Clinical features of patients with homozygous complement C4A or C4B deficiency

Inka Liesmaa1*, Riitta Paakkanen2-3*, Asko Järvinen1, Ville Valtonen1, Marja-Liisa Lokki2

1 Division of Infectious Diseases, Inflammation Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, 2 Transplantation Laboratory, Medicum, University of Helsinki, Helsinki, Finland, 3 Division of Cardiology, Department of Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

☯ These authors contributed equally to this work.
* inka.liesmaa@hus.fi

Abstract

Introduction
Homozygous deficiencies of complement C4A or C4B are detected in 1–10% of populations. In genome-wide association studies C4 deficiencies are missed because the genetic variation of C4 is complex. There are no studies where the clinical presentation of these patients is analyzed. This study was aimed to characterize the clinical features of patients with homozygous C4A or C4B deficiency.

Material and methods
Thirty-two patients with no functional C4A, 87 patients with no C4B and 120 with normal amount of C4 genes were included. C4A and C4B numbers were assessed with genomic quantitative real-time PCR. Medical history was studied retrospectively from patients’ files.

Results
Novel associations between homozygous C4A deficiency and lymphoma, coeliac disease and sarcoidosis were detected. These conditions were present in 12.5%, (4/32 in patients vs. 0.8%, 1/120, in controls, OR = 17.00, 95%CI = 1.83–158.04, p = 0.007), 12.5% (4/32 in patients vs. 0%, 0/120 in controls, OR = 1.14, 95%CI = 1.00–1.30, p = 0.002) and 12.5%, respectively (4/32 in patients vs. 2.5%, 3/120 in controls, OR = 5.571, 95%CI = 1.79–2.32, p = 0.036). In addition, C4A and C4B deficiencies were both associated with adverse drug reactions leading to drug discontinuation (34.4%, 11/32 in C4A-deficient patients vs. 14.2%, 17/120 in controls, OR = 3.174, 95%CI = 1.30–7.74, p = 0.009 and 28.7%, 25/87 in C4B-deficient patients, OR = 2.44, 95%CI = 1.22–4.88, p = 0.010).

Conclusion
This reported cohort of homozygous deficiencies of C4A or C4B suggests that C4 deficiencies may have various unrecorded disease associations. C4 gene should be considered as a candidate gene in studying these selected disease associations.
Introduction

The complement system is an essential humoral defence mechanism that is involved in maintaining tissue homeostasis, innate immune reactions, activation and propagation of adaptive immune reactions as well as in non-immune functions such as lipid metabolism, synapsis maturation and blood coagulation. The complement cascade can be activated by three pathways; classical, alternative or lectin pathway. To maintain these varied functions, the complement system is meticulously regulated. Dysfunctions in the complement system have been linked with risk of various infections, autoimmune conditions such as systemic lupus, rheumatoid arthritis and asthma, as well as sepsis, ischemia-reperfusion injury and age-related macular degeneration [1].

The complement component C4 plays a role in the activation of classic and lectin pathways, leading to cleavage of C2, C3 and C5. The C4 protein is encoded by two slightly different adjacent genetic loci, C4A and C4B, located within the major histocompatibility complex (MHC) class III region on the short arm of chromosome 6 (MIM +120810 and *120820, respectively) [2–4]. Consequently, there are both C4A and C4B proteins circulating in the blood and tissues, which together form the commonly measured C4 concentration. Most individuals have four C4 genes. Approximately 60% of healthy individuals have two C4A and two C4B genes [5, 6]. In the Finnish population, 59% individuals have one C4A and one C4B gene in a chromosome [7]. However, the total number of C4 genes may vary between 2 and 8 [8].

The presence of no functional C4A or C4B genes causes complete C4A or C4B deficiency and is called homozygous C4 deficiency. The presence of one C4A or C4B gene is called heterozygous C4A or C4B deficiency [8]. Compared to deficiencies of other complement components of the classical pathway, homozygous C4A and C4B deficiencies are rather common [9]. Homozygous C4A deficiency is detected in 1–6% and homozygous C4B deficiency in 1–10% of studied healthy populations [7, 10–15]. Heterozygous C4 deficiency is more common; 12–21% of healthy populations are heterozygously C4A-deficient and 20–41% of the populations have a heterozygous deficiency of C4B [8, 10, 11, 13–15]. Deficiency of all C4 genes (neither functional C4A nor C4B genes present) is extremely rare and to date, only 28 patients have been described in the literature [9, 16, 17]. Patients with no functional C4 genes have been reported to have SLE (n = 17), SLE-like disease (n = 5), kidney diseases (n = 6), and repeated or invasive infections (n = 7) [9].

In disease association studies, homozygous and heterozygous C4A or C4B deficiencies are usually grouped together under the general term of "C4A or C4B deficiency". The studies distinguishing homozygous C4 deficiencies have repeatedly reported association between homozygous C4A deficiency and systemic lupus erythematosus (SLE)[10, 18–20]. There is also one study reporting association with pulmonary tuberculosis [21] and one with capillary leak syndrome during cardiopulmonary bypass in children [22]. Homozygous C4B deficiency has been associated with coronary artery disease [23], glomerular disease and infections in case reports [24, 25]. However, the number of cases in these reports is very low, ranging from three to twenty-six.

This study was aimed to systematically assess the clinical features and characteristics as well as disease associations of patients with either homozygous C4A or C4B deficiency. We detected novel, previously unrecorded links with disease conditions and confirmed previously known associations.

