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The role of C1q in (auto) immunity
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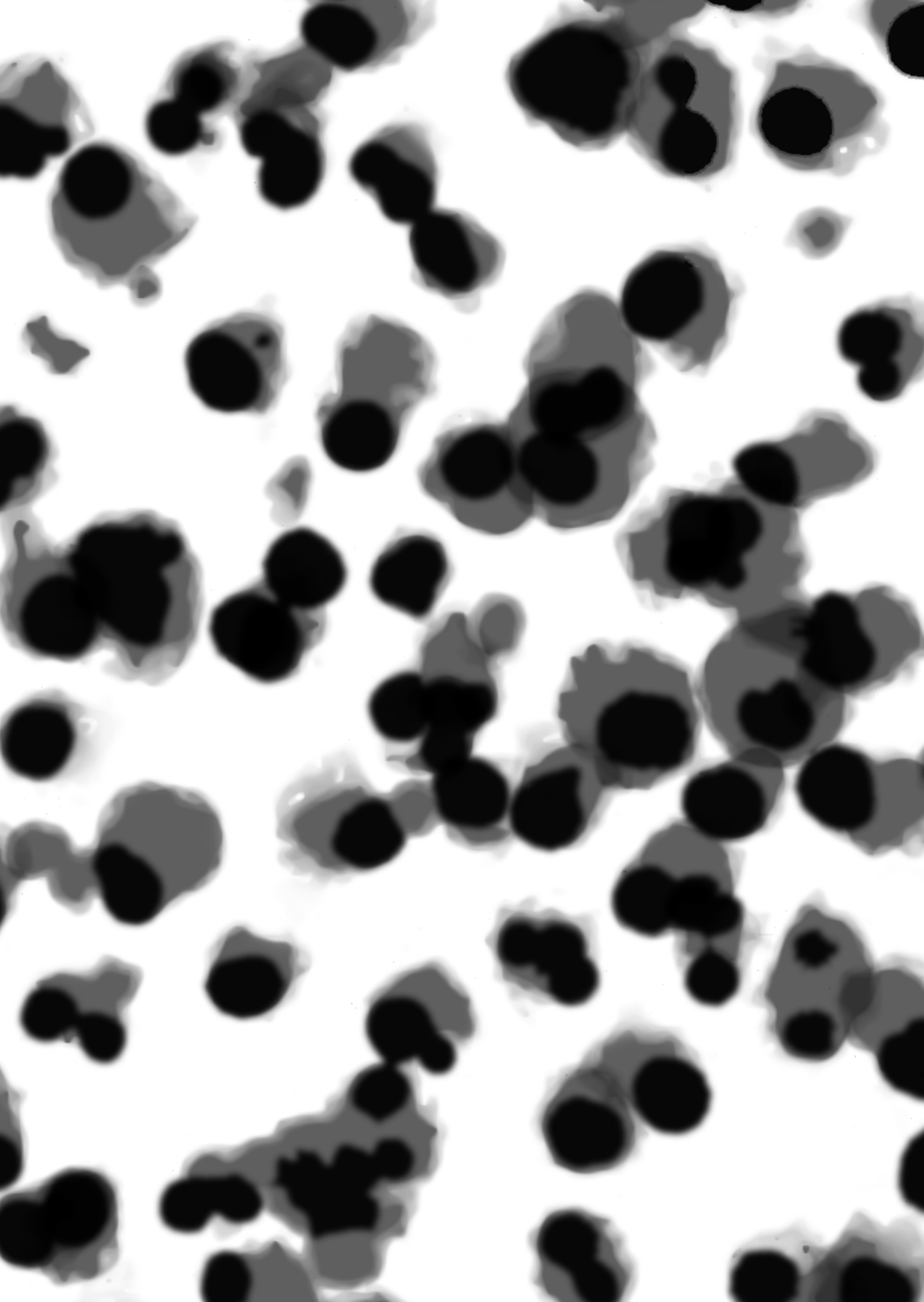
Part I

The clinical association of C1q and anti-C1q



Chapter 2

Identification of a novel non-coding mutation in C1qB in a Dutch child with C1q deficiency associated with recurrent infections

