Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Genetic variant in complement receptor 1 (CR1, CD35) is associated with a cluster of severe fatal COVID-19 in a family

Dear Editor,

We read with great interest the study by Quiles-Jiménez et al., showing that severely ill COVID-19 patients have altered circulating levels of proteins controlling the epitranscriptome.[1] While severe COVID-19 can occur in otherwise healthy individuals of any age, it predominantly occurs in adults with advanced age or certain underlying medical comorbidities.[2] However, during the early stages of the pandemic, it was already noted that these risk factors did not fully explain the clinical variability of COVID-19 with reports of severe and fatal illness among otherwise healthy individuals, sometimes clustering in families, suggesting a role for human genetics as a risk factor for disease severity. This study used whole exome sequencing (WES) to explore whether genetic variants could be identified in a family that was severely affected by COVID-19 with four members succumbing to COVID-19 and two others suffering from severe and near-fatal infection. We included 23 members belonging to a family of Arabic origin (8 males and 15 females) of which there were 11 siblings (8 females and 3 males) (see pedigree chart in Fig. 1). Exome sequencing results were analyzed using 2 panels; a customized COVID-19 gene panel which included 46 gene loci that were associated with SARS-CoV-2 infection susceptibility or COVID-19 severity based on recent large-scale population-based association studies.[3] Second, through immune deficiency gene panel that in-

