Molecular Analysis of Hereditary Deficiency of the Third Component of Complement (C3) in Two Sisters

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Abstract

We report two sisters with hereditary deficiency of the third complement component (C3) and a homozygous mutation at C3303G (Tyr1081Stop) of the gene. They developed systemic lupus erythematosus-like symptoms during adolescence. Their C3 were not detected in serum immunochemically. Their mother and a brother had half of the normal C3 levels and a heterozygous mutation in the same position. Western blot analysis of murine L-cells transfected with the mutant C3 cDNA showed no C3 protein, however mRNA was detectable using reverse-transcriptase polymerase chain reaction. To the best of our knowledge, this is the first report of C3 deficiency due to a stop codon in the gene.

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Key words: stop codon, systemic lupus erythematosus

Introduction

Inherited third component of complement (C3) deficiency is a rare disease, which is characterized by recurrent infections with pyogenic encapsulated bacteria and certain immune complex-related disorders including membranoproliferative glomerulonephritis, systemic lupus erythematosus (SLE), and vasculitis. Since 1972, over 20 inherited C3 deficiency cases have been described, however the responsible gene mutations, such as point mutation, gene deletions and splice site mutations in the C3 genome, have been reported in only a few of these families (1–3). We previously reported two sisters with hereditary C3 deficiency exhibiting SLE-like symptoms (4). To clarify the molecular mechanism in these sisters, we analyzed the sequence of the C3 gene and the expression of the gene in culture cells.

Patients' Report

The details of clinical and laboratory findings in this family have been reported previously (4). Briefly, patient 1 was a 36-year-old woman born by normal delivery after an uneventful pregnancy. During her early childhood, she was in good health and showed no signs of proteinuria or hypertension. However, at 16 years of age, she developed SLE-like symptoms. The diagnosis of C3 deficiency was made at the age of 19, due to the absence of C3 as determined immunochemically (Table 1). However, a slight amount of C3 activity as well as total complement activity was present. She developed high fever, butterfly rash, Raynaud's phenomenon, intermittent arthralgia and photosensitivity. Patient 2 was a 34-year-old woman (the younger sister of patient 1) with an almost identical clinical course and symptomatology. She was diagnosed with C3 deficiency at 14 years of age. The patients were treated with corticosteroid hormone with relatively good control of symptoms. Both the mother and brother were in good health despite their C3 concentrations being approximately half of normal serum C3 levels as determined immunochemically.

Sequence Analysis of C3 and Expression Study of C3 Protein

Methods

cDNA synthesis and sequence

First strand cDNA was obtained by using RNase H⁻ reverse transcriptase (SuperscriptTM preamplification system for first strand cDNA synthesis, Invitrogen, Groningen, The Netherlands) from Epstein-Barr virus transformed lymphoblasts. The cDNA of C3 was amplified and its sequence determined by direct sequencing of both sense and antisense strands with ABI PRISMTM 310 Genetic Analyzer using the fluorescence-labeled primers.

Genomic DNA

Genomic DNA was isolated from whole blood obtained from

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Table 1. Complement Component Profiles in the Sisters with C3 Deficiency (4)

	Patient 1	Patient 2	Pooled normal human serum
Hemolytic activities			
CH50 (μ/ml)	6.3	5.8	40.0
C1 (SFU/ml)	90,400 (87.9%)*	100,000 (97.3%)*	102,800
C4	26,400 (39.8%)	26,400 (39.8%)	66,400
C2	430 (43.9%)	380 (38.8%)	980
C3	320 (0.7%)	270 (0.6%)	47,000
C5	229,000 (110.1%)	229,000 (110.1%)	208,000
C6	7,900 (148.0%)	7,200 (134.8%)	5,340
C7	22,000 (183.3%)	19,800 (165.0%)	12,000
C8	22,400 (81.8%)	21,800 (79.6%)	27,400
C9	29,600 (197.3%)	30,000 (200.0%)	15,000
Protein concentrations			
C1q**	141	141	
C4	11.7	11.7	
C3	ND	ND	
C5	110	130	
Factor B	24.9	25.9	
C1-inactivator	115	119	
C3b-inactivator	100	110	

*Numbers in parentheses are % of pooled normal human serum (NHS). **Normal ranges: C1q=60–130% NHS; C4=20–50 mg/dl; C3=60–110 mg/dl; C5=85–125% NHS; factor B=13–23 mg/dl; C1-inactivator=75–145 %NHS; C3b-inactivator=60–140 %NHS. SFU: site forming units, ND: not detected.

the patients, the mother and the brother in order to compare and confirm the sequence of *C3*. Also, genomic DNA isolated from 50 healthy volunteers served as controls after informed consent for participation in this study had been obtained.

