CD21 deficiency in two siblings with recurrent respiratory infections and hypogammaglobulinemia

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Short summary

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

Keywords

Primary antibody deficiency, CD21, respiratory infections

Abbreviations

CVID: common variable immunodeficiency

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 (FDC) 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 (BCR)-Igαβ. The coligation of a C3d-bound antigen to the BCR and CD21 increases the strength of the activating signal delivered to B cells[1]. Only two 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 two siblings were born to consanguineous French parents (**Figure E1**). The proband (P1), a 14-year-old girl, was first referred at the age of seven years, for recurrent otitis media and bronchitis, which persisted despite three adenoidectomies and multiple grommet insertions. She also had undocumented lobar pneumonia at the age of 12 years. Immunological tests performed at the age of seven years revealed low levels of immunoglobulin (Ig) G1, resulting in low levels of IgG. IgG and IgG1 levels were normal at the age of 10 years. IgG to tetanus and diphtheria were reduced despite routine immunization (**Table E1)**. 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 (**Figure E2**). P1's 11-year-old brother (P2) was first referred at the age of five years, for recurrent otitis media, rhinopharyngitis and bronchitis, which have begun at the age of four months. He underwent one adenoidectomy and two 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 two patients, whereas those for anti-diphtheria toxoid antibodies remained low (**Table E1** and **Figure E2**). None of the two patients received immunoglobulin substitution. P1 received prophylaxis by cotrimoxazole during one winter season at the age of 7 which reduced the frequency of infections. A flow-cytometry study of B cells from the two 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 one-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/mm^3), 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 two 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 two 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 1**). The first patient reported was diagnosed at the age of 26 years. He had recurrent upper respiratory tract infections before the age of six 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 two patients reported here had more severe clinical and immunological phenotypes than the first two patients to be described[2,3] (**Table 1**). As our patients were born to consanguineous parents, we cannot rule out the existence of 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 underlie 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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Table 1 – Clinical, immunological and genetic characteristics of CD21-deficients patients

	Thiel *et al.*, 2012[2]	Wentink *et al.*, 2015[3]	P1	P2
Age at diagnosis	26 y	13 y	14 y	11 y
Sex	M	M	F	M
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*

*assessed by blood group isohemagglutin production; URT: upper respiratory tract; LRT: lower respiratory tract; SMG: splenomegaly