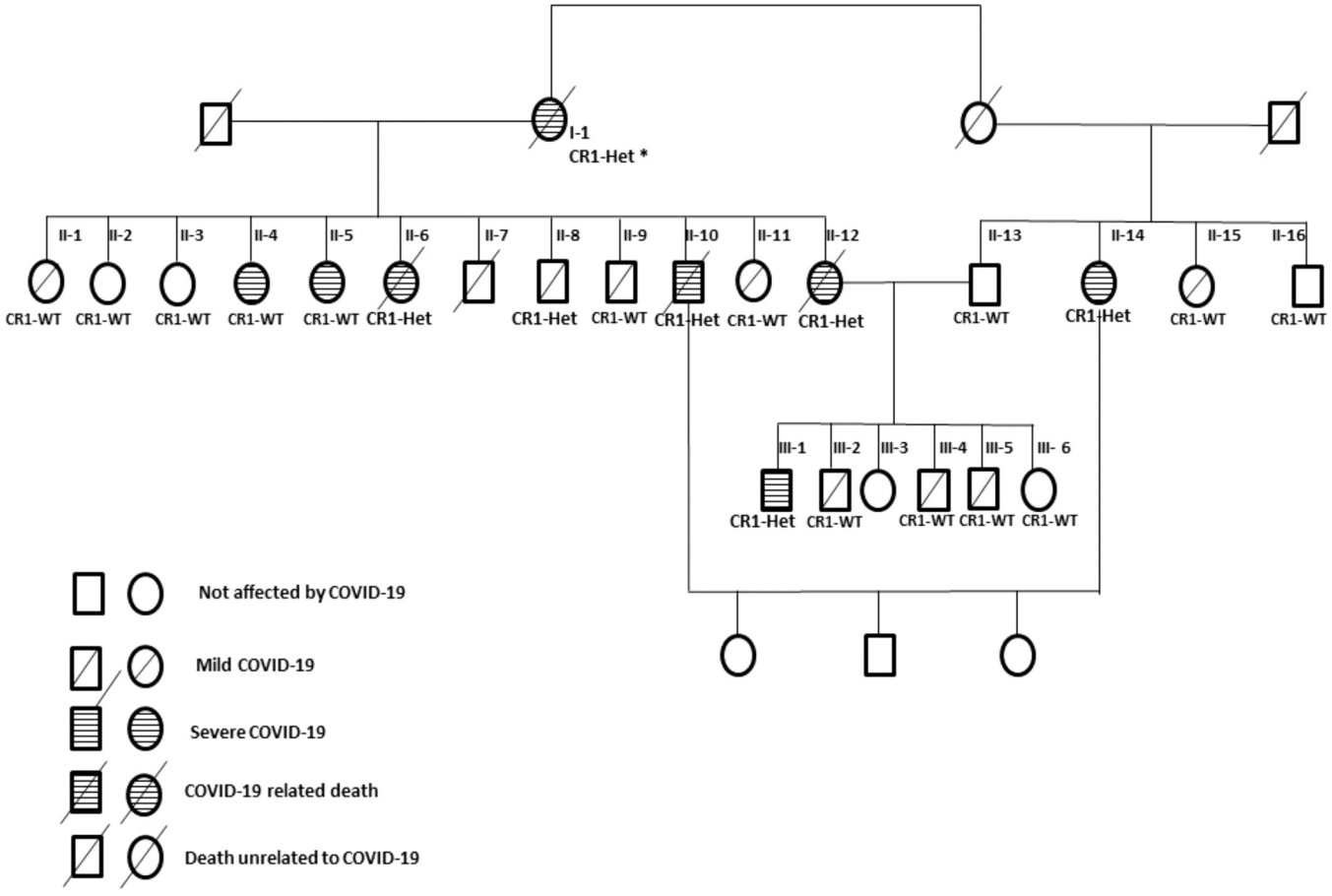

Fig. 1. Pedigree chart of the affected family (three generations). * het; heterozygote for the variant c.5302+1G > A in *CR1* gene (NM_000651.5.).

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cludes genes that were previously associated with abnormal immune response (for more details, see Supplementary materials).

The family analyzed in this study was severely affected by the pandemic. Four members suffered from critical illness and died from COVID-19-related complications (mother and three siblings, members; I-1, II-6, II-10, II-12), two siblings suffered from severe COVID-19 (members; II-4, II-5), and two other members of the extended family suffered from severe near-fatal disease (members II-14 and III-1) (Fig. 1). Three members died prior to the introduction of SARS-CoV-2 vaccines (siblings II-6, II-10, II-12) and one received two immunizations one year prior to her fatal illness (member I-1). Segregation analysis using the COVID-19 gene panel and immune deficiency gene panel did not show any segregation among the severely affected family members. However, by analyzing all coding genes, a potential pathogenic variant: c.5302+1G > A in *CR1* gene (complement receptor 1, CD35) [NM_000651.6, chr1:207,755,349G > A, rs756221326, (GRCh37)] was detected. The *CR1* gene encodes complement receptor 1 (CD35), the receptor for C3b/C4b complement peptides. CR1 has been identified as an inhibitor of the complement cascade by promoting the dissociation of the alternative pathway C3 convertase C3b,Bb and the cleavage of C3b by C3b/C4b inactivator. CR1 also inactivates the C3 and C5 convertases of the classical pathway and inhibits the consumption of C3 by C3 convertase EAC142 and enhances the decay of C4b,2a sites.[4] Given the regulatory role of CR1 in the complement system we hypothesized that the identified variant in *CR1* gene may provide insights into the catastrophic outcome of the affected family members. Therefore, we completed Sanger sequencing and segregation analysis for the rest of the family. We found a high correlation between severely affected individuals and heterozygote alteration. The variant c.5302+1G > A is an extremely rare variant in the general population (< 0.01%), absent from in-house database (1320 alleles), and very low (< 0.001%) in the Genome Aggregation Database (gnomAD). *In silico* analysis predicted dramatic decrease of donor splice-site strength**.** CR1 gene expression analysis identified two isoforms expressed for carries: one with full exon 32 inclusion (wild-type allele) and second with exon skipping (without exon 32). A major outcome of exon skipping is abolishing normal reading frame and creating early stop gain, leading to truncated protein (31 exons compared to a wild-type protein that includes 47 exons). (Fig. 2). Quantitative allele expression by qPCR of the wild-type allele, using primers targeting exon 32, among carriers and wild-type samples revealed significantly decreased levels of the wild-type allele (see Supplementary material).

Patient I-1, who was a heterozygous of the variant c.5302+1G > A in *CR1* gene, died from complications of severe COVID-19 during January 2022. We had obtained blood samples from the patient prior to that admission as part of the workup that was carried out to investigate the clustering of several COVID-19 related deaths in the family early in the pandemic (August 2020). During the patient's admission with severe COVID-19, we obtained weekly blood samples and compared mRNA *CR1* expression to preadmission levels and to other patients (wild-type controls) with severe COVID-19. The relative expression of mRNA *CR1* of the heterozygous carrier during severe COVID-19 was ~ fourfold higher compared to preadmission levels but not significantly different from wild-type carriers admitted with severe COVID-19 (see Supplementary material).

