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.

Medical Hypotheses 145 (2020) 110342

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

NCAM protein and SARS-COV-2 surface proteins: In-silico hypothetical evidence for the immunopathogenesis of Guillain-Barré syndrome

Sara Morsy

Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

ARTICLE INFO

Keywords:
Guillain-Barré syndrome
COVID-19
SARS-COV-2
NCAM
Autoimmunity
Major histocompatibility complex

ABSTRACT

This study aimed at identifying human neural proteins that can be attacked by cross-reacting SARS-COV-2 antibodies causing Guillain-Barré syndrome. These markers can be used for the diagnosis of Guillain-Barré syndrome (GBS). To achieve this goal, proteins implicated in the development of GBS were retrieved from literature. These human proteins were compared to SARS-COV-2 surface proteins to identify homologous sequences using Blastp. Then, MHC-I and MHC-II epitopes were determined in the homologous sequences and used for further analysis. Similar human and SARS-COV-2 epitopes were docked to the corresponding MHC molecule to compare the binding pattern of human and SARS-COV-2 proteins to the MHC molecule. Neural cell adhesion molecule is the only neural protein that showed homologous sequence to SARS-COV-2 envelope protein. The homologous sequence was part of HLA-A68 and HLA-DQA/HLA-DQB epitopes had a similar binding pattern to SARS-COV-2 envelope protein. Based on these results, the study suggests that NCAM may play a significant role in the immunopathogenesis of GBS. NCAM antibodies can be used as a marker for Guillain-Barré syndrome. However, more experimental studies are needed to prove these results.

Introduction

Currently, few studies reported that severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection was associated with the occurrence of Guillain-Barré syndrome that is considered the most frequent post-infection acute paralytic neuropathy [1–4]. Guillain-Barré syndrome did not have the best prognosis since only 20% survive with disabilities and a 5% mortality rate [5]. Moreover, it is estimated that 20% to 30% of cases had a respiratory failure [6]. Usually, Guillain-Barré syndrome is preceded by infections that cause an autoimmune response against peripheral nerves [5,6].

It is not the first time that the SARS-COV-2 infection caused neurologic manifestations. In a cohort of 214 hospitalized patients, it was estimated that neurologic symptoms appeared in 36% of the patients [7]. Guillain-Barré syndrome is reported in different coronaviruses' infection, however, the exact mechanism is not well understood [8,9].

In Northern Italy, five patients were diagnosed as COVID-19 developed symptoms of Guillain-Barré syndrome within 5 – 10 days after infection. Three patients were tested negative for gangliosides antibodies. All cases had poor to moderate outcomes with two of them remained in intensive care units [2]. 61 years old Chinese female developed Guillain-Barré syndrome after seven days of COVID-19 diagnosis. The patient had a full recovery after 30 days of infection, however, the patient was not tested for any antibodies [3]. Another case in the United States developed Guillain-Barré syndrome and tested positive for SARS-COV-2. The patients had not recovered completely but patients had weakness in the lower extremities [4]. Another case in Iran developed symptoms and signs, however, no antibody tests were done for the patient [1].

The mechanism of the induction of Guillain-Barré syndrome is still not understood in the SARS-COV-2 infection as many studies did not perform diagnostic tests to identify the neural antibodies. Understanding the mechanism would provide an insight into the disease immunopathogenesis.

Hence, in this study, through the computational approach, the possibility of similar epitopes between SARS-COV-2 surface proteins and neuronal proteins was investigated.