Patients and methods

Samples

Individuals from Helsinki University Hospital with homozygous C4A (n = 32) or C4B (n = 87) deficiency were identified from our Laboratory’s database between years 2004 and 2011 by...
screening 2173 individuals. Randomly selected individuals with two functional C4A and C4B genes (n = 120) from the same record served as controls. Cases with two C4A and C4B genes were chosen as controls as this is the most common genetic combination of the background population and in order to make the control population as homogenous as possible to ease the comparisons between cases and controls. The controls were selected over the same time range as the first and middle sample of a given month. The Local Ethics Committee’s approval was not needed due to anonymous register-like nature of the study.

Medical history

The medical records were retrospectively evaluated for diagnosed clinical diseases and descriptive symptoms. The medical history and diagnoses were individually retrieved as defined by the treating clinician. The searched diagnoses/conditions are listed below.

Autoimmune conditions: SLE, rheumatoid arthritis, spondylarthrits encyclopaetica, seronegative spondylarthropathy (SSA), type I diabetes mellitus, coeliac disease, autoimmune gastritis, primary biliary cirrhosis, autoimmune hepatitis, autoimmune haemolytic anaemia, autoimmune neutropenia, idiopathic thrombocytopenic purpura, glomerulonephritis, IgA nephropathy and asthma.

Infections were categorized as 1) invasive infections including bacteremia, pneumonia and meningitis, 2) recurrent central nervous system Herpes simplex virus (HSV) manifestations, and 3) recurrent tonsillitis or sinusitis which required operative treatment.

Neurological diagnoses and signs: Epilepsy, multiple sclerosis, vertigo, migraine or severe headache, spasticity or ataxia of unknown causes, numbness, myalgia, paresthesias and paralyses of unknown causes.

Gastrointestinal diseases and signs: inflammatory bowel disease, abdominal pain necessitating hospital investigations, irritable bowel syndrome, and recurrent Clostridium difficile colitis.

Other symptoms: reactive arthritis, hypothyroidism.

Adverse drug effects: Any mention of adverse drug reactions leading to drug discontinuation (and avoidance), toxic reactions and side effects were recorded.

Allergies: Reported by patients included mainly pollen and/or animal epithelium, nickel and iodine.

Psychological conditions: Psychosis, schizophrenia, severe depression and anxiety.

Gene analyses

The copy numbers of C4A, C4B and the most common silencing mutation of C4A, CTins, were recorded with a validated real-time quantitative polymerase chain reaction as described in detail elsewhere [7]. In brief, genomic DNA was amplified with SYBRR Green labelling using isotype-specific primers (Rotor-Gene 3000, Qiagen, Austria). The resulting fluorescence curve was analysed in relation to controls and housekeeping gene fluorescence recordings (Rotor-Gene, software v 6.0, Qiagen). The number of functional C4A was determined by reducing the number of CTins copies from the total number of C4A. Besides the CTins, no other C4 mutations were screened.

Human leucocyte antigen (HLA) alleles (HLA-DRB1*01, *03, *04, *15) were characterized with similar quantitative real-time PCR procedure using allele-specific primers and performed in our HLA-laboratory having accreditation by the European Federation for Immunogenetics (EFI). HLA-genotyping data was available from 50% (16/32) of C4A-deficient patients, 51% (44/87) of C4B-deficient patients and 46% (55/120) of the controls. The HLA data was available if the treating physician had requested for these assays based on clinical judgement.
Laboratory parameters

Immunoglobulin levels (IgG, M, A and E) and antibody levels against *Herpes simplex* viruses (HSV, types 1 and 2), *Varicella zoster* virus (VZV), nuclear antigens (ANA), neutrophilic cytoplasmic components (ANCA) and rheumatic factor (RF) were collected from medical reports. An abnormal value was indicated if differed from reference values (6.8–15 g/l for IgG, 0.36–2.59 g/l for IgM, 0.88–2.84 g/l for IgA, <1 g/l for IgE, 0.71–1.41 g/l for C3, 0.15–0.5 g/l for C4, positive titre recording (95th percentile) for HSV or VZV, 1:320 for ANA and 1:20 for ANCA, all positive titres represented about 95th percentile level in Finnish population). All laboratory recordings were done by the standard procedures of the accredited diagnostic laboratory of Helsinki and Uusimaa Hospital District (HUSLAB).

Statistical analyses

Statistical analyses, Chi-square, Student’s t- and Mann–Whitney U test, were performed when appropriate using PASW statistics (v. 20.0). The continuous variables that were normally distributed were also tested with Student’s t-test. Two-tailed p-value <0.05 was considered statistically significant. Odd’s ratios (OR) and their 95% confidence intervals (95%CI) were assessed with Chi-square statistics. The number of autoimmune conditions was assessed with Linear-by-linear association. Sex, decreased complement activity of mannan binding lectin pathway, genetic backgrounds of C4 deficiencies and the simultaneous presence of HLA-B*35 and HLA-DRB1*01 were studied in subgroup analyses of C4A or C4B deficiency and compared with the same subgroups in controls. In most cases, all available laboratory parameters were included except for complement activity analyses, where the cases were excluded listwise.

Results

Age- and sex distributions were similar in all groups. The majority (>75%) of the samples were from Helsinki University Hospital, Division of Infectious Diseases, corresponding to our Laboratory’s general distribution (Table 1).

Both patient groups with homozygous C4A or C4B deficiency had lower blood C4 levels (Table 1). Patients with homozygous C4A deficiency had autoantibodies more often than controls (33.3%, 10/32 vs. 16.5%, 17/120, p = 0.044 for positive ANCA or ANA autoantibodies, Table 1). In addition, the IgG and IgM immunoglobulin levels seemed to be more often abnormal (elevated or lowered) among patients with homozygous C4A deficiency as compared to controls. Patients with homozygous C4B deficiency were more often recorded to have statistically lower complement C3 activation values (Table 1), but the frequency of abnormally low C3 (i.e. below the laboratory reference range) was similar in all groups (data not shown).