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Abstract

C1q deficiency is a rare genetic disorder that is strongly associated with development of Systemic Lupus Erythematosus (SLE). Several mutations in the coding regions of the C1q genes have been described that result in stop-codons or other genetic abnormalities ultimately leading to C1q deficiency. Here we report on a Dutch boy suffering from recurrent infections with a complete C1q deficiency, without any SLE symptoms.
The presence of C1q in serum was assessed using ELISA and hemolytic assay. By western blot we examined the different C1q chains in cell lysates. We identified the mutation using deep-sequencing. By qPCR we studied the mRNA expression of C1qA, C1qB and C1qC in the PBMCs of the patient.
Deep-sequencing revealed a homozygous mutation in the non-coding region of C1qB in the patient, whereas both parents were heterozygous. The mutation is located two nucleotides before the splice site of the second exon. In-silico analyses predict a complete abrogation of this natural splice site. Analyses of in vitro cultured cells from the patient revealed a lack of production of C1q and intracellular absence of C1qB in the presence of C1qA and C1qC peptides. Quantitative PCR analysis revealed total absence of C1qB mRNA, a reduced level of C1qA mRNA and normal levels of C1qC mRNA.
In this study we report a new mutation in the non-coding region of C1qB that is associated with C1q deficiency.

Introduction

C1q is the recognition molecule of the classical pathway of complement activation [1]. Next to its role in activation of the complement system, C1q has also been shown to bind to apoptotic and necrotic cells to facilitate their clearance [2, 3]. In the absence of C1q, accumulation of apoptotic material was noted, which led to the formulation of the 'waste disposal hypothesis'[4, 5]. Likewise, in the absence of C1q also immune complexes cannot be cleared effectively resulting in their accumulation. Over the recent years more insight has been generated in the role of C1q in modulating the adaptive immune response [6-8]. Collectively these data indicate that absence of C1q may not only impact on clearance mechanisms for e.g. apoptotic cells and immune complexes, but also on the adaptive immune responses [9]. In most C1q deficient individuals the balance is shifted towards autoimmunity and the development of Systemic Lupus Erythematosus (SLE). Complete genetic

deficiency of C1q is strongly associated with development of SLE, but in some individuals the disease mainly presents with recurrent infections or, in exceptional cases, remains largely unnoticed [10-12].
The C1 complex is composed of 3 different proteins; one C1q molecule, two C1r molecules and two C1s molecules. C1q is the recognition molecule of the C1 complex and is composed of 18 polypeptide chains: 6 C1qA, 6 C1qB and 6 C1qC. Each chain has a collagen-like region (N-terminal region) and a globular head region. The C1qA chains associate with the C1qB chains as heterodimers. C1qC chains first will form homodimers and finally associate with the A-B heterodimers to eventually form a tulip-like structure [13]. The genes encoding these three subunits are located on chromosome 1 within a genomic region of ~25kb and have an ACB orientation [14]. C1q is thought to be produced predominantly by immature dendritic cells and macrophages [15, 16]. C1q production can be upregulated via IFN-γ stimulation in which the expression of the three chains is suggested to be synchronized via transcription factors PU.1 and IRF8 [17].
Nowadays around 65 cases of C1q deficiencies have been reported [18]. These deficiencies are mostly caused by homozygous mutations in one of the chains. The most common mutation is in the A-chain caused by a nonsense mutation whereby a transition of C to T occurs in a codon for Glu-186 [13]. Next to mutations causing C1q deficiency also several genetic variations in C1q have been associated with increased risk for developing autoimmunity [19, 20]. To which extent mutations in different C1q chains affect the clinical presentation, e.g. infection versus SLE, is currently unclear.
Here we report on a non-coding homozygous mutation in an RNA splice site that leads to complete lack of expression of C1qB and hence lack of secretion of C1q.

Materials and Methods

Patient and controls

We have analyzed in detail one C1q-deficient Caucasian patient and studied materials obtained from both his parents as controls as well as a reference panel of 48 healthy adult controls collected in the LUMC. Informed consent was obtained from the parents and the controls in compliance with the Helsinki declaration.

Samples

Blood was collected from the patient and both parents in order to obtain serum and to isolate DNA. From the patient also PBMCs were collected using Ficoll-Paque

density gradient centrifugation. Control serum samples were chosen matched by age of the parents.

