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Molecular analysis of a novel hereditary C3 deficiency with systemic lupus erythematosus

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Abstract

A case of inherited homozygous complement C3 deficiency (C3D) in a patient with systemic lupus erythematosus (SLE) and the molecular basis for this deficiency are reported. A 22-year-old Japanese male was diagnosed as having SLE and his medical history revealed recurrent tonsillitis and pneumonia. He was diagnosed as having C3D because of undetectable serum C3 level. His parents were consanguineous. Sequence analysis of C3D cDNA revealed a homozygous deletion of exon 39 (84 bp). A single base substitution (AG to GG) in the 3′-splice acceptor site of intron 38 was identified by sequencing the genomic DNA. Expression of C3Δ(ex39) cDNA, the C3cDNA lacking exon 39, in COS-7 cells revealed that C3Δ(ex39) was retained in endoplasmic reticulum–Golgi intermediate compartment because of defective secretion. These data indicate that a novel AG → GG 3′-splice acceptor site mutation in intron 38 caused aberrant splicing of exon 39, resulting in defective secretion of C3.

Keywords: Human; Immunodeficiency diseases; Lupus; Complement C3; Molecular biology

The complement system is part of the innate immune system and plays an important role in host defense mechanisms. The complement system is activated via the classical pathway, which is initiated by antigen–antibody complexes, or by the mannose-binding lectin pathway, which is initiated by mannose on the surface of the bacterial wall. The complement system can be also activated via the alternative pathway, which is initiated by microbial surfaces [1]. Complement component C3 is the most abundant protein in the complement system and is also the most essential for the critical steps of the complement cascade, such as opsonization, generation of anaphylatoxins, and formation of the membrane attack complex [2].

C3 is synthesized as a precursor form consisting of a single polypeptide with a molecular size of approximately 200 kDa. This is then processed by proteolysis to yield the mature protein, which consists of two disulfide-linked subunits, α and β, with a molecular size of 115 and 70 kDa, respectively. C3 protein is translated from a 5.2 kb mature mRNA that is transcribed from a 42-kb gene of 41 separate exons [3] on chromosome

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19 [4]. C3 is synthesized predominantly by hepatocytes and also by cells at extrahepatic sites including monocytes [5], fibroblasts [6], endothelial cells [7], and smooth muscle cells [8].

An inherited deficiency of C3 has been described in humans, dogs, and guinea pigs [2,9,10]. Each of the homozygous C3 deficient individuals suffers from recurrent systemic infections with encapsulated pyogenic bacteria, and some cases have additional immune complex-related disorders [2].

This paper reports a case of hereditary C3 deficiency (C3D) and its novel molecular basis in a patient with systemic lupus erythematosus (SLE). AG → GG point mutation was identified in the 3′-splice acceptor site of intron 38 causing exon-skipping of exon 39, resulting in impaired secretion of C3. SLE or SLE-like disease is a complication of hereditary homozygous C3D in Japanese individuals, but is rare outside Japan.

Case report

A 23-year-old Japanese male had suffered from photosensitivity, recurrent fever, and facial erythema from childhood. He had been hospitalized due to tonsillitis and recurrent pneumonia in his late teens. At the age of 20 years, he was diagnosed as having SLE at the Dermatology Department of Kyushu University Hospital based on the presence of a butterfly rash, photosensitivity, leukopenia, and positive antinuclear antibody (ANA). He was treated with 60 mg prednisone daily. He was subsequently admitted to Kyushu University Hospital due to steroid psychosis at 21 years of age. Laboratory findings at this time were as follows: ANA, titer 1/40; RAHA, titer 1/320; IgG, 1108 mg/dl (normal range 900–2300: IgG1 42.3%, IgG2 48.0%, IgG3 9.6%, and IgG4 <0.2%); IgA, 540 mg/dl (80–450); and IgM, 444 mg/dl (80–300). Anti-dsDNA and the LE-test were negative. HLA was typed serologically as A2, A33, B52, B44, DR9, and DR6. From the data of complement assay presented below, he was also diagnosed with inherited C3D.

Materials and methods

Complement assay. CH50 was determined by the method of Mayer [11]. Serum concentrations of complement proteins were measured by laser nephelometry. C3 hemolytic activity was measured as described previously [12]. C3 concentrations in the supernatants of transfected COS-7 cells were measured by ELISA as described previously [13].

Cultures of fibroblasts. Fibroblast cell lines were established from the patient as described previously [14]. Normal skin fibroblasts (NHSF46) were purchased from the RIKEN Cell Bank (Tsukuba, Ibaraki, Japan).

Preparation of RNA and DNA. Normal fibroblasts and C3D fibroblasts were stimulated with 10 ng IL-1β/ml (Sigma Chemical, St. Louis, MO), 20 h prior to the preparation of RNA. Total RNA was prepared from the normal and C3D fibroblasts as described previously [15]. DNA was prepared from peripheral blood mononuclear cells of the patient and a normal control as described previously [15].

