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BRIEF REPORT

C1q deficiency: identification of a novel missense mutation and treatment with fresh frozen plasma

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Abstract A Turkish patient with C1q deficiency presented with a lupus-like disease, and a new missense mutation at A chain is presented. To characterize the genetic defect, all exons of the genes for the A, B, and C chains of C1q were sequenced in the patient. This revealed a missense mutation in the collagen-like domain of the A chain, p.Gly31Arg. No other sequence variants, including the common silent mutations, were found in the three chains. Exon 1 of the C1q A chain was sequenced in 105 samples from healthy controls for this particular mutation. None of these carried the mutation. The C1q-deficient patient was treated with fresh frozen plasma infusions. Our findings showed that Turkish patients may have different mutations than the previously described common mutation, and once again, not only nonsense mutations but also missense mutations cause hereditary C1q deficiency. Regular fresh frozen plasma infusions to the patient have been clinically and therapeutically successful.

Keywords C1q deficiency · Fresh frozen plasma · Missense mutation · Novel mutation · SLE · Treatment

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Introduction

The complement system is one of the key components of the immune system. The complement cascade is activated through the classical, alternative, and lectin-dependent pathways. C1q is the very first subcomponent of the classical pathway of the complement activation cascade. C1q is comprised of three different chains A, B, and C, and each contains six polypeptides. Overall, 3 chains and 18 polypeptides form a C1q molecule. C1q is associated with complement activation and furthermore facilitates the removal of immune complexes and necrotic and apoptotic cells, stimulates the production of some cytokines, and modulates the function of lymphocytes [1–4]. The A, B, and C chains of C1q are encoded by genes *C1qA, C1qB, and C1qC*, respectively. They are located on the short arm of chromosome 1 [1]. Few nonsense disease-causing mutations involving all three chains of C1q gene have been reported in hereditary C1q deficiency [5–9]. Later, it has been shown that not only nonsense but also missense mutations of C1q gene may also cause C1q deficiency [10–12]. Up to now, 12 disease-causing mutations in 68 patients have been reported [5–14] (Table 1). Hereditary C1q deficiency has been closely associated with recurrent bacterial infections and the development of systemic lupus erythematosus (SLE) [3, 5, 15]. In terms of treatment, these patients may benefit from regular plasma infusion, which helps in the transient normalization of C1q activity [13]. In this report, we described a Turkish case with a new missense mutation of C1q gene in the A chain who presented with SLE-like disease and benefited from fresh frozen plasma infusions.

Case report

A girl aged 6 years presented with fatigue, arthralgia, recurrent infections, and butterfly rash to a medical center, and

Table 1 Previously reported C1q mutations

C1q chain/exon	Mutation
C1qA/exon 2	Glu53fs
C1qA/exon 3	Gln64X
C1qA/exon 3	Gln108X
C1qA/exon 3	Trp216X
C1qB/exon 2	Gly42Asp
C1qB/exon 3	Arg177X
C1qB/exon 3	Gly244Arg
C1qC/exon 2	Gly34Arg
C1qC/exon 2	Gly55fsX83
C1qC/exon 3	Arg69X
C1qC/exon 3	Gly71fsX137
C1qC/exon 3	Gly76Arg

she was diagnosed with SLE and followed with steroids, azathioprine, and hydroxychloroquine for 9 years. Her parents were second-degree cousins. She had a sister and a brother. All the family members were healthy and have not complained of recurrent infection or SLE-like signs and symptoms. She presented to our center at age 15 years with photosensitive skin eruption, oral aphthous lesions, and Reynaud's phenomenon.

On physical examination, she was noted to have erythematous, desquamative skin lesions. Her blood pressure was normal, and the rest of the physical examination was unremarkable. Blood tests revealed hemoglobin, 11.5 g/dl; WBC, 6,200/mm^3; and platelets, 303,000/μL. Renal and liver function tests, direct Coombs test, anti-DNA, C3, C4 immunoglobulins, anticardiolipins, and antiphospholipis were normal or negative. ANA was positive with a titer of 1/160; furthermore, ENA Sm, ENA Sm RNP, ENA SSA were positive. Classical pathway hemolytic complement activity (CH50) measured by modified Mayer method [16] was zero, and no C1q was detectable by single radial immunodiffusion. With her clinical presentation and the complement assay, she was diagnosed with C1q deficiency, and the diagnosis is confirmed by genetic analysis.

DNA sequencing of the C1q genes and results

Genomic DNA was extracted from EDTA anticoagulated peripheral blood according to a standard method. The sequence of primers and the PCR conditions were set up according to the published data for the amplification of A, B, and C chain genes (C1QA, C1QB, and C1QC) of C1q [5, 7, 10]. All exons and the exon–intron boundaries of C1QA, C1QB, and C1QC were sequenced in all five members of the family. Additionally, in 105 healthy controls, exon 1 of the C1qA gene was sequenced. Sequencing was performed with BigDye Terminator sequencing kit 3.1 (PE Applied Biosystems, CA, USA).