Functional reconstitution complement activity assays

Using an erythrocyte lysis assay the complement-mediated hemolytic activity was measured. Rabbit antibody coated sheep erythrocytes were used and different dilutions of serum of the patient or normal human sera (NHS) were added to C1q-, C2- or C4-depleted serum. Hemolysis was assessed by measuring OD at 414 nm, and specific complement-mediated lysis was calculated as described before [21]. To exclude the possibility that next to C1q deficient the patients sample would also be deficient for C1r or C1s we performed assays to measure activation of the classical pathway of the patient serum by reconstitution of purified C1q. Plates coated with human IgG were incubated with 1% serum of the patient (diluted in GVB++; 0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN3, 0.15 mM calcium chloride, 0.5 mM magnesium chloride, pH 7.3) with or without addition of purified C1q (Quidel) in different concentrations. As a read-out C4 deposition was measured.

Cell stimulation

PBMCs (1 x 106 cells/ml) were stimulated in a 48 wells plate (Corning Inc.) for 72 hours using RPMI (Gibco) supplemented with IFN-γ (200 U/ml, Peprotech) and 10 μM dexamethasone (Pharmacy LUMC, Leiden, The Netherlands) as described before to increase the C1q secretion (Walker, 1998;Moosig et al., 2006;Kaul & Loos, 2001). After 72 hours the supernatants and the cells were collected. The supernatants were used for ELISA and the cells for extraction of mRNA for qPCR and for western blot analysis of cell lysates.

Detection of C1q by ELISA

C1q levels in serum and culture supernatants were analyzed by ELISA. Maxisorp plates (Nunc) were coated with rabbit anti-human C1q (DAKO) in coating buffer (0.1 M NA2CO3, 0.1 M NaHCO3, pH 9.6) overnight at 4°C. Plates were washed in PBS/0.05% Tween (PBS-T, Sigma). Then the wells were blocked with PBS/1% BSA for 1 hour at room temperature. After washing, the standard of C1q (Quidel), applied in a two-fold dilution series starting from 150 ng/ml, control samples and patient samples in a 1:8000 dilution in PBS/1% BSA/0.05% Tween (Sigma) were added to the wells. The standard and the serum samples were incubated for 1 hour at 37°C. After incubation the plates were washed and rabbit anti-C1q DIG (Nephrology, LUMC) was added to the wells and the plate was incubated for 1

hour at 37°C. As detection antibody sheep anti-DIG HRP (Roche diagnostics) was added for 1 hour at 37°C, following final washing a substrate reaction was performed using ABTS (Sigma). C1q levels in the cell supernatants were measured with minor adjustments.

Detection of C1q by western blot

The presence of C1q in serum and in lysates of cultured PBMCs was analyzed by western blot. Serum samples of the patient and his parents were analyzed using reducing conditions at a dilution of 1:100 of the samples. Cell lysates were generated from PBMCs after 72 hours of stimulation. Cells were lysed on ice in lysis buffer (Roche Diagnostics) supplemented with a protease inhibitor (Sigma) for 30 minutes on ice. To avoid contamination of cell debris, cell lysates were centrifuged. The cell lysates were diluted 4x with Laemmli sample buffer (Bio-Rad)/ 5% ß-mercaptoethanol (Merck) and boiled for 5 minutes on 95°C to reduce the samples. Proteins were separated by SDS/PAGE on a mini-protean TGX precast gel (4-15%, Bio-Rad) followed by protein transfer on a Trans-Blot Turbo Transfer pack: mini, 0.2 μM PVDF (Bio-Rad). The membrane was blocked one hour in PBS containing 0.05% Tween and 3% skimmed milk (Fluka) on room temperature. Next, the blot was incubated with rabbit anti-human C1q (DAKO) overnight on 4°C. After washing with PBS/0.1% Tween the membrane was incubated with goat anti-rabbit HRP (DAKO) for 1 hour at room temperature. Then the blot was washed and C1q was visualized using ECL Western Blotting Analysis system (GE Healthcare).

Sequencing

Genomic DNA was extracted from blood collected with tubes supplemented with EDTA. Sequencing of the complete C1q genes (C1qA, C1qB and C1qC), of both introns and exons was performed. Deep-sequencing was performed using the 454 NGS Roche GS FLX Titanium platform. Data were compared to internal controls and to Human Genome build 19 as well as Human_v37_2 de dbSNP database v132 using the NextGENe software package for Next Generation Sequence Analysis (NGS) from Softgenetics. The effect of the mutation on splicing was in-silico analyzed using the NetGene2 Server http://www.cbs.dtu.dk/services/NetGene2/. To confirm the mutation Sanger sequencing was performed on C1qB using standard protocol.