Hsypothesis/theory

The epitope mimicry between surface proteins of SARS-COV-2 and human neural proteins is responsible for the autoimmune mechanism underlying Guillain-Barré Syndrome. In this study, the NCAM protein was identified as the target proteins responsible for the development of Guillain-barré Syndrome in these patients. Besides, two HLA molecules either HLA-A*68 and HLA-DQA1/HLA-DQB1 could bind the

E-mail address: sara.morsy@med.tanta.edu.eg.

https://doi.org/10.1016/j.mehy.2020.110342
Received 11 September 2020; Received in revised form 1 October 2020; Accepted 6 October 2020
Available online 08 October 2020

homologous sequence in both humans and SARS-COV-2. These specific HLA distributions would explain the occurrence of Guillain-Barré Syndrome in a specific population and not in all COVID-19 patients. NCAM is not only expressed in neural tissues but also expressed in cardiac and skeletal muscles [10]. In different myopathies, decreased NCAM protein in muscles is considered important pathogenesis in these diseases as it was linked to denervation of the muscles [11]. This is considered crucial for more understanding of the respiratory distress in COVID-19 patients as respiratory muscle weakness can be considered one of the causes of respiratory distress [12,13]. That is why this hypothesis is important for more understanding of the immunopathogenesis of COVID-19.

Evaluation of the hypothesis

The hypothesis was evaluated using a bioinformatics approach through molecular mimicry analysis to identify possible neural proteins that can be attacked by cross-reacting immune cells. Besides, molecular docking was performed to confirm the strength of binding of cross-reacting immune cells to the human neural protein.

The SARS-COV-2 surface protein sequence

The amino acid sequence of the membrane glycoproteins, envelope proteins, and surface glycoprotein of SARS-COV-2 reference proteins were used as query proteins (RefSeq ID: NC_045512) from the NCBI virus database [14].

Proteins involved in Guillain-Barré syndrome

Neuronal proteins implicated in the development of Guillain-Barré syndrome were retrieved from the literature. Neurofascin (NFASC), neuronal cell adhesion molecule (NCAM), contactin-1 (CNTN1), contactin-associated protein-like 1 (CNTP1), contactin-associated protein-like 2 (CNTP2), moesin (MOES), gliomedin (GLDN), and heat shock proteins 27, 60, 70, and 90 (HSPB1, CH60, HS90A) were reported in different studies and antibodies against them were detected in Guillain-Barré syndrome patients [15–20]. The amino acid sequence of each protein was retrieved from the Uniprot database [21]. The search was refined to reviewed proteins and expressed in Homo Sapiens.

The homology search

BLASTp program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to find the homologous sequence between the human proteins implicated in Guillain-Barré syndrome and structural proteins of SARS-COV-2 [22]. Only homologous peptides of five amino acids or more were selected for epitope prediction and docking [20].

The prediction of potential T cell epitopes

The detected homologous sequences were then used in the IEDB database (http://www.iedb.org) to find immune epitopes in both human and SARS-COV-2 homologous sequences for MHC-I and MHC-II molecules [23]. For the epitope to be selected for further analysis, the epitope should contain the homologous sequence as well as has the same binding HLA molecule for both human and SARS-COV-2 proteins. The IEDB recommended models and parameters were used. For HLA selection, the HLA allele reference set for both MHC-I and MHC-II was selected [23].

Peptide-MHC docking

To confirm the results, the homologous epitopes recovered from the IEDB database were docked to corresponding MHC molecules using GalaxyPepDock (http://galaxy.seoklab.org/) server [24].

The docked models were analyzed using UCSF Chimera 1.14 [25] and LigPLOT + [26] for similarity in hydrogen bonds.

The crystal structure of HLA-A*68 and HLA-DQ1 were obtained from the Protein Data Bank database (PDB ID: 4I48, and 3PL6). These crystal structures were inspected and modified to remove any bound ligands and make the groove available for the docking with SARS-COV-2 and human proteins using UCSF- Chimera 1.14 [25].

Results

Results of the homology search

The only human neural protein that had a homologous sequence to surface SARS-COV-2 proteins was the neuronal cell adhesion molecule (NCAM) which was homologous to envelop proteins. The envelope proteins are 85% identical to NCAM. The homologous sequence is TGTLIIN for NCAM corresponding to TGTLIVN for envelope proteins.

The homologous sequence acting as HLA-I motifs inducing an immune response and inactivating the human homologous protein

For the MHC-I molecule, HLA-A*68 was the MHC-I molecule that could bind both immune epitopes of NCAM (TGTLIINIM) and envelope proteins (TGTLIVNSV).

