

## GENOMIC STUDY OF COVID-19 CORONA VIRUS EXCLUDES ITS ORIGIN FROM RECOMBINATION OR CHARACTERIZED BIOLOGICAL SOURCES AND SUGGESTS A ROLE FOR HERVs IN ITS WIDE RANGE SYMPTOMS

A.M. EL-SHEHAWI<sup>1,2\*</sup>, SAQER S ALOTAIBI<sup>1</sup>, M.M. ELSEEHY<sup>2</sup>

<sup>1</sup> Department of Biotechnology, Faculty of Sciences, Taif University, 888 Taif, Kingdom of Saudi Arabia

<sup>2</sup> Department of Genetics, Faculty of Agriculture, University of Alexandria, Alexandria 21527, Egypt  
E-mail: elshehawi@hotmail.com, saqer-20@hotmail.com, monaahmedma@yahoo.com

*The COVID-19 corona virus has become a world pandemic which started in December 2019 in Wuhan, China with no confirmed biological source. Various countries reported the genomic sequence of different isolates obtained from infected patients. This allowed us to obtain a number of 38 isolates of full genomic sequences. Alignment of nucleotide (nt) sequence was carried out using Clustal Omega multiple alignment service at the EBI website. Alignment of nt sequence and phylogenetic relationship revealed that the COVID-19 is a new viral strain and its biological source has not been yet detected. The expected orf pattern was different among isolates obtained from the same country or different countries as well as from SARS-CoV isolates or bats CoV suggesting different virus human interaction possibilities during infection and severity. All isolates had the main five orfs (1ab, S, M, N, E), whereas they differed in the expected accessory orfs. Being with the biological source of COVID-19 undetected, the role of human endogenous retrovirus (HERVs) in the regulation of the host cell gene expression or the encoding for products that could modulate COVID-19 infection and the spectrum of its symptoms is discussed.*

**Key words:** COVID-19, genome, nucleotide sequence alignment, Human endogenous retroviruses (HERVs).

ГЕНОМНЕ ДОСЛІДЖЕННЯ КОРОНАВІРУСА COVID-19 ВИКЛЮЧАЄ ЙОГО ПОХОДЖЕННЯ ВІД РЕКОМБІНАЦІЇ ЧИ З ОПИСАНИХ БІОЛОГІЧНИХ ДЖЕРЕЛ І ПРИПУСКАЄ РОЛЬ HERV У ШИРОКОМУ ДІАПАЗОНІ ЙОГО СИМПТОМІВ

COVID-19 – це викликане коронавірусом захворювання, яке переросло у всесвітню пандемію,

початок якої було зафіксовано у грудні 2019 р. у місті Ухань, Китай, але біологічне джерело якого не було підтверджено. У різних країнах повідомляли про геномну послідовність різних ізолятів, отриманих від інфікованих пацієнтів. Це дозволило нам загалом отримати 38 ізолятів повних геномних послідовностей. Вирівнювання нуклеотидної (nt) послідовності проводили за допомогою множинного вирівнювання Clustal Omega на веб-сайті Європейського інституту біоінформатики (EBI). Вирівнювання нуклеотидної послідовності і філогенетичний зв'язок показали, що COVID-19 – це новий штам вірусу, біологічне походження якого ще не було встановлено. Очікувана структура orf була різною серед ізолятів, отриманих з однієї країни або різних країн, а також ізолятів SARS-CoV чи CoV кажанів, що дозволяє припустити різні можливості взаємодії вірусу та людини під час інфікування та складності захворювання. Всі ізоляти мали п'ять основних orfs (1ab, S, M, N, E), однак, відрізнялися очікуваними допоміжними orfs. Оскільки біологічне джерело COVID-19 залишається невстановленим, було обговорено роль ендегенних ретровірусів людини (HERV) у регуляції експресії генів клітин господаря чи кодування продуктів, які можуть модулювати інфекцію COVID-19 і спектр її симптомів.