qPCR

RNA was isolated from the cultured cells using the mirVana RNA isolation kit (Life Technologies) and analyzed with the NanoDrop (NanoDrop Technologies), followed by DNase treatment (Invitrogen) and cDNA synthesis with superscript III (200U/μl,

Invitrogen). Real time SYBR Green I qPCR was performed with primers specific for C1q genes C1qA/B/C separately (see table 1 for primer sequences) to analyze expression on transcriptional level. ß-actin was used as reference gene, CD14 gene expression was used as control for cellular input. The cDNA was diluted 1:50 and qPCR was performed using SensiFast Sybr (Bio-line) and primers with a start concentration of 10 pmol. The qPCR was performed on the real time PCR system (BioRad CFX-384) with an activation step of 3 min on 95°C (hot start polymerase activation), a melting temperature of 95°C for 5 seconds and an annealing temperature of 60.5 °C for 5 seconds and an elongation step for 20 seconds on 72°C for 40 cycles. At the end of the protocol melting curves were performed from 65°C to 95°C to test specific binding of SensiFast Sybr. The qPCR products were analyzed on a 2% agarose gel and visualized with nancy 520 (Sigma).

Gene	5' Forward	3' Reverse
ß-actin	GCAATGAGCGGTTCCGCTGC	CGATCCACACGGAGTACTTG
C1qA exon 3	TGGAGTTGACAACAGGAGGC	CGATATGGCCAGCACACAGA
C1qB exon 1 - 2	GACCGAGGGCAGTAGGCTC	TCATCATACTGTGTCAGACGCC
C1qC exon 2 - 3	AAGGARGGGTACGACGGACT	GTAAGCCGGGTTCTCCCTTC
CD14	GCCGCTGTGTAGGAAAGAAG	AGGTTCGGAGAAGTTGCAGA

Table 1. Primers used in the qPCR analysis

Statistical analysis

Statistical analysis on the qPCR data was performed using paired sample t-test. P-value's <0.05 were considered significant (Graphpad Prism software version 5.01).

Results

Patient

Here we describe a Dutch boy born from two Caucasian, healthy, non-consanguineous parents. During the first years of life he has suffered from recurrent upper airway infections. At the age of three he developed redness and swelling of the left ankle combined with fever (39°C) after an injury. Initially a cellulitis was suspected and treated with flucloxacillin. Due to persistent complaints he was admitted to a local hospital. A subsequent skeletal scintigraphy yielded a hotspot of the distal tibia compatible with the diagnosis osteomyelitis. Blood cultures remained negative. After intravenous antibiotics he recovered completely.

At the age of 5, he was admitted due to seizures and high fever. The analysis of cerebrospinal fluid yielded 470 x10E6 leukocytes/liter and both blood and CSF cultures showed a pneumococcal infection. Treatment with cephalosporin and dexamethasone resulted in complete recovery.
Examination of his medical history revealed that he had frequent upper airway infections and analysis for immune deficiencies showed a low classical pathway activity of 2% (reference range > 74 %) and a low level of IgG4 (0.007 g/l) (reference range 0.017 – 1.58 g/l), whereas C3 (1.1 mg/L, reference range: 0.9-2.0mg/L) and C4 (267 mg/L, reference range: 95-415 mg/L) were in the normal range. The alternative pathway (74%, reference range: >39%) showed no abnormalities and no antinuclear antibodies and rheumatoid factor were detected. Using an activity assay we investigated which complement protein from the classical pathway was defective. Complete absence of functional complement protein C1q was detected by reconstitution experiments in a C1q-specific hemolytic assay (Figure 1A). Similar reconstitution experiments revealed that the patient has a normal C2 and C4 activity (Figure 1B-C). With a reconstitution assay we could rescue the activity of the classical pathway by adding purified C1q to the patient serum (Figure 1D). At follow-up at the age of 8 years he was healthy without infectious problems or signs of autoimmunity.

2

Complete absence of C1q in the serum

Using ELISA we confirmed a complete C1q deficiency in the patient. Sera of both parents were also analyzed and their level of circulating C1q was in the same range as values obtained in a set of healthy adult controls (Figure 2A). In addition we have used western blot to analyze the presence of C1q in sera of the patient, parents and controls, which also confirmed the complete absence of circulating C1q in the patient, with normal levels in the parents (Figure 2B).

