

CD21 deficiency in 2 siblings with recurrent respiratory infections and hypogammaglobulinemia



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Clinical Implications

- We report the clinical history, immunological phenotype, and causal mutation for 2 patients with CD21 deficiency.

TO THE EDITOR:

The complement system plays a major role in humoral immunity via activation of the classical pathway and the development of adaptive responses. This function is ensured principally by CD21 (or CR2), a receptor that recognizes C3 cleavage fractions containing a C3d moiety bound to an antigen.¹ CD21 is primarily expressed by follicular dendritic cells (FDCs) and B cells. The CD21 on FDCs traps soluble C3d-bound antigens for their presentation to B cells in the lymphoid tissues. The CD21 on B cells forms part of the CD19 complex, along with CD81, CD225, and CD19, and acts as a coreceptor for the B-cell receptor-Ig $\alpha\beta$. The coligation of a C3d-bound antigen to the B-cell receptor and CD21 increases the strength of the activating signal delivered to B cells.¹ Only 2 patients with a common variable immunodeficiency syndrome (CVID) phenotype caused by autosomal-recessive CD21 deficiency have been reported to date.^{2,3} We report here the first multiplex kindred with CD21 deficiency.

The 2 siblings were born to consanguineous French parents (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). The proband (P1), a 14-year-old girl, was first referred at the age of 7 years, for recurrent otitis media and bronchitis, which persisted despite 3 adenoidectomies and multiple grommet insertions. She also had undocumented lobar pneumonia at the age of 12 years. Immunological tests performed at the age of 7 years revealed low levels of IgG₁, resulting in low levels of IgG. At the age of 10 years, IgG and IgG₁ levels were normal. IgG to tetanus and diphtheria were reduced despite routine immunization (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). This patient developed partial conductive deafness, despite the systematic treatment of otitis media with antibiotics. Histological analysis of an adenoid sample showed a hypertrophic mucosa with florid hyperplasia of the secondary lymphoid follicles. Germinal centers contained large numbers of macrophages, the cell bodies of which were readily stained. Interfollicular areas contained lymphocytes, marginal cells, immunoblasts, and plasmocytes, with no cellular atypia (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). P1's 11-year-old brother (P2) was first referred at the age of 5 years, for recurrent otitis

media, rhinopharyngitis, and bronchitis, which have begun at the age of 4 months. He underwent 1 adenoidectomy and 2 grommet insertions. Initial immunological explorations showed normal levels of IgG but slightly low levels of IgG₁. P2 then developed mild hypogammaglobulinemia between the ages of 7 and 11 years. IgG to tetanus and diphtheria were reduced despite routine immunization. After booster injections, the titers of antibodies specific for tetanus toxoid reached protective levels in the 2 patients, whereas those for antidiphtheria toxoid antibodies remained low (see Table E1 and Figure E2 in this article's Online Repository at www.jaci-inpractice.org). None of the 2 patients received immunoglobulin substitution. P1 received prophylaxis by cotrimoxazole during one winter season at the age of 7 years which reduced the frequency of infections. A flow-cytometry study of B cells from the 2 patients showed a decrease in class-switched memory B cells and a complete loss of CD21 cell surface expression (Figure E1). CD21 immunohistochemistry of the adenoid tissue of P1 revealed a complete absence of staining (Figure E2). Genomic sequencing of *CR2*, the gene encoding CD21, revealed a homozygous 1-base pair deletion (c.234delC) predicted to lead to a frameshift and the insertion of a premature stop codon (p.T209HfsX10) (Figure E1). Both parents were heterozygous for this mutation. The father was asymptomatic and had a normal immunophenotype, with slightly lower levels of CD21 expression on B cells than observed in the healthy control (Figure E1). The mother was asymptomatic but had had recurrent otitis media during childhood. Laboratory assessments revealed profound B lymphopenia (0.9%; 3/mm³), but normal levels of immunoglobulins and a protective titer of antibodies against toxoid tetanus in the mother at the age of 35 years.

We report here complete CD21 deficiency due to a new homozygous *CR2* mutation in 2 siblings. Both patients were referred for recurrent upper respiratory tract infections beginning in early childhood. Both have mild hypogammaglobulinemia and low levels of memory class-switched B cells, with impaired production of antibodies specific for certain protein antigens. The adenoid tissue of P1 had hyperplastic germinal centers with a normal architecture but no CD21-positive FDCs. The number and size of germinal centers within splenic follicles are smaller after immunization in *cr2*^{-/-} mice than in wild-type mice,⁴ probably because *cr2*^{-/-} mice are also CD35-deficient.¹ CD35 is another receptor of cleaved complement fractions.⁴ Only 2 other CD21-deficient patients have been described to date.^{2,3} Both have mild hypogammaglobulinemia associated with low levels of memory B cells, but they have a normal antibody response to protein antigens^{2,3} (Table I). The first patient reported was diagnosed at the age of 26 years. He had recurrent upper respiratory tract infections before the age of 6 years and remained asymptomatic for 20 years following adenoidectomy.² At the age of 26 years, he developed symptoms suggestive of CVID. The second reported case was a 16-year-old boy with a history of autoimmune disease with no history of recurrent infection.³ The 2 patients reported here had more severe clinical and immunological phenotypes than the first 2 patients to be described^{2,3} (Table I). As our patients were born to consanguineous parents, we cannot rule out the existence of