**Ключові слова:** COVID-19, геном, вирівнювання нуклеотидної послідовності, ендегенні ретровіруси людини (HERV).

### REFERENCES

1. Kahn, J.S., McIntosh, K., History and recent advances in coronavirus discovery. *Pediatr. Infect. Dis. J.* 2005, vol. 24, no. 11, pp. S223–S226.
2. Fehr, A.R., Perlman, S., Coronaviruses: an overview of their replication and pathogenesis. *Meth. Mol. Biol.* 2015, vol. 1282, pp. 1–23.
3. Drosten, C., et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N. Engl. J. Med.* 2003, vol. 348, no. 20, pp. 1967–76.
4. Ksiazek, T.G., et al. A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 2003, vol. 348, no. 20, pp. 1953–66.
5. Peiris, J.S., et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*, 2003, vol. 361, no. 9366, pp. 1319–25.
6. Paules, C.I., Marston, H.D., and Fauci, A.S., Coronavirus Infections More Than Just the Common Cold. *JAMA*. 2020, vol. 323, no. 8, pp. 707–8. doi:10.1001/jama.2020.0757.
7. Guan, Y., et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science*, 2003, vol. 302, no. 5643, pp. 276–8.

8. Lau, S.K., et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc. Natl. Acad. Sci. USA*. 2005, vol. 102, no. 39, pp. 14040–5.
9. Cui, J., Li, F., Shi, Z.L., Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* 2018, vol. 17, no. 3, pp. 181–92. <https://doi.org/10.1038/s41579-018-0118-9>.
10. Zaki, A.M., et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* 2012, vol. 367, no. 19, pp. 1814–20.
11. Hajjar, S.A., Memish, Z.A., and McIntosh, K., Middle East Respiratory Syndrome Coronavirus (MERS-CoV): a perpetual challenge. *Ann. Saudi Med.* 2013, vol. 33, no. 5, pp. 427–36.
12. Alagaili, A.N., et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *mBio*. 2014, vol. 5, no. 2, e00884–14.
13. Ithete, N.L., Stoffberg, S., Corman, V.M., et al. Close relative of human Middle East respiratory syndrome coronavirus in bat. South Africa. *Emerg. Infect. Dis.* 2013, vol. 19, pp. 1697–9.
14. Paraskevis, D., Kostaki, E.G., Magiorkinis, G., Panayiotakopoulos, G., Sourvinos, G., and Tsiodras, S., Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect. Genet. Evol.* 2020, vol. 79, pp. 104212. doi: 10.1016/j.meegid.2020.104212.
15. Zhu, N., et al. A novel coronavirus from patients with pneumonia in China, 2020. *N. Engl. J. Med.* 2020, vol. 382, pp. 727–33.
16. Perlman, S., Another decade, another coronavirus. *N. Engl. J. Med.* 2020, vol. 382, pp. 760–2.
17. World Health Organization (WHO), 2020. Geneva, Switzerland.
18. Hui D.S., et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - the latest 2019 novel coronavirus outbreak in Wuhan, China. *Int. J. Infect. Dis.* 2020, vol. 91, pp. 264–6.
19. World Health Organization (WHO), 2020. Novel Coronavirus (2019-nCoV) Situation report–162, June30, 2020. Geneva, Switzerland.
20. Chan, J.F., Kok, K.H., Zhu, Z., Chu, H., To, K.K., Yuan, S., and Yuen, K.Y., Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microb. Infect.* 2020, vol. 9, no. 1, pp. 221–36. doi: 10.1080/22221751.2020.1719902.
