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CASE REPORT Open Access

A case report of complement *C4B* deficiency in a patient with steroid and IVIG-refractory anti-NMDA receptor encephalitis

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Abstract

Background: Complement C4A or C4B deficiency has never been reported in autoantibody-associated encephalitides patient. Here we present a case of anti-*N*-methyl- D-aspartate (NMDA) receptor encephalitis associated with homozygous C4B deficiency, who did not respond to intravenous immunoglobulin and pulse methylprednisolone but plasmapheresis and rituximab.

Case presentation: A fourteen-year-old boy presented to our unit with subacute onset of behavioral changes and confusion, and was later confirmed to be anti-NMDA receptor encephalitis. He was initially managed with intravenous immunoglobulin (IVIG) and pulse methylprednisolone but did not achieve any clinical improvement. Seven sessions of plasmapheresis was commenced with remarkable improvement after the second session, and was followed by four doses of rituximab. His neurological and cognitive functioning gradually returned to baseline. Immunological investigations demonstrated persistently low C4 levels below 8 mg/dL. A more in-depth complement analysis of the patient and his family showed that he has homozygous C4B deficiency. Genetic analysis revealed that the index patient has homozygous deficiency in complement C4B and he carries one non-functioning mutant *C4B* gene inherited from his mother.

Conclusions: Low levels of serum C4 correlate with reduced functions of the classical and lectin pathways, leading to the impairment of immune-complexes removal. Plasmapheresis ameliorates complement deficiency and removes the offending immune-complexes leading to clinical improvement that was not achieved by IVIG and steroids. We postulate that serum C4 levels may serve as a biomarker for the need of plasmapheresis upfront rather than only after non-response to steroid and IVIG in treating anti-NMDA-receptor encephalitis.

Keywords: Anti-NMDA receptor encephalitis, Homozygous C4B deficiency, Plasmapheresis

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Background

Complement C4 plays a crucial role in the activation of the classical and lectin complement pathways. It has two isotypes, C4A and C4B. While deficiency of C4A and C4B are detectable at low frequency in healthy populations, they have also been reported to be associated with various autoimmune and inflammatory diseases [1, 2]. However, a C4A or C4B deficiency has never been reported in autoantibody-associated encephalitides patient. Here we report a case of anti-*N*-methyl- D-aspartate (NMDA) receptor encephalitis associated with homozygous C4B deficiency.

Case presentation

Our patient is a fourteen-year-old boy with background of mild attention-deficit and hyperactive disorder. He has insignificant family history of neurological, psychological or immunological disorders. Preceded by two weeks of viral illness-like prodromal symptoms, he progressively developed hypersomnolence, confused speech with echolalia, self-muttering, dysarthria, mood fluctuation, bilateral upper limbs tremor, and headache. Physical examination was unremarkable, apart from having confusion, inappropriate speech with foul languages, urine and fecal incontinence. Investigations showed normal complete blood count, liver and renal function tests. Autoimmune markers including anti-nuclear antibodies and anti-double stranded DNA antibody were negative. His serum C3 levels were within normal range between 109 and 115 mg/dL, but his C4 levels were persistently below 8 mg/dL (Table 1). MRI brain was normal. Electroencephalogram showed a mild slowing of background activities. Cerebrospinal fluid (CSF) analysis demonstrated a total white cell count of 33 cells/mm^2, with normal protein and glucose levels. Oligoclonal protein was detected in the CSF. Microbiological investigations including bacterial cultures and viral polymerase chain reactions for herpes simplex virus, varicella zoster virus and enteroviruses were all negative. Anti-NMDA receptor antibodies based on commercial assay (Euroimmune®, Lueback, Germany) were detectable (titer < 1:10) in the CSF but not the serum.

The diagnosis of anti-NMDA receptor encephalitis was made based on the diagnostic criteria published by Graus et al. [3] He was immediately given intravenous immunoglobulin (IVIG) 1 g/kg for on day 2 of admission for two consecutive days followed by pulse methylprednisolone 1 g on day 6 of admission for five days with gradual taper. However, he did not demonstrate any clinical improvement after IVIG and pulse methylprednisolone.

Plasmapheresis was therefore performed for a total of 7 sessions between day 14 and 29 of admission, with sedation required for the initial two sessions. He demonstrated significant improvement after the second session and was able to complete the remaining plasmapheresis without sedations. Nevertheless, his cognitive improvement remained slow and four doses of rituximab at 375 mg/m^2/dose at weekly intervals were given between day 35 and 56 of admission. Accelerated improvement was observed after the second dose in terms his self-care abilities, behavior and cognitive functions. He was discharged after eight weeks of admission with some residual cognitive impairment requiring rehabilitation.