Autoimmune manifestations

Homozygous C4A deficiency was associated with increased number of autoimmune conditions (p = 0.047, Table 2). The specific autoimmune conditions were SLE (18.8%, 6/32 vs. 5.8%, 7/120 in C4A deficient patients and controls, respectively, OR = 3.75, 95%CI = 1.16–12.01, p = 0.031) and coeliac disease (12.5%, 4/32 vs. 0%, 0/120 in C4A deficient patients and controls, p = 0.002).

Homozygous C4B deficiency was not associated with autoimmune condition(s). Although there was increased incidence of symptoms detected post-infectiously reminiscent of autoimmune diseases (13.8% vs. 4.17% in C4B deficient patients and controls, respectively, OR = 3.68, 95% CI = 1.25–10.87, p = 0.013). These conditions are listed in detail in S1 Table.
Infections

No differences in rate of recurrent or invasive infections were apparent between patients with homozygous C4A or C4B deficiency and controls. Central nervous system Herpes simplex virus infections were marginally (but not statistically) increased in both C4 deficient groups (6.3%, 2/32, 5.7%, 5/87 and 1.7% 2/120 in C4A deficient, C4B deficient and in controls, respectively, data not shown). HSV type 1 was the causative agent in two C4A-deficient and HSV 2 in five C4B-deficient patients.

Other conditions

In patients with homozygotic C4A deficiency, lymphomas were significantly more common than in other groups (12.50%, 4/32 in C4A deficient vs. 0.8%, 1/120 in controls OR = 17.00, 95%CI = 1.83–158.04, p = 0.007, Table 3). However, no specific lymphoma type could be identified. In addition, sarcoidosis was more commonly diagnosed in C4A deficient patients (12.50%, 4/32 vs. 2.50%, 3/120 OR = 5.71, 95%CI = 1.1789–26.3, p = 0.036, for C4A deficient patients and controls, respectively). No differences in frequencies of recorded neurological, ocular or psychiatric diagnoses were apparent.

Table 1. Baseline characteristics.

|                  | Homozygous C4A deficiency (n = 32) | Homozygous C4B deficiency (n = 87) | Controls (n = 120) | P = (C4A-deficient vs. Controls) | P = (C4B-deficient vs. Controls) |
|------------------|------------------------------------|------------------------------------|--------------------|--------------------------------|--------------------------------|
| Age (years)      | 45.25 (18.0)                       | 44.08 (14.6)                       | 43.92 (15.2)       | 0.778                           | 0.900                           |
| Females          | 22 (68.8)                          | 59 (67.8)                          | 72 (60.0)          | 0.365                           | 0.181                           |
| Samples ordered  |                                    |                                    |                    | 0.036                           | 0.615                           |
| Infectious       | 25 (78.2)                          | 75 (86.2)                          | 108 (90.0)         |                                |                                |
| Internal medicine| 1 (3.1)                            | 7 (8.0)                            | 6 (5.0)            |                                |                                |
| Other departments| 6 (18.1)                           | 5 (5.7)                            | 6 (5.0)            |                                |                                |
| Complement values|                                    |                                    |                    |                                |                                |
| Plasma C3 g/l    | 1.19 (0.4)                         | 1.11 (0.34)                        | 1.21 (0.4)         | 0.263                           | 0.025                           |
| Low C3 (%)       | 2 (6.9)                            | 7 (8.5)                            | 4 (3.6)            | 0.604                           | 0.209                           |
| Plasma C4 g/l    | 0.17 (0.1)                         | 0.2 (0.1)                          | 0.27 (0.1)         | <0.0001                         | <0.0001                         |
| Low C4 (%)       | 5 (17.2)                           | 15 (18.5)                          | 2 (1.8)            | 0.005                           | 0.0001                          |
| Abnormal immunoglobulin levels | | | | | |
| IgG (%)          | 10 (35.7)                          | 17 (20.7)                          | 19 (17.0)          | 0.029*                          | 0.461                           |
| IgM (%)          | 8 (28.6)                           | 11 (13.3)                          | 11 (9.9)           | 0.026**                         | 0.436                           |
| IgA (%)          | 6 (21.4)                           | 11 (13.4)                          | 13 (11.7)          | 0.218                           | 0.682                           |
| Positive autoimmune antibodies | | | | | |
| ANA or ANCA (%)  | 10 (33.3)                          | 14 (17.5)                          | 17 (16.5)          | 0.044***                        | 0.836                           |

Data is presented as median (SD) for age, plasma C3, plasma C4 and as n (%) for other variables.C4A, complement component C4A; C4B, Complement component C4B; C3, complement component 3; Abnormal immunoglobulin levels, either elevated or lowered; IgG, Immunoglobulin class G; IgM, Immunoglobulin class M; IgA Immunoglobulin class A; ANA, anti-nuclear antibodies; ANCA, anti-neutrophilic cytoplasmic antibodies.

* OR = 2.719, 95%CI = 1.087–6.804
** OR = 3.636, 95%CI = 1.299–10.181
*** OR = 2.53, 95%CI = 1.00–6.35
**** For cut-offs for abnormal C3, C4, IgG, IgM and IgA levels, see text for Laboratory Parameters
Adverse drug reactions

Patients with homozygous C4A or C4B deficiency were recorded to have more adverse effects leading to drug discontinuation than controls (34.38%, 11/32 in C4A deficient, 28.74%, 25/87 in C4B deficient vs. 14.17%, 17/120 in controls, p = 0.009 for C4A-deficient vs. controls, p = 0.010 for C4B-deficient vs. controls, Table 4). The discontinued medications were most often antibiotics. For C4A deficient patients, statistical significance was not attained for any individual category. Patients with C4B deficiency, however, had significantly more often discontinued antimicrobial agents than the controls (25%, 22/87 vs. 10%, 12/120, p = 0.003). This difference seemed to be explained by the markedly increased intolerance to sulphonamides (12.8%, 11/87 vs. 0.8%, 1/120, OR = 17.45, 95%CI = 2.21–138.0, p < 0.001) and doxycycline (8.1%, 7/87 vs. 0.8%, 1/120, OR = 10.544, 95%CI = 1.27–87.37, p = 0.001 for C4B-deficient vs. controls).