Reverse transcriptase (RT)-PCR. The six pairs of primers used for this analysis were as follows: 5′-GCCTGCTGCTCCTGCTACTAA-3′ and 5′-GGTCTTCTGCTCGGAGGTTCT-3′ for fragment 1 (nt 83–1015, product size 933 bp), 5′-GGGAAGAAAGTGGAGGGAAC-3′ and 5′-GGGAAGAAAGTGGAGGGAAC-3′ and 5′-AAGGAAGGGATGAAGTCGGT-3′ for fragment 2 (nt 844–1646, 803 bp), 5′-GAGACCCTCAACGTCAACTTCC-3′ and 5′-CATGAGCTTCGTAGAGATTCCAT-3′ for fragment 3 (nt 1465–2433, 969 bp), 5′-TGTGGAACGTTGAGGACTTGAA-3′ and 5′-TGAGGTTGACAGCCAGAGAGAA-3′ for fragment 4 (nt 2375–3337, 963 bp), 5′-ACCGCCTACGTGGTCAAGGT-3′ and 5′-ACATAGTGGCATCCTGGTCTCC-5′ for fragment 5 (nt 3295–4261, 967 bp), and 5′-CAGGATGCCAAGAACACTATGA-3′ and 5′-CAAAGAACTCCAGACACGTGAG-3′ for fragment 6 (nt 4192–5117, 926 bp).

Primers were synthesized on the basis of the nucleotide sequence of C3 complementary DNA (cDNA) to overlap with each other and were used to amplify the entire coding region of C3 cDNA. A schematic representation of C3 cDNA along with the positions of the primers is shown in Fig. 1. Total RNA was reverse transcribed using the Super Script Preamplification System (Gibco-BRL, Rockville, MD) according to the manufacturer's instructions. PCRs were

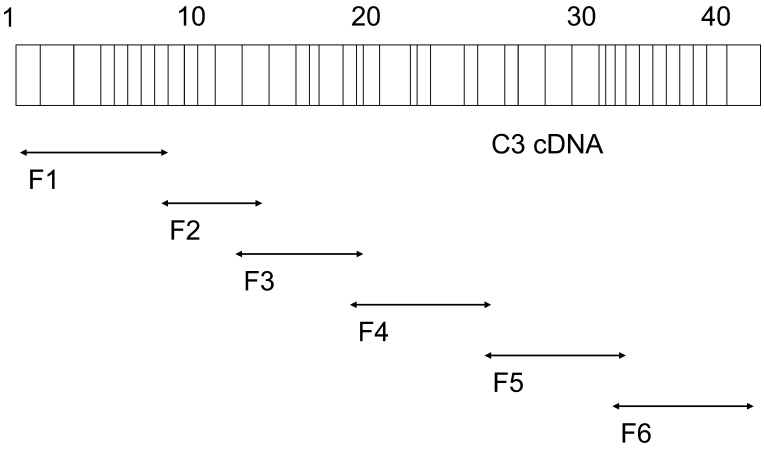

Fig. 1. Structure of C3 complementary DNA (cDNA) and location of the primers for PCR, which split C3 cDNA into six fragments. F, fragment.

conducted for 40 cycles consisting of 0.5 min at 95 °C, 1 min at 58 °C, and 1.5 min at 72 °C using TAKARA Ex *Taq* (Takara Shuzo, Tokyo, Japan). PCR products were subjected to electrophoresis on 1% agarose gel.

Nucleotide sequencing. Fragment 6 was purified using RECOCHIP (Takara Shuzo) and directly sequenced using an Amplicycle Sequencing Kit (Applied Biosystems, Foster City, CA) and radiolabeled primers according to the manufacturer's instructions. Primers were labeled with T4 polynucleokinase (New England Biolabs, Beverly, MA) and [γ-^{32}P]dATP(ICN Radiochemicals, Irvine, CA) at 37 °C for 20 min. PCR products of genomic DNA were fluorescein-labeled with a BigDye terminator cycle sequencing kit (Applied Biosystems) and sequenced with an ABI PRISM 310 genetic analyzer (Applied Biosystems).

Construction of full-length C3D cDNA. Wild-type (wt) C3 cDNA (from Dr. David E. Isenman, Toronto University School of Medicine, Toronto, Canada) was cloned into an expression vector pcDNA3 (Invitrogen, Carlsbad, CA). The portion of C3D cDNA which included exons 38 and 40 was amplified by RT-PCR and recombined with wt C3 cDNA at the restriction site of *Sfi*I and *Xba*I. The pair of primers used was: 5′-ACCGCCTACGTGGTCAAGGT-3′ and 5′-GC TCTAGATATAACTGAAGCTTTATCTGG-3′. The constructed C3DcDNA was named C3Δ(ex39) cDNA.

