CASE REPORT

Homozygosity For a Novel Mutation in the C1q C Chain Gene in a Turkish Family With Hereditary C1q Deficiency

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■ Abstract

Hereditary complete deficiency of complement component C1q is associated with a high prevalence of systemic lupus erythematosus and increased susceptibility to severe recurrent infections. An 11-year-old girl was screened for immunodeficiency due to a history of recurrent meningitis and pneumonia. Immunologic studies revealed absence of classic pathway hemolytic activity and undetectable levels of C1q. Exon-specific amplification of genomic DNA by polymerase chain reaction followed by direct sequence analysis revealed a novel homozygous missense mutation at codon 48 in the C1q C gene causing a glycine-to-arginine substitution affecting the collagen-like region of C1q. No changes were seen in the exons of the A and B chains. The mutation affected both the formation and the secretion of C1q variant molecules. We describe a novel mutation in the C1q C chain gene that leads to an interchange in amino acids resulting in absence of C1q in serum.

■ Resumen

La deficiencia completa hereditaria del componente C1q del complemento está asociada a una alta prevalencia de lupus eritematoso sistémico y a un aumento de la sensibilidad a infecciones recurrentes graves. Una niña de 11 años de edad fue sometida a pruebas de inmunodeficiencia debido a su historial de meningitis y neumonía recurrentes. Los estudios inmunológicos revelaron la ausencia de actividad hemolítica de la vía clásica y niveles inapreciables de C1q. La amplificación específica del exón del ADN genómico mediante reacción en cadena de la polimerasa seguida de un análisis directo de secuencias revelaron una nueva mutación de sentido equivocado homocigótica en el codón 48 del gen de la cadena C C1q. Esta mutación era la causante de una sustitución de glicina por arginina en la región tipo colágeno de C1q. No se observaron cambios en los exones de las cadenas A y B. La mutación afectó a la formación y la secreción de moléculas variantes de C1q. En este estudio se describe una nueva mutación en las cadenas C C1q cuya consecuencia es un intercambio de aminoácidos que provoca la ausencia de C1q en el suero.

Palabras clave: Hereditaria. Complemento. Inmunodeficiencia primaria. Deficiencia de C1q. Nueva mutación

Introduction

C1q is a subcomponent of C1, the first component of complement. It is composed of 3 distinct subunits—C1q, C1r, and C1s—which are present in a molecular ratio of 1:2:2. C1 triggers the classic pathway of complement activation by binding to antigen-antibody complexes or to microbial ligands. Several other functions have been assigned to C1q, including clearance of immune complexes and apoptotic cell debris [1] and modulation of cytokine expression [2].

Hereditary deficiency of the complement subcomponent C1q is associated with a high prevalence of systemic lupus erythematosus (SLE) and increased susceptibility to severe recurrent infections [3]. In this report, we describe a C1q-deficient patient from a Turkish family that was homozygous for a previously unreported mutation in the C1q C chain gene.

Case Description

The index case was an 11-year-old girl who was admitted with fever and loss of consciousness. Her medical history included bacterial meningitis at the age of 8 and pneumonia at the age of 10. Neither the parents nor the 5 siblings had a history of increased susceptibility to pyogenic infections. The parents were first-degree cousins.

Meningitis caused by *Streptococcus pneumoniae* was diagnosed by examination of cerebrospinal fluid. Vancomycin combined with ceftriaxone was administered. The patient had been vaccinated against meningococci, pneumococci, and *Haemophilus influenzae* type B, and penicillin prophylaxis was administered. Her antinuclear antibody and rheumatoid factor titers were negative after repeated investigations. During a 5-year follow-up, she was asymptomatic for autoimmune diseases and infections.

Immunologic studies performed when the patient had recovered revealed normal immunoglobulin (Ig) levels. Alternative pathway-dependent hemolytic complement activity was normal, although no classic pathway activity was detected using the hemolysis in gel (HIG) assay [4]. Levels of complement proteins C2, C3, and C4 were within normal ranges (C2, 115% of normal concentration [reference range, 77%-159%]; C3, 1.04 g/L [reference range, 0.77-1.38 g/L]; and C4, 0.20 g/L [reference range, 0.12-0.33 g/L]). The serum concentration of C1q was below the detection limit when measured by electroimmunoassay (<6% of normal concentration; reference range, 78%-131%). These findings led to the diagnosis of C1q deficiency. The parents and 5 siblings, who were well and had no history to suggest increased susceptibility to infection, all had normal classic and alternative pathway-dependent hemolytic function, as well as normal C3 and C4 concentrations. However, in 4 of the siblings the serum concentrations of C1q were below the normal range (Table).

