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CHAPTER 7

COMPLEX MEDICAL HISTORY OF A PATIENT WITH A COMPOUND HETEROZYGOUS MUTATION IN *C1QC*

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ABSTRACT

Introduction: C1q is an essential part of the classical pathway of complement activation. Genetic deficiencies, caused by homozygous mutations in one of the C1q genes are rare and are strongly associated with development of Systemic Lupus Erythematosus (SLE). Here we describe a C1q deficient patient with a compound heterozygous mutation.

Material and methods: Serum was analysed with ELISA and western blot for the presence of C1q and DNA and RNA sequencing was performed to identify the mutations and confirm that these were located on different chromosomes.

Results: The medical history of the patient includes SLE diagnosis at the age of eleven with cerebral involvement at the age of thirteen, various infections, osteonecrosis and hemophagocytic syndrome. Using ELISA and western blot we confirmed the absence of C1q in the serum of the patient. Using DNA sequencing two mutations in the *C1QC* gene were identified: c.100G>A p.(Gly34Arg) ; c.205C>T p.(Arg69X). With RNA sequencing we confirmed that the mutations are located on different chromosomes.

Discussion: The patient described in this case report has a compound heterozygous mutation in *C1QC* resulting in C1q deficiency.

INTRODUCTION

The complement system consists of both soluble and membrane bound proteins. Activation can occur via three different pathways: the classical pathway (CP), the lectin pathway and the alternative pathway. The CP is activated when C1q is bound to IgM, immunocomplexes or among others pentraxins[1]. Many complement proteins are produced by hepatocytes, but also cells of the immune system produce several complement proteins[2]. Importantly, C1q is not produced by hepatocytes but largely by cells from the myeloid lineage. C1q has been described to have other functions outside of the complement system cascade: in the remodelling of the maternal decidua during pregnancy, during embryonic development, in neurological synapse function and in the coagulation process[3]. The C1q protein is a molecule of 480kDa which has six identical arms. Each arm consists out of three combined peptide chains: A, B and C. These peptide chains are generated from three different genes: *C1QA*, *C1QB* and *C1QC* which have a synchronized transcription[4].

C1q deficiency is a rare condition with just over 70 documented cases from at least 45 different families[5]. These deficiencies all are the result of homozygous mutations in one of the three C1q genes, except in one case with a compound heterozygous mutation in *C1QA*[6]. Patients with C1q deficiency have various clinical presentations and outcome[7]. Most common is the diagnosis of Systemic Lupus Erythematosus (SLE) in early childhood and recurrent infections[6-8]. Other common clinical manifestations are alopecia, Raynaud's phenomenon, involvement of the central nervous system, and also Sjögren's syndrome and Hyper IgM syndrome have been reported[5-7]. Treatment of C1q deficient patients mainly focuses on the treatment of the symptoms. For combating the C1q deficiency itself, intravenous administration of fresh frozen plasma (FFP) is used. In rare cases allogenic hematopoietic stem cell transplantation has been performed[9, 10].

Here we describe a patient with C1q deficiency based on a compound heterozygous mutation in the *C1QC* gene. This patient was treated with FFP for over a decade, over time it has resulted in various adverse reactions ranging from mild to anaphylactic which led to discontinuation of FFP therapy.

MATERIAL AND METHODS

Patient

The patient is a 29 years old Dutch woman, diagnosed with C1q deficiency since early childhood. Blood was obtained from the patient upon signing an informed consent in compliance with the Helsinki declaration.

Samples
Blood was collected from the patient in order to obtain serum as well as Peripheral Blood Mononuclear Cells (PBMCs) using Ficoll-Paque density gradient centrifugation.

Western blot
With western blot the availability of C1q was examined by detection of the three chains of the C1q protein. Serum of the patient and normal human serum (NHS), which was used as a positive control, were applied in reduced and non-reduced SDS conditions. The western blot was performed using previously described methods[11].

ELISA
C1q measurement by an in-house developed ELISA was performed as previously described[12]. In short, plates were coated with mouse anti-human C1q (2204), Nephrology department, LUMC) in coating buffer (0.1M Na_2CO_3, 0.1M $NaHCO_3$, pH9.6), samples were incubated at 37°C and detection was performed with rabbit anti-human C1q (Dako cat#A0136) for one hour at 37°C and subsequently a goat anti-rabbit HRP (Dako cat#P0448) which was also incubated for one hour at 37°C. The substrate was added to the plates using 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) and the signal was measured at an absorbance level of 415 nm using Biorad iMark Microplate Absorbance Reader.