TABLE I. Clinical, immunological, and genetic characteristics of CD21-deficient patients

Characteristic	Thiel et al ²	Wentink et al ³	P1	P2
Age at diagnosis (y)	26	13	14	11
Sex	Male	Male	Female	Male
Country of residence	Germany	The Netherlands	France	France
Consanguinity	No	No	Yes	Yes
Clinical history	<6 y: URT infections 6-26 y: asymptomatic 26 y: URT and LRT infections, SMG, diarrhea, fever	No recurrent infection Possible autoimmune disease	Recurrent URT and LRT infections since early childhood	Recurrent URT infections since early childhood
CR2 mutations	c.1225+1G>C/p.W766X	p.R142X/p.I926SfsX14	p.T209HfsX10/p.T209HfsX10	p.T209HfsX10/p.T209HfsX10
Immunophenotyping				
CD21 expression	Absent	Absent	Absent	Absent
Class-switched memory B cells	Decreased	Decreased	Decreased	Decreased
Immunoglobulin				
IgG	Low	Low	Low to normal	Low to normal
IgA	Low	Low	Normal	Normal
IgM	Normal	Low	Normal	Subnormal
Serology				
Protein antigens	Normal	Normal	Low	Low
Polysaccharide antigen	Low	Normal	Normal*	Low*

LRT, Lower respiratory tract; SMG, splenomegaly; URT, upper respiratory tract.

*Assessed by blood group isohemagglutinin production.

other pathogenic variants in their genomes, although laboratory investigations detected no other immunological abnormality.

Low levels of calcium flux have been reported in CD21-deficient B cells exposed to a suboptimal quantity of C3d-bound antigen.² This finding suggests that the impaired B-cell response in CD21-deficient patients is due to decreased stimulation of B cells from the germinal center in the absence of positive signals mediated via the CD21 coreceptor.¹ The absence of CD21 expression on FDCs may also be responsible for impaired antigen retention in lymphoid follicles. Taken together, these data suggest that the absence of CD21 expression on B cells and FDCs could lead to an inability to sustain specific humoral responses after antigen challenge. This finding highlights the importance of CD21 for the development of humoral adaptive immunity and the induction of memory B cells in humans. CD21 deficiency may however be surmountable in case of high concentration or repeated antigen exposure.² Other deficiencies in components of the CD19 complex (CD19 and CD81 deficiencies) are also associated with an impaired response to protein antigens, albeit with a more severe clinical and immunological phenotype than in CD21 deficiency.^{3,5-9} This underlies that CD21 may be more redundant than CD19 and CD81. Additional clinical reports for CD21-deficient patients and their long-term follow-up are now required to improve our understanding of the natural course of this deficiency. The defect observed in these patients could easily be screened by flow-cytometry panel for CVID, which should be offered to patients with an impairment of humoral immunity.

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A

I. 1 2

II. 1 2 3 4

III. 1 2

IV. 1 (P1) 2 (P2)

B

C.234delC (p.T209HfsX10)

Patients (P1, P2)

Father, Mother (III.1, III.2)

Healthy control

C

R142X

T209HfsX10

W766X

I926SfsX14

1225+1G>C

CCP1 CCP2 CCP3 CCP4 CCP5 CCP6 CCP7 CCP8 CCP9 CCP10a CCP10b CCP11 CCP12 CCP13 CCP14 CCP15 Trans Cyp

D

Cell number

CD21

P2

P1

Father (III.1)

HC

E

Titre (U/mL)

Age (years)

Toxoid tetanus

Toxoid diphtheria

Vaccine administration

FIGURE E1. A, Pedigree of the family. **B**, Chromatograms for the study of the *CR2* gene in the 2 patients, their parents, and a healthy control. **C**, Structure of the CD21 propeptide (1092 amino acids (aa)) with reported mutations associated with CD21 deficiency (including those reported here). CD21 is composed of a signal peptide (SP), an extracellular domain consisting of 15 to 16 complement control protein (CCP) domains, a transmembrane domain (Trans), and a short cytoplasmic tail (Cytop). **D**, Flow cytometric analysis of CD21 expression (clone B-ly4, BD Biosciences) on CD19⁺ cells from the 2 patients, their father (III.1), and a healthy control (HC). **E**, Follow-up of IgG to tetanus and diphtheria toxoids in P1 and P2. Booster vaccinations are depicted with an arrow. Dotted lined represent protective IgG titers to tetanus and diphtheria toxoids (0.6 and 1.2 UI/mL, respectively).

