Case report

CD21 deficiency in 2 siblings and frequency of the associated mutation in the Danish population

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The clinical presentation of CD21 deficiency in 2 siblings caused by a novel mutation in the CD21 gene is reported, and the frequency of this mutation in the Danish population is explored. Successful treatment with IgG replacement in both patients with CD21 deficiency is also reported. (J Allergy Clin Immunol Global 2024;3:100274.)

Key words: *Immunodeficiency, CD21, clinical presentation, novel mutation, allele frequency, immunoglobulin replacement therapy, CR2*

Abbreviation used
CR2: Complement receptor 2

CD21-mediated costimulation of the B-cell receptor is important for B-cell activation and the production of IgG antibodies against foreign antigens.[1] CD21, also known as complement receptor 2 (CR2) or Epstein-Barr virus receptor, is expressed on both B cells and follicular dendritic cells. CD21 recognizes the C3 cleavage fractions containing a C3d moiety bound to an antigen.[2,3]

To date, only 4 patients with complete CD21 deficiency have been reported.[4-6] Two of these patients were diagnosed at ages 26 and 13 years, and CD21 deficiency in both patients was caused by a compound heterozygous mutation in *CR2*,c.1225+1G>C/p.Trp766Ter and p.R142X/p.I926SfsX14.[4,5] Furthermore, 2 siblings diagnosed at ages 14 and 11 years were reported to have CD21 deficiency due to a homozygous 1-bp deletion, resulting in a stop codon (p.T209HfsX10).[6] The phenotypes of these patients varied. Here, we report the fifth and sixth patients with CD21 deficiency: a 10-year-old Danish male patient and his 5-year-old sister.

Our patients are the only children of a Danish couple with no family history of recurrent infections or autoimmune diseases. The brother (patient 1) was referred to the outpatient clinic at the Department of Pediatrics, Aalborg University Hospital, for evaluation owing to recurrent infections since early childhood. The sister (patient 2) was initially less affected than her brother, but as she grew older, she experienced symptoms of the skin and gastrointestinal canal, as well as recurrent infections. In addition to the children, the father, mother, grandmother, and aunt were tested for CD21 expression.

Patient 1 has been attending the pediatric department since he was 3 weeks old. In the first year, he showed failure to thrive, recurrent fever episodes, urinary tract infections, and upper and lower respiratory tract infections. By age 6 years, recurrent acute otitis media had led to 16 tympanostomy tube insertions. Loose stools and frequent bowel movements have been persistent complications. A microbiologic cause was identified only once (*Yersinia enterocolitica*), and further investigation of the intestinal symptoms did not provide clarification. Lower and especially upper respiratory tract infections recurred primarily during nonsummer and were accompanied by fatigue and a very distinct dark coloring of the surroundings of the boy's eyes. At age of 2, he developed a papular rash located mainly on the face and chest. Skin biopsy samples showed interface dermatitis. Subsequently, he developed impetigo and erythema nodosum, both of which resolved without treatment. Patient 1 had approximately 20 hospital visits per year throughout his first 5 years of life. During the year before this report was written, patient 1 had been treated with subcutaneous IgG (human normal immunoglobulin, 500 mg/kg per month). During this period, no respiratory symptoms have occurred, and the number of infections has been reduced to 1 episode of an upper airway infection that required antibiotic treatment. His sleep has improved, and his ability to engage in social and physical activities has improved significantly.

Until the age of 4 years, patient 2 presented with mild symptomatology. She mostly experienced loose stools and recurrent acute otitis media, resulting in insertion of tympanostomy tubes 4 times. Recently, she developed a papular rash. At

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The parents of the 2 children described have signed an informed statement of consent to publish the medical case history of their 2 CD21-deficient children.