Analysis of binding patterns between HLA-A*68, NCAM epitopes, and envelope epitopes revealed that both epitopes share binding sites in the groove of HLA-A*68 Supplementary Video 1. They share hydrogen bonds with Tyrosine 99, Asparagine 66, and Asparagine 63. The difference between the binding of both human and NCAM epitopes to HLA-A*68 was 1.02 A° Fig. 1.

The homologous sequence acting as HLA-II motifs inducing an immune response and inactivating the human homologous protein

HLA-DQA1/HLA-DQB1 was the binding MHC-II molecules for the envelope proteins (TGTLIVNSVLL) and NCAM (TGTLIINIMSE) Supplementary Video 2. Both epitopes share hydrogen bonds with Glycine 55, and Asparagine 71 on the A chain of HLA-DQA1/HLA-DQB1 Fig. 2. On the B chain, Histidine 261, Asparagine 262, and Tyrosine 189 were involved in hydrogen bonds between MHC molecules and the epitopes of both envelope proteins and NCAM Fig. 3. The difference between the binding of both epitopes to HLA-DQA1 was 0.44 A°. The small difference between the binding patterns indicated a strong similarity between both epitopes for binding patterns.

Discussion

Based on the present study results, the cause of Guillain-Barré syndrome in SARS-COV-2 is epitope mimicry between envelope proteins and NCAM. In addition, two HLA molecules were involved either HLA-A*68 and HLA-DQA1/HLA-DQB1.

The Neural cell adhesion molecule is a neuronal surface adhesion protein that is widely expressed in neural cells. In addition, NCAM was expressed in myelinating Schwan cells, cardiac and skeletal muscles [27]. It is important for cell function, adhesion, and differentiation. It has many isoforms depending on mRNA splicing [10]. Another study found that it was overexpressed in cases of neuronal regeneration and remyelination [27]. It was implicated in other diseases as Alzheimer's disease, multiple sclerosis, and neuroblastoma [28–31].

The NCAM is expressed in nodal tissues like nodes of Ranvier. A study found that nodal and paranodal membrane injury is one of the main pathologies in Guillain-Barré syndrome resulting in an axonal injury that clinically will lead to paralysis [32]. It also assessed antibodies against NCAM in Guillain-Barré syndrome patients found that 29 out of 150 had positive antibodies [32]. Peripheral neuropathy was also evident in leprosy which is caused by *Mycobacterium leprae* (*M. Leprae*)