RNA isolation and cDNA preparation
PBMCs (1 x 10^6 cells/ml) of the patient were cultured for 72 hours in RPMI (Gibco) culture medium supplemented with +1% Penicillin/streptomycin, +1%Glutamax and +8%FCS. PBMCs were either cultured in medium alone or in the presence of Dexamethason (10 µM, Pharmacy LUMC) and IFN-γ (200 U/ml, Peprotech), to increase *C1QA/B/C* expression[13]. After 72 hours RNA was isolated from the PBMCs using the mirVana miRNA isolation kit (Life Technologies cat# AM1561) according to manufacturer's protocol. The isolated RNA was subsequently treated with DNase I, Amplification Grade (Invitrogen) and cDNA was synthesized using superscript III (200U/µl, Invitrogen).

Ion Torrent sequencing
An Ampliseq™ custom panel (Thermo Fisher Scientific, Waltham, MA USA) was used to sequence the coding regions of the following genes: *C4A*, *C2*, *C1S*, *C1R*, *DNASE1L3*, *TREX1*, *MASP2*, *C4B*, *C1QA*, *C1QB*, *C1QC*, *PLG* and *SERPING1*. Library preparation and sequencing was performed according to manufacturer protocols on a S5 system (Thermo Fisher Scientific).

PCR
To confirm mutations found by Ion Torrent sequencing and to test whether the mutations found were on different alleles, mutation (allele) specific PCR was used on cDNA of the patient. We used the Rapid Cycler technology (BioFire Diagnostics, Salt Lake

City, UT USA) with 50 cycles of 5s at 95°C, 30s at 65°C and 60s at 72°C, in 15 µl of PCR buffer containing 2 U of Taq polymerase (Promega Benelux, Leiden, The Netherlands), 2 U of TaqStart antibody (Takara, Mountain View, CA, USA), 50 ng of each primer 200 µM for each of the dNTPs, 50 mM KCl, 1.5 mM MgCl2, 0.1% (v/v) Triton X-100, 10 mM Tris, pH 9.0 at 25°C, in 10-µl glass capillaries (BioFire Diagnostics). The *C1QC* primer sequences for cDNA are in **Table 1**.

Table 1. *C1QC* primer sequences

Name primer	sequence
C1QC-exon2-fw	GCCCCTCAGGGGCCAAGCCAACAC
C1QC -exon2-mut-fw	GGGCCAAGCCAACACAGGCTGCTTCA
C1QC -exon2-wt-fw	GGGCCAAGCCAACACAGGCTGCTTCG
C1QC -exon3-rev	GGTAAGCCGGGTTCTCCCTTCTGC
C1QC -exon3-mut-rev	TCCCTTCTGCCCTTTGGGTCCACA
C1QC -exon3-wt-rev	TCCCTTCTGCCCTTTGGGTCCACG

Sanger sequencing

PCR products from genomic DNA and cDNA were sequenced on an automated fluorescent sequencer (ABI 3730; Thermo Fisher Scientific) with the use of Big Dye Terminator (v.1.1) chemistry (Thermo Fisher Scientific). Primers used for sequencing were the same as those used for the PCR. For cDNA numbering, the A of the ATG translation initiation codon was taken as 1. This codon is codon 1. NM_172369.4 was used as reference sequence.

RESULTS

Patient medical history

Here we describe a Dutch woman born from two Caucasian non-consanguineous parents. She has two half-brothers who are reported to be healthy. C1q deficiency was diagnosed at the age of five. The patient has encountered many different clinical problems including infections, neurological-, vascular- and bone complications. The case will be presented per disease manifestation and not in chronological order. The use of FFP throughout her medical history will be discussed separately.

Infections

During the first year of her life she suffered from recurring otitis and gingivitis. She was reported to be a non-responder for Hepatitis B vaccination. Additionally, at the age of six she developed sepsis caused by *Streptococcus pneumoniae*. She experienced a herpes zoster infection when she was 12 years old. During adulthood, she was hospitalized with hemophagocytic lymphohistiocytosis (confirmed with a bone marrow biopsy) and pancytopenia which was potentially induced by co-trimoxazole. During this

hospitalization various infections were also diagnosed and treated accordingly; *Escheria coli* and candidiasis.