To reveal the putative genetic defect, the C1q genes from all 8 family members were sequenced. Genomic DNA was amplified using polymerase chain reaction (PCR) and sequenced in both directions using the Big Dye Terminator sequencing kit 3.1 (PE Applied Biosystems, Foster City, California, USA) and analyzed on an ABI prism 3100 genetic analyzer with the DNA Sequencing Analysis software version 3.4 (Applied Biosystems). Sequences were aligned and compared with GenBank sequence AL158086 using Bioedit Sequence Alignment Editor version 7.0.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Sequencing of the C1q genes in the index case revealed a novel homozygous missense mutation at codon 48 that changed codon GGA to AGA and caused a glycine-to-arginine exchange in the part of the C1q C chain belonging to the collagen-like region of the C1q molecule. The mutation was reported to the National Center for Biotechnology Information single-nucleotide polymorphism database (rs35049192). The patient was also homozygous for the common T allele of the silent CCT/CCC mutation in proline at position 14 of the C chain, as investigated by Petry and Loos [5], while no base changes were observed in the A and B chains compared with the reference. Four siblings and both parents were

Table. Concentration of Complement Proteins and Complement Function in the Family Members

	CP[a]	AP[a]	C3, g/L	C4, g/L	C1q, % of normal[b]
Index case	0	N	1.04	0.20	<0.1
Brother 1	N	N	1.49	0.26	49
Brother 2	N	N	1.30	0.19	53
Brother 3	N	N	1.48	0.29	52
Brother 4	N	N	1.44	0.27	109
Brother 5	N	N	1.15	0.23	77
Father	N	N	1.37	0.31	86
Mother	N	N	1.15	0.20	86

Abbreviations: AP, alternative pathway hemolytic complement activity; CP, classic pathway hemolytic complement activity.
[a]Qualitative functional assay by hemolysis in gel.
[b]Measured by electroimmunoassay and, in the index case, also by enzyme-linked immunosorbent assay.
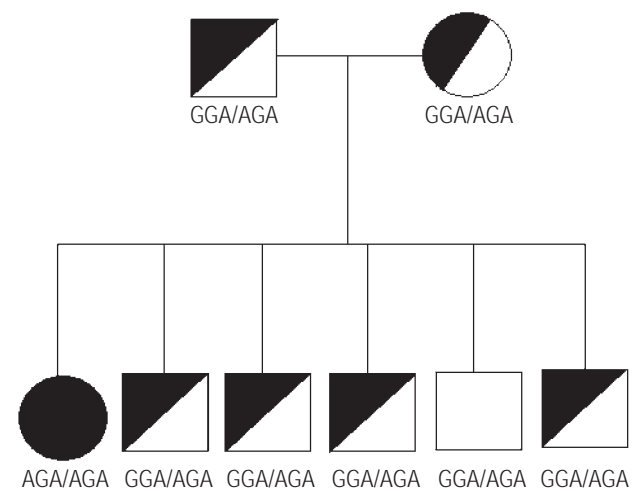

Figure. Pedigree showing the presence of a novel missense mutation at codon 48 in the C1q C chain gene (codon GGA to AGA). The mutation causes a glycine-to-arginine exchange in a position of the C1q C chain located in the collagen-like region of the C1q molecule. The index case (filled circle) was homozygous for this mutation.

heterozygous for the glycine-to-arginine mutation, while 1 sibling (brother 4) was homozygous for the wild-type allele (Figure). The affected glycine residue is conserved between the human C1q A, B, and C chains [6].