Construction of full-length normal and mutant *C3* cDNA

Human liver cDNA library (Takara Shuzo, Tokyo) was screened by colony hybridization and full-length *C3* cDNA was selected. The *C3* cDNA was subcloned into pAP3neo vector (Takara Shuzo, Tokyo) using *NotI* restriction site. We amplified the cDNA of the present cases including the proposed mutation site and ligated the amplified product into the full-length *C3* cDNA clone using *SfiI* and *NarI* restriction enzymes.

Cell culture and transfection

The subclone of murine L-cell used in this study expressed no endogenous *C3* protein or mRNA (data not shown). Murine L-cells were cultured in 5%CO₂ incubator at 37°C in Dulbecco's modified Eagle's medium (Nissui, Tokyo) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Murine L-cells were stabilized and transfected with 5–35 μg of *C3* plasmid DNA together with *C3* mutant and Lipofectin reagent (Invitrogen, Groningen, The Netherlands) according to the manufacturer's protocol. Three days after transfection, selection was started with neomycin (500 μg/ml, Life Technologies) and indepen-

dent clones were randomly chosen after 3 weeks of selection.

Expression of *C3* protein and mRNA

Murine L-cells, transfected with normal and mutant *C3* cDNA, were harvested and the expression of *C3* proteins was examined by Western blot method using a monoclonal antibody for the β-chain of *C3* (Progen, Heidelberg). Expression of normal *C3* and mutant *C3* mRNA were investigated by the reverse-transcriptase polymerase chain reaction (RT-PCR) method using the primers 5'-ATGGGACCCACCTCAGGTC-3' and 5'-CACCTTCTCCACCACTTGGG-3' according to the standard protocol.

Results

By sequencing the cDNA obtained from the Epstein-Barr virus transformed lymphoblasts, we found a homozygous point mutation (exon 26, C 3303 G, Try 1081 Stop) in the *C3* gene of the two sisters and a heterozygous mutation in their mother and brother. This mutation, which generated a new recognition site for a restriction enzyme *BlnI*, was confirmed by sequencing genomic DNA isolated from whole blood using primers, 5'-CTGCAGGGTACACCCAGCAG-3' and 5'-TTCTTGGTG-TATCACGGGCG-3', corresponding to exon 25 and exon 26, respectively. Using the primers described above, the PCR products of the patients, mother, brother and normal controls were

Figure 1. Sequence and restriction enzyme digestion analysis of C3 gene in patients with C3 deficiency. Upper panel. The direct sequence (reverse sequence) of the gene showed a homozygous point mutation (exon 26, C 3303 G, Tyr 1081 Stop) in the two sisters (A) and heterozygous mutation in their mother and a healthy brother (B). This substitution generated a new *BlnI* recognition site (right arrow). Lower panel. PCR products of C3 were digested with *BlnI*. The digestion products of the patients showed two DNA fragments (412 bp and 147 bp, arrows), while those of the mother and brother showed three DNA fragments. However, the PCR products of healthy volunteers remained undigested. +: digestion with the enzyme, -: no digestion with the enzyme.

digested by *BlnI*. The digestion products of the patients showed two DNA fragments (412 bp and 147 bp) while those of the mother and brother showed three DNA fragments. However, the PCR products of healthy volunteers remained undigested (Fig. 1). To evaluate the expression of the mutant C3 cDNA, we constructed full-length normal and mutant C3 cDNA and transfected into murine L-cells. After selection with neomycin, murine L-cells transfected with normal or mutant C3 cDNA were harvested and analyzed by Western blot method. Although murine L-cells transfected with mutant C3 did not produce C3 protein, a low level of mRNA was detectable (Fig. 2).