Transfection, immunoprecipitation, and Western blot analysis. COS-7 cells were grown to 70–80% confluence in 25 mm petri dish at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (Gibco-BRL) with 10% fetal bovine serum. Wt C3 or C3Δ(ex39) cDNA ligated in the expression vector pcDNA3 was transfected into COS-7 cells using lipofectamine plus reagent (Gibco-BRL) according to the manufacturer's instructions. Supernatants were harvested 48 h after transfection. The cells were detached by trypsinization, lysed in 40 μl of 1× SDS–PAGE sample buffer consisting of 62 mM Tris–HCl (pH 7.5), 2% SDS, 1 mM EDTA, 10% glycerol, and 5% mercaptoethanol, and sonicated with Sonifier 250 (Branson Ultrasonics, Danbury, CT). The supernatants were incubated with rabbit anti-human C3 antibody (MBL, Nagoya, Japan) overnight at 4 °C. Protein A–Sepharose was added and incubated for 3 h. Immunoprecipitates were washed and redissolved in 40 μl of 1× SDS–PAGE sample buffer. The samples from the lysates and supernatants were loaded onto 7.5% SDS–polyacrylamide gel. After SDS–PAGE, separated proteins were electrotransferred onto nitrocellulose filters (Bio-Rad Laboratories, Hercules, CA) by the semidry method. The membranes were incubated for 1 h in PBS containing 5% non-fat milk. After washing with 0.05% Tween 20 in PBS (T-PBS), goat anti-human C3 antibody (MBL) was added and incubated overnight at 4 °C. After washing with T-PBS, peroxidase-conjugated anti-goat IgG (MBL) was added and incubated for 2 h at room temperature. The nitrocellulose membranes were then washed in T-PBS and binding of the second antibody was visualized with the enhanced chemiluminescence system (ECL; Amersham, Arlington Heights, IL).

Subcellular localization of of C3Δ(ex39) and wt C3 in transfected COS-7 cells by immunofluorescence. Single label immunofluorescence was used to investigate the subcellular distribution of wt and mutant C3. COS-7 cells were cultured on glass coverslips and transfected as described above. They were fixed 48 h later in 75% methanol/25% acetic acid at −20 °C for 30 min followed by postfixation with 3% formaldehyde in PBS for 15 min at room temperature. After coverslips were blocked with 1% BSA in PBS for 10 min, the cells were incubated with FITC-conjugated goat anti-human C3 (MBL) for 30 min at room temperature. Following staining, the cells were counterstained for 3 min with the DNA dye, Hoechst 33258 (20 mg/ml in PBS, Sigma Chemical), rinsed with PBS, and mounted on glass slides in Permount (Fisher Scientific, Pittsburgh, PA). Slides were examined on fluorescence microscope (Carl Zeiss, Thornwood, NY) equipped with imaging system (Axio imager, Carl Zeiss).

Statistics. The Wilcoxon signed rank test was used to determine statistical significance.

Results

Complement assay and family study

No CH50 or C3 protein was detected in the proband. Other complement components were within the normal range. A family study revealed that the parents of the proband were consanguineous (Fig. 2). The parents and two of the patient's three brothers had 50% of the normal serum C3 concentration. The proband had only a trace amount of C3 hemolytic activity, and his parents and one of his brothers had 50% C3 hemolytic activity (Table 1). These results show that the proband had a hereditary homozygous C3D, and his parents and two of his brothers had heterozygous C3D.

RT-PCR analysis of C3mRNA

To study the molecular basis of this C3D, total RNA was prepared from IL-1β-stimulated primary fibroblasts obtained from the C3D patient. C3 mRNA from the C3D patient and a normal control were amplified in six fragments by RT-PCR as described. As shown in Fig. 3, there was no difference in the sizes of fragments 1–5 between the patients with C3D and the normal con-

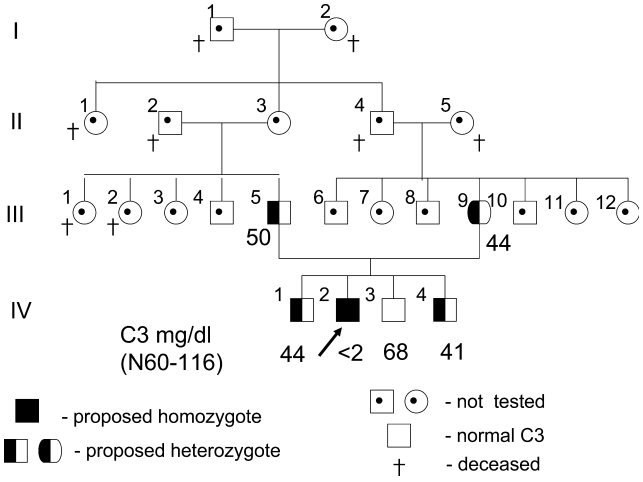

Fig. 2. Pedigree of the family with C3 deficiency. The proband is indicated by the arrow.