Identification of a splice site mutation in C1qB

Deep sequencing of the C1q genes (C1qA,B,C), revealed a mutation in homozygous state in the patient and in heterozygous state in both parents (data not shown). This mutation is located 2 nucleotides before the mRNA splice site of the second exon of C1qB (g.6251A>C). In-silico prediction programs indicate a complete absence of splicing when the mutant form is present. As a confirmation also Sanger sequencing was performed on the C1qB region identified by deep-sequencing. Using this technology, it was confirmed that the patient has a nucleotide change replacing adenine into a cytosine (Figure 3).

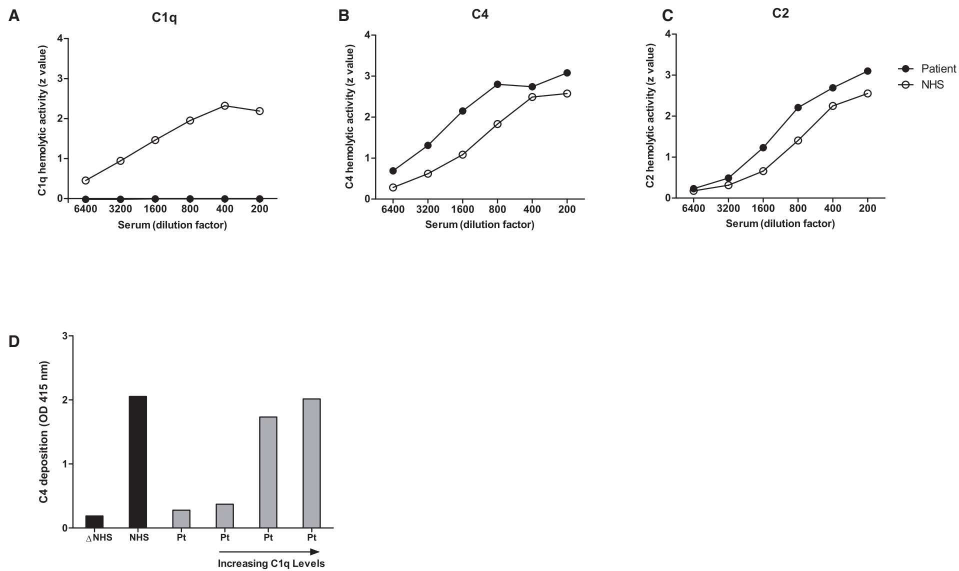

Figure 1. Functional reconstitution complement activity assays.

A. The addition of patient serum or NHS in a dilution range to C1q depleted serum. The total lysis is used as read-out. As positive control (100% lysis) distilled water was used. **B.** The addition of patient or NHS to C2 depleted serum. **C.** The addition of patient or NHS to C4 depleted serum. **D.** Reconstitution of the classical pathway by adding 0.05 μg/ml, 0.5 μg/ml and 2.5 μg/ml purified C1q to the patient serum. As a positive control normal human serum was used (NHS) and as a negative control heat inactivated NHS (ΔNHS) was used.

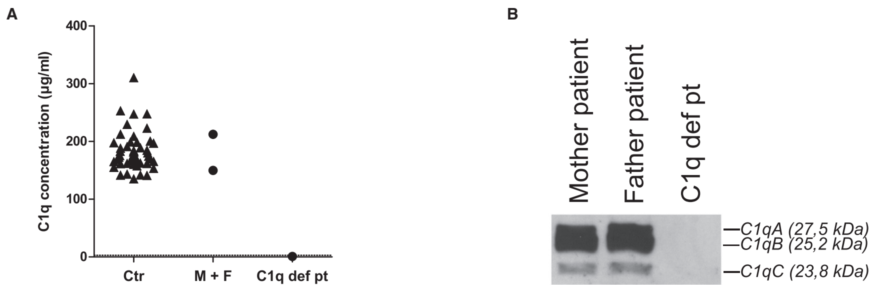

Figure 2. ELISA and western blot analysis of patient serum.

A. The presence of C1q in the serum was analyzed by ELISA. Diluted serum from the C1q-deficient patient (C1q def pt), mother and father (M + F) were compared with healthy controls (n=48) (Ctr). Dashed horizontal line indicates the lower detection limit of the assay, which was 0.01 μg/ml. **B.** Serum samples from the C1q-deficient patient together with the mother and father were analyzed by western blotting for the presence of the peptides C1qA, C1qB and C1qC in the serum using an antibody staining for C1q.