Systemic Lupus Erythematosus From the age of four, clinical symptoms were compatible with SLE-like disease, with butterfly rash. At the age of eleven, the diagnosis of SLE was established based on butterfly rash, oral ulcers, thrombocytopenia and positive antibodies (positive ANA, positive anti-Sm and positive anti SSA). Anti-dsDNA and antiphospholipid antibodies were not present. Furthermore, she experienced recurrent fevers with lymphadenopathy and vasculitis lesions of hand and feet. Initially, the SLE was treated with hydroxychloroquine and prednisolone, with serious side effects, including weight gain (Cushingoid), osteoporosis and bone infarctions. Furthermore, symptoms of fatigue, headache and arthralgia occurred during attempts to taper prednisolone treatment. On the basis of the diagnosis SLE in combination with the earlier established C1q deficiency, and both the side effects and the inability to taper prednisolone, treatment with FFP was initiated.

Cerebral involvement
When the patient was 14 years old she was hospitalized with a fever, paraesthesia and difficulties with speech. Infectious causes were excluded. MRI scans of the brain was normal. EEG showed left parieto-occipital irritative abnormalities. Liquor analysis revealed enhanced protein content without elevated cell count. With the working diagnosis of TIA / partial epileptic seizure due to cerebral vasculitis she was treated with prednisolone and carbasalate calcium. Subsequent visits at the age of 24, 25 and 28 at the Leiden University Medical Center multidisciplinary neuropsychiatric SLE (NPSLE) clinic [14] because of anxiety and difficulty in speech, did not reveal signs of active inflammatory NPSLE.

Vascular problems
At the age of 26 she developed a deep venous thrombosis, although no anti phospholipid antibodies were detected (ACA IgM/IgG, anti-B2GPI IgM/IgG and LAC). When the patient was 27 years old, she experienced a spontaneous abortion at a gestation of 8 weeks.

Bone lesions
At the age of nine she developed an avascular necrosis of the humerus which led to a destructed right shoulder. During the recent years she developed extensive bone infarctions around the knee. Because of the osteonecrosis she underwent total hip replacement surgery at the age of 29. Osteoporosis was identified already during childhood.

Therapy
Initially, prednisolone and hydroxychloroquine were used to treat the SLE. Because she was unable to taper the prednisolone and she was already diagnosed with

C1q deficiency, FFP treatment was started at the age of 11 with 15 mL/kg. The FFP infusions were administered 1-4 times a month and were preceded by clemastine and prednisolone intravenously. CP activity was measured preceding each FFP infusion. When FFP took place each week the CP activity was 80-90%, when the FFP was every two weeks the CP activity dropped below 50%, which was in line with previous reports[15]. Anti-C1q antibodies were detectable, though not increased. There were several adverse reactions to the FFP therapy, ranging from mild urticarial to anaphylactic reaction. Despite these adverse reactions, the patient preferred the FFP therapy, because of reduction of fatigue, arthralgia and number of infections. However, because of a serious anaphylactic reaction at the age of 25, FFP treatment was discontinued and her current treatment regimen consists of: hydroxychloroquine, azathioprine, low dose prednisolone, clopidogrel, bisphosphonates and cholecalciferol.

Complete absence of C1q in the serum

With western blot analysis sera of both the patient and normal human serum (NHS; pool of four healthy adults) were analysed for the presence of C1q. The same amount of serum was applied in native, denaturing or reducing conditions. Only in the NHS lane C1q was detected (**Figure 1 A-C**). Therefore, we confirmed the absence of circulating C1q in the serum of the patient. Additionally, patient serum was tested for C1q in ELISA format, next to 21 healthy female controls (age (26-32). The C1q levels in the healthy control had an average of 171 µg/mL C1q, while in the sera of the patient the C1q level was below the detection limit of 0,065 µg/mL (**Figure 1D**).

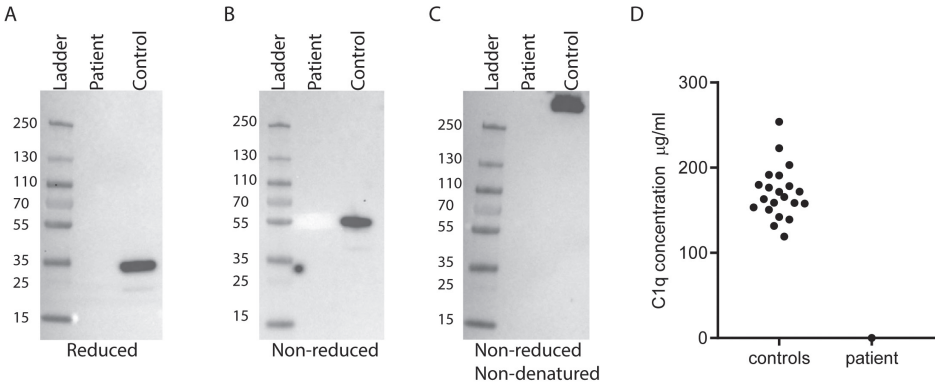

Figure 1. Western blot analysis shows no C1q in serum patient

Western blot analysis of serum from the patient and a control (normal human serum). The serum samples were either prepared under reducing condition (A), denaturing and non-reduced condition (B) or non-reducing and non-denaturing conditions (C). Measurement of C1q with ELISA in the sera of healthy female age matched controls (n=21) and the patient (D).