21. Wan, Y., Shang, J., Graham, R., Baric, R.S., and Li, F., Receptor recognition y novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *J. Virol. Pii.* 2020, JVI.00127-20. doi: 10.1128/JVI.00127-20.
22. Duffy, S., Shackelton, L.A., and Holmes, E.C., Rates of evolutionary change in viruses: patterns and determinants. *Nat. Rev. Genet.* 2008, vol. 9, no. 4, pp. 267–76.
23. Sanjuan, R., Nebot, M.R., Chirico, N., Mansky, L.M., Belshaw, R., Viral mutation rates. *J. Virol.* 2010, vol. 84, no. 19, pp. 9733-4.
24. Retel, C., Markle, H., Becks, L., and Feulner, P.G.D., Ecological and Evolutionary Processes Shaping Viral Genetic Diversity. *Viruses.* 2019, vol. 11, no. 3, pp. 220. doi: 10.3390/v11030220.
25. Armbruster, V., Sauter, M., Krautkraemer, E., et al. A novel gene from the human endogenous retrovirus K expressed in transformed cells. *Clin. Cancer Res.* 2002, vol. 8, pp. 1800–7.
26. Bannert, N., Kurth, R., The evolutionary dynamics of human endogenous retroviral families. *Ann. Rev. Genom. Hum. Genet.* 2006, vol. 7, pp. 149–73.
27. Vargiu, L., Rodriguez-Tomé, P., Sperber, G.O., et al. Classification and characterization of human endogenous retroviruses; mosaic forms are common. *Retrovirol.* 2016, vol. 13, pp. 7. doi: 10.1186/s12977-015-0232-y.
28. Lander, E.S., Linton, L.M., Birren, B., et al. Initial sequencing and analysis of the human genome. *Nature.* 2001, vol. 412, pp. 860–921. doi: 10.1038/35057062.
29. Bronson, D.L., Fraley, E.E., Fogh, J., Kalter, S.S., Induction of retrovirus particles in human testicular tumor (Tera-1) cell cultures: An electron microscopic study. *J. Natl. Cancer Inst.* 1979, vol. 63, pp. 337–9.
30. Jern, P., Sperber, G.O., Blomberg, J., Use of endogenous retroviral sequences (ERVs) and structural markers for retroviral phylogenetic inference and taxonomy. *Retrovirol.* 2005, vol. 2, pp. 50. doi: 10.1186/1742-4690-2-50.
31. Blomberg, J., Benachenhou, F., Blikstad, V., Sperber, G., Mayer, J., Classification and nomenclature of endogenous retroviral sequences (ERVs): problems and recommendations. *Gene.* 2009, vol. 448, pp. 115–23. doi: 10.1016/j.gene.2009.06.007.
32. Grandi, N., Tramontano, E., Human Endogenous Retroviruses Are Ancient Acquired Elements Still Shaping Innate Immune Responses. *Front. Immunol.* 2018a, vol. 9, pp. 2039. doi: 10.3389/fimmu.2018.02039.
33. Esposito, F., Corona, A., and Tramontano, E., HIV-1 reverse transcriptase still remains a new drug target: structure, function, classical inhibitors, and new inhibitors with innovative mechanisms of actions. *Mol. Biol. Int.* 2012, pp. 586401. doi: 10.1155/2012/586401.
34. Esposito, F., Tramontano, E., Past and future. Current drugs targeting HIV-1 integrase and reverse transcriptase-associated ribonuclease H activity: single and dual active site inhibitors. *Antivir. Chem. Chemother.* 2013, vol. 23, pp. 129–44. doi: 10.3851/IMP2690.