Table 2. Autoimmune conditions in patients with homozygous C4A or C4B deficiency and in patients without C4 deficiencies.

| Condition                        | Homozygous C4A deficiency (n = 32) | Homozygous C4B deficiency (n = 87) | Controls (n = 120) | P = (C4A-deficient vs. controls) | P = (C4B-deficient vs. controls) |
|----------------------------------|-----------------------------------|-----------------------------------|--------------------|----------------------------------|----------------------------------|
| Any autoimmune condition        | 21 (65.6)                         | 42 (48.3)                         | 55 (45.8)          | 0.047*                           | 0.728                            |
| Systemic Lupus Erythematosus     | 6 (18.8)                          | 8 (9.20)                          | 7 (5.8)            | 0.031**                          | 0.357                            |
| Coeliac disease                  | 4 (12.5)                          | 0 (0)                             | 0                  | 0.002***                         | 1.000                            |
| Hypothyroidism                   | 2 (6.3)                           | 6 (6.9)                           | 10 (8.3)           | 1.000                            | 0.702                            |
| Seronegative spondyloarthritis   | 3 (9.4)                           | 7 (8.0)                           | 4 (3.3)            | 0.162                            | 0.208                            |
| Post-infective prolonged symptoms| 3 (9.4)                           | 12 (13.8)                         | 5 (4.2)            | 0.241                            | 0.013****                        |
| Reactive arthritis               | 1 (3.13)                          | 4 (4.6)                           | 1 (0.8)            | 0.312                            | 0.164                            |

* OR (95%CI) = 2.26 (1.00–5.09)
** OR (95%CI) = 3.75 (1.16–12.01)
*** OR (95%CI) = 1.14 (1.00–1.30)
**** OR (95%CI) = 3.68 (1.25–10.87)

https://doi.org/10.1371/journal.pone.0199305.t002

Table 3. Other clinical conditions associated with homozygous C4 deficiencies.

| Condition                        | Homozygous C4A deficiency (n = 32) | Homozygous C4B deficiency (n = 87) | Controls (n = 120) | P = (C4A-deficient vs. controls) | P = (C4B-deficient vs. controls) |
|----------------------------------|-----------------------------------|-----------------------------------|--------------------|----------------------------------|----------------------------------|
| Any malignancy                   | 6 (18.8)                          | 9 (10.3)                          | 8 (6.7)            | 0.077*                           | 0.442                            |
| Lymphoma                         | 4 (12.5)                          | 1 (1.1)                           | 1 (0.8)            | 0.007**                          | 1.000                            |
| Sarcoidosis                      | 4 (12.5)                          | 4 (4.6)                           | 3 (2.5)            | 0.036***                         | 0.453                            |
| Ischemia                         | 2 (6.3)                           | 4 (4.6)                           | 3 (2.5)            | 0.283                            | 0.394                            |
| Epilepsy                         | 1 (3.1)                           | 4 (4.6)                           | 4 (3.3)            | 1.000                            | 0.621                            |
| Migraine                         | 0 (0.0)                           | 2 (2.3)                           | 3 (2.5)            | 1.000                            | 1.000                            |
| Psychiatric diagnosis            | 0 (0.0)                           | 4 (4.6)                           | 3 (2.5)            | 1.000                            | 0.453                            |

* OR = 3.231, 95%CI = 1.032–10.115
** OR = 17.00, 95%CI = 1.83–158.04
*** OR = 5.57, 95%CI = 1.18–26.3

https://doi.org/10.1371/journal.pone.0199305.t003
Complement activity
The activities of complement activation pathways were assessed by the treating clinician in one
fourth of the study populations. This number is too small to draw conclusions. The available
data is depicted in S2 Table.

C4 deficiency and remaining C4 genes
The genetic background for homozygous C4 deficiency was variable (S3 Table). Almost all
patients with homozygous C4A deficiency had two copies of C4B genes (93.75%, 30/32). Most
patients with homozygous C4B deficiency had three copies of C4A genes (68.97%, 60/87)
whereas the rest had two C4A genes and one patient had four C4A genes.

Discussion
In this relatively large cohort of patients with homozygous C4A or C4B deficiency, we were
able to discover novel disease associations as well as replicate some previously known associa-
tions. Where the previous genetic studies of C4 deficiency have included patients with homo-
zygous and heterozygous C4 deficiency, this is one of the largest cohorts gathering only
patients with homozygous C4A or C4B deficiency (i.e. no functional C4A or C4B genes at all).
Because the serum levels and the gene numbers do not correlate well, in selecting patients

Table 4. Adverse drug reactions with homozygous C4 deficiency.

