24++ CD38++	Transitional	5	7
2	CD24– CD38++	Plasmablasts	6	2
2	CD21 ^{low} CD38 ^{low}		1	3

A multicolor flow cytometric analysis was carried to assess the frequency of B-cell subpopulations within the CD20+ B-cell compartments. Two antibodies combinations were used: anti-CD20-PC5/anti-CD27-FITC/anti-IgD-PE (tube 1) and anti-CD20-PC5/anti-CD21-FITC/anti-CD24-APC/anti-CD38-PE (tube 2). All fluorochrome-conjugated antibodies were purchased from Beckman Coulter (Villepinte, France) and used according to the manufacturer's recommendations. Cells were analyzed on a Navios flow cytometer (Beckman Coulter). Briefly, total lymphocytes were gated on forward scatter and side scatter as previously described (6). Accordingly, total B cells were gated on side scatter and CD20 positive expression. Then, percentages of naive, non-switch memory, switched memory and CD27 negative memory were obtained on tube 1 when CD27 vs. IgD fluorescence were plotted. Percentages of transitional, plasmablasts and CD21^{low}/CD38^{low} B cells were obtained by plotting CD24 vs. CD38 and CD21 vs. CD38 on tube 2. Age-matched control percentages were obtained from Ref. (6).

patient reported by Kanegane had a compound heterozygote mutation consisting of a maternal IVS5-1G>T allele and large deletion of paternal origin on chromosome 16 involving the *CD19*, *ATP2A1* and *NFATC2IP* genes (2). The existence of the same mutation in two children of different ethnic origins presumably indicates that this gene region is highly unstable and that exon–intron boundary sequences should be carefully analyzed.

Immunologic findings of our patient are in agreement with eight of nine previously reported CD19 deficient cases that were characterized by hypogammaglobulinemia, impaired antibody responses to vaccination, normal B lymphocyte frequency, absence of CD19+ expression and decreased numbers of CD27+ memory B cells (1–5). Another case was reported to have selective IgG1 deficiency, normal frequency of CD27+ memory B cells and positive ANA (4).

The evaluation of the results yielded by the first lymphocyte phenotyping of the patient did not take into account that the sum of the percentages of NK cells (CD3–CD56+CD16+) and B cells (CD19+) did not equal 100% of the CD3 negative lymphocytes, leading to the misdiagnosis of B-cell lymphocytosis and the false examination of several targeted genetic defects associated with Bruton and CVID diseases. This indicates the usefulness of a double CD19/CD20 staining for lymphocyte phenotyping for patients with a suspicion of

primary immunodeficiency, especially when the percentage of CD3 negative cells does not recapitulate that of NK cells.

Regarding clinical manifestations of CD19 deficiency, children aged from 1 to 13 yr at onset mostly suffered from recurrent bacterial respiratory infections. Except from one case with selective IgG1 deficiency and ANA positivity that developed nephrotic syndrome leading to end stage renal disease (4), no other complications related to CVID including autoimmunity, granulomatous disease, lymphoproliferation and malignancy were reported. Moreover, all the aforementioned patients seem to have a favorable disease outcome after immunoglobulin replacement therapy, although the duration of follow-up is not available. All these cases indicate that deficiency of CD19 receptor represents a rather homogeneous syndrome (Table S1).

In comparison with other CD19 deficiency cases, our patient developed progressive airway lung disease despite the administration of intravenous immunoglobulins. This can probably be explained by the very early onset of the disease, at the age of 3 months, compared with the other cases. Moreover, initial misdiagnosis of asthma resulted in a considerable 7-yr delay for immunoglobulin administration. In fact, asthma-mimicking symptoms, such as dry coughing, expiratory wheeze and dyspnea, may be triggered by recurrent respiratory infections. On the other hand, asthma patients are more susceptible to protracted respiratory infections, which might delay a possible diagnosis of antibody immunodeficiency (9). In addition, insufficient patient compliance related to his precarious low socio-familial situation may facilitate this clinical phenotype.

