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Complement C4 gene copy numbers modulate serum immune protein profiles in patients with first-episode psychosis and healthy controls in a sex-specific manner

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ABSTRACT

Background: The copy number of complement component C4 genes (*C4A* and *C4B*) has been associated with schizophrenia risk, particularly in men. Psychotic disorders are associated with alterations in serum immune protein levels, but whether the *C4A/B* copy numbers are related to circulating immune protein levels is not known.

Methods: Two Finnish first-episode psychosis (FEP) cohorts were studied, comprising 105 FEP patients and 71 controls who were assessed at baseline and at two-month and one-year follow-ups. The *C4A/B* copy numbers were analyzed by genomic RT-PCR. We measured immune protein concentrations using a 38-plex Luminex assay at all available time points and correlated the results with the *C4A/B* copy numbers using Spearman's rank correlations.

Results: The median *C4A* and *C4B* copy numbers did not differ between patients and controls. *C4A* copy number correlated broadly and positively with serum cytokine and chemokine levels across all measurement points in FEP patients, while correlations with *C4B* copy numbers were negative. Correlations in controls were weaker and less consistent. When analyzed separately in males and females, the broadest and most significant positive correlations of immune protein levels with *C4A* copy number and negative correlations with *C4B* copy number were observed in male FEP patients.

Conclusions: *C4A* and *C4B* gene copy variations influence immunoinflammatory responses and serum levels of many immune proteins. This phenomenon was pronounced in male FEP patients, suggesting that they may be more vulnerable to tissue injury or infections. The results underscore the importance of investigating sex-specific effects of *C4* gene variations in psychotic disorders.

1. Introduction

Psychotic disorders are severe mental disorders with a lifetime prevalence of 3.5 % (Perälä et al., 2007) and multifactorial etiology with high heritability (Tandon et al., 2024). It has been suggested that psychotic disorders should be called multisystem disorders, since patients with first-episode psychosis (FEP) already show alterations in parameters involving multiple organ systems, including immune and cardiometabolic systems, gut microbiome and the hypothalamic–pituitary–adrenal axis (Pillinger et al., 2019, Sen et al., 2024).

Schizophrenia and other psychotic disorders have been associated

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with multiple immunological changes. Alterations in the concentrations of several cytokines and chemokines have been reported in patients with psychotic disorders, already at the first psychotic episode (Laurikainen et al., 2020; Dunleavy et al., 2022; Halstead et al., 2023; Solmi et al., 2021). Some cytokines have been presented as possible state markers for acute psychosis, and others have been seen as possible trait markers that are not affected by current symptom severity or anti-psychotic medication (Halstead et al., 2023). However, metabolic comorbidities and unhealthy lifestyle also contribute to these changes in patients with long duration of illness (Suvisaari et al., 2011).

Some environmental risk factors of psychotic disorders indicate a potential role of the immune system. Exposure to childhood infections requiring hospital treatment is associated with an increased risk of schizophrenia spectrum psychoses (Debost et al., 2022). Several autoimmune diseases are also associated with an increased risk of schizophrenia spectrum disorders, whereas a few associate with a decreased risk (Cullen et al., 2019). Moreover, persons with schizophrenia have an increased risk of respiratory infections and asthma (Suetani et al., 2021). All these findings suggest abnormalities in immune system response to various antigens in people with schizophrenia.

The complement system, a crucial part of innate immunity, consists of over 50 proteins including soluble proteins, membrane regulators and receptors. Apart from a role in innate immunity complement acts as a trigger for the adaptive immune response. Complement functions in causing inflammation and targeting microbes for phagocytosis and direct killing, but also in cell and tissue development and clearance of injured tissue components. One of the complement system proteins is complement C4, which functions in the activation of the classical and lectin pathways of the complement system (Wang and Liu, 2021). C4 is one of the most polymorphic plasma proteins. In humans, there are two distinct *C4* genes, *C4A* and *C4B*, located in the MHC class III region in chromosome 6 (6p 21.3). *C4A* and *C4B* genes encode protein allotypes that differ slightly by sequence, while showing different binding affinities to specific molecular groups. C4A and C4B have interesting functional differences (Law et al., 1984; Isenman and Young, 1984). C4A prefers binding covalently to amino groups, typically in proteins, whereby it can bind effectively to proteins and immune complexes. Therefore, C4A is important in clearance functions that involve endogenous targets in addition to those in viruses and bacteria. C4B prefers covalent binding to hydroxyl groups, commonly present in carbohydrates, like on bacterial capsules.

There is copy number variation in both *C4A* and *C4B* genes, and each individual has a combination of them. The most common combination is composed of two *C4A* genes and two *C4B* genes. However, variations and deficiencies are common. Both *C4A* and *C4B* genes can present as either long or short ones depending on the presence or absence of a human endogenous retroviral (HERV) insertion at intron 9 (Dangel et al., 1994; Wu et al., 2008). A high proportion (76 %) of *C4* genes, and the majority of *C4A* genes, include an endogenous retrovirus (HERV) in intron 9 of the gene (Blanchong et al., 2001; Sekar et al., 2016).