|                      | Homozygous C4A deficiency (n = 32) | Homozygous C4B deficiency (n = 87) | Controls (n = 120) | P = (C4A-deficient vs. controls) | P = (C4B-deficient vs. controls) |
|----------------------|----------------------------------|----------------------------------|-------------------|---------------------------------|---------------------------------|
| All adverse drug     | n (%)                            | n (%)                            | n (%)             | 0.009*                          | 0.010**                         |
| reactions            |                                  |                                  |                   |                                 |                                 |
| Antimicrobial        |                                  |                                  |                   |                                 |                                 |
| discontinuation      |                                  |                                  |                   |                                 |                                 |
| Sulfonamides         | 2 (6.9)                          | 11 (12.8)                        | 1 (0.8)           | 0.097                           | 0.0003***                       |
| Doxycycline          | 1 (3.5)                          | 7 (8.1)                          | 1 (0.8)           | 0.352                           | 0.010***                        |
| Beta-lactamases      | 4 (13.8)                         | 11 (12.8)                        | 8 (6.7)           | 0.250                           | 0.134                           |
| Macrolides           | 0 (0)                            | 6 (7.0)                          | 2 (1.7)           | 1.000                           | 0.070                           |
| Other                | 0 (0)                            | 4 (4.7)                          | 2 (1.7)           | 1.000                           | 0.238                           |
| Any antimicrobial    | 6 (20.7)                         | 22 (25.6)                        | 12 (10.0)         | 0.121                           | 0.003****                       |
| Anti-inflammatory    |                                  |                                  |                   |                                 |                                 |
| NSAID                | 2 (6.9)                          | 3 (3.4)                          | 2 (1.7)           | 0.195                           | 0.652                           |
| ASA                  | 1 (3.5)                          | 5 (5.7)                          | 4 (3.3)           | 1.000                           | 0.497                           |
| Other                |                                  |                                  |                   |                                 |                                 |
| Any other            | 0 (0)                            | 8 (9.2)                          | 5 (4.2)           | 0.585                           | 0.141                           |

NSAID, non-steroidal anti-inflammatory drug; ASA, acetylsalicylic acid
* OR (95%CI) = 3.17 (1.3–7.7)
** OR (95%CI) = 2.44 (1.22–4.88)
*** OR (95%CI) = 17.45 (2.21–137.96)
**** OR (95%CI) = 10.54 (1.27–87.37)
***** OR (95%CI) = 3.09 (1.44–6.67)
****** Other intolerated medicines for C4B deficient (barium, alendronate, metamizole + pitophenone, tramadol, antibiotics for tuberculosis, valaciclovir, methylprednisolone). For controls the intolerated medicines (angiotensinogen II inhibitor, beta-blocker, lidocaine and myasthenia combination drugs).

https://doi.org/10.1371/journal.pone.0199305.1004
without any C4A or C4B genes, we are hoping to better clarify disease associations of these conditions.

**Biological effect of homozygous C4 deficiencies**

Although C4A and C4B proteins differ only by 4 amino acids, they exhibit marked differences in chemical reactivity to substrates. Activated C4A protein forms a covalent amide bond with amino groups on peptide antigens, whereas activated C4B binds more efficiently to hydroxyl group containing substrates of carbohydrate antigens [2]. Accordingly, homozygous deficiency of C4A has been reported to associate with increased frequency of autoimmune diseases, whereas homozygous C4B deficiency has been associated with increased susceptibility of bacterial and enveloped viral infections. However, the reported disease associations of genetic C4B deficiency (homozygous and heterozygous deficiency combined) include also common and complex diseases such as psychiatric disorders and atherosclerosis [26–28].

In our study, somewhat surprisingly, the homozygous deficiency of C4A or C4B were not associated with significantly increased frequency of infectious or autoimmune disease burden (except for the association between SLE and C4A deficiency), at least when compared with hospitalized controls. If homozygous C4A deficient patients would have significantly aberrant immune clearance, the patients could be expected to exhibit features of aberrant autoimmune reactions, despite the redundancy of the immune system. Correspondingly, the homozygous C4B deficient patients would be expected to have at least some kind of signal of increased rate of infections. We could not see such differences, but due to the lack of healthy controls, we were not able to reliably assess the role of infections in this material.

For homozygous C4A deficient patients in this study population, the majority of patients had two copies of C4B. Thus, C4B copy number did not seem to compensate for the lacking C4A genes. However, it is not known, to which extent the local production of C4B could be increased due to increased demand. In the homozygous C4B deficient patients, the majority did have one “extra” copy of C4A (three copies in total). Whether this “increase” in C4A copies could explain for the small amount of disease associations with homozygous C4B deficiency detected in this study remains unknown. In addition, the plasma C4 protein concentration is only partly determined by C4 gene copy number and is also affected by the size variations as well [29, 30]. Unfortunately, the size variation of C4 genes and the activities of different complement activation routes were not routinely assessed in this study. In a steady stage, this would naturally be very informative. In addition, a C4-independent activation route of the complement system has been described [31].

As with many other studies assessing the HLA genes, we must account for the surrounding gene region. HLA genes are inherited in tightly connected “haploblocks”, in which the normal laws or genetic recombination do not apply [32, 33]. Without extensive and detailed data or some kind of biological modelling, in mere association studies, the causative nature of the gene marker in question can only be contemplated. Correspondingly, even with the strongest known association between C4A deficiency and SLE, the surrounding genetic landscape is playing an essential role in disease susceptibility [10]. In addition, it has recently been shown that despite the linkage with the surrounding genes, C4 deficiency is not reliably assessed in GWAS [10]. This makes both the disease association studies and analyses more challenging.

**Homozygous C4A deficiency**

We discovered that patients with homozygous C4A deficiency had a significantly increased prevalence of lymphomas, with an OR of 17. To our knowledge, this association has not been previously reported. However, the HLA gene region has been linked with various types of
haematological malignancies in multiple studies. Unfortunately, we could not reliably classify
the subtypes of these lymphomas and therefore, were unable to further assess the specific dis-
 ease associations.

It has to be borne in mind that our material consists of relatively small number of patients.
In addition, our patients were suffering from difficult or recurrent infections, and the majority
was treated in the Department of Infectious Diseases. Even though the control population con-
sisted of similar patients and did not show increased incidence on lymphomas, we cannot
speculate, whether the association between homozygous C4A deficiency and haematological
malignancy could be mediated or underlined by infections the patients were suffering from.