Discussion

Since the first case of inherited C3 deficiency described in 1972 (5), over 20 cases, including our original report, have been reported with a variety of ethnic and national origins (4).

The clinical features are characterized by recurrent infection and/or certain immune complex-related disorders, the pathomechanisms of which still remain to be identified (4–7). After the full genomic organization of the human C3 gene was characterized (8), the molecular mechanisms for C3 deficiency were then studied. Five families, including the present cases, were examined for the molecular defects causing C3 deficiency (Table 2). The C3 gene in two of the patients had a splicing defect. A G-A substitution of the 5' splice donor site of intron 18 was found in the first patient, which resulted in a 61 bp deletion of exon 18 (9). The second patient had a G-T substitution in the splice donor site of intron 10. This substitution resulted in exon 10 skipping during transcription and has been predicted to cause premature truncation of the C3 protein as a result of a frameshift mutation (10). A third patient had 800 bp deletion leading to the loss of exon 22 and 23 of the α -chain (11). Similarly, premature truncation of the protein has been

Figure 2. Expression study of C3 in murine L-cells transfected with wild and mutant types C3 cDNA. The L-cells transfected with mutant C3 cDNA did not produce C3 protein (A: Western blot analysis), however they expressed mRNA of C3 (B: RT-PCR analysis). C: control C3 protein (Chemicon International, Temecula, CA, USA), 1: murine L-cells transfected with normal C3 cDNA, 2: murine L-cells transfected with mutant C3 cDNA, 100 bp: 100 bp ladder marker.

Table 2. Reported Cases of Hereditary C3 Deficiency with Molecular Analysis

Author (ref #)	Patient	Gene abnormality	Symptoms
Botto M et al (9)	10 y.o. male	61 bp deletion (exon 18)	Recurrent otitis media, convulsion, erythema multiforme
Huang JL et al (10)	22 y.o. female	skipped exon 10 due to G-T substitution (intron 10)	Recurrent meningitis, pneumonia
Botto M et al (11)	34 y.o. female	800 bp deletion (exon 22 & 23)	Recurrent meningitis, facial rash
Singer L et al (12)	18 y.o. male	exon 13, G1705A (Asp549Asn)	Periorbital cellulitis, recurrent pneumonia
Present cases/2001	36 y.o. female	exon 26, C3303G (Tyr1081Stop)	SLE-like symptoms
	34 y.o. female	exon 26, C3303G (Tyr1081Stop)	SLE-like symptoms

predicted to be the result of a frameshift mutation. The fourth patient had a critical amino acid substitution resulting in a secretion defect. In this patient, an Asp549Asn substitution was thought to interfere with the secretion of the C3 protein (12). In the present study, a stop codon was detected in exon 26 in the sisters. Initially we expected this mutation to result in the

production of a truncated C3 protein, however the L-cells, transfected with mutant C3 cDNA, expressed no protein while mRNA was detected at low levels. We thought of two possibilities to explain why the C3 protein was not detectable while mRNA was present. The first is that due to the mutation, the C3 protein is unstable and is easily degraded, therefore it be-

comes undetectable. The second possibility is that due to the mutation, the three-dimensional configuration of the C3 protein is changed, therefore it becomes undetectable. In either case, we suggest that this unstable and fragile or unusual form of mutant C3 protein may be responsible for the SLE-like symptoms such as arthritis.

The present cases did not show recurrent infections such as those previously reported (1–3, 5–7). Kitamura et al clearly showed that C5 compensated for the genetic lack of C3 and served a protective function (13). It may be possible that the patients' adaptive immune system is able to compensate for defective innate complement activity as previously reported (11).

C3 deficiency has been identified in about 20 unrelated patients, however the responsible mutations have been identified in only five of the families, including our cases. Continued investigation of the molecular basis of C3 deficiency will provide useful information concerning the biosynthesis, processing, and structure of the C3 protein as well as insight into the function of C3 and its products in the immune system.

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