A high copy number of *C4A* genes has been identified as a potential risk factor for schizophrenia (Sekar et al., 2016; Kamitaki et al., 2020), progressive supranuclear palsy (Farrell et al., 2024) and bipolar disorder with psychotic features (Hörbeck et al., 2024), whereas a higher number of *C4A* and to a lesser degree *C4B* is protective against autoimmune diseases, such as SLE and Sjögren's syndrome (Kamitaki et al., 2020). Conversely, *C4A* deficiency, and to a lesser degree *C4B* deficiency, is associated with increased risk of autoimmune diseases, while *C4B* deficiency is associated with susceptibility to invasive bacterial infections (Bishof et al., 1990; Lundtoft et al., 2022; Wang and Liu, 2021). The effect of *C4A* and *C4B* copy number to the risk of SLE, Sjögren's syndrome and schizophrenia is sexually dimorphic, being stronger in men than in women (Kamitaki et al., 2020). Interestingly, the incidence of Sjögren's syndrome and SLE is several-fold higher in women than in men (Mariette and Criswell, 2018, Barber et al., 2023), while men are 1.4-times more likely to be diagnosed with schizophrenia, and schizophrenia tends to be more severe in men (Abel et al., 2010).

Consistent with genetic findings, patients with first-episode psychosis (FEP) have been found to have elevated mRNA levels of *C4A* and higher serum concentrations of C4 and other complement components in several studies (Hatzimanolis et al., 2022; Yu et al., 2023; Cao et al., 2023). In postmortem studies of patients with schizophrenia, overexpression of the complement component C4 has also been observed in several brain areas (Rey et al., 2020; Jenkins et al., 2023).

The complement system has been indicated in synaptic pruning during the development of the brain (Stephan et al., 2012). Excessive synaptic pruning has been suggested as a potential mechanism how the increase in the *C4A* copy number affects schizophrenia risk (Sekar et al., 2016). However, given the wide spectrum of associations of schizophrenia to the functions of the immune system, other mechanisms are also possible. Here, we examined whether *C4A* and *C4B* copy number variation might be linked to peripheral immune protein levels, similarly as observed in some earlier studies on autoimmune diseases (Hou et al., 2013).

We set out to investigate potential *C4A* and *C4B* copy number differences in a longitudinal follow-up study of Finnish patients with FEP and controls. We studied whether the copy number of *C4* genes affects the peripheral immune system by analyzing the correlation between the copy number of *C4A* and *C4B* genes and serum immune protein, mostly cytokine and chemokine, levels. We further explored whether these correlations show longitudinal stability and whether they are similar in patients with FEP and healthy controls. We also investigated potential sex differences in these associations.

2. Materials and methods

2.1. Study populations

The recruited patients were in- or out-patients with first treatment contact for a psychotic episode (FEP) between December 2009 and November 2017. In the Helsinki Early Psychosis Study (HEPS), the patients were recruited from the in- and outpatient psychiatric services of the City of Helsinki and the Helsinki University Hospital. The Turku Early Psychosis Study (TEPS) cohort was recruited from the City of Turku clinics and the Hospital District of Southwest Finland. All primary psychotic disorders were included, while patients with psychotic disorders due to a general medical condition were excluded. In this study we included 105 patients, 91 of whom were from HEPS and 14 from TEPS. Seventy-one controls were recruited through the Population Register Center, and were matched by sex, age and the place of residence. 52 of the control participants were from Helsinki, and 19 from Turku. All the participants were aged between 18 and 40 years of age. The protocol in Helsinki included baseline visit as soon as the patient was able to give informed consent to the study, and follow-up visits at two and 12 months, while the control participants had two visits, 12 months apart. The protocol in Turku included two visits both for patients and controls, 12 months apart. The studies have been described in detail in Laurikainen et al. (2020).

As a second control group for gene copy number analysis, we used the results of previously collected sample of 149 people from the general population (Paakkanen et al., 2012).

2.2. Ethics statement

The HEPS and TEPS studies were approved by the Ethics Committees of the Hospital Districts of Helsinki and Uusimaa (diary numbers 257/12/03/03/2009 and 226/13/03/03/2013), and Southwest Finland (diary numbers 64/180/2011 and 65/1801/2013), respectively. Capacity to consent was assessed by the psychiatrist responsible for the patient's care, and all participants gave written informed consents before participation.

2.3. Laboratory analytical methods

DNA extraction, dilution and aliquoting was done at the DNA Extraction and Storage Facility in Biomedicum Helsinki. The *C4A* and *C4B* gene copy numbers were determined by using genomic RT-PCR at the HLA laboratory, Department of Pathology, University of Helsinki, as previously described (Paakkanen et al., 2012).

Fasting blood samples were collected at 8–10 a.m., coagulated at room temperature (max. 2 h) and then centrifuged. Sera were aliquoted and stored at −80 °C. The serum concentrations of 38 cytokines, chemokines and growth factors were analyzed using the 38-plexed Milliplex MAP Kit (cat. no. HCYTMAG-60 K-PX38) according to the manufacturer's recommendations (Merck-Millipore, Billerica, MA, USA) at the University of Helsinki (Laurikainen et al., 2020). Samples from follow-up time points were included whenever available. All analyses were done simultaneously using previously unthawed samples. Altogether, serum inflammatory marker measurements were available for 86 FEP patients at baseline, for 66 patients at 2-month follow-up and for 51 FEP patients at 1 year follow-up. For the control group, the baseline concentrations were available for 68 persons and the 1-year measurements for 33 persons.