We found the increased incidence of coeliac disease in patients with homozygous C4A defi-
ciency. HLA class II alleles are known to confer strong risk for coeliac disease and HLA testing
is recommended in exclusion of coeliac disease in certain patients [34]. The C4A deficiency
has been linked with coeliac disease, most likely due to linkage disequilibrium with the sur-
rounding HLA-alleles [35]. The common ancestral haplotype (AH8.1) has been associated
with coeliac disease and various other autoimmune conditions including sarcoidosis [36, 37].
We were able to replicate the finding that the patients with homozygous C4A deficiency had
almost six-fold more sarcoidosis corresponding to the previous study in Finnish sarcoidosis
patients [38]. As with many other disease association studies, based on the data at hand, it is
difficult to state, which of the associated gene markers, if any, comprises the true causative
factor.

We also were able to confirm the previously reported associations between homozygous
C4A deficiency and autoimmune condition SLE [10, 18, 19, 20]. Homozygous deficiency of
C4A is a predisposing factor for SLE, but not all homozygous C4A deficient patients develop
SLE [10, 18]. The increased incidence of autoantibodies in the homozygous C4A deficient
patients supports the role of C4A deficiency in autoimmunity.

In previous studies in Finland, heterozygous C4A deficiency has been shown to associate
with recurrent or chronic respiratory tract infections [39]. We were not able to replicate these
findings in our material with patients with diseases necessitating hospital treatment. Whether
there would be an association if the control material would have consisted of general popula-
tion, remains unknown.

Homozygous C4B deficiency

In our study, patients with homozygous C4B deficiency seemed to have more intolerance to
sulphonamides and doxycycline as well as suffering more often from various post-infectious
symptoms. The association between drug discontinuation was strong; ORs ranged from 10 to
17. Although the term “post-infectious symptoms” is anything but exact it is an existing and
clinically difficult entity that is difficult to verify. The recorded symptoms ranged from vasculi-
tis to prolonged fever, arthralgia and myocarditis after infection. It is impossible to determine
the role of infection in these cases but in all of these cases, the symptomatology had started soon
after bacterial or viral infection and could not be corrected by medical treatment. This corre-
sponds to our previous finding from a patient with persistent arthralgia and myalgia 6 months
after acute SINV infection [40].

The data on infection-proneness of C4 deficiency is controversial [41]. In the previous stud-
ies, (mostly homo- and heterozygous deficiency combined) C4B deficiency has been linked
with increased rate of invasive infections [27]. However, recent association studies have chal-
lenged this by inverse associations [21,41–43]. In our cohort, homozygous C4B deficient
patients did not have increased rate of invasive infections. Due to the biased nature of our data
with similar background of controls, we cannot reliably assess the role of homozygous C4B
deficiency in meningitis or in invasive infections. According to our data, it seems however, that homozygous \textit{C4B} deficiency alone is not significant in causing invasive infections as only a small part of \textit{C4B}-deficient patients suffered from such infections.

**Limitations**

The limitations of our study are the relatively small number of homozygous \textit{C4A} deficient patients although this is among the largest clinical studies on homozygous C4 deficiencies, the lack of complete HLA-types and of follow-up data. In addition, another limitation is that the patient data was retrospectively retrieved from medical recordings. We cannot therefore evaluate the medical conditions based on diagnostic criteria.

Due to the rarity of this genetic phenomenon (1–6%), a very large population cohort (1666–10 000) should be screened to attain a population of 100 patients.

In addition, multiple hypotheses in multiple conditions were tested without correcting the p-value, thus the validity of our findings has to be replicated in an independent patient sample. However, as this study is hypothesis-forming rather than hypothesis-confirming, the p-value correction for multiple testing does not need to be stringently followed. Although the background population is highly selected, both patients and controls were drawn from the same cohort. Population- based screening and follow-up studies are needed to determine causality and the applicability to general population. We do not intend to elucidate the disease associations, but merely characterize this population and launch new hypotheses.

**Conclusion**

Homozygous \textit{C4A} and \textit{C4B} deficiencies differ in their clinical characteristics and disease associations. In addition to the previous disease associations, we discovered that homozygous \textit{C4A} deficiency might be associated with lymphoma and sarcoidosis and that homozygous \textit{C4B} deficiency might be associated with prolonged post-infectious symptoms and drug discontinuation due to adverse reactions, which occurred mainly with antibiotics. These notions may open novel pathways to assess the role of C4 in these conditions. Therefore, novel and larger studies are warranted.

**Supporting information**

S1 Table. Post-infectious symptoms in 20 patients with homozygous \textit{C4B} deficiency.

(S1 Table)

S2 Table. Available complement activities in study populations.

(S2 Table)

S3 Table. Number of C4 genes in the study populations.

(S3 Table)

**Author Contributions**

Writing – original draft: Inka Liesmaa, Riitta Paakkanen, Marja-Liisa Lokki.

Writing – review & editing: Asko Järvinen, Ville Valtonen.

**References**

1. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: A key system for immune surveillance and homeostasis. Nat Immunol. 2010; 11: 785–797. https://doi.org/10.1038/ni.1923 PMID: 20720586
2. Yu CY, Belt KT, Giles CM, Campbell RD, Porter RR. Structural basis of the polymorphism of human complement components C4A and C4B: Gene size, reactivity and antigenicity. EMBO J. 1986; 5: 2873–2881. PMID: 2431902

3. Yu CY. The complete exon-intron structure of a human complement component C4A gene. DNA sequences, polymorphism, and linkage to the 21-hydroxylase gene. Journal of Immunology (Baltimore, Md.: 1950). 1991; 146: 1057–1066.