In view of his low C4 protein levels, an in-depth genetic analysis of the index patient, his parents and his maternal stepbrother was performed (Fig. 1; Table 1, Supplementary Fig. 1). Long-range mapping by pulsed field gel electrophoresis of *Pme*I-digested genomic DNA revealed heterozygosity with bimodular (LL) and monomodular (L) *RP-C4-CYP21-TNX* (RCCX) haplotypes (*panel* A). Regular Southern blots of *Taq*I digested genomic DNA revealed the presence of three long *C4* genes in LL/L configurations (*panel* B), by which two *C4* genes belong to the *C4A* isotype and one *C4* gene belongs to the *C4B* isotype, as shown by *Psh*AI/*Pvu*II Southern blot (*panel* C). Immunofixation experiment of EDTA-plasma showed that the patient expressed C4A protein but no C4B protein (*panel* D) [4]. In other words, the patient contained a *C4B* gene that did not produce a C4B protein (*panels* C and D, *arrows*) and thus assigned a *C4B*

Table 1 Complement genetic profiles of the patient and his family members

	Plasma C4 (mg/dL)	Plasma C3 (mg/dL)	RCCX-C4 haplotypes	Total C4 GCN	C4 Long GCN	C4 Short GCN	C4A GCN	C4B GCN*	C4A Protein	C4B Protein	C4 protein Haplotype1	C4 protein Haplotype2
Patient	7.7	118.5	LL / L	3	3	0	2	(1)	A3A3	Q_0	A3Q_0	A3
Mother	20.2	106.4	LL / LLS	5	4	1	3	2 (1)	A3A3A3	B2, Q_0	A3Q_0	A3A3B2
Father	19.2	167.6	L / L	2	2	0	2	0	A3A3		A3	A3
Maternal Step Brother	10.0	91.1	LL / L	3	3	0	1	2 (1)	A3	B1, Q_0	A3Q_0	B1

GCN gene copy number, *L* C4 long gene, *S* C4 short gene, *LL* long-long, *LS* long-short, *LLS* long-long-shortm, *A3 C4A* allotype 3
B1 C4B allotype 1, *B2* C4B allotype 2, Q_0 zero quantity of C4 protein from the corresponding gene;
* C4B GCN in brackets indicates the number of mutant gene

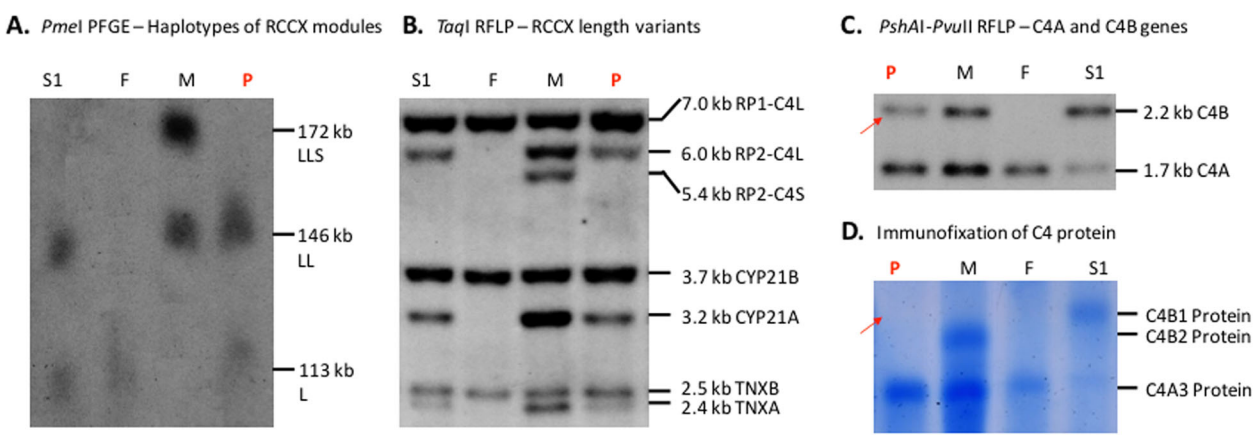

Fig. 1 Molecular genetic characterization of the encephalitis patient withantibodies against NMDA-receptor and his family members. **a**. Pulsed field gel electrophoresis (PFGE) of PmeI digested genomic DNA to show RP-C4-CYP21-TNX (RCCX) haplotypes; **b**. TaqI restriction fragment polymorphism (RFLP) to show details of RCCX structures with long (L) and short (S) C4 genes; **c**. PshAI/PvuII RFLP to show the presence and ratios of C4B and C4A genes; **d**. Immunofixation of EDTA-plasma to show polymorphisms of C4A and C4B proteins. A red arrow showed the presence of a C4B gene in panel C but no C4B protein in panel D. Data interpretation is tabulated in Table 1. Abbreviations: P, patient; F, father; M, mother; S1, stepbrother.

mutant. Concurrent study of the patient's parents and maternal stepbrother suggested the patient (Fig. 1, Table 1) inherited the mutant *C4B* gene originated from their mother with a LL haplotype consisting of one long *C4A* gene coding for C4A3 protein, and one long mutant *C4B* gene with no product.