2.4. Statistical analyses

We present the *C4A* and *C4B* copy number distributions in patients and controls and tested the difference in the median copy numbers using the Mann-Whitney test. In addition, we tested with the chi-squared test whether the distribution of copy numbers differed between patients and controls, combining the most infrequent combinations, i.e. for *C4A* zero and one copy as one group as well as three and four copies as one group, and for *C4B* two and three copies as one group.

We analyzed the correlation of *C4A* and *C4B* copy numbers with serum immune protein levels using the Spearman's rank correlation coefficient. These correlations are presented as Heatmaps for the different time points available. We also analyzed these correlations separately by gender in the patient and control groups.

The analysis was done with IBM SPSS Statistics 27 software (IBM corp. Armonk, NY, USA). P value <0.05 in a two-tailed test was considered significant. Heatmap analysis was performed using the R statistical software (version 4.4.1) in RStudio with the gplots package. Hierarchical clustering was applied to the rows to produce dendrograms on each heatmap.

3. Results

Descriptive information on the study sample is provided in Table 1.

Table 1
Descriptive information on the sample.

	FEP patients (N = 105)	Controls (N = 71)
Male / female	69/36	39/32
Helsinki / Turku	91/14	52/19
Age (years; mean (SD)	26.3 (5.8)	28.1 (7.2)
DSM-IV main diagnosis:		
Schizophrenia	35	
Schizophreniform disorder	25	
Schizoaffective disorder	6	
Brief psychotic disorder	4	
Psychotic disorder NOS	17	
Delusional disorder	1	
Bipolar I disorder with psychotic features	9	
Major depressive disorder with psychotic features	6	
Substance-induced psychotic disorder	2	
Any lifetime DSM-IV non-psychotic disorder		20

There were more men than women in both the patient and the control groups. The most common diagnoses in FEP patients were schizophrenia and schizophreniform disorder. At the baseline assessment, 43 patients (41 %) were using olanzapine, 33 risperidone (31 %), 21 quetiapine (20 %), 7 aripiprazole (7 %) and 7 other antipsychotics (7 %). A few patients were using more than one antipsychotic, while there were 8 patients (8 %; four men and four women) who were not using any antipsychotic medication.

3.1. C4A and C4B gene copy numbers in FEP patients and controls

The *C4* gene copy numbers of FEP patients and controls are presented in Table 2. No differences were observed in the median numbers of *C4A* and *C4B* genes between FEP patients and controls (Mann-Whitney test for *C4A* $P = 0.56$ and for *C4B* $P = 0.21$). There were no gender differences in the median copy number of either gene in the whole group or in patients and in controls separately. When the distribution of *C4A* and *C4B* copy numbers was compared, combining zero and one copy as one group and three and four copies as one group for *C4A* and two and three copies as one group for *C4B*, the distribution was different between patients and controls for *C4A* (χ^2(d.f. 2) = 8.27, $P = 0.016$). This was due to both low and high copy numbers being more common in controls than in patients. While the most common combination in patients was having two copies of both *C4A* and *C4B*, in controls it was three copies of *C4A* and one copy of *C4B* (Table 3). However, when a larger, historical control sample from the Finnish general population was used, neither *C4A* copy number distribution ($P = 0.71$) nor *C4B* copy number distribution ($P = 0.18$) differed significantly from controls (Table 2).

3.2. Correlations of serum immune protein concentrations with C4A and C4B gene copy numbers

The levels of serum inflammatory markers correlated with *C4* gene copy numbers in both FEP patients and controls. Hierarchical cluster analysis of the correlations is presented in Fig. 1a in patients and Fig. 1b in controls. A general trend of positive correlation with *C4A* gene copy numbers and a negative correlation of *C4B* gene numbers with serum immune protein concentrations across different time points was evident in patients (Fig. 1a). In controls, the positive correlation with *C4A* copy number was less consistent, while negative correlation with *C4B* copy number was seen at both time points (Fig. 1b).

When stratified by sex and examined separately in the patient and control groups, these correlations revealed sex-specific patterns (see supplementary tables 1 and 2 for a full presentation of results). In the

Table 2
The frequencies of C4 gene copy numbers in first-episode psychosis patients and controls.

	C4 gene number	C4A	C4B
FEP patients	0	1 (1.0 %)	6 (5.7 %)
	1	8 (7.6 %)	39 (37.1 %)
	2	64 (61.0 %)	59 (56.2 %)
	3	31 (29.5 %)	1 (1.0 %)
	4	1 (1.0 %)	0 (0 %)
Controls	0	1 (1.4 %)	5 (7.0 %)
	1	11 (15.5 %)	37 (52.0 %)
	2	28 (39.4 %)	27 (38.0 %)
	3	27 (38.0 %)	2 (2.8 %)
	4	4 (5.6 %)	0 (0 %)
External reference controls[1]	0	1 (0.7 %)	15 (10.1 %)
	1	15 (10.1 %)	46 (30.9 %)
	2	86 (57.7 %)	87 (58.4 %)
	3	43 (28.9 %)	1 (0.7 %)
	4	4 (2.7 %)	0

[1] Paakkanen R, Vauhkonen H, Eronen KT, Järvinen A, Seppänen M, Lokki ML. Copy number analysis of complement C4A, C4B and C4A silencing mutation by real-time quantitative polymerase chain reaction. PLoS One. 2012;7(6):e38813.