No secretion of C1q by PBMCs of the patient in-vitro

With western blot we confirmed that C1q is present in serum from both parents whereas the child is completely C1q-deficient (Figure 2B). As absence of circulating C1q in serum could be the result of either lack of production or consumption, we studied the secretion and intracellular accumulation of C1q using cells of the patient and control. Analysis of culture supernatant from PBMC's of the patient collected after 72 hours of stimulation with IFN-γ and dexamethasone revealed no secretion of C1q, whereas significant amounts of C1q were present in the supernatant of control PBMC in unstimulated and in the stimulated conditions (Figure 4A). Lack of secretion of C1q can be the result of intracellular accumulation of C1q. Therefore we also investigated the intracellular presence of C1q, using western blot analysis on cell lysates. Following 72 hours stimulation with IFN-γ and dexamethasone the three peptides of C1q were clearly detectable in the lysates of the control PBMC's. However, in the lysates of PBMC's of the patient only expression of C1qA and C1qC was detected, but no C1qB peptide (Figure 4B). Taken together, the results show that C1qA and C1qC peptides are transcribed and translated and that the C1q deficiency is due to the absence of the C1qB peptide.

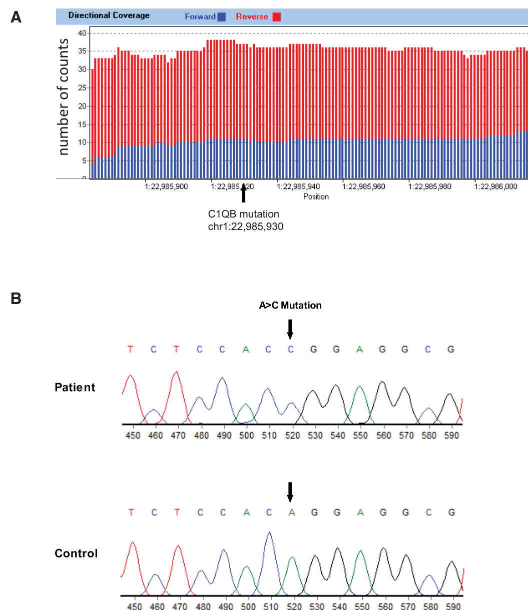

Figure 3. DNA sequencing data from the C1q-deficient patient.

A. Data obtained from deep sequencing showing a C1qB mutation on location chr1:22,985,930, g.6251A > C. **B.** As confirmation Sanger sequencing is performed indicating a nucleotide change in the intron before exon 2 of the C1qB gene. The C1q deficient patient is compared to a healthy donor.

qPCR analysis of expression of C1qA, C1qB and C1qC

A set of intron spanning primers was designed (Table 1) to analyze the expression levels of C1qA, C1qB and C1qC relative to the expression of the reference gene ß-actin. RNA isolated from the in-vitro stimulated PBMCs of patient and control was used as input material. We observed, as expected, complete lack of expression of C1qB in the patient (Figure 5), but also a partial reduction in the expression of C1qA by qPCR. The expression level of C1qC was not different between patient and control (Figure 5A). With the agarose gel we confirm that the primers for the qPCR are specific (Figure 5B). Expression levels of CD14, as a control for the input of monocytes, were similar in both control and patient (data not shown).

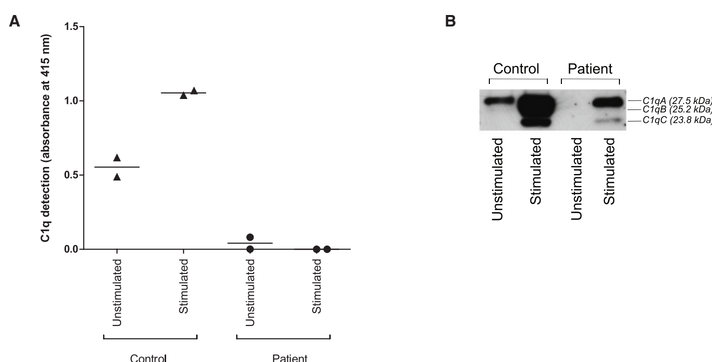

Figure 4. ELISA and western blot of stimulated cells from the C1q-deficient patient.
A. Undiluted cell supernatants were analyzed in duplicate by ELISA for the presence of C1q with or without stimulation of PBMC by IFN-γ (200 U/ml) together with DXM (10 µM). PBMCs of the C1q-deficient patient were stimulated for 72 hour to increase the C1q production. PBMCs from a healthy person were used as a control. **B.** After stimulation, the presence of intracellular C1qA, C1qB and C1qC peptides were examined in reduced cell lysates. The three peptides were detected closely together: C1qA at 27.5 kDa, C1qB at 25.2 kDa and C1qC at 23.8 kDa.