To our knowledge, the variant identified in this case series has not been described previously. This genetic variant impaired CR1 expression; however, a considerable upregulation of *CR1* gene expression occurred during severe infection. To date, there are no known individuals with innate CR1 deficiency. Acquired CR1

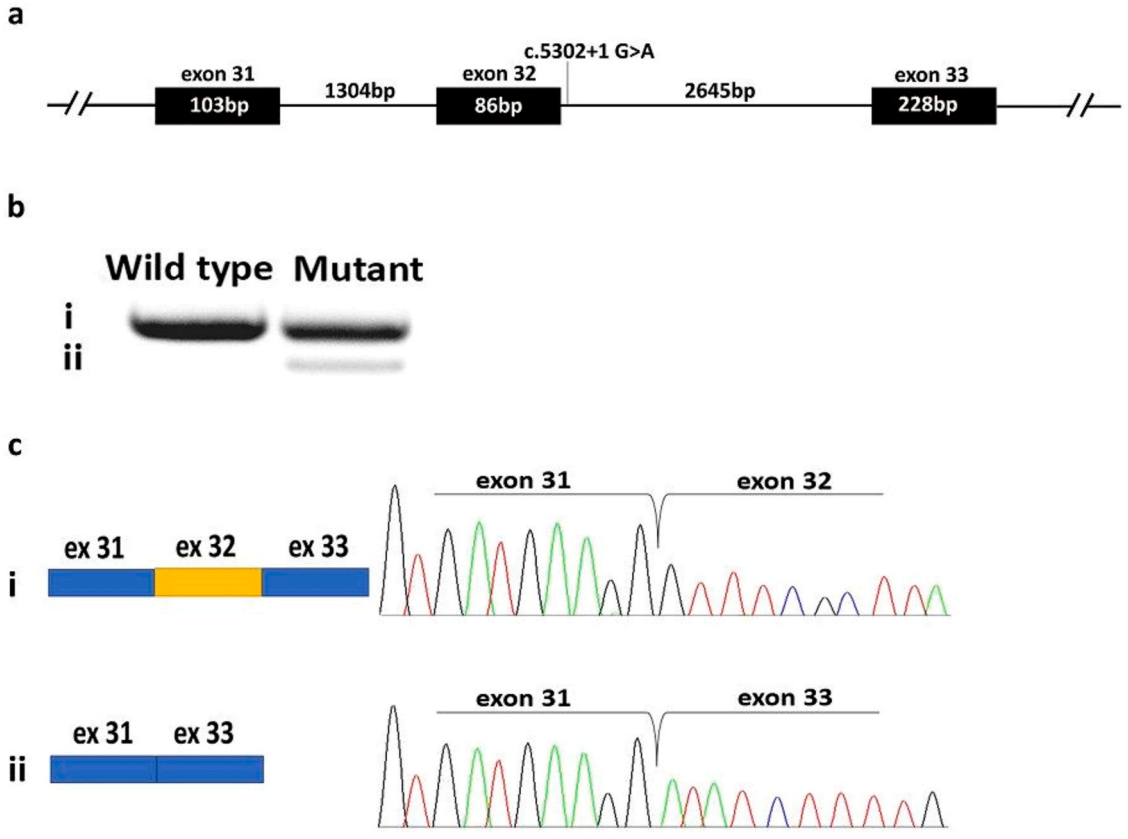

Fig. 2. RNA expression analysis of *CR1*. (a) Genomic region encompassing the detected variant in CR1. (b) Gel electrophoresis of wild-type and heterozygote PCR products. (c) Sanger sequencing results of extracted PCR products from the gel confirming normal expression for wild-type sample (i) and aberrant transcript presenting exon 32 skipping for heterozygote sample (ii).

deficiency, demonstrated by low expression of CR1 on erythrocytes, has been described in pregnancy and in acute or chronic infectious and inflammatory conditions.[5] In addition, temporary decrease in CR1 levels has been reported during the acute phase of COVID-19 with normalization during recovery.[6,7]

Emerging data indicate that complement activation plays a critical role in pathogenesis and disease severity of coronavirus infections.[8,9] Given that CR1 plays a critical role in controlling complement activity by acting on all three complement pathways as a membrane-bound receptor of C3b and C4b, a decay accelerator for C3/C5, and a cofactor for factor I-mediated cleavage of C3b and C4b, it is intriguing to speculate that decreased CR1 expression among heterozygotes of the genetic variant may have contributed to the evolution of severe and fatal COVID-19 in this family.

In conclusion, in this family that was tragically affected by COVID-19, we detected a unique genetic variant in *CR1* gene that impaired CR1 expression. Our findings suggest a significant role for CR1 in the pathogenesis of COVID-19. Future research will focus on direct mechanisms by which CR1 affects the complement system in COVID-19.

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Declaration of Competing Interest

Nothing to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.03.014.

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