Table 3
The combinations of C4A and C4B in FEP patients and controls.

	FEP patients					
		C4B				
C4A		0	1	2	3	Total
	0	0	0	1	0	1
	1	0	2	6	0	8
	2	0	17	46	1	64
	3	6	20	5	0	31
	4	0	0	1	0	1
	Total	6	39	59	1	105

	Controls					
		C4B				
C4A		0	1	2	3	Total
	0	0	0	1	0	1
	1	0	2	8	1	11
	2	1	11	16	0	28
	3	1	23	2	1	27
	4	3	1	0	0	4
	Total	5	37	27	2	71

FEP group, statistically significant positive correlations of several serum immune protein levels with *C4A* copy number were seen in men (Fig. 2a). Altogether 11 immune markers correlated positively with *C4A* copy numbers in men with FEP, with Spearman correlation coefficients ranging from 0.26 to 0.34. In women with FEP, the only marginally significant finding was a negative correlation between CCL2 serum levels and *C4A* copy number. However, while men in the FEP sample had *C4A* copy numbers ranging from 0 to 4, women only had two or three copies. This lack of variation due to small sample size may have affected the results seen in women with FEP. For *C4B* copy number, the correlations were negative (Fig. 2b). In men with FEP, *C4B* copy numbers had significant negative correlations with G-CSF and several interleukins, including IL-2, IL-3, IL-4, IL-10, IL-12p40 and IL-15, with Spearman correlation coefficients ranging from −0.28 to −0.45. In women with FEP, there were no significant correlations between *C4B* copy numbers and immune markers, although *C4B* copy numbers in women with FEP ranged from 0 to 3.

In controls, statistically significant positive correlations with inflammatory markers and *C4A* copy number were seen in women with six immune markers, with Spearman correlation coefficients ranging from 0.37 to 0.51 (Fig. 2c). In men from the control group, *C4A* copy number correlated negatively with three immune markers with correlation coefficient ranging from −0.38 to −0.43. Statistically significant findings with *C4B* among controls were scarce: in men, a negative correlation with IL-3 (−0.34, 95 % CI -0.60- -0.02) and in women, a negative correlation with IL-8 (−0.39, 95 % CI -0.67 - -0.03) and CCL3 (−0.40, 95 % CI -0.67- -0.04) was seen.

4. Discussion

The main finding of this study was that complement *C4* copy number variation appears to modulate the peripheral immunoinflammatory system, particularly in patients with first psychotic episode. In patients with FEP, this effect was consistently seen at baseline, during the acute psychotic episode, and during the follow-up when over half of the patients had achieved symptomatic remission (Laurikainen et al., 2020). *C4A*, and to a smaller extent *C4B*, gene copy numbers correlated broadly with serum levels of cytokines and chemokines. These correlations were largely positive for *C4A* and negative for *C4B* gene copy numbers and were seen consistently in all measurement points from which we had data on serum immune markers. In controls, negative correlations with the *C4B* gene copy number were also consistently seen, whereas the findings regarding *C4A* copy number were less consistent.

When these correlations were analyzed separately by sex, we observed sex-specific differences. Among patients with FEP, statistically significant positive correlations of *C4A* gene copy number and negative correlations of *C4B* gene copy number with several immune proteins were mostly seen in male patients. In the control group positive correlations with *C4A* gene copy number were seen in females, while negative correlations with the *C4B* gene copy number were seen both in females and in males.

Our results suggest that complement C4 modulates peripheral immune activity, with *C4A* and *C4B* having to some extent opposite and potentially sex-specific effects. While there are previous studies on age- and sex-specific differences in the levels of complement proteins (Gaya da Costa et al., 2018) and in the association of *C4* copy number to C4 protein serum concentration (Borbye-Lorenzen et al., 2023), few studies have investigated whether the gene copy numbers of *C4A* or *C4B* affect the serum levels of a broader spectrum of immune proteins. One previous study examined the correlation of *C4A* copy number with 6 cytokines and 2 chemokines in healthy controls and found that a higher *C4A* copy number was associated with higher serum IL-6 level (Hou et al., 2013). Another study in patients with schizophrenia found a significant correlation between cerebrospinal fluid C4A protein and IL-1β concentrations; however, this study had adjusted for the copy number variation of *C4A* so these results are not comparable with ours (Gracias et al., 2022).

Larger *C4* copy numbers are associated with higher C4 serum or plasma concentrations (Yang et al., 2003, Kamitaki et al., 2020, Borbye-Lorenzen et al., 2023), with *C4A* copy numbers having a larger effect than *C4B* copy numbers (Borbye-Lorenzen et al., 2023). In a study based on neonatal blood samples Borbye-Lorenzen et al. (2023) found that although males and females did not differ in the gene copy number, females had significantly higher serum C4 protein concentrations than males (Borbye-Lorenzen et al., 2023), while the sex difference in C4 plasma protein concentrations was reversed in young adults (Kamitaki et al., 2020). The landmark study on the role of *C4* copy number variation to autoimmune diseases and schizophrenia showed that the copy number variation in *C4A* and *C4B* genes has a stronger effect on the risk of SLE, Sjögren's syndrome and schizophrenia in males than in females (Kamitaki et al., 2020). These studies support the view that the copy number variation of *C4A* and *C4B* genes has sex-specific effects.