The sequencing of all nine exons and splice sites of the C1q molecule revealed a missense mutation in the collagen-like domain of the A chain in the patient, c.91G>A (p.Gly31Arg). No other sequence variants were found in the three chains. The parents of the patient and her brother were found to be heterozygous for this mutation, while her sister was not carrying the mutation (Fig. 1a and b).

Heterozygous carriers (mother, father, brother) of the mutation were asymptomatic; their CH50 levels were normal. To explore whether the coding p.Gly31Arg mutation was seen in the normal population, exon 1 of the C1q A

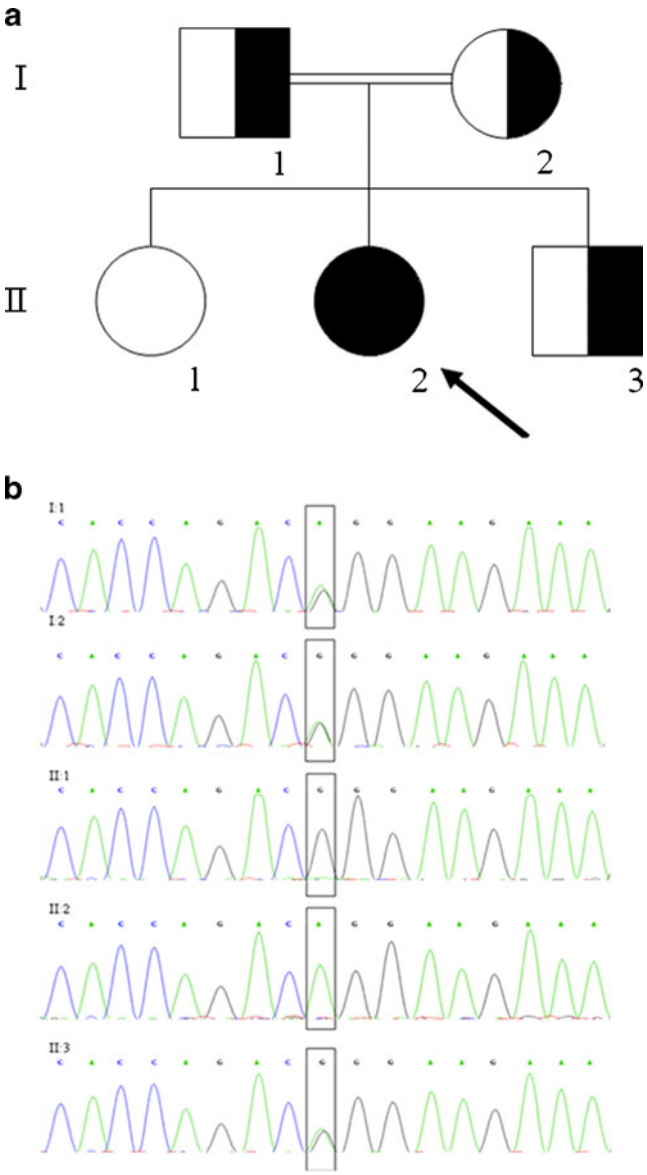

Fig. 1 Pedigree of the family. Circles represent females; squares, males. Half-solid symbols are heterozygous carriers of the mutation (**a**), extracts of DNA sequences from all family members are shown. Both parents and one of the children (II:3) are heterozygous carriers of the mutation. The proband have two mutant alleles. The sister of the patient (II:1) is healty (**b**)

chain was sequenced in 105 healthy controls. None of these controls carried the mutation.

In the absence of crystal structures for human or other mammalian species, we attempted to infer the relative impact that non-synonymous replacement may have by using a comparative protein-sequence approach. In order to accomplish this, homologous sequences representative of eukaryotic and prokaryotic species were extracted from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) database and aligned using PolyPhen2 software (http://genetics.bwh.harvard.edu/pph2/) (Fig. 2). We observed that the disease-associated glycine residue lies in a strongly conserved position. According to PolyPhen software, Gly31Arg mutation was predicted to be probably damaging with a score of 0.999 (sensitivity, 0.11; specificity, 0.99).

Treatment and follow-up of the patient

Since, she was diagnosed with SLE and followed with steroids, azathioprine, and hydroxychloroquine for 9 years elsewhere. At our center for the management and treatment of the patient, azathioprine was stopped, and she continued to have hydroxychloroquine and low-dose prednisolone (10 mg/day). Furthermore, fresh frozen plasma infusions are commenced. During follow-up, she highly benefited from the fresh frozen plasma transfusion. She followed with the CH50 level (normal, >15 units/ml) after plasma infusion. While her CH50 levels was 0, right after plasma infusion the CH50 level was 14 units/ml, it rose up to 24 units/ml on the 1st day and decreased to 0 on the 7th day. According to CH50 levels, she seemed to need fresh frozen plasma infusions every week, but her skin lesions were under control up to 3 weeks. She was scheduled to receive 2 units of plasma every 3 weeks. Eventually, hydroxychloroquine and prednisolone were stopped. She is fine only with the plasma infusions for every 3 weeks for the last 3 years.