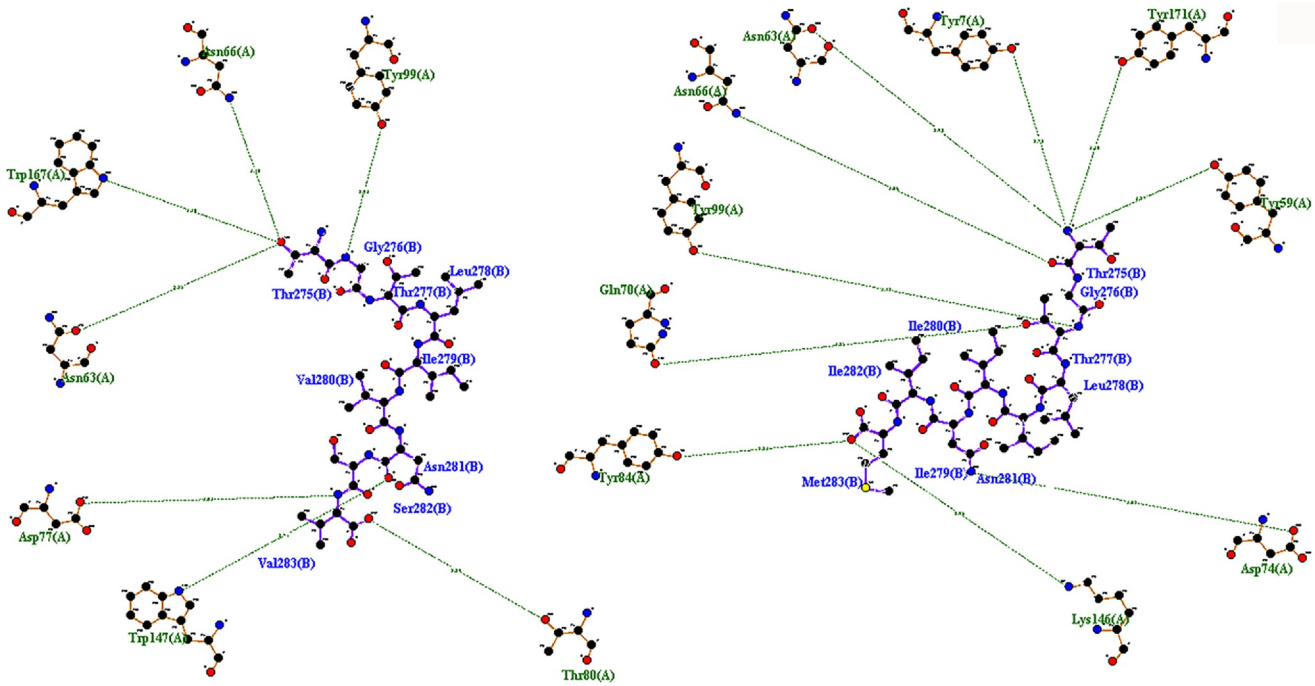

Fig. 1. The binding pattern of the HLA-A68 molecule to envelope proteins (left) and NCAM (right); green lines are hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that alter the expression of NCAM [33]. Furthermore, MHC-II attack Schwann cells infected with *M. Leprae* implying similar immunopathogenesis as suggested in the current study [33]. Another study found that sera from patients diagnosed with demyelinating disorders including Guillain-Barré syndrome reacted against NCAM, however, it was non-significant even though it was detected in around half of the patients [29].

This is not the first time that viral infection causes Guillain-Barré syndrome. It is well-evident in the Zika infection and herpes virus infection [34,35]. In addition, neuropathies were also evident in SARS and MERS infection [8,36,37].

There is also an evident association between HLA antigens and Guillain-Barré syndrome [38,39]. There was a significant association between HLA-DQ in Guillain-Barré syndrome caused by campylobacter jejune [38,40]. Another study found that HLA-DQ1 was associated with severe forms of Guillain-Barré syndrome which is consistent with the current study [41]. Blum et al. discovered that HLA is also an important factor for the development of Guillain-Barré syndrome through the activation of Natural killer cells which had a crucial role in the development of Guillain-Barré syndrome [42].

