Immunobiology 219 (2014) 241–246

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Short Communication

New C1q mutation in a Tunisian family

Hend Jlajla[a], Maryam Kallel Sellami[a,*], Imen Sfar[b], Lilia Laadhar[a], Yousr Zerzeri[a], Mohamed Slim Abdelmoula[c], Yousr Gorgi[b], Marie-Françoise Dridi[c], Sondes Makni[a]

[a] Laboratoire d'immunologie, Hôpital la Rabta, 1007 Tunis, Tunisia
[b] Laboratoire d'immunologie, Hôpital Charles Nicolle, 1007 Tunis, Tunisia
[c] Service de Pédiatrie, Hôpital La Rabta, 1007 Tunis, Tunisia

ARTICLE INFO

Article history:
Received 29 April 2013
Received in revised form 22 October 2013
Accepted 28 October 2013
Available online 6 November 2013

Keywords:
Autoimmunity
Classical pathway
Complement
C1q deficiency
Mutation
Primary immunodeficiency

ABSTRACT

Hereditary C1q deficiency (C1qD) is the most penetrant genetic factor predisposing to the development of lupus pathology with more than 93% of C1q deficient patients developing this autoimmune pathology throughout their life. It is a rare autosomal recessive deficiency, with only 67 cases reported so far including one Tunisian girl who died at the age of three from complications resulting from severe systemic lupus erythematosus. Although C1qD was confirmed in the serum of this patient using C1q ELISA and classical pathway specific functional assays, no DNA sample had been obtained from this patient. Here we report the analysis of sera and DNA of members of this patient's closer family. Our analysis identified a homozygous mutation within the gene encoding the C-chain of C1q leading to a deficiency of C1q in an older sister of our original patient. This mutation, termed g.5580G4C, represents a single basepair substitution in exon 1 of the C1q C chain gene which changes the codon of Gly61 to Arg 61. Amongst the other 14 mutations leading to C1qD, g.5580G4C represents the first reported transversion leading to human C1qD.

Introduction

The classical pathway of complement activation (CP) is the first of 3 different activation pathways of complement, a plasma based defense system bridging the innate with the adaptive immune response. While the CP is primarily initiated on antigen/antibody complexes formed during an immune response, the other two activation pathways, the lectin pathway (LP) and the alternative pathway (AP) can be initiated in absence of antibodies as components of the innate immune system (as reviewed by Schwaeble et al. 2002). The CP is initiated when C1, a multi-molecular complex (formed out to the 18-chain recognition subcomponent C1q (composed of a hexamer of heterotrimeric subunits C1qA, C1qB and C1qC) and a tetramer of the C1q associated serine proteases C1r and C1s) binds to immune complexes and perhaps other activating surfaces which in turn leads a conformational change within C1q that catalyses the subsequent enzymatic activations of the C1q associated serine proteases C1r and C1s (Gaboriaud et al., 2004; Wallis et al., 2010). Binding of the serine protease tetramer C1s-C1r-C1r-C1s, is strictly calcium-dependent. All activators of the classical pathway are recognized by the C1q subunit of C1, a process that generates a conformational signal that triggers self activation of C1r, which in turn activates C1s, the enzyme that mediates specific cleavage of C4 and C2, the C1 substrates (Arlaud et al., 2002).

Each of the six subunits of C1q is composed of a heterotrimer comprising three polypeptides chains A, B and C composed of either 223, 226 and 217 amino acids (AA) respectively, which share an identical domain structure, as previously described for all effector enzymes of the CP and the LP (Schwaeble et al. 2002; Wallis et al. 2010). Each of these chains has a short N-terminal region followed by a collagen-like region and a C-terminal globular region that form the globular C1q domain. Association between 2 heterodimers A–B and a homodimer C–C results in ABC–CBA structural unit with two heterotrimeric globular heads. Three of these structural units join in a bouquet of tulip-like structure of C1q (Kishore et al., 2004). These 3 chains are encoded by the *C1qA* (MIM*120550), *C1qB* (MIM*120570) and *C1qC* (MIM*12575) genes, respectively.

C1q is the only recognition subcomponent of the CP. Beside its role as activator of the CP, C1q is considered to be a critical scavenger molecule which may mediate the clearance of cellular debris and of apoptotic cells even in absence of complement activation via the binding to C1q/collectin receptors on phagocytes (Eggleton et al., 2000). Accordingly, *in vivo* and *in vitro* experiments demonstrated that C1q deficiency (C1qD) induced impaired clearance of apoptotic cells and exposition of nuclear autoantigens to autoreactive B and T cells, thus promoting the onset of autoimmunity.

Abbreviations: AA, amino acid; C1qD, C1q deficiency; SLE, systemic lupus erythematosus; LE, lupus erythematosus.

* Corresponding author. Tel.: +216 71578716; fax: +216 71564983.
E-mail address: maryam_kallel@yahoo.com (M.K. Sellami).