Table 1
C3 hemolytic activity of the proband, his parents, and his younger brothers

	C3 hemolytic activity (SFU/ml)	% of normal human serum (NHS)
Proband	24	0.1
Father	10,470	52.1
Mother	8660	43.1
Brother 1	10,100	50.2
Brother 2	13,720	68.3
NHS	20,100	100

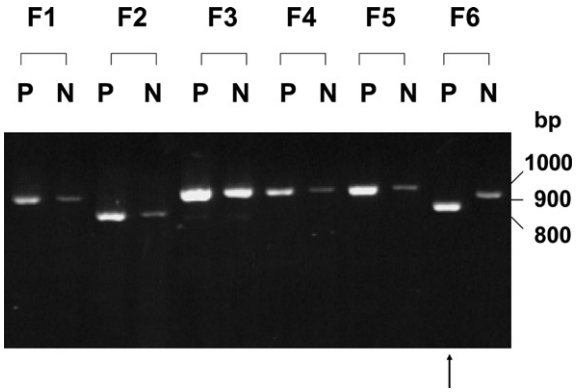

Fig. 3. RT-PCR analysis of C3 cDNA. Fragment 6 (arrow) of the patient with C3 deficiency (C3D) was 80–90 bp smaller than that of the normal control. F, fragment; P, C3D; and N, normal control.

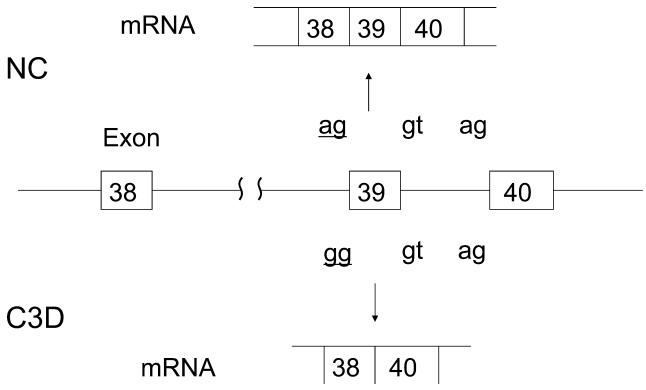

Fig. 5. Molecular basis for the C3 deficiency. A single base substitution (AG → GG) in the 3′-splice acceptor site of intron 38, causing skipping of exon 39, was identified.

trol. However, the size of the fragment 6 (nt 4192–5117) of the C3D patient was 80–90 bp smaller than that of the normal control. Since fragment 6 of the patient with C3D migrated as a single band, this deletion was considered to be homozygous.

Sequence analysis of C3 cDNA and genomic DNA

Sequence analysis of fragment 6 of the patient with C3D revealed a homozygous deletion of exon 39 (84 bp, Fig. 4). To determine the mechanism of this deletion in C3cDNA, C3 genomic DNA between exons 38 and 40 was amplified by PCR and directly sequenced (Fig. 5). The sequence of the splice acceptor site in intron 38 was changed from AG to GG. This mutation caused use of the splice acceptor site of intron 39 instead, resulting in skipping of exon 39. Since the deletion of exon 39 resulted in a 84 bp deletion in-frame, a 28 amino acid shorter mutant of the C3 polypeptide should be translated.

Expression of C3Δ(ex39) and wt C3 cDNA in COS-7 cells

To determine whether the deletion of exon 39 in C3 mRNA caused the C3D or not, C3 cDNA lacking exon 39, named C3Δ(ex39) cDNA, was constructed from wt C3 cDNA. C3Δ(ex39) and wt C3 cDNA were transiently transfected into COS-7 cells using the expression vector pcDNA3. The concentration of C3Δ(ex39) measured by ELISA in the supernatants of transfected COS cells was extremely and significantly low (mean ± SEM; 2.3 ± 0.5 ng/ml) compared with that in wt C3 (120.2 ± 40.0 ng/ml, $p < 0.03$). Western blot analysis was used to investigate the level of expression and fate of C3Δ(ex39) (Fig. 6). Intracellular wt C3 was visualized as a single, 185-kDa polypeptide of pro-C3. Wt C3 in the supernatant consisted of two bands of α-chain (115 kDa) and β-chain (70 kDa). In contrast, intracellular C3Δ(ex39) was detected as a single, 180-kDa polypeptide corresponding to pro-C3. However, no extracellular C3Δ(ex39) was detected. C3Δ(ex39) was retained intracellularly because of defective secretion.