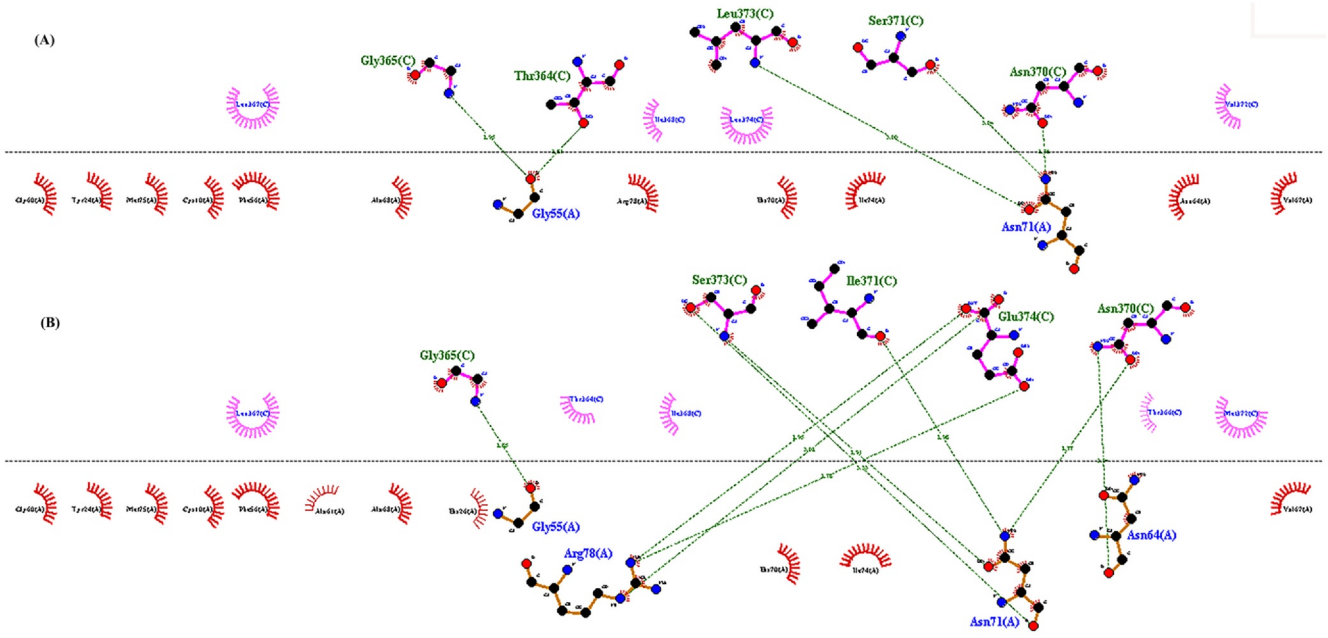

Fig. 2. The binding pattern of the HLA-DQA1 molecule to envelope proteins (A) and NCAM (B); green lines are hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

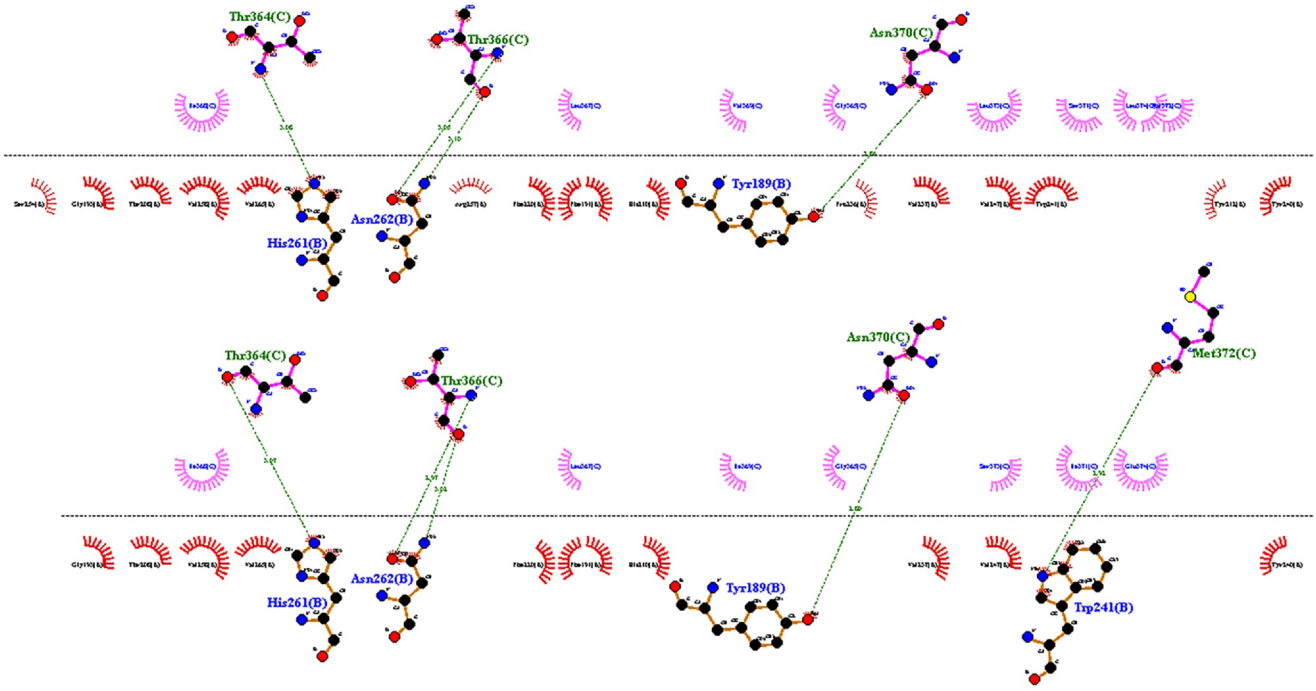

Fig. 3. The binding pattern of the HLA-DQA1 molecule to envelope proteins (Top) and NCAM (Bottom); green lines are hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Funding

None.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110342.