Sequencing

To determine what the mutation(s) are in this patient and where they are located, DNA and RNA sequencing was performed. Two previously described mutations were identified in the *C1QC* gene of the patient: c.100G>A p.(Gly34Arg) ; c.205C>T p.(Arg69X). With RNA sequencing we confirmed that both mutations were heterozygous, meaning these mutations are compound heterozygous (**Figure 2**).

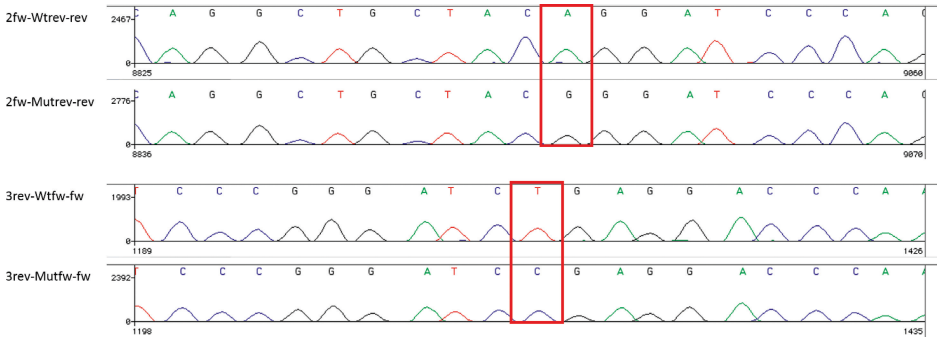

Figure 2. Compound heterozygous mutation located in *C1QC* gene

RNA sequence analysis revealed heterozygous mutations of C1QC highlighted by the red box: c.100G>A p.(Gly34Arg) ; c.205C>T p.(Arg69X).

DISCUSSION

C1q deficiency is a rare genetic disorder which is often accompanied with development of SLE. The patient described here was already diagnosed with both C1q deficiency and SLE in early childhood. She has suffered from recurrent infections, which is also a hallmark for C1q deficiency[12]. Another part of her medical history involves bone lesions. Interestingly, osteoclasts are able to produce and secrete C1q, which could be suggestive for a direct relation between C1q deficiency and development of bone lesions, although the function of C1q is unknown in this environment[16]. However, it is unclear and difficult to determine whether these bone lesions and recurrent infections are a consequence of the C1q deficiency, the SLE, the steroid treatment, or a combination of these factors. C1q is important in the clearance of apoptotic material and immune complexes. Next to activation of the CP of the complement system, C1q has various functions independent of CP activation[3].

Previously, 15 C1q deficient patients with NPSLE have been described in literature, with the most frequent presenting symptom being seizures (67%). which is much higher than observed in conventional SLE patients[12]. Cerebral vasculitis has also been reported in 27% of the C1q deficient NPSLE patients. The patient described here has experienced neurological symptoms in adolescence and was diagnosed as a TIA / partial epileptic seizure due to cerebral vasculitis. It is known that C1q inhibits interferon production.

In patients with C1q deficiency high interferon levels are observed in serum and cerebrospinal fluid. Recently, it was found that type I interferon stimulates microglia to engulf synaptic material, resulting in synaptic loss in the central nervous system [17, 18]. This could contribute to neuropsychiatric involvement in C1q deficiency.

This patient has been treated with FFP for almost 14 years. Shortly after infusion C1q levels reach their maximum and rapidly decline, the CP activity was sustained for a longer period of time. Even though the C1q levels and CP activity effects were relatively short lived, the symptomatic relief and substantial improvement in quality of life of the FFP treatment was sustained for various weeks. Empirically it had been established, for this patient, that two units of FFP every two weeks was most optimal. The FFP therapy has been accompanied by adverse events upon infusion, to even anaphylactoid reactions, although anti-C1q antibodies were not increased in this patient.

From the C1q deficient patients that have been described so far, all except one have been reported to have a homozygous mutation in on the C1q genes. Here, we report the second case of C1q deficiency with a compound heterozygous mutation, in this case located in *C1QC*: c.100G>A p.(Gly34Arg) ; c.205C>T p.(Arg69X).

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