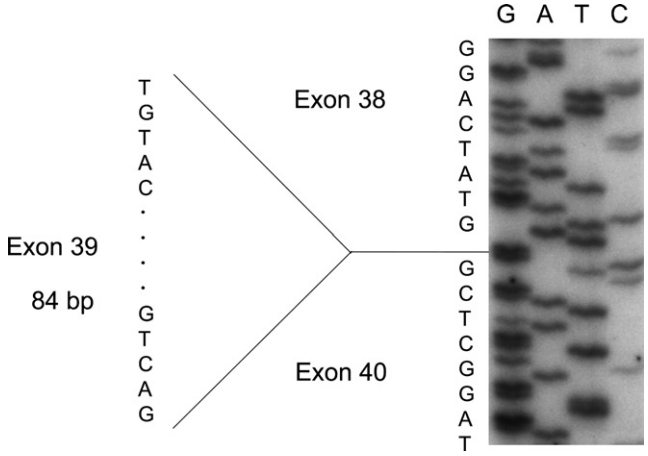

Fig. 4. Sequence analysis of fragment 6 of the patient with C3 deficiency.

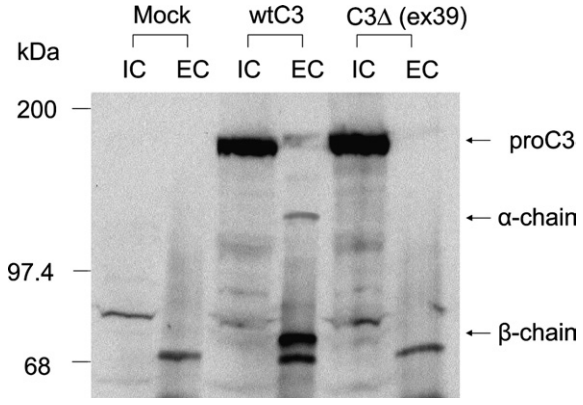

Fig. 6. Western blotting of wt C3 and C3Δ(ex39). IC, intracellular; EC, extracellular.

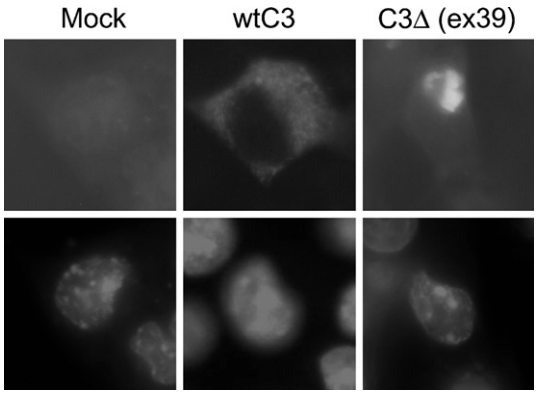

Fig. 7. Indirect immunofluorescence microscopy of transfected COS-7 cells. (Upper panel) COS-7 cells stained with anti-C3/FITC. (Lower panel) COS-7 cells counterstained with DNA dye. 1000× magnification.

These data indicate that an AG → GG 3′-splice acceptor site mutation in intron 38 caused aberrant splicing of exon 39, resulting in defective secretion of C3 in this case of C3D.

Intracellular distribution of C3Δ(ex39) and wt C3 by immunofluorescence

Indirect immunofluorescence staining of transfected COS-7 cells showed that wt C3 was predominantly distributed in a diffuse reticular pattern, typical of endoplasmic reticulum (ER) localization (Fig. 7). In contrast, C3Δ(ex39) was predominantly distributed in a punctate ring in the perinuclear area, which, as indicated by the previous study [16], corresponds to ER–Golgi intermediate compartment (ERGIC). Mock-transfected with vector alone showed only minimal background immunofluorescence. These results indicate that C3Δ(ex39) is arrested in ERGIC in the secretary pathway of C3.

Discussion

In this paper, a Japanese case of inherited homozygous C3D and the molecular basis for this deficiency are reported in a patient with SLE.

Hereditary homozygous C3D has been reported in 17 kindreds of different ethnic backgrounds and from different geographic regions [17]. Each of the homozygous C3 deficient individuals suffered from recurrent systemic infections with encapsulated pyogenic bacteria, and some of them had additional immune complex-related disorders, including membranoproliferative glomerulonephritis, IgA nephropathy, and maculopapular rashes [18]. Although SLE is recognized as an immune complex disease, C3D with SLE is not common outside Japan.

A novel AG → GG point mutation was identified in the 3′-splice acceptor site of intron 38 in this patient. The molecular basis for hereditary C3D has been identified in six kindreds including the present case. Other mutations were two 5′-donor splice site mutations (introns 10 and 18) [18,19], a large gene deletion [20], a single amino acid change (Asp549Asn) [21], and point mutations resulting in a termination codon (Tyr1081-Stop) [22]. All of the mutations were different, indicating that the molecular genetic basis for hereditary C3D is heterogeneous.