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

Characteristics	Thiel et al[4]	Wentink et al[5]	Rosain et al[6]	Rosain et al[6]	Patient 1	Patient 2
Age (y)	26	13	14	11	9	4
Sex	Male	Male	Female	Male	Male	Female
Country of residence	Germany	The Netherlands	France	France	Denmark	Denmark
Clinical history	<6 y: URT infections	No recurrent infections	Recurrent URT and LRT infections	Recurrent URT infections	Early onset of recurrent infections	Recurrent infections
	6-26 y: asymtomatic	Possible autoimmune disease	since early childhood	since early childhood	from several organ systems	increasingly after age 5 y
	26 y: URT and LRT infections				Frequent bowel movement	Frequent bowel movement
	SMG, diarrhea, fever				Papular rash, face and chest	Papular rash, face and chest
B cells						
CD19-positive B-cells	Unknown	Normal	Normal	Normal	Normal 0.63 (0.3-0.7 × 10^9/L)	Normal 1.2 (0.4-1.5 × 10^9/L)
CD21 expression	**Absent**	**Absent**	**Absent**	**Absent**	**Absent 0.00**)*	**Absent 0.00***
Class-switched memory B cells	**Decreased**	**Decreased**	**Decreased**	**Decreased**	**Decreased 0.6%** (1.0%-2.2% of PBLs)	**Decreased 0.8%** (1.0%-2.2% of PBLs)
B-cell somatic hypermutation	N/A	N/A	N/A	N/A	**17%** (27%-88%)	N/A
Immunoglobulin						
IgG	**Low**	**Low**	**Low to normal**	**Low to normal**	**4.8** (5.4-13.6 g/L)	4.3 (3.2-11.5 g/L)
IgG1	N/A	N/A	N/A	N/A	**2.61** (4.32-10.2 g/L)	**2.58** (3.15-9.45 g/L)
IgG2	N/A	N/A	N/A	N/A	1.06 (0.72-4.3 g/L)	0.81 (0.36-2.25 g/L)
IgG3	N/A	N/A	N/A	N/A	0.79 (0.13-0.85 g/L)	0.32 (0.17-0.68 g/L)
IgG4	N/A	N/A	N/A	N/A	**0.005** (0.019-0.932 g/L)	**0.005** (0.01-0.537 g/L)
IgA	**Low**	**Low**	Normal	Normal	1.3 (0.47-2.21g/L)	0.64 (0.26-1.47 g/L)
IgM	Normal	**Low**	Normal	Normal	1.1 (0.48-1.86 g/L)	0.75 (0.48-1.86 g/L)
IgE	N/A	N/A	N/A	N/A	**482** (<150 kU/L)	N/A
Complement						
Complement activity, alternative	N/A	N/A	N/A	N/A	62% (50-150%)	N/A
Complement activity, classic	N/A	N/A	N/A	N/A	78% (50-150%)	N/A
C3	N/A	N/A	N/A	N/A	**1.62** (0.85-1.60)	N/A
C4	N/A	N/A	N/A	N/A	0.31 (0.10-0.40)	N/A
Specific antibodies, after vaccination						
Haemophilus influenzae type B	N/A	N/A	N/A	N/A	**<0.11** (>0.99 μg/mL)	**0.18** (>0.99 μg/mL)
Hepatitis A	N/A	N/A	N/A	N/A	**Neg** (pos)	Not vaccinated
Diphtheria	N/A	N/A	N/A	N/A	2.066 (>0.1kIU/L)	0.113 (>0.1 kIU/L)
Tetanus	N/A	N/A	N/A	N/A	0.276 (>0.2 kIU/L	**0,038** (>0,2 kIU/L)
Streptococcus pneumoniae	N/A	N/A	N/A	N/A	**Neg** (pos)	**Neg** (pos)
Measles/morbilli	N/A	N/A	N/A	N/A	**Neg** (pos)	**Neg** (pos)
Rubella	N/A	N/A	N/A	N/A	**Low 11** (>15 IU/mL)	**Low 12** (>15 IU/mL)
Genetics						
CR2 mutations	c.1225+1G>C/p.W766X	p.R142X/p.I926SfsX14	p.T209HfsX10/ p.T209HfsX10	p.T209HfsX10/ p.T209HfsX10	c.2298G>A/p.Trp766Ter	c.2298G>A/p.Trp766Ter
CR2 mRNA expression	N/A	N/A	N/A	N/A	Positive	Positive
HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1	N/A	N/A	N/A	N/A	Haploidentical	Haploidentical