Since the mutation gave rise to an exchange in amino acids, a variant C1q C chain could have been synthesized. A sensitive enzyme-linked immunosorbent assay (ELISA) for C1q was therefore developed. F(ab´)2 fragments of in-house rabbit antihuman C1q at a concentration of 20 µg/mL in phosphate-buffered saline (PBS; pH 7.2) were adsorbed at a volume of 100 µL/well on Microtiter plates (Nunc

Maxisorp A/S, Nunc, Denmark) and incubated overnight at 4°C. The plates were then washed 3 times in the same buffer before incubation with samples serially diluted in PBS (pH 7.2) containing 0.05% Tween 20 (PBS-T) with 0.85 M NaCl and 1 mM EDTA. Volumes of 100 µL per well were added in duplicate to coated wells and to 1 uncoated control well. After incubation at room temperature for 2 hours, the wells were washed 3 times with PBS-T. Alkaline phosphatase–conjugated F(ab´)2 fragments of rabbit antihuman C1q were added (100 µL/well) and appropriately diluted in PBS-T with 0.85 M NaCl and 1 mM EDTA. After incubation for 1 hour at room temperature, the plates were washed 3 times with PBS-T and developed with 100 µL disodium-p-nitrophenyl phosphate (Sigma, St Louis, Missouri, USA) 1 mg/mL dissolved in 10% (w/v) diethanolamine (pH 9.8) containing 50 mM $MgCl_2$. Absorbance was measured at 405 nm, and values were calculated using duplicate wells with the absorbance of the control well subtracted. The C1q concentration was calculated from titration curves obtained with a normal serum pool. With this ELISA, the serum from the patient contained <0.1% of normal.

Discussion

A consistent clinical feature of hereditary C1q deficiency is increased susceptibility to infections with pyogenic organisms, thus reflecting an inability to activate the classic pathway and efficiently opsonize bacteria [7,8]. In the present case, selective C1q deficiency was associated with recurrent meningitis and pneumonia.

Deficiencies of all the classic pathway complement components are associated with SLE [9], although the strongest association is seen with C1q. Of the 41 C1q-deficient patients described in the literature up to the year 2000, 38 presented with SLE or SLE-like symptoms [10,11]. The basis for this predisposition is believed to be the result of defective immune complex clearance and/or deficient regulation of apoptosis [12], both of which events are at least partly dependent on C1q. Increased deposition of immune complexes and apoptotic material could promote the initiation of a local inflammatory response in C1q-deficient patients [3,10,13,14]. Other suggested explanations for the increased risk of development of SLE in C1q deficiency include impaired tolerance and aberrations in cytokine regulation [9]. However, the index case was negative for antinuclear antibody and rheumatoid factor, and at 5 years of follow-up she revealed no autoimmune symptoms.

C1q is composed of 3 polypeptide chains—A, B, and C—which are all products of the corresponding individual genes clustered in a 24-kb stretch of DNA on human chromosome 1 [15]. Mutations have been identified in each of the 3 chains. Single base mutations leading to a termination codon, a frame shift, or an amino acid residue exchange were thought to be responsible for these defects. In patients in whom stop codons occur in any of the 3 genes, no C1q protein can be detected in serum. In patients in whom the point mutation leads to an amino acid exchange, dysfunctional low-molecular-weight forms of C1q can be identified [5,7,8,16-18]. Recently, Petry and Loos reported the presence of silent mutations (C1q A exon 2 Gly70 and C1q C exon 1 Pro14) in C1q genes from families with different types of known hereditary C1q deficiencies [5].

A point mutation in the codon for glutamine at position 186 of the A chain that led to a termination codon was first identified in members of a Gypsy family from the Slovak Republic by Petry et al [19]. The same mutation is common among C1q-deficient patients from the Mediterranean area and has been found in all previously reported cases of C1q deficiency from Turkey [10,11,16,18]. In our patient, exon-specific amplification of genomic DNA by PCR followed by direct sequence analysis showed a novel homozygous missense mutation causing a glycine-to-arginine exchange at codon 48 in the C1q C chain. This replacement of glycine in the collagen amino acid triplet probably prevents formation of the normal C1q molecule structure and explains the absence of secreted C1q. Four siblings and both parents were heterozygous for this previously undescribed mutation, and 1 sibling was homozygous for the normal C gene. No changes were observed in the A and B chains. No trace amounts of C1q could be detected in serum using a sensitive method. Thus, the mutation affected either the formation of intracellular C1q variant molecules or completely inhibited secretion of any formed C1q. This mutation represents the same class of C1q mutations previously reported in an Inuit family by Marquart et al [20], where missense mutations cause a lack of detectable C1q antigen in serum. In conclusion, we describe a child suffering from severe infections due to C1q deficiency caused by homozygous presence of a previously unreported C1q C chain mutation.

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