We observed notable sex differences in the correlations of *C4A* and *C4B* gene copy numbers with serum immune protein levels in patients with FEP. Although several recent studies have investigated the role of complement component C4 and *C4A* and *C4B* gene copy numbers in psychotic disorders (Gracias et al., 2022, Cropley et al., 2023, Yu et al., 2023, Hörbeck et al., 2024), potential sex differences have received less attention. However, a recent study examined whether *C4A* and *C4B* copy numbers were associated with symptom severity and global functioning in patients with schizophrenia or schizoaffective disorder, finding an association between *C4B* copy number and symptom severity and between *C4A* copy number and global functioning in female patients (Chen et al., 2024).

Collectively, the molecules upregulated in male FEP patients are involved in immune system signaling, inflammation and cell recruitment. For example, the chemokines CCL2, CCL4, CCL7 can attract monocytes, T cells and dendritic cells to sites of inflammation or tissue injury. IL-1β is a pro-inflammatory cytokine, while IL-1RA is its natural inhibitor. The cytokine IL-12p70 mediates differentiation of T cells into Th1 cells, whereas IL-4 and IL-5 favor more Th2-responses by promoting differentiation of B cells and allergic reactions. The other cytokines also have cell specific effects: G-CSF stimulates the production granulocytes, IL-15 of NK cells, while FGF2 is involved in wound healing and angiogenesis. TNF-beta is a lymphotoxin that plays a role in inflammation and regulation of immune cells. Overall, it is possible that men with FEP are more likely to face events of tissue injury or infections, where some of the above-mentioned cytokines are involved.