Reference values are in brackets. Values out of range are highlighted in bold. Values for patient 1 and patient 2 are before immunoglobulin substitution.
LRT, Lower respiratory tract; *N/A*, not available; *pos*, positive; *PBL*, peripheral blood lymphocyte; *SMG*, splenomegaly; *URT*, upper respiratory tract.
*More than 90% of CD19-positive cells are expected to express CD21 in healthy controls.

age 5 years, her symptoms worsened significantly, with increased infections, requiring several hospital admissions and a very frequent need for antibiotic treatment. Patient 2 was treated with IgG (human normal immunoglobulin, 400 mg/kg per month) for more than 5 months. During treatment, she experienced no infections and her sleep and quality of daily life improved.

Patient 1 had remarkably fluctuating levels of IgG1, IgG2, IgG3, and IgG4, and his total IgG level (4.8 g/L) was below the normal range before immunoglobulin substitution. It was assumed that diarrhea did not substantially influence immunoglobulin levels, as the patient's albumin levels were normal. After 1 year of immunoglobulin treatment, the patient's total IgG level increased to 9.0 g/L.

Both children received vaccination according to the Danish Childhood Immunization Program; however, their overall vaccination responses were inadequate (Table I).

Both patients shared *HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1,* and *HLA-DQB1* haplotypes, confirming their relationship (Table I).

Flow cytometric immunophenotyping of B cells using 3 different CD21 clones (BL13, B-ly4, and Bu32), revealed complete extracellular and intracellular CD21 deficiency in both siblings, whereas their parents showed only marginally decreased CD21 expression (for data on immunophenotyping and immunologic analysis, see Fig E1 in the Online Repository at www.jaci-global.org).

Sequencing of *CR2* revealed a homozygous c.2298G>A/p.Trp766Ter mutation, which leads to a stop codon and is located in exon 13 (rs151093663), in both patients. Sequencing the rest of the family revealed that both parents and the aunt were heterozygous for the same mutation, whereas the grandmother was wild-type for the same c.2298G>A/p.Trp766Ter mutation (see Figs E2 and E3 in the Online Repository at www.jaci-global.org). A next-generation sequencing panel of 264 immunodeficiency genes revealed only CD21 mutation in the 2 patients. The results were confirmed by Sanger sequencing. Finally, the mRNA transcription levels of CR2 indicated that all exons were transcribed (Table I).

A group of 1000 randomly selected blood donors from the Danish Bone Marrow Registry were analyzed by direct sequencing of exon 13 and the flanking area. We found 5 individuals heterozygous for the rs151093663 mutation. The calculated allele frequency of the minor allele among Danes is 0.0025, which implies that the risk of 2 Danes having an affected child would be approximately 1 in 160,000 births. Our allele frequency is similar to the frequency of 0.0020 found in a North Swedish population but greater than the global minor allele frequency of 0.0004 described in the National Center for Biotechnology Information's ClinVar database.

SUMMARY

Here we have described a novel genetic variant associated with CD21 deficiency and immunodeficiency in 2 siblings who were successfully treated with IgG. The reported infections, IgG levels, decreased numbers of class shift memory B cells, and low specific antibody titers confirm the pivotal role of CD21 in relation to B-cell activation and IgG. The frequency of this genetic mutation in the general population is surprisingly high, and clinicians should consider CD21 deficiency in patients presenting with recurrent infections and antibody deficiency accompanied by normal B cell counts.

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