35. Chuong, E.B., Elde, N.C., and Feschotte, C., Regulatory evolution of innate immunity through co-option of endogenous retroviruses. *Science*. 2016, vol. 351, pp. 1083–7. doi: 10.1126/science.aad5497.
36. Frank, J.A., Feschotte, C., Co-option of endogenous viral sequences for host cell function. *Curr. Opin. Virol.* 2017, vol. 25, pp. 81–9. doi: 10.1016/j.coviro.2017.07.021.
37. Feschotte, C., Gilbert, C., Endogenous viruses: insights into viral evolution and impact on host biology. *Nat. Rev. Genet.* 2012, vol. 13, pp. 283–96. doi: 10.1038/nrg3199.
38. Lavialle, C., Cornelis, G., Dupressoir, A., et al. Paleovirology of “syncytins”, retroviral env genes exapted for a role in placentation. *Philos. Trans R. Soc. Lond. B. Biol. Sci.* 2013, vol. 368, pp. 20120507. doi: 10.1098/rstb.2012.0507.
39. Mi, S., Lee, X., Li, X., Veldman, G.M., et al. Syncytin is a captive retroviral envelope protein involved. *Nature*. 2000, vol. 403, pp. 785–9. doi: 10.1038/35001608.
40. Malassine, A., Handschuh, K., Tsatsaris, V., et al. Expression of HERV-WEnv glycoprotein (syncytin) in the extravillous trophoblast of first trimester human placenta. *Placenta*. 2005, vol. 26, pp. 556–62. doi: 10.1016/j.placenta.2004.09.002.
41. Mangeney, M., Renard, M., Schlecht-Louf, G., et al. Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc. Natl. Acad. Sci. USA*. 2007, vol. 104, pp. 20534–9. doi: 10.1073/pnas.0707873105.
42. Van de Lagemaat, L.N., Landry, J.R., Mager, D.L., Medstrand, P., Transposable elements in mammals promote regulatory variation and diversification of genes with specialized functions. *Trends Genet.* 2003, vol. 19, pp. 530–6. doi: 10.1016/j.tig.2003.08.004.
43. Bourque, G., Leong, B., Vega, V.B., et al. Evolution of the mammalian transcription factor binding repertoire via transposable elements. *Genome Res.* 2008, vol. 18, pp. 1752–62. doi: 10.1101/gr.080663.108.
44. Sundaram, V., Cheng, Y., Ma, Z., et al. Widespread contribution of transposable elements to the innovation of gene regulatory networks. *Genome Res.* 2014, vol. 24, pp. 1963–76. doi: 10.1101/gr.168872.113.
45. Trizzino, M., Park, Y., Holsbach-Beltrame, M., et al. Transposable elements are the primary source of novelty in primate gene regulation. *Genome Res.* 2017, vol. 27, pp. 1623–33. doi: 10.1101/gr.218149.116.
46. Ito, J., Sugimoto, R., Nakaoka, H., et al. Systematic identification and characterization of regulatory elements derived from human endogenous retroviruses. *PLoS Genet.* 2017, vol. 13, pp. e1006883. doi: 10.1371/journal.pgen.1006883.