0171-2985/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.imbio.2013.10.010

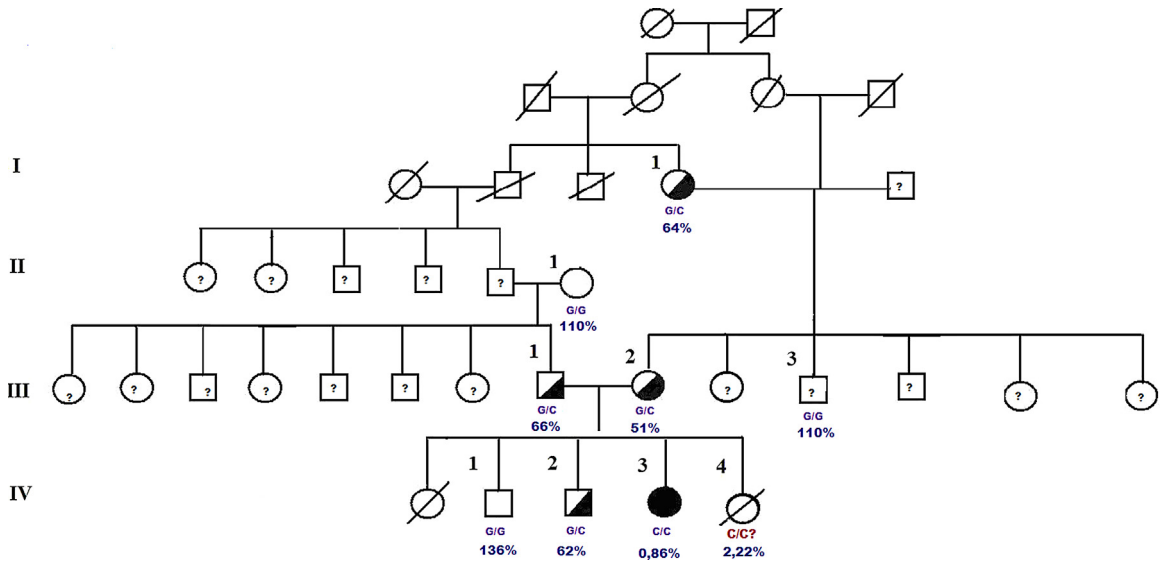

Fig. 1. Pedigree of the Tunisian family with genotypes and C1q level.

This mechanism explains the link between disorders involving the classical pathway and autoimmune diseases (Botto et al., 1998). In human systemic lupus erythematosus (SLE), all deficiencies of the classical pathway could precipitate the disease. Both, the severity and the strength of this association are greatest for homozygous hereditary C1qD. Approximately, 93% of individuals with C1qD have SLE or lupus-like illness (Pickering et al., 2000).

C1qD is an extremely rare autosomal recessively inherited complement deficiency with only 67 cases reported worldwide. Among these, we reported the case of a Tunisian girl, Sibling 1 (S1), with a severe SLE, who died by a severe digestive hemorrhage (Kallel-Sellami et al., 2007). Our study now presents to most likely same defect in the older sister, Sibling 2 (S2), who has developed subacute cutaneous lupus. We present the distribution of the underlying mutation leading to C1qD in homozygosity in this Tunisian family and provide a brief summary of all *C1q* molecular defects described so far on the background of their clinical impact.

Materials and methods

Patients and family members

Full clinical details and laboratory findings of S1 have been described previously (Kallel-Sellami et al., 2007). S1 was initially diagnosed as cutaneous lupus at the age of two. Transition to SLE was associated with arthritis and glomerulonephritis. High systemic corticosteroid treatment was started; however the patient died by a severe digestive hemorrhage at the age of three. A whole blood sample for DNA extraction was unfortunately not available for this patient.

S2 is a thirteen-year-old-girl. Her past history revealed recurrent respiratory infections. Physical examination showed photosensitivity and malar rash. A diagnosis of discoid lupus erythematosus was performed according to histological and direct immunofluorescence test of skin biopsy. Anti-nuclear antibodies (ANA) and anti-DNA autoantibodies were detected by indirect immunofluorescence (IIF) on HEp-2 cells and *Crithidia liciliae*, respectively. The presence of autoantibodies to extractable nuclear antigens (ENA) was determined by a commercial ELISA (BMD, Marne la Vallée, France). By IIF, ANA were positive at a titer of 1/100 with speckled fluorescence. Tests for anti-ds DNA and anti-ENA autoantibodies were negative.

Preliminary complement studies revealed an elevated 3rd and 4th complement components and a complete lack of hemolytic complement activity (CH50). Alternative pathway hemolytic activity was normal. Antigenic levels of classical complement proteins were as follows: C1q undetectable by ELISA (0, 86%), normal C1r and C1s levels by radial immuno diffusion. Cutaneous lupus complicating C1qD was therefore diagnosed. She was treated by anti-malaria drugs with rapid relief of symptoms.

The family was originated from Beja, Tunisia (north-west). Parents were first cousins. Seven family members participated in this study: the 2 parents, 2 grand-mothers, 1 maternal uncle and 2 brothers (Fig. 1). No similar disease was reported in family members except the history of siblings' sister who died at age of 6 months.

To investigate whether genetic defects, detected in S2, are mutations or polymorphisms, samples were also obtained from 100 Tunisian controls collected from healthy blood donors and healthy volunteer subjects.

After informed consent, EDTA whole blood was collected from relatives and controls. Plasma was immediately extracted and frozen at −80 °C. DNA was extracted and frozen at −20 °C.

Complement assay

Functional activities of the classical pathway (CH50), as well as C1 haemolytic activity, were measured according to standard procedures (Kazatchkine et al. 1985). Results were expressed as a percentage of standard human plasma pool. Serum concentration of C3 and C4 were determined by nephelometry using the MININEPH TM kit (The Binding Site, Birmingham, UK). Normal values were ranged between 0.74 and 1.62 g/l for C3 and 0.16 and 0.53 g/l for C4.

C1q concentration was assessed by double-ligand ELISA using a polyclonal sheep anti-C1q antibody (DAKO, Glostrup, Denmark) as described previously (Perissutti and Tedesco 1994). C1q level were expressed in percentage according to a standard curve containing 5 dilutions of a pool of 100 healthy subjects sera. Normal values range between 85 and 130%. C1r and C1s were measured by radial immunodiffusion (The Binding Site, Birmingham, UK). Normal threshold values were 34 μg/ml and 31 μg/ml, respectively.