References

[1] Sedaghat Z, Karimi N, Guillain Barre syndrome associated with COVID-19 infection: a case report, J Clin Neurosci (2020), pp. S0967-5868(0920)30882-30881.
[2] Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, Franciotta D, Baldanti F, Daturi R, Postorino P, Cavallini A, Micieli G. Guillain–Barré Syndrome Associated with SARS-CoV-2. N Engl J Med 2020;382(26):2574–6.
[3] Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? The Lancet Neurology 2020;19:383–4.
[4] Virani A, Rabold E, Hanson T, et al. Guillain-Barré Syndrome associated with SARS-CoV-2 infection. IDCases 2020;20:e00771.
[5] Yuki N, Hartung H-P. Guillain-Barré Syndrome. N Engl J Med 2012;366:2294–304.
[6] Sejvar JJ, Baughman AL, Wise M, Morgan OW. Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis. Neuroepidemiology 2011;36:123–33.
[7] Mao L, Jin H, Wang M, Hu Yu, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu Bo. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020.
[8] Kim JE, Heo JH, Kim HO, et al. Neurological complications during treatment of middle east respiratory syndrome. J Clin Neurol 2017;13:227–33.
[9] Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, Liu C, Yang C. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18–22.
[10] Kolkova K. Biosynthesis of NCAM. In: Berezin V, editor. Structure and function of the neural cell adhesion molecule NCAM. New York, New York, NY: Springer; 2010. p. 213–25.
[11] Winter A, Bornemann A. NCAM, vimentin and neonatal myosin heavy chain expression in human muscle diseases. Neuropathol Appl Neurobiol 1999;25:417–24.
[12] Dinglas VD, Aronson Friedman L, Colantuoni E, et al. Muscle weakness and 5-year survival in acute respiratory distress syndrome survivors. Crit Care Med 2017;45:446–53.
[13] Macklem PT, Roussos CS. Respiratory muscle fatigue: a cause of respiratory failure? Clin Sci Mol Med 1977;53:419–22.
[14] Hatcher EL, Zhdanov SA, Bao Y, et al. Virus Variation Resource – improved response to emergent viral outbreaks. Nucl Acids Res 2017;45:D482–90.
[15] Devaux JJ, Odaka M, Yuki N. Nodal proteins are target antigens in Guillain-Barre syndrome. J Peripheral Nervous System: JPNS 2012;17:62–71.
[16] Querol L, Illa I. Paranodal and other autoantibodies in chronic inflammatory neuropathies. Curr Opin Neurol 2015;28.
[17] Sawai S, Satoh M, Mori M, et al. Moesin is a possible target molecule for cytomegalovirus-related Guillain-Barre syndrome. Neurology 2014;83:113–7.
[18] Stathopoulos P, Alexopoulos H, Dalakas MC. Autoimmune antigenic targets at the node of Ranvier in demyelinating disorders. Nature Rev Neurol 2015;11:143–56.
[19] Wanschitz J, Ehling R, Loscher WN, et al. Intrathecal anti-alphaB-crystallin IgG antibody responses: potential inflammatory markers in Guillain-Barre syndrome. J Neurol 2008;255:917–24.
[20] Lucchese G, Kanduc D. Zika virus and autoimmunity: from microcephaly to Guillain-Barré syndrome, and beyond. Autoimmun Rev 2016;15:801–8.
[21] The UniProt C. UniProt: a worldwide hub of protein knowledge. Nucl Acids Res 2018;47:D506–15.
[22] Altschul SF, Wootton JC, Gertz EM, et al. Protein database searches using compositionally adjusted substitution matrices. FEBS J 2005;272:5101–9.
[23] Vita R, Mahajan S, Overton JA, et al. The Immune Epitope Database (IEDB): 2018 update. Nucl Acids Res 2018;47(2019):D339–43.
[24] Lee H, Heo L, Lee MS, Seok C. GalaxyPepDock: a protein–peptide docking tool based on interaction similarity and energy optimization. Nucl Acids Res 2015;43:W431–5.
[25] Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera–a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605–12.
[26] Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51:2778–86.
[27] Saporta MA, Shy ME. Chapter 12 – Peripheral neuropathies. In: Zigmond MJ, Rowland LP, Coyle JT, editors. Neurobiology of brain disorders. San Diego: Academic Press; 2015. p. 167–88.
[28] Ziliotto N, Zivadinov R, Jakimovski D, et al. Plasma levels of soluble NCAM in multiple sclerosis. J Neurol Sci 2019;396:36–41.
[29] Poltorak M, Steck AJ, Schachner M. Reactivity with neural cell adhesion molecules in sera from patients with demyelinating diseases. Neurosci Lett 1986;65:199–203.
[30] Leshchyns'ka I, Liew HT, Shepherd C, et al., Aβ-dependent reduction of NCAM2-mediated synaptic adhesion contributes to synapse loss in Alzheimer's disease, Nat Commun 6 (2015), pp. 8836-8836.
[31] Valentiner U, Muhlenhoff M, Lehmann U, Hildebrandt H, Schumacher U. Expression of the neural cell adhesion molecule and polysialic acid in human neuroblastoma cell lines. Int J Oncol 2011;39:417–24.