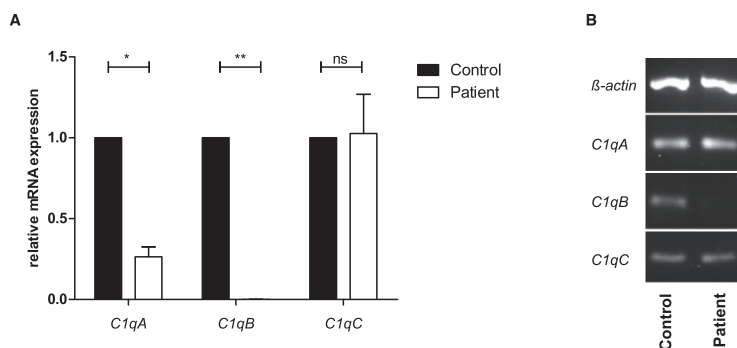

Figure 5. Expression levels of C1qA, C1qB and C1qC
A. Relative mRNA expression of the three C1q chains after stimulation with IFN-γ and DXM for 72 hours. As reference gene ß-actin was used. **P < 0.01, *** P < 0.001. **B.** qPCR products from control PBMCs and the C1q-deficient patient were analyzed on a 2% agarose gel.

Discussion

C1q deficiency is a rare hereditary condition associated with a high prevalence of SLE. The combination of reduced clearance of dying cells and immune complexes and an altered balance in the adaptive immune system likely increases the chance to develop the autoimmune disease SLE [7, 22, 23]. Globally around 65 patients have been described with a C1q deficiency [10]. Interestingly these patients display substantial variation in their clinical presentation, disease progression and outcome [24].

The patient described in this manuscript showed a clear absence of circulating C1q, but is not suffering from lupus, as evidenced by a lack of antinuclear antibodies and rheumatoid factor, but rather from recurrent infections. Although the patient is currently not suffering from lupus, the patient may develop lupus in the future. The IgG4 levels were lower compared to normal levels in this age category. This is often observed in patients with a complement deficiency in the classical pathway and may correspond with the recurrent infections of the upper airways [25].

A common phenomenon in C1q deficiency is that the parents are consanguineous, but the parents of this patient reported not to be related to each other (although not confirmed by genetic analyses). In the vast majority of C1q-deficient patients the mutation is located in the coding region of one of the chains, with only one report on a non-coding mutation [10, 26]. The current patient also harbors a mutation in a non-coding region. The mutation, located in C1qB, results in the abolition of a splice site with as a consequence complete C1q deficiency. This splice site in intron 1 in front of exon 2, which influences the splicing of intron 1. Intron 1 has a size of 6kb, which is too large to be quantified by qPCR and therefore the product of C1qB will conceivably be too large for efficient transcription or an unstable protein will be translated. By qPCR the C1qA and C1qC chains are still detected. This suggests that in the absence of C1qB the transcription of C1qA and C1qC will remain.

By western blot we could not detect any peptide of C1q in serum, but in cell lysates of activated PBMC's, C1qA and C1qC chains, but not C1qB chains could be detected. The protein levels for the C1qA and C1qC chains were lower compared to the control. Because the C1qB chain is not produced, a complete C1q protein cannot be generated, possibly followed by degradation of C1qA and C1qC. This is confirmed by ELISA, where no secreted C1q could be detected after stimulation of PBMCs of the patient.

In conclusion, we have identified a novel non-coding mutation in a splice site for C1qB that is associated with complete C1q deficiency. The patient showed several infectious problems but is currently doing well and shows no signs of autoimmunity.

He is receiving prophylactic antibiotics to protect him from severe infections and will stay under close control to detect possible development of autoimmunity in an early stage.