In conclusion, we suggest early assessment of serum immunoglobulin levels and B-cell immunophenotyping in infants and children with clinical phenotypes attributed to refractory asthma. Furthermore, the delay in diagnosis and initiation of appropriate immunoglobulin replacement treatment in this patient underlines the importance of high awareness of the warning signs of primary immune deficiency in children and the

necessity of close collaboration between first line pediatricians, immunologists and geneticists.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Epidemiological, genetic and clinical characteristics of all CD19 deficiency cases that have been reported to date.

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Reference	N	CD19 mutation	Sex	Descent	Age at onset	Age at diagnosis	Presenting manifestations	Clinical manifestations before diagnosis	Clinical manifestations at diagnosis	Treatment	Outcome
[1]	4	c.972insA	F	Turkish^	1 year	10 year	LRI	Recurrent LRI and meningitis	COPD, postinfectious GN	IVIGs	Good
		c.1384delGA	M	Colombian*	7 year	35 year	URI	Recurrent LRI, chronic sinusitis	LRI, bacterial conjunctivitis, gastritis	IVIGs, Sinus surgery	Good
		c.1384delGA	F	Colombian*	6 year	33 year	URI	LRI, herpes zoster, chronic sinusitis	Recurrent bacterial conjunctivitis /dacryocystitis, diarrhea	IVIGs, Sinus surgery	Good
		c.1384delGA	F	Colombian*	5 year	49 year	URI	Recurrent LRI, recurrent skin abscesses, chronic sinusitis	LRI, recurrent bacterial conjunctivitis, chronic diarrhea	IVIGs, Sinus surgery	Good
[2]	1	Maternal allele: IVS5-1G>T Paternal allele: gross deletion on chromosome 16	M	Japanese	5 year	8 year**	Pyelonephritis, bronchitis, gastritis, thrombocytopenia		Pyelonephritis, bronchitis, gastritis, thrombocytopenia	IVIGs	Good
[3]	1	c.972insA	M	Turkish^	NR	12 year	Recurrent URI/LRI	NR	Recurrent URI/LRI	IVIGs	Good
[4]	2	c.1464delC 1653_167119del2 8pbins23pb	F F	Kurdish Moroccan	5 year 13 year	11 year 31 year	URI, giardiasis Failure to thrive, microscopic hematuria, proteinuria	Recurrent URI Chronic sinusitis, pneumococcal pneumonia	Pneumococcal meningitis IgA nephropathy, nephrotic syndrome	NR Sinus surgery	Good Nephrotic syndrome /ESRD
[5]	1	c.156G>C	M	Moroccan	NR	6 year	URI/LRI	Recurrent URI/LRI, <i>S. pneumoniae</i> septicemia	Recurrent URI/LRI	IVIGs, SCIGs	Good
This report	1	IVS5-1G>T	M	France	3 months	11 year***	RSV bronchiolitis, Asthma mimicking symptoms	Asthma resembling symptoms Recurrent URI/LRI	Bronchiectasis, lobar atelectasis, COPD	IVIGs, Lobectomy	Chronic lung disease

Supplementary Table: Epidemiological, genetic and clinical characteristics of all CD19 deficiency cases that have been reported to date.

*Siblings, ^ Cousins

** Diagnosis of CVID and IVIG therapy at age of 5, genetic diagnosis of CD19 deficiency at age of 8.

*** Diagnosis of hypogammaglobulinemia and IVIG therapy at age of 7, genetic diagnosis of CD19 deficiency at age of 11

COPD: chronic obstructive pulmonary disease, ESRD: end-stage renal disease, F: female, GN: glomerulonephritis, IVIGs: intravenous immunoglobulins, LRI: lower respiratory infection, M: male, N: number of patients, NR: non-reported, RSV: Respiratory Syncytial Virus, SCIGs: subcutaneous immunoglobulins, URI: upper respiratory infection