In the present case, exon 39 was spliced out in the mRNA of C3Δ(ex39) by the AG → GG substitution. C3D mRNA lacks the 84-bp region encoded by exon 39, which encodes a C3 polypeptide 28 amino acid shorter than normal C3. The polypeptide encoded by exon 39 is located near the C-terminus in the α-chain and does not involve the thioester bond, the cleavage site of C3 convertase, or factor I, disulfide bonds, or the glycosylation site. If C3Δ(ex39) is secreted, it should have C3 hemolytic activity.

The intracellular pathways of C3 synthesis, transport, post-translational modification, and secretion are largely unknown. During translation, the pre-pro-C3 molecules are processed into pro-C3, which is then properly folded and glycosylated [23]. Expression of C3Δ(ex39) cDNA in COS-7 cells revealed that the mutant protein, C3Δ(ex39), was retained intracellularly because of defective secretion. By immunofluorescence, the steady-state intracellular distribution of wt C3 was visualized as an ER localization as previously reported [24]. In contrast, C3Δ(ex39) was predominantly accumulated in ERGIC, a compartment localized between ER and *cis*-Golgi. In another case of C3D, secretion of C3 was disturbed by a single amino acid substitution, Asp549Asn, and mutant C3, C3 (Asp549Asn) showed perinuclear localization in transfected L-cells [21]. In contrast, wild-type C3 was distributed throughout the cytoplasm [21]. Pro-C3 is cleaved into C3 α and β chains by a dibasic directed proprotein convertase of the furin/PAGE family in the Golgi network [25]. C3Δ(ex39) was not cleaved into α- and β-chain because it was retained in ERGIC and was not transported to the Golgi complex. It should be noted that cleavage of pro-C3 to C3 α and β chains is not necessary for secretion and that the C3 processing in the Golgi complex is not involved in the quality control system [26]. C3Δ(ex39) may be trapped by an ER chaperone such as calnexin and BiP. Since normal C3 was shown to be bound to calnexin [27], C3Δ(ex39) is likely to be trapped by calnexin that was reported to be transported up to ERGIC. The incorrectly folded protein generally distributes in ER and the further studies on the mechanism for ERGIC localization of C3Δ(ex39) are warranted.

The strongest susceptibility genes for the development of SLE are null mutants of classical pathway complement proteins [17,28]. In addition, gene-targeted C1q-deficient mice also develop a lupus-like disease

[29]. Complement has both inflammatory and anti-inflammatory functions, the latter reflected by its role in clearing immune complexes. Complement also helps to eliminate apoptotic cells that are thought to be a major source of autoantigens in SLE. Deficiency of the early components of classical pathway causes the defect of the clearance of immune complexes and apoptotic cells, resulting in generating an inflammation and autoimmune response [28].

It is believed that there is a hierarchy of disease susceptibility according to the position of the missing protein in the activation pathway, with the strongest susceptibility linked to C1q deficiency [30]. This idea is supported by the observation that there is a similar hierarchical role for classical pathway complement proteins in vivo in the clearance of apoptotic cells by macrophages [30]. Gene-targeted C3-deficient mice do not develop lupus-like disease. Human hereditary homozygous C3-deficient individuals have less than three symptoms and signs of the criteria for SLE outside Japan although a patient is classified with SLE if he or she has four or more of the revised criteria for the classification of SLE [31]. In contrast, it is of interest that two of the five cases of Japanese homozygous C3D in three kindreds including the present case had four or more of the criteria for SLE and that the other two Japanese patients who were considered to have SLE-like disease had three of the criteria [32,33]. As shown in Table 2, the prevalence of SLE or SLE-like disease in hereditary homozygous C3D is as high as that in hereditary homozygous C1q deficiency among Japanese patients [34,35]. There is no correlation between the mutation sites of C3 gene and clinical appearance [18–22]. Two Japanese sisters with C3D, whose mutation was detected (Tyr1081Stop), developed facial erythema, photosensitivity, and arthralgia. Two other Japanese patients with C3D developed SLE without nephropathy and IgA nephropathy, respectively [32], however their mutation sites in the C3 gene were not determined. There may be a specific genetic background, which, combined with C3D, induces susceptibility to SLE in the Japanese population. In C1q-deficient mice, it was also demonstrated that background genes were a significant factor for the expression of autoimmunity [36]. Since C3D may cause the impairment of the clearance of apoptotic cells, it may induce the susceptibility to SLE when it combines with the other defects of the clearance of apoptotic cells, such as the defects of DNase1 and serum amyloid protein. However, there have been no genes identified to make Japanese people be easier suffering SLE than non-Japanese.