Acknowledgements

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Reference list

1. Daha, N.A., et al., Complement activation by (auto-) antibodies. Mol.Immunol., 2011. 48(14): p. 1656-1665.
2. Korb, L.C. and J.M. Ahearn, C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J.Immunol., 1997. 158(10): p. 4525-4528.
3. Nauta, A.J., et al., Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. Eur.J.Immunol., 2002. 32(6): p. 1726-1736.
4. Trouw, L.A., A.M. Blom, and P. Gasque, Role of complement and complement regulators in the removal of apoptotic cells. Mol.Immunol., 2008. 45(5): p. 1199-1207.
5. Walport, M.J., Complement. Second of two parts. N.Engl.J.Med., 2001. 344(15): p. 1140-1144.
6. Fossati-Jimack, L., et al., C1q deficiency promotes the production of transgenic-derived IgM and IgG3 autoantibodies in anti-DNA knock-in transgenic mice. Mol.Immunol., 2008. 45(3): p. 787-795.
7. Baruah, P., et al., C1q enhances IFN-gamma production by antigen-specific T cells via the CD40 costimulatory pathway on dendritic cells. Blood, 2009. 113(15): p. 3485-3493.
8. Jiang, K., et al., T cell activation by soluble C1q-bearing immune complexes: implications for the pathogenesis of rheumatoid arthritis. Clin.Exp.Immunol., 2003. 131(1): p. 61-67.
9. Santer, D.M., et al., C1q deficiency leads to the defective suppression of IFN-alpha in response to nucleoprotein containing immune complexes. J.Immunol., 2010. 185(8): p. 4738-4749.
10. Schejbel, L., et al., Combined IL-12 receptor and IgA deficiency in an adult man intestinally infested by an unknown, non-cultivable mycobacterium. Scand.J.Immunol., 2011. 74(6): p. 548-553.
11. Vassallo, G., et al., Clinical variability and characteristic autoantibody profile in primary C1q complement deficiency. Rheumatology.(Oxford), 2007. 46(10): p. 1612-1614.
12. Walport, M.J., K.A. Davies, and M. Botto, C1q and systemic lupus erythematosus. Immunobiology, 1998. 199(2): p. 265-285.
13. Petry, F., Molecular basis of hereditary C1q deficiency. Immunobiology, 1998. 199(2): p. 286-294.
14. Sellar, G.C., D.J. Blake, and K.B. Reid, Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subcomponent C1q. The complete derived amino acid sequence of human C1q. Biochem.J., 1991. 274 (Pt 2): p. 481-490.
15. Loos, M., H. Martin, and F. Petry, The biosynthesis of C1q, the collagen-like and Fc-recognizing molecule of the complement system. Behring Inst.Mitt., 1989(84): p. 32-41.
16. Castellano, G., et al., Maturation of dendritic cells abrogates C1q production in vivo and in vitro. Blood, 2004. 103(10): p. 3813-20.
17. Chen, G., et al., Molecular mechanisms for synchronized transcription of three complement C1q subunit genes in dendritic cells and macrophages. J.Biol.Chem., 2011. 286(40): p. 34941-34950.
18. Schejbel, L., et al., Molecular basis of hereditary C1q deficiency--revisited: identification of several novel disease-causing mutations. Genes Immun., 2011. 12(8): p. 626-634.
19. Martens, H.A., et al., Analysis of C1q polymorphisms suggests association with systemic lupus erythematosus, serum C1q and CH50 levels and disease severity. Ann Rheum Dis, 2009. 68(5): p. 715-20.
20. Trouw, L.A., et al., Genetic variants in the region of the C1q genes are associated with Rheumatoid Arthritis. Clin.Exp.Immunol., 2013.
21. Roos, A., et al., Specific inhibition of the classical complement pathway by C1q-binding peptides. J.Immunol., 2001. 167(12): p. 7052-7059.
22. Trendelenburg, M., et al., Monocytosis and accelerated activation of lymphocytes in C1q-deficient

autoimmune-prone mice. Immunology, 2004. 113(1): p. 80-88.
23. Gullstrand, B., et al., Complement classical pathway components are all important in clearance of apoptotic and secondary necrotic cells. Clin.Exp.Immunol., 2009. 156(2): p. 303-311.
24. Al-Mayouf, S.M., H. Abanomi, and A. Eldali, Impact of C1q deficiency on the severity and outcome of childhood systemic lupus erythematosus. Int.J.Rheum.Dis., 2011. 14(1): p. 81-85.
25. Bird, P. and P.J. Lachmann, The regulation of IgG subclass production in man: low serum IgG4 in inherited deficiencies of the classical pathway of C3 activation. Eur.J.Immunol., 1988. 18(8): p. 1217-1222.
26. Higuchi, Y., et al., The identification of a novel splicing mutation in C1qB in a Japanese family with C1q deficiency: a case report. Pediatr.Rheumatol.Online.J., 2013. 11(1): p. 41.