Discussion

Thus, all previously published Turkish patients with C1q deficiency were homozygous for the same nonsense mutation, C to T change at codon 186 in the A chain (Gln208X); a recently reported new Turkish case had a novel homozygous missense mutation in the C1qC chain (Gly76Arg) as a disease-causing mutation [5, 7, 9, 12]. Genetic analysis of our case also revealed a different mutation from the common mutation. She was homozygous for a novel missense mutation in the C1qA chain gene. With the recent report and our case, it was shown that there might be different disease-causing mutations including missense ones in the Turkish population. The novel p.Gly31Arg missense mutation described here is positioned in the start site of the collagen region. It is highly likely that the exchange of the nonpolar glycine to the hydrophilic, bulky, and positively charged arginine at this position represents a major alteration at the collagen-like amino-terminus of the Clq molecule. The interruption of the collagen-like triplet repeats in the A chain consequently disputes the heterotrimeric assembly of the C1q chains Therefore, the C1q deficiency in this patient may be caused by instability or reduced assembly of C1q because of the A chain Gly31Arg mutation.

Another aspect of the case was her clinic presentation. It was not severe but mild which could be related to her missense mutation. Previously reported cases with the nonsense mutation have had severe clinical disease with recurrent infections with encapsulated bacteria and lupus-like disease [5–10].

In general, for the management and treatment of the hereditary complement deficiencies, no specific treatment is available. Only supportive treatment such as preventing and treating the infections and skin lesions is offered [17, 18]. All routine vaccines are recommended in complement deficiencies. Meningococcal vaccine is recommended for patients with early or terminal complement component deficiencies and pneumococcal vaccines for deficiency of early components [17, 18]. As the more specific treatment modalities, gene treatment for the complement deficiencies could be a future approach, and bone marrow transplantation could be a choice of treatment for C1q deficiency with severe lupus [19]. On the other hand, fresh frozen plasma infusions are proposed and used for the replacement of complement components including in C1q-deficient patients [13, 17, 18]. We used fresh frozen plasma infusions in our patient. Another aspect of our case was her successful management and treatment. The disease activity of the patient was well controlled with regular plasma infusions that were thought to help in the short-lived normalization of C1q activity [13].

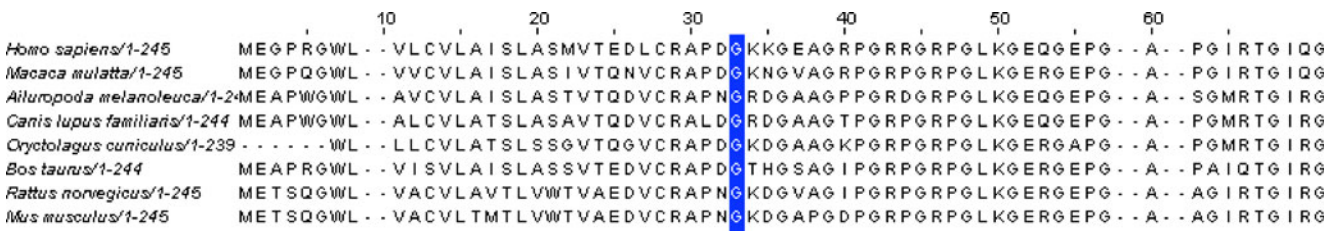

Fig. 2 Multiple sequence alignment of the first 70 residues of C1QA protein throughout evolution. Mutant residue was shown in blue line

Complement is critical for the physiological processing of immune complexes (IC). In the hereditary complement deficiency, reconstituting of the deficient complement component with FFP is likely to be at least in some extent temporarily restoring the biological role of complement in the processing of IC. This hypothesis could be supported by the reports showing while the IC uptake by the spleen is absent in complement C2 deficiency but it is restored after administration of FFP, and circulating IC levels fell in a C2-deficient patient following plasma infusion [13, 19–21]. While the amelioration of CH50 levels lasted for less than 48 h, symptomatic relief could be longer such as 3–4 weeks following plasma infusion. It is thought that the short-time normalization of complement activity may be sufficient to reduce circulating IC to a level that prevents tissue damage.

Administration of plasma every 3 weeks in our patient has been clinically successful over 4 years. Although this may be a valid treatment option in this patient, one has to be aware of the development of C1q antibodies. Our patient is an example of successful treatment of FFP.

Disclosures None.

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