4. Dangel AW, Mendoza AR, Menachery CD, Baker BJ, Daniel CM, Carroll MC, et al. The dichotomous size variation of human complement C4 genes is mediated by a novel family of endogenous retroviruses, which also establishes species-specific genomic patterns among old world primates. Immunogenetics. 1994; 40: 425–436. PMID: 7545960

5. Chung EK, Yang Y, Rupert KL, Jones KN, Rennebohm RM, Blanchong CA, et al. Determining the one, two, three, or four long and short loci of human complement C4 in a Major Histocompatibility Complex haplotype encoding C4A or C4B proteins. Am J Hum Gene. 2002; 71: 810–822.

6. Chung EK, Wu YL, Yang Y, Zhou B, Yu CY. Human complement components C4A and C4B genetic diversities: Complex genotypes and phenotypes. Curr Protoc Immunol. 2005; Chapter 13: Unit 13 18.

7. Paakkonen R, Vauhkonen H, Järvinen A, Seppänen M, and Lokki M-L. Copy number analysis of complement C4A, C4B and C4A silencing mutation by real-time quantitative polymerase chain reaction. PLoS One. 2012; 7: e38813. https://doi.org/10.1371/journal.pone.0038813 PMID: 22737222

8. Blanchong CA, Zhou B, Rupert KL, Chung EK, Jones KN, Sotos JF, et al. Deficiencies of human complement component C4A and C4B and heterozygosity in length variants of RP-C4-CYP21-TNX (RCCX) modules in Caucasians. The load of RCCX genetic diversity on Major Histo-compatibility Complex-associated disease. The Journal of experimental medicine. 2000; 191: 2183–2196. PMID: 10859342

9. Lipsker D, Hauptmann G. Cutaneous manifestations of complement deficiencies. Lupus. 2010; 19: 1096–1106. https://doi.org/10.1177/0961203310373370 PMID: 20693203

10. Boteva L, Morris DL, Cortes-Hernandez J, Martin J,Vyse TJ and Fernando MM. Genetically determined partial complement C4 deficiency states are not independent risk factors for SLE in UK and Spanish populations. Am J Hum Genet. 2012; 90: 445–456. https://doi.org/10.1016/j.ajhg.2012.01.012 PMID: 22387014

11. Seppanen M, Suvilehto J, Lokki ML, Notkola I-L, Järvinen A, Jarva H, et al. Immunoglobulins and complement factor C4 in adult rhinosinusitis. Clin Exp Immunol. 2006; 145: 219–227. https://doi.org/10.1111/j.1365-2249.2006.03134.x PMID: 16879240

12. Boteva L, Imagen, Wu YL, Cortes-Hernandez J, Martin J, Vyse TJ, et al. Determination of the loss of function complement C4 exon 29 CT insertion using a novel paralog-specific assay in healthy UK and Spanish populations. PLoS one. 2011; 6: e22128. https://doi.org/10.1371/journal.pone.0022128 PMID: 21857912

13. Szi1agi1, Blasko B, Szi1assy D, Fust G, Sasvari-Sz1ekely M and Ronai Z. Real-time qpcr quantification of human complement C4A and C4B genes. BMC genetics. 2006; 7: 1.

14. Wahrman M, Dohler B, Ruhenstroth A, Haslacher H, Perkman T, Exner M, et al. Genotypic diversity of complement component C4 does not predict kidney transplant outcome. J Am Soc Nephrol. 2011; 22: 367–376. https://doi.org/10.1681/ASN.2010050513 PMID: 21164027

15. Wouters D, van Schouwenburg P, van der Horst A, De Boer M, Schooneman D, Kuijpers TW, et al. High-throughput analysis of the C4 polymorphism by a combination of MLPA and isotype-specific ELISA's. Mol Immunol. 2009; 46: 592–600. https://doi.org/10.1016/j.molimm.2008.07.028 PMID: 19062096

16. Wu YL, Hauptmann G, Viguier M, Yu CY. Molecular basis of complete complement C4 deficiency in two North-African families with systemic lupus erythematosus. Genes and immunity. 2009; 10: 433–445. https://doi.org/10.1038/gene.2009.10 PMID: 19279649

17. Lokki ML, Circolo A, Ahokas P, Rupert KL, Yu CY and Colten HR. Deficiency of human complement protein C4 due to identical frameshift mutations in the C4A and C4B genes. J Immunol. 1999; 162: 3687–3693. PMID: 10092831

18. Yang Y, Chung EK, Wu YL, Savelli SL, Nagaraja HN, Zhou B, et al. Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): Low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in European Americans. Am J Hum Genet. 2007; 80: 1037–1054. https://doi.org/10.1086/518257 PMID: 17503323

19. Ittiprasert W, Kantachuvessi S, Pavasuthapisit K, Verasertniyom O, Chaomthum L, Totemchokchakarn K, et al. Complete deficiencies of complement C4A and C4B including 2-bp insertion in codon 1213 are genetic risk factors of systemic lupus erythematosus in thai populations. J Autoimmun. 2005; 25: 77–84. https://doi.org/10.1016/j.jauto.2005.04.004 PMID: 15998580
20. Man XY, Luo HR, Li XP, Yau YG, Mao CZ and Zhang YP. Polymerase chain reaction based C4A*q0 and C4Bq0 genotyping: Association with systemic lupus erythematosus in southwest Han Chinese. Ann Rheum Dis. 2003; 62: 71–73. https://doi.org/10.1136/ard.62.1.71 PMID: 12480675

21. Senbagavalii P, Kumar N, Kaur G, Mehra NK, Geetha ST and Ramanathan VD. Major Histocompatibility Complex class III (C2, C4, factor B) and C3 gene variants in patients with pulmonary tuberculosis. Hum Immunol. 2011; 72: 173–178. https://doi.org/10.1016/j.humimm.2010.11.002 PMID: 21093518