A point of interest regarding the influence of sex on the effects of *C4*

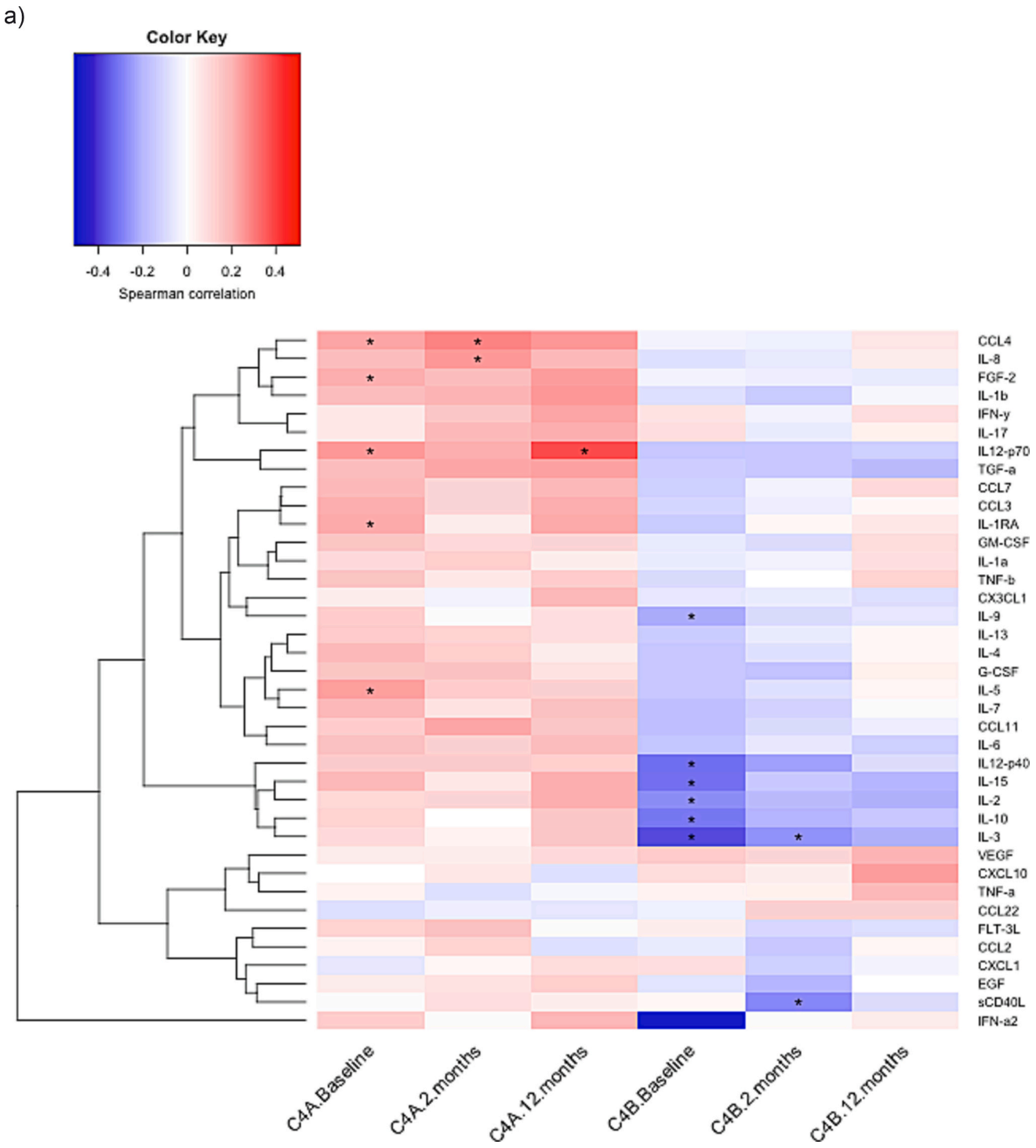

Fig. 1. a. Heatmap of Spearman rank order correlations of *C4A* and *C4B* copy numbers with immune protein levels in patients with first-episode psychosis. Baseline represents recruitment to the study, and 2 and 12 months were the respective follow-up points. Correlations with *P* value <0.05 are marked with asterisk. b. Heatmap of Spearman rank order correlations of *C4A* and *C4B* copy numbers with immune protein levels in controls. Baseline represents recruitment to the study, and 12 months was the follow-up point available for controls. **Correlations with *P* value < 0.05 are marked with asterisk.** CCL2 = C-C motif chemokine 2 (Monocyte chemoattractant protein 1 (MCP-1)); CCL3 = C-C motif chemokine 3 (macrophage inflammatory protein 1-alpha (MIP-1α)); CCL4 = C-C motif chemokine 4 (macrophage inflammatory protein 1-beta (MIP-1β)); CCL7 = C-C motif chemokine 7 (monocyte-chemotactic protein 3 (MCP-3)); CCL11 = C-C motif chemokine 11 (eotaxin); CCL22 = C-C motif chemokine 22 (macrophage derived chemokine (MDC)); CX3CL1 = chemokine (C-X3-C motif) ligand (fractalkine); CXCL1 = chemokine (C-X-C motif) ligand 1 (GROα); CXCL10 = chemokine (C-X-C motif) ligand 10 (Interferon gamma-induced protein 10 (IP10)); EGF = Epidermal growth factor; FGF-2 = basic fibroblast growth factor; FLT-3 L = Fms-related tyrosine kinase 3 ligand; G-CSF = Granulocyte-colony stimulating factor; GM-CSF = human granulocyte-macrophage colony–stimulating factor; IFN = Interferon; IL = interleukin; IL-1RA = interleukin 1 receptor antagonist; IL-8 = chemokine (C-X-C motif) ligand 8; IL12-p40 = Subunit beta of interleukin 12 (common subunit for IL-12 and IL-23; IL12B); IL12-p70 = the active heterodimer of IL-12; sCD40L = soluble CD-40 ligand; TGF-α = Transforming growth factor alpha; TNF-α = tumor necrosis factor-alpha; TNF-β = tumor necrosis factor-beta; VEGF = vascular endothelial growth factor.