47. Feschotte, C., The contribution of transposable elements of the evolution of regulatory networks. *Nat. Rev. Genet.* 2008, vol. 397–405. doi: 10.1038/nrg2337.
48. Goke, J., Ng, H.H., CTRL+INSERT: retrotransposons and their contribution to regulation and innovation of the transcriptome. *EMBO Rep.* 2016, vol. 17, pp. 1131–44. doi: 10.15252/embr.201642743.
49. Wolff, F., Leisch, M., Greil, R., Risch, A., and Pleyer, L., The double-edged sword of (re)expression of genes by hypomethylating agents: from viral mimicry to exploitation as priming agents for targeted immune checkpoint modulation. *Cell Commun. Signal.* 2017, vol. 15, pp. 13. doi: 10.1186/s12964-017-0168-z.
50. Kamp, C., Hirschmann, P., Voss, H., Huellen, K., and Vogt, P.H., Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum. Mol. Genet.* 2000, vol. 9, pp. 2563–72. doi: 10.1093/hmg/9.17.2563.
51. Wang, Y., Xu, Z., Jiang, J., et al, Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Dev. Cell.* 2013, vol. 25, pp. 69–80. doi: 10.1016/j.devcel.2013.03.002.
52. Durruthy-Durruthy, J., Sebastiano, V., Wossidlo, M., et al. The primate-specific noncoding RNA HPAT5 regulates pluripotency during human preimplantation development and nuclear reprogramming. *Nat. Genet.* 2016, vol. 48, pp. 44–52. doi: 10.1038/ng.3449.
53. Grow, E.J., Flynn, R.A./, Chavez. S.L., et al. Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells. *Nature*. 2015, vol. 522, pp. 221–5. doi: 10.1038/nature14308.
54. Mikuni, T., Uesaka, N., Okuno, H., et al. Arc/Arg3.1 is a postsynaptic mediator of activity-dependent synapse elimination in the developing cerebellum. *Neuron*. 2013, vol. 78, pp. 1024–35. doi: 10.1016/j.neuron.2013.04.036.
55. Zhang, W., Wu, J., Ward, M.D., et al. Structural basis of arc binding to synaptic proteins: implications for cognitive disease. *Neuron*. 2015, vol. 86, pp. 490–500. doi: 10.1016/j.neuron.2015.03.030.
56. Pastuzyn, E.D., Day, C.E., Kearns, R.B., et al. The neuronal gene arc encodes a repurposed retrotransposon gag protein that mediates intercellular RNA transfer. *Cell*. 2018, vol. 172, pp. 275–88.
57. Chuong, E.B., Elde, N.C., Feschotte, C., Regulatory activities of transposable elements: from conflicts to benefits. *Nat. Rev. Genet.* 2017, vol. 18, pp. 71–86. doi: 10.1038/nrg.2016.139.
58. Wang, T., Zeng, J., Lowe, C.B., et al. Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53. *Proc. Natl. Acad. Sci. USA*. 2007, vol. 104, pp. 18613–8. doi: 10.1073/pnas.0703637104.
59. Andersson, G., Svensson, A.C., Setterblad, N., Rask,