Discussion and conclusions

Deficiency of C4B proteins, low gene copy number or mutation of *C4B* gene has not been reported in any autoimmune encephalitides. Therefore, its potential role in the development of anti-NMDA receptor encephalitis remains to be established. Classical pathway complement proteins such as C1q and C4 have been implicated in the wiring of neurons, or the formation and pruning of synapses in the brain [5]. High copy number of *C4A*, which can also be presented as low copy number of *C4B* because a *C4* gene either codes for C4A or C4B protein [2], are associated with schizophrenia [6].

Low levels of serum C4 correlate with reduced functions of the classical and lectin pathways, leading to the impairment of immune-complexes removal [7]. Our patient was a poor responder to IVIG and pulse glucocorticoids but achieved significant improvement after plasmapheresis and rituximab. While a study by Martinez-Hernandez et al. demonstrated that complement-mediated cytotoxicity, i.e. cellular destruction through deposition of C3b and formation of membrane attack complexes C5b-9, was not readily detectable in the pathogenesis of anti-NMDA receptor encephalitis, their study did not examine the activation of early classical pathway complement components such as C1q and C4 [8]. This would be relevant because complement activation on autologous cells are tightly controlled and mostly stop after the deposition of C4b. We postulate that C4B deficiency in our patient might have led to the defective removal of immune complexes and possibly impairment of yet to be characterized neurogenic pathways, which has a substantial role in autoimmune encephalitis [9–11]. Complement deficiency is ameliorated by plasmapheresis, which normally correct the complement deficiency, but not by IVIG. Plasmapheresis also effectively removes offending autoantibodies and immune-complexes [12]. The use of rituximab, an anti-CD20 monoclonal antibodies, depletes $CD20^+$ B cells, preventing further autoantibody production and immune complex formation [8]. These strategies lead to a sustained clinical improvement. It remains to be determined if routine plasmapheresis is required to keep patient's disease in remission.

Further studies investigating the complement profiles and circulating immune complexes in patients with anti-NMDA receptor and other autoimmune encephalitides would clarify the role of *C4B* deficiency. A study by Shu et al. demonstrated that southern Chinese patients with anti-NMDA receptor encephalitis had higher C4 levels among female patients ($N = 19$) than female healthy controls ($N = 16$), the sample sizes (for controls) were relatively small and required validation. Moreover, genetic deficiency of C4B was not examined [13]. While the best first-line therapeutic option (steroid, IVIG or plasmapheresis) to treat anti-NMDA receptor encephalitis remains debatable [14], inheritently low C4 levels or C4B deficiency may be a biomarker for the need of plasmapheresis upfront rather than only after non-response to steroid and IVIG.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12883-020-01906-x.

Additional file 1.

Abbreviations

A3: *C4A* allotype 3; B1: *C4B* allotype 1; B2: C4B allotype 2; CSF: Cerebrospinal fluid; GCN: Gene copy number; IVIG: Intravenous immunoglobulin; NMDA: Anti-*N*-methyl-D-aspartate; L: C4 long gene; LL: Long-long; LS: Long-short; LLS: Long-long-short; S - C4: Short gene.; PFGE: Pulsed field gel electrophoresis; Q_0: Zero quantity of C4 protein from the corresponding gene; RFLP: Restriction fragment polymorphism

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Authors' contributions

GTC, YLL, ACCH, SHSC made the diagnosis and performed clinical studies of the patient. DZ and CYY performed complement studies. GTC, YLL, DZ and CYY wrote the manuscript draft. All authors approved the final version of the manuscript.

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Availability of data and materials

The laboratory data for this study were shown in Table 1 and Fig. 1. Further clinical data would be available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed after approved IRB protocol (ID# MOD00003296) entitled "The Major Histocompatibility Complex (MHC) and Gene Copy Number Variations (CNVs) in Human Disease Associations" from The Nationwide Children's Hospital. All of the family members involved in this study signed informed consents to participate.

Consent for publication

Written consent for publication is obtained from the parents of index patient, with ascent obtained from the patient. The patient and first-degree relatives were deidentified.

Competing interests

The authors declare that they have no competing interests.

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