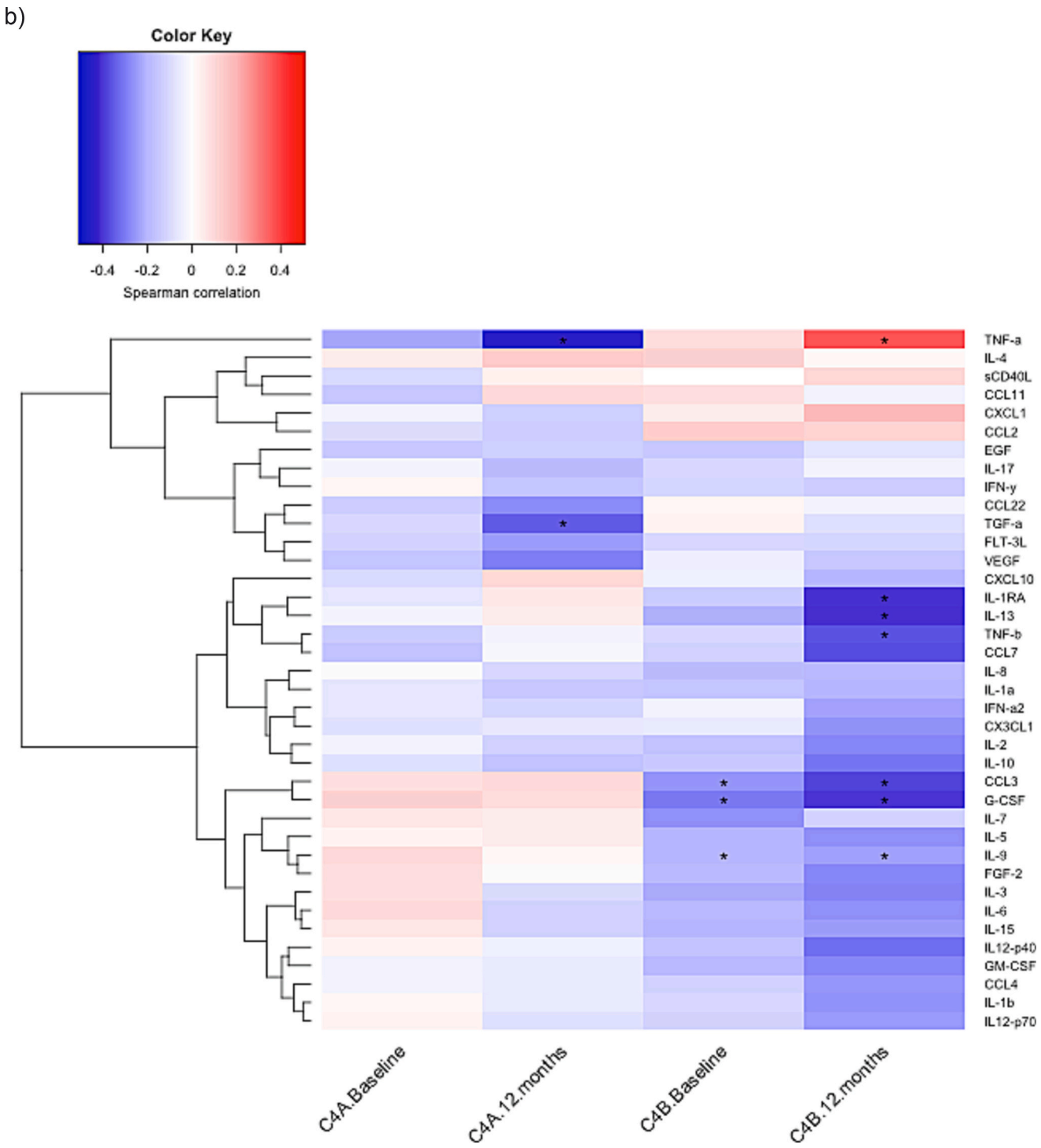

Fig. 1. (*continued*).

copy number variation is the connection of human *C4A* to mouse *Slp*, sex-limited protein gene. Mouse *C4* gene is similar to human *C4B*, and *Slp* gene is similar to human *C4A*. As mouse *Slp* is expressed only in adult males, it serves as yet another evolutionally interesting connection between sex, *C4* expression and its effects. The functionality and the androgen effect on mouse *Slp* is still debated. However, there is a possibility of a similar androgen-related mechanism influencing human expression of *C4A*. In mice, it has been reported that Slp expression can be induced by testosterone in female mice (Nelson and Robins, 1997). In previous human studies, sex has in healthy individuals affected the levels of C4 in the cerebrospinal fluid as well as in plasma in an age-dependent manner (Kamitaki et al., 2020).

Our study was limited by relatively small sample size. We did not control for multiple testing, and to avoid aggravating the multiple testing problem, we did not formally test interactions when analyzing sex differences or differences between FEP patients and controls. However, the correlations between *C4A* and *C4B* gene copy numbers and peripheral immune protein levels were consistent over time and the effects were quite broad, although individual correlation coefficients are fairly low.

In conclusion, *C4* genes contribute to the risk of complement-mediated disease in a sex-dependent manner. Our study shows that