In conclusion, a case of Japanese hereditary homozygous C3D is reported in a patients with SLE. The point mutation at the 3′ splice site of intron 38 caused the deletion of 28 amino acids of the C3 polypeptide, resulting in impaired secretion of C3. SLE or SLE-like disease is a complication of hereditary homozygous C3D in Japan, but is not common outside Japan.

Table 2
Prevalence of SLE or SLE-like disease in hereditary homozygous complement deficiencies [33,34]

Type of complement deficiency	Prevalence of SLE or SLE-like disease	
	Non-Japanese	Japanese
C1q	92% (34/37)	100% (5/5)
C1r, C1s	54% (7/13)	50% (1/2)
C4	75% (18/24)	—
C2	32% (24/77)	—
C3	0% (0/19)	80% (4/5)[a]

[a] Including the present case.

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References

[1] M.J. Walport, Complement. First of two parts, N. Engl. J. Med. 344 (2001) 1058–1066.
[2] L. Singer, H.R. Colten, R.A. Wetsel, Complement C3 deficiency: human, animal, and experimental models, Pathobiology 62 (1994) 14–28.
[3] K.Y. Fong, M. Botto, M.J. Walport, A.K. So, Genomic organization of human complement component C3, Genomics 7 (1990) 579–586.
[4] A.S. Whitehead, E. Solomon, S. Chambers, W.F. Bodmer, S. Povey, G. Fey, Assignment of the structural gene for the third component of human complement to chromosome 19, Proc. Natl. Acad. Sci. USA 79 (1982) 5021–5025.
[5] L.P. Einstein, P.J. Hansen, M. Ballow, A.E. Davis, J.S. Davis, C.A. Alper, F.S. Rosen, H.R. Colten, Biosynthesis of the third component of complement (C3) in vitro by monocytes from both normal and homozygous C3-deficient humans, J. Clin. Invest. 60 (1977) 963–969.
[6] Y. Katz, R.C. Strunk, Synovial fibroblast-like cells synthesize seven proteins of the complement system, Arthritis Rheum. 31 (1988) 1365–1370.
[7] A. Ueki, T. Sai, M. Tabata, K. Hosokawa, Y. Mochizuki, Biosynthesis and secretion of the third component of complement by human endothelial cells in vitro, Immunology 67 (1987) 11–14.
[8] Y. Ueda, K. Nagasawa, H. Tsukamoto, T. Horiuchi, H. Nishizaka, K. Ikeda, Y. Niho, Production of the third and fourth component of complement(C3, C4) by smooth muscle cells, Immunology 89 (1996) 183–188.
[9] R. Ameratunga, J.A. Winklestein, L. Brody, M. Binns, L.C. Cork, P. Colombani, D. Valle, Molecular analysis of the third component of canine complement (C3) and identification of the mutation responsible for hereditary canine C3 deficiency, J. Immunol. 160 (1998) 2824–2830.