22. Zhang S, Wang S, Li Q, Yao S, Zeng B, Ziegelstein RC, et al. Capillary leak syndrome in children with C4A deficiency undergoing cardiac surgery with cardiopulmonary bypass: A double-blind, randomised controlled study. Lancet. 2005; 366: 556–562. https://doi.org/10.1016/S0140-6736(05)67099-7 PMID: 16099291

23. Szalai C, Fust G, Duba J, Kramer J, Romics L, Prohászka Z, et al. Association of polymorphisms and allelic combinations in the tumour necrosis factor-alpha-complement MHC region with coronary artery disease. J Med Genet. 2002; 39: 46–51. https://doi.org/10.1136/jmg.39.1.46 PMID: 11826025

24. Jaatinen T, Ruuskanen O, Truedsson L, Lokki ML. Homozygous deletion of the CYP21a-TNX-C4B gene region conferring C4B deficiency associated with recurrent respiratory infections. Hum Immunol. 1999; 60: 707–714. PMID: 10439316

25. Soto K, Wu YL, Ortiz A, Aparicio SR, Yu CY. Familial C4B deficiency and immune complex glomerulonephritis. Clin Immunol. 2010; 137: 166–175. https://doi.org/10.1016/j.clinim.2010.06.003 PMID: 20580617

26. Mougey R. A review of the Chido/Rodgers blood group. Immunohematology. 2010; 26: 30–38. PMID: 20795316

27. Samano ES, Ribeiro Lde M, Gorescu RG, Rocha KC, Grumach AS. Involvement of C4 allotypes in the pathogenesis of human diseases. Revista do Hospital das Clinicas. 2004; 59: 138–144. PMID: 15286835

28. Szilagyi A, Fust G. Diseases associated with the low copy number of the C4B gene encoding C4, the fourth component of complement. Cytogenetic and genome research. 2008; 123: 118–130. https://doi.org/10.1159/000184699 PMID: 19287146

29. Margery-Muir AA, Wetherall JD, Castley AS, Hew M, Whidborne RS, Mallon DF, et al. Establishment of gene copy number-specific normal ranges for serum C4 and its utility for interpretation in patients with chronically low serum C4 concentrations. Arthritis Rheumatol. 2014; 66: 2512–2520. https://doi.org/10.1002/art.38680 PMID: 24757030

30. Saxena K, Kitzmiller KJ, Wu YL, Zhou B, Esack N, Hiremath L, et al. Great genotypic and phenotypic diversities associated with copy-number variations of complement C4 and RP-C4-CYP21-TNX (RCCX) modules: A comparison of Asian-Indian and European American populations. Mol Immunol. 2009; 46: 1289–1303. https://doi.org/10.1016/j.molimm.2008.11.018 PMID: 19135723

31. Schwaeble WJ, Lynch NJ, Clark JE, Marber M, Samani NJ, Ali YM, et al. Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and gastrointestinal ischemia/reperfusion injury. Proc Natl Acad Sci U S A. 2011; 108: 7523–7528. https://doi.org/10.1073/pnas.1101748108 PMID: 21502512

32. Klein J, Sato A. The HLA system. Second of two parts. N Engl J Med. 2000; 343: 782–786. https://doi.org/10.1056/NEJM200009143431106 PMID: 10984567

33. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med. 2000; 343: 702–709. https://doi.org/10.1056/NEJM200009073431006 PMID: 10974135

34. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R, et al. European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012; 54: 136–160. https://doi.org/10.1097/MPG.0b013e31821a23d0 PMID: 22197856

35. Wittner C, DeMarchi M, Mollenhauer E, Carbonara A. Colonic disease and C4A*Q0: An association secondary to HLA-DR3. Tissue Antigens. 1984; 23: 130–134. PMID: 6608806

36. Candore G, Lio D, Colonna Romano G, Caruso C. Pathogenesis of autoimmune diseases associated with B1 ancestral haplotype: Effect of multiple gene interactions. Autoimmun Rev. 2002; 1: 29–35. PMID: 12849055

37. Grunewald J, Lofgren’s syndrome: Human Leukocyte Antigen strongly influences the disease course. Am J Respir Crit Care Med. 2009; 179: 307–312. https://doi.org/10.1164/rccm.200807-1082OC PMID: 18996998

38. Wennersrom A, Pietinalho A, Vauhkonen H, Lahtela L, Palikhe A, Herman J, et al. HLA-DRB1 allele frequencies and C4 copy number variation in Finnish sarcoidosis patients and associations with disease prognosis. Hum Immunol. 2012; 73: 93–100. https://doi.org/10.1016/j.humimm.2011.10.016 PMID: 22074998
39. Kainulainen L, Peltola V, Seppanen M, Viander M, He Q, Lokki ML, et al. C4A deficiency in children and adolescents with recurrent respiratory infections. Hum Immunol. 2012; 73: 498–501. https://doi.org/10.1016/j.humimm.2012.02.015 PMID: 22406254

40. Sane J, Kurkela S, Lokki ML, Miettinen A, Helve T, Vaheri A, et al. Clinical sindbis alphavirus infection is associated with HLA-DRB1*01 allele and production of autoantibodies. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2012; 55: 358–363.

41. Skattum L, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. Mol Immunol. 2011; 48: 1643–1655. https://doi.org/10.1016/j.molimm.2011.05.001 PMID: 21624663

42. Mostafa GA, Shehab AA. The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. J Neuroimmunol. 2010; 223: 115–119. https://doi.org/10.1016/j.jneuroim.2010.03.025 PMID: 20452682

43. Rigby WF, WuYL, Zan M, Zhou B, Rosengren S, Carlson C, et al. Increased frequency of complement C4B deficiency in rheumatoid arthritis. Arthritis Rheum. 2012; 64: 1338–1344. https://doi.org/10.1002/art.33472 PMID: 22076784