[32] Devaux JJ, Odaka M, Yuki N. Nodal proteins are target antigens in Guillain-Barré syndrome. J Peripheral Nervous System 2012;17:62–71.
[33] Scollard DM. The biology of nerve injury in leprosy. Lepr Rev 2008;79:242–53.
[34] do Rosário MS, de Jesus PAP, Vasilakis N, et al. Guillain-Barré syndrome after zika virus infection in Brazil. Am J Trop Med Hyg 2016;95:1157–60.
[35] Kang J-H, Sheu J-J, Lin H-C. Increased risk of Guillain-Barré syndrome following recent herpes zoster: a population-based study across Taiwan. Clin Infect Dis 2010;51:525–30.
[36] Chao CC, Tsai LK, Chiou YH, et al. Peripheral nerve disease in SARS: report of a case. Neurology 2003;61:1820–1.
[37] Stainsby B, Howitt S, Porr J. Neuromusculoskeletal disorders following SARS: a case series. J Can Chiropr Assoc 2011;55:32–9.
[38] Rees JH, Vaughan RW, Kondeatis E, Hughes RAC. HLA-class II alleles in Guillain-Barré syndrome and Miller Fisher syndrome and their association with preceding Campylobacter jejuni infection. J Neuroimmunol 1995;62:53–7.
[39] Jin P-P, Sun L-L, Ding B-J, et al., Human Leukocyte Antigen DQB1 (HLA-DQB1) Polymorphisms and the Risk for Guillain-Barré Syndrome: A Systematic Review and Meta-Analysis, PloS one 10 (2015), pp. e0131374-e0131374.
[40] Hayat S, Jahan I, Das A, et al. Human leukocyte antigen-DQB1 polymorphisms and haplotype patterns in Guillain-Barré syndrome. Ann Clin Transl Neurol 2019;6:1849–57.
[41] Schirmer L, Worthington V, Solloch U, et al. Higher frequencies of HLA DQB1*05:01 and anti-glycosphingolipid antibodies in a cluster of severe Guillain-Barré syndrome. J Neurol 2016;263:2105–13.
[42] Blum S, Csurhes P, McCombe P. The frequencies of Killer immunoglobulin-like receptors and their HLA ligands in chronic inflammatory demyelinating polyradiculoneuropathy are similar to those in Guillian Barre syndrome but differ from those of controls, suggesting a role for NK cells in pathogenesis. J Neuroimmunol 2015;285:53–6.