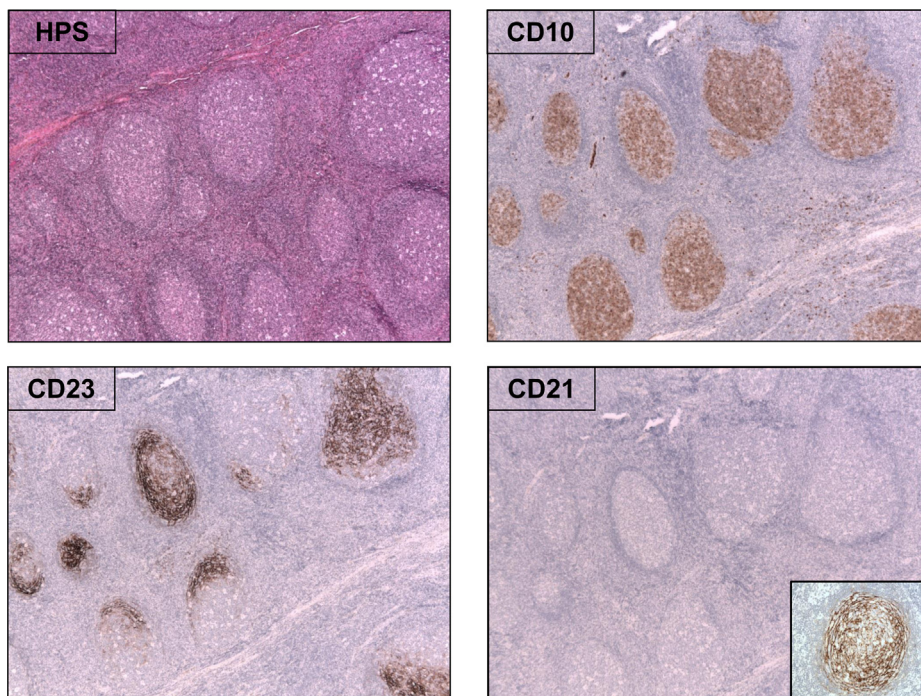


FIGURE E2. Adenoid tissue sections from P1 stained with hematoxylin-phloxin-saffron (HPS) and processed for anti-CD10 (clone 56C6), anti-CD23 (clone 1B12), or anti-CD21 (clone 2G9, all antibodies from Novocastra) immunohistochemistry. The inset shows a CD21-positive external control. Immunohistochemistry showed the germinal centers to have a normal architecture, with CD10-positive centrofollicular cells associated with a CD23-positive network of FDCs. The lymphoid tissue showed a normal physiological distribution of B and T cells with CD20 and CD3 staining, no viral Epstein–Barr virus latent membrane protein 1, and the presence of polytypic plasmacytes labeled with anti- κ and anti- λ light-chain antibodies (data not shown).

TABLE E1. Most recent immunological analysis of P1 and P2

Immunological work-up	P1 (14 years)	P2 (11 years)	Normal values for age
Lymphocytes (/mm ³)	2587	1250	
T cells (%)			
CD3 ⁺	75	73	56-84
CD4 ⁺	43	33	31-52
CD31 ⁺ CD45RA ⁺ /CD4 ⁺	44	29	43-55
CD45RA ⁺ /CD4 ⁺	57	43	58-70
CD8 ⁺	23	25	18-35
CCR7 ⁺ CD45RA ⁺ /CD8 ⁺	42	46	52-68
CCR7 ⁺ CD45RA ⁻ /CD8 ⁺	4	4	3-4
CCR7 ⁻ CD45RA ⁻ /CD8 ⁺	46	42	11-20
CCR7 ⁻ CD45RA ⁺ /CD8 ⁺	8	8	16-28
B cells			
CD19 ⁺ (/mm ³)	336	188	193-628
CD19 ⁺ (%)	13	15	4.8-24.3
IgD ⁺ CD27 ⁻ /CD19 ⁺ (%)	90	97	58.5-87.4
IgD ⁺ CD27 ⁺ /CD19 ⁺ (%)	7	2.7	2.6-21.1
IgD ⁻ CD27 ⁺ /CD19 ⁺ (%)	2	0.3	4-21.2
CD21 ⁺ (%)	0	0	
CD24 ⁺⁺ CD38 ⁺⁺ /CD19 ⁺ (%)	5	6	
CD24 ⁻ CD38 ⁺⁺ (%)	0.1	0.5	
NK cells (%)	12	9	3-22
Immunoglobulin levels (g/L)			
IgG	7.36	4.8	7-16
IgG ₁	4.05	2.6	4-11
IgG ₂	1.6	0.85	0.50-5
IgG ₃	0.98	1.21	0.17-0.70
IgG ₄	0.07	0.09	0.002-1.400
IgA	1.91	1.25	0.7-4
IgM	2.11	0.44	0.4-2.4
Specific antibodies			
Blood group and allohemagglutinin	Normal	Decreased	
Tetanus toxoid	Low to normal*	Low to normal*	
Diphtheria toxoid	Low	Low	
EBV	Negative at 7 y	Isolated positive IgG anti-VCA	

VCA, Viral capsid antigen.

Unless otherwise mentioned, analyses were performed at age 14 y for P1 and 11 y for P2.

*Antibody titers against the tetanus and diphtheria toxoids have been followed since the age of 7 y for P1 and the age of 5 y for P2.

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