[10] H.S. Auerbach, R. Burger, A. Dodds, H.R. Colten, Molecular basis of complement C3 deficiency in guinea pigs, J. Clin. Invest. 86 (1990) 96–106.
[11] M.M. Mayer, Complement and complement fixation, in: E.A. Kabat, M.M. Mayer (Eds.), Experimental Immunochemistry, C.C. Thomas Publisher, Illinois, 1961, pp. 133–240.
[12] S. Inai, H. Kitamura, S. Hiramatsu, K. Nagaki, Deficiency of the ninth complement component in man, J. Clin. Lab. Immunol. 2 (1979) 85–87.
[13] H. Tsukamoto, A. Ueda, K. Nagasawa, Y. Tada, Y. Niho, Increased production of the third component of complement (C3) by monocytes from patients with systemic lupus erythematosus, Clin. Exp. Immunol. 82 (1990) 257–261.
[14] Y. Katz, R.C. Strunk, IL-1 and tumor necrosis factor. Similarities and differences in stimulation of expression of alternative pathway of complement and IFN-beta 2/IL-6 genes in human fibroblasts, J. Immunol. 142 (1989) 3862–3867.
[15] T. Horiuchi, K.J. Macon, V.J. Kidd, J.E. Volanakis, Translational regulations of complement protein C2 expression by differential utilization of the 5′-untranslated region of the mRNA, J. Biol. Chem. 265 (1990) 6521–6524.
[16] H. Tsukamoto, A. Tousson, R.B. Marchase, J.E. Volanakis, Down-regulation of secretion of human complement component C2 by the product of an alternatively spliced C2 messenger RNA, J. Immunol. 156 (1996) 4901–4908.
[17] L. Singer, H.R. Colten, R.A. Wetsel, Complement C3 deficiency: human, animal, and experimental models, Pathobiology 62 (1994) 14–28.
[18] J.-L. Huang, C.-Y. Lin, A hereditary C3 deficiency due to aberrant splicing of exon 10, Clin. Immunol. Immunopathol. 73 (1994) 267–273.
[19] M. Botto, K.Y. Fong, A.K. So, A. Rudge, M.J. Walport, Molecular basis of hereditary C3 deficiency, J. Clin. Invest. 86 (1990) 1158–1163.
[20] M. Botto, K.Y. Fong, A.K. So, R. Barlow, R. Routier, B.J. Morley, M.J. Walport, Homozygous hereditary C3 deficiency due to a partial gene deletion, Proc. Natl. Acad. Sci. USA 89 (1992) 4957–4961.
[21] L. Singer, W.T. Whitehead, H. Akama, Y. Katz, Z. Fishelson, R.A. Wetsel, Inherited human complement C3 deficiency. An amino acid substitution in the α-chain (ASP549 to ASN) impairs C3 secretion, J. Biol. Chem. 269 (1994) 28494–28499.
[22] W. Matsuyama, M. Nakagawa, H. Takashima, F. Muranaga, Y. Sano, M. Osame, Identification of a novel mutation(Tyr1081Ter) in sisters with hereditary component C3 deficiency and SLE-like symptoms, Human Mutat. 17 (2001) 79.
[23] J.D. Lambris, A. Sahu, R.A. Wetsel, The chemistry and biology of C3, C4 and C5, in: J.E. Volanakis, M.M. Frank (Eds.), The Human Complement System in Health and Disease, Marcel Dekker, New York, 1998, p. 83.
[24] H. Tsukamoto, A. Tousson, A. Circolo, R.B. Marchase, J.E. Volanakis, Calnexin is associated with and induced by overexpressed human complement protein C2, Anat. Rec. 267 (2002) 7–16.
[25] K. Oda, Y. Misumi, Y. Ikehara, S.O. Brennan, K. Hatsuzawa, K. Nakayama, Proteolytic cleavages of proalbumin and complement Pro-C3 in vitro by a truncated soluble form of furin, a mammalian homologue of the yeast Kex2 protease, Biochem. Biophys. Res. Commun. 189 (1992) 1353–1361.
[26] K. Oda, Calcium depletion blocks proteolytic cleavages of plasma protein precursors which occur at the Golgi and/or *trans*-Golgi network. Possible involvement of Ca(2+)-dependent Golgi endoproteases, J. Biol. Chem. 267 (1992) 17465–17471.
[27] W.J. Ou, P.H. Cameron, D.Y. Thomas, J.J. Bergeron, Association of folding intermediates of glycoproteins with calnexin during protein maturation, Nature 364 (1993) 771–776.
[28] M.J. Walport, Complement. Second of two parts, N. Engl. J. Med. 344 (2001) 1140–1144.
[29] M. Botto, C. Dell'Agnola, A.E. Bygrave, E.M. Thompson, H.T. Cook, F. Petry, M. Loos, P.P. Pandolfi, M.J. Walport, Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies, Nat. Genet. 19 (1998) 56–59.
[30] P.R. Taylor, A. Carugati, V.A. Fadok, H.T. Cook, M. Andrews, M.C. Carroll, J.S. Savill, P.M. Henson, M. Botto, M.J. Walport, A hierarchial role for classical pathway complement proteins in the clearance of apoptotic cell in vivo, J. Exp. Med. 192 (2000) 359–366.
[31] E.M. Tan, A.S. Cohen, J.F. Fries, A.T. Masi, D.J. McShane, N.F. Rothfield, J.G. Schaller, N. Talal, R.J. Winchester, The 1982 revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 25 (1982) 1271–1277.
[32] K. Imai, K. Nakajima, K. Eguchi, M. Miyazaki, M. Endoh, Y. Tomino, Y. Nomoto, H. Sakai, Y. Hyodo, Homozygous C3 deficiency associated with IgA nephropathy, Nephron 59 (1991) 148–152.
[33] Y. Sano, H. Nishimukai, H. Kitamura, K. Nagaki, S. Inai, Y. Hamasaki, I. Maruyama, A. Igata, Hereditary deficiency of the third component of complement in two sisters with systemic lupus erythematosus-like symptoms, Arthritis Rheum. 24 (1981) 1255–1260.
[34] M.C. Pickering, M.J. Walport, Links between complement abnormalities and systemic lupus erythematosus, Rheumatology 39 (2000) 133–141.
[35] H. Tsukamoto, T Horiuchi, Genetic susceptibility factors in systemic lupus erythematosus, Recent Res. Dev. Immunol. 4 (2002) 479–494.
[36] D.A. Mitchell, M.C. Pickering, J. Warren, L. Fossati-Jimack, J. Cortes-Hernandez, H.T. Cook, M. Botto, M.J. Walport, C1q deficiency and autoimmunity: the effects of genetic background on disease expression, J. Immunol. 168 (2002) 2538–2543.