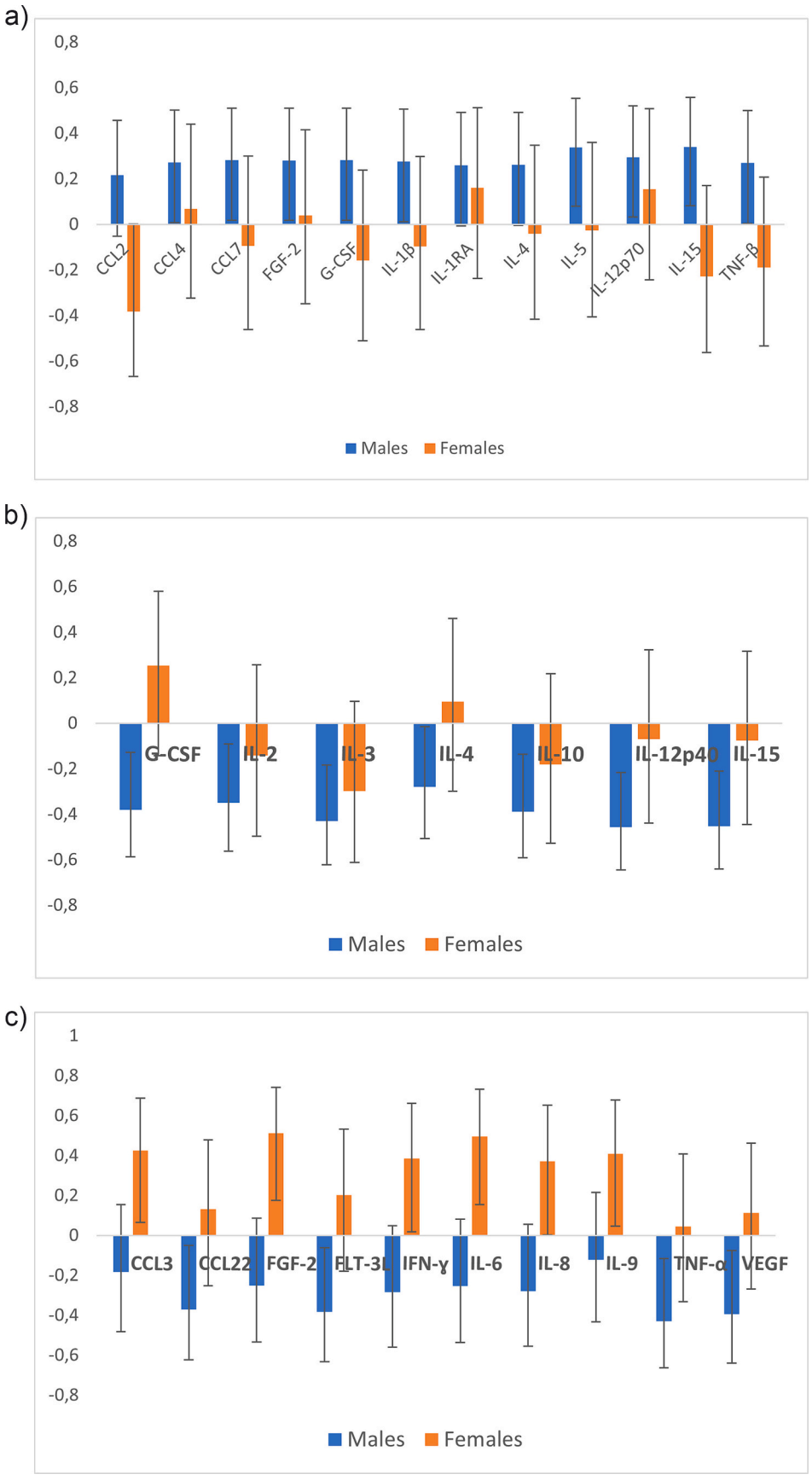

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Fig. 2. a. Spearman rank correlations between C4A copy numbers and immune protein levels in male and female patients with FEP at baseline. For the correlation coefficients, 95 % confidence intervals are presented. Immune proteins where the correlation was statistically significant in either males or females are presented in the figure. b. Spearman rank correlations between C4B copy numbers and immune protein levels in male and female patients with FEP at baseline. For the correlation coefficients, 95 % confidence intervals are presented. Immune proteins where the correlation was statistically significant in either males or females are presented in the figure. c. Spearman rank correlations between C4A copy numbers and immune protein levels in male and female controls at baseline. For the correlation coefficients, 95 % confidence intervals are presented. Immune proteins where the correlation was statistically significant in either males or females are presented in the figure. CCL2 = C-C motif chemokine 2 (Monocyte chemoattractant protein 1 (MCP-1)); CCL3 = C-C motif chemokine 3 (macrophage inflammatory protein 1-alpha (MIP-1α)); CCL4 = C-C motif chemokine 4 (macrophage inflammatory protein 1-beta (MIP-1β)); CCL7 = C-C motif chemokine 7 (monocyte-chemotactic protein 3 (MCP-3)); CCL11 = C-C motif chemokine 11 (eotaxin); CCL22 = C-C motif chemokine 22 (macrophage derived chemokine (MDC)); CX3CL1 = chemokine (C-X3-C motif) ligand (fractalkine); CXCL1 = chemokine (C-X-C motif) ligand 1 (GROα); CXCL10 = chemokine (C-X-C motif) ligand 10 (Interferon gamma-induced protein 10 (IP10)); EGF = Epidermal growth factor; FGF-2 = basic fibroblast growth factor; FLT-3 L = Fms-related tyrosine kinase 3 ligand; G-CSF = Granulocyte-colony stimulating factor; GM-CSF = human granulocyte-macrophage colony–stimulating factor; IFN = Interferon; IL = interleukin; IL-1RA = interleukin 1 receptor antagonist; IL-8 = chemokine (C-X-C motif) ligand 8; IL12-p40 = Subunit beta of interleukin 12 (common subunit for IL-12 and IL-23; IL12B); IL12-p70 = the active heterodimer of IL-12; sCD40L = soluble CD-40 ligand; TGF-α = Transforming growth factor alpha; TNF-α = tumor necrosis factor-alpha; TNF-β = tumor necrosis factor-beta; VEGF = vascular endothelial growth factor.

the effects of *C4* copy number variation influence the peripheral immune protein levels in individuals with FEP and to a lesser degree also in healthy controls. This suggests that when the potential mechanisms of how particularly *C4A* affects the risk of psychotic disorder are studied, more attention should be paid to the effects of complement *C4* copy number variation on peripheral immune system. The modulating effect on peripheral immune protein levels could affect immune response broadly or to specific antigens. Moreover, the stronger effect of *C4A* and *C4B* copy number on peripheral immune protein levels seen in men with FEP may offer clues to why *C4A* copy number variation affects schizophrenia risk more in men than in women.

CRediT authorship contribution statement

Uni Rankka: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Marja-Liisa Lokki:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Vesa Vahermaa:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Heikki Laurikainen:** Writing – review & editing, Investigation. **Jarmo Hietala:** Writing – review & editing, Investigation, Funding acquisition. **Jarno Honkanen:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Seppo Meri:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jaana Suvisaari:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

Role of the funding source

The funding organizations had no role in the design or execution of the study; in the collection, management, analysis, or interpretation of data; in the preparation, review, or approval of the manuscript; or in the decision to submit the manuscript for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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