- L., Retroelements in the human MHC class II region. *Trends Genet.* 1998, vol. 14, pp. 109–114. doi: 10.1016/S0168-9525(97)01359-0.
60. Grandi, N., Cadeddu, M., Pisano, M.P., Esposito, F., Blomberg, J., Tramontano, E., Identification of a novel HERV-K(HML10): Comprehensive characterization and comparative analysis in non-human primates provide insights about HML10 proviruses structure and diffusion. *Mob. DNA.* 2017a, vol. 8, pp. 15. doi: 10.1186/s13100-017-0099-7.
61. Mack, M., Bender, C., Schneider, P.M., Detection of retroviral antisense transcripts and promoter activity of the HERV-K(C4) insertion in the MHC III region. *Immunogenet.* 2004, vol. 56, pp. 321–32. doi: 10.1007/s00251-004-0705-y.
62. Nehyba, J., Hrdličková, R., Bose, H.R., Dynamic evolution of immune system regulators: the history of the interferon regulatory factor family. *Mol. Biol. Evol.* 2009, vol. 26, pp. 2539–50. doi: 10.1093/molbev/msp167.
63. Katoh, I., Kurata, S., Association of endogenous retroviruses and long terminal repeats with human disorders. *Front. Oncol.* 2013, vol. 3, pp. 234. doi: 10.3389/fonc.2013.00234.
64. Manghera, M., Ferguson-Parry, J., Lin, R., and Douville, R.N., NF- $\kappa$ B and IRF1 induce endogenous retrovirus K expression via interferon-stimulated response elements in its 5' long terminal repeat. *J. Virol.* 2016, vol. 90, pp. 9338–49. doi: 10.1128/JVI.01503-16.
65. Hurst, T.P., Magiorkinis, G., Activation of the innate immune response by endogenous retroviruses. *J. Gen. Virol.* 2015, vol. 96, pp. 1207–18. doi: 10.1099/vir.0.000017.
66. Grandi, N., Tramontano, E., HERV envelope proteins: physiological role and pathogenic potential in cancer and autoimmunity. *Front. Microbiol.* 2018b, vol. 9, pp. 462. doi: 10.3389/fmicb.2018.00462.
67. Grandi, N., Tramontano, E., Type W human endogenous retrovirus (HERVW) integrations and their mobilization by L1 machinery: contribution to the human transcriptome and impact on the host physiopathology. *Viruses.* 2017b, vol. 9, pp. 162. doi: 10.3390/v9070162.
68. Trela, M., Nelson, P.N., and Rylance, P.B., The role of molecular mimicry and other factors in the association of human endogenous retroviruses and autoimmunity. *APMIS.* 2016, vol. 124, pp. 88–104. doi: 10.1111/apm.12487.
69. Nelson, P., Rylance, P., Roden, D., Trela, M., and Tugnet, N., Viruses as potential pathogenic agents in systemic lupus erythematosus. *Lupus.* 2014, vol. 23, pp. 596–605. doi: 10.1177/0961203314531637.
70. Mameli, G., Erre, G.L., Caggiu, E., et al. Identification of a HERV-K env surface peptide highly recognized in Rheumatoid Arthritis (RA) patients: a cross-sectional case–control study. *Clin. Exp. Immunol.* 2017, vol. 189, pp. 127–31. doi: 10.1111/cei.12964.
71. Manghera, M., Douville, R.N., Endogenous retrovirus-K promoter: a landing strip for inflammatory transcription factors? *Retrovirol.* 2013, vol. 10, pp. 16. doi: 10.1186/1742-4690-10-16.
72. Hurst, T.P., Magiorkinis, G., Epigenetic control of human endogenous retrovirus expression: focus on regulation of long-terminal repeats (LTRs). *Viruses.* 2017, vol. 9, pp. 1–13. doi: 10.3390/v9060130.
73. Nellaker, C., Yao, Y., Jones-Brando, L., et al, Transactivation of elements in the human endogenous retrovirus W family by viral infection. *Retrovirol.* 2006, vol. 3, pp. 44. doi: 10.1186/1742-4690-3-44.
74. Li, F., Nelleker, C., Sabunciyan, S., et al. Transcriptional derepression of the ERVWE1 locus following influenza A virus infection. *J. Virol.* 2014, vol. 88, pp. 4328–37. doi: 10.1128/JVI.03628-13.
75. Young, G.R., Mavrommatis, B., and Kassiotis, G., Microarray analysis reveals global modulation of endogenous retroelement transcription by microbes. *Retrovirol.* 2014, vol. 11, pp. 59. doi: 10.1186/1742-4690-11-59.
76. Gurtler, C., Bowie, A.G., Innate immune detection of microbial nucleic acids. *Trends Microbiol.* 2013, vol. 21, pp. 413–20. doi: 10.1016/j.tim.2013.04.004.
77. Roulois, D., Loo, Y.H., Singhania, R., et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell.* 2015, vol. 162, pp. 961–73. doi: 10.1016/j.cell.2015.07.056.
78. Ramasamy, R., Joseph, B., Whittall, T., Potential molecular mimicry between the human endogenous retrovirus W family envelope proteins and myelin proteins in multiple sclerosis. *Immunol. Lett.* 2017, vol. 183, pp. 79–85. doi: 10.1016/j.imlet.2017.02.003.
79. Dupressoir, A., Lavalie, C., and Heidmann, T., From ancestral infectious retroviruses to bona fide cellular genes: role of the captured syncytins in placenta. *Placenta.* 2012, vol. 33, pp. 663–71. doi: 10.1016/j.placenta.2012.05.005.
80. Hummel, J., Kammerer, U., Müller, N., Avota, E., Schneider-Schaulies, S., Human endogenous retrovirus envelope proteins target dendritic cells to suppress T-cell activation. *Eur. J. Immunol.* 2015, vol. 45, pp. 1748–59. doi: 10.1002/eji.201445366.
81. Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel V Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. *Nat. Immunol.* 2011, vol. 12, no. 2, pp. 137–43. doi: 10.1038/ni.1979.

Received July 07, 2020

Received July 12, 2020

Accepted November 18, 2020