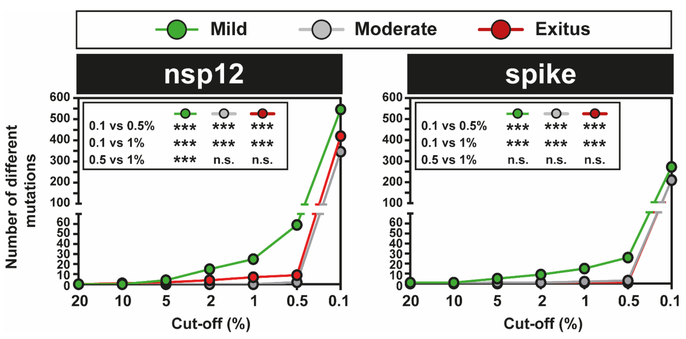
SARS-CoV-2 MUTANT SPECTRA REVEAL DIFFERENCES BETWEEN COVID-19 SEVERITY CATEGORIES

Brenda Martínez-González[1,2], María Eugenia Soria[2,3,4], Lucía Vázquez-Sirvent[2], Cristina Ferrer-Orta[5], Rebeca Lobo-Vega[2], Pablo Mínguez[1,6], Lorena de la Fuente[1,6], Carlos Llorens[7], Beatriz Soriano[7], Ricardo Ramos-Ruíz[8], Marta Cortón[1,6], Rosario López-Rodríguez[1,6], Carlos García-Crespo[3,4], Pilar Somovilla[3,9], Antoni Durán-Pastor[3], Isabel Gallego[3,4], Ana Isabel de Ávila[3,4], Soledad Delgado[10], Federico Morán[11], Cecilio López-Galíndez[12], Jordi Gómez[4,13], Luis Enjuanes[2], Llanos Salar-Vidal[2], Mario Esteban-Muñoz[2], Jaime Esteban[2], Ricardo Fernandez-Roblas[2], Ignacio Gadea[2], Carmen Ayuso[1,6], Javier Ruíz-Hornillos[14], Nuria Verdaguer[5], Esteban Domingo[3,4] and Celia Perales[1,2,4]

[1]Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM) Av. Reyes Católicos 2, 28040 Madrid, Spain. [2]Centro Nacional de Biotecnología (CNB-CSIC), Consejo Superior de Investigaciones Científicas (CSIC), Campus de Cantoblanco, 28049 Madrid, Spain. [3]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Consejo Superior de Investigaciones Científicas (CSIC), Campus de Cantoblanco, 28049 Madrid, Spain. [4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, 28029 Madrid, Spain. [5]Institut de Biología Molecular de Barcelona CSIC, 08028 Barcelona, Spain. [6]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, 28029 Madrid, Spain. [7]Biotechvana, "Scientific Park", Universidad de Valencia, 46980 Valencia, Spain. [8]Scientific Park of Madrid, Campus de Cantoblanco, 28049 Madrid, Spain. [9]Departamento de Biología Molecular, Universidad Autónoma de Madrid, Campus de Cantoblanco, 28049, Madrid, Spain. [10]Escuela Técnica Superior de Ingeniería de Sistemas Informáticos (ETSISI), Universidad Politécnica de Madrid, Madrid, Spain. [11]Departamento de Bioquímica y Biología Molecular, Universidad Complutense de Madrid, Madrid, Spain. [12]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain. [13]Instituto de Parasitología y Biomedicina 'López-Neyra' (CSIC), Parque Tecnológico Ciencias de la Salud, Armilla, 18016 Granada, Spain. [14]Hospital Infanta Elena, Valdemoro, Madrid, Spain.

RNA virus populations are composed of complex mixtures of genomes that are termed mutant spectra. SARS-CoV-2 replicates as a viral quasispecies, and mutations that are detected at low frequencies in a host can be dominant in subsequent variants. We have studied mutant spectrum complexities of SARS-CoV-2 populations derived from thirty nasopharyngeal swabs of patients infected during the first wave (April 2020) in the Hospital Universitario Fundación Jiménez Díaz. The patients were classified according to the COVID-19 severity in mild (non-hospitalized), moderate (hospitalized) and exitus (hospitalized with ICU admission and who passed away due to COVID-19). Using ultra-deep sequencing technologies (MiSeq, Illumina), we have examined four amplicons of the nsp12 (polymerase)-coding region and two amplicons of the spike-coding region. Ultra-deep sequencing data were analyzed with different cut-off frequency for mutation detection. Average number of different point mutations, mutations per haplotype and several diversity indices were significantly higher in SARS-CoV-2 isolated from patients who developed mild disease. A feature that we noted in the SARS-CoV-2 mutant spectra from diagnostic samples is the remarkable absence of mutations at intermediate frequencies, and an overwhelming abundance of mutations at frequencies lower than 10%. Thus, the decrease of the cut-off frequency for mutation detection from 0.5% to 0.1% revealed an increasement (50- to 100-fold) in the number of different mutations. The significantly higher frequency of mutations in virus from patients displaying mild than moderate or severe disease was maintained with the 0.1% cut- off frequency. To evaluate whether the frequency repertoire of amino acid substitutions differed between SARS-CoV-2 and the well characterized hepatitis C virus (HCV), we performed a comparative study of mutant spectra from infected patients using the same bioinformatics pipelines. HCV did not show the deficit of intermediate frequency substitutions that was observed with SARS-CoV-2. This difference was maintained when two functionally equivalent proteins, the corresponding viral polymerases, were compared. In conclusion, SARS-CoV-2 mutant spectra are rich reservoirs of mutants, whose complexity is not uniform among clinical isolates. Virus from patients who developed mild disease may be a source of new variants that may acquire epidemiological relevance.

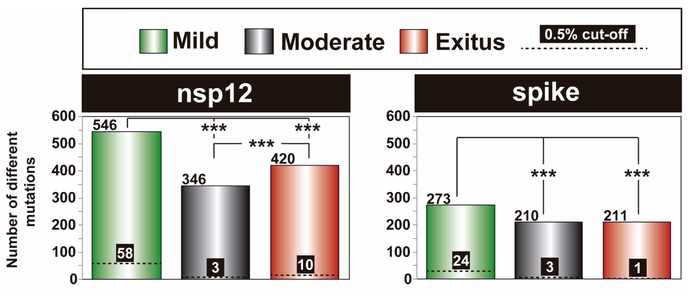
Overwhelming abundance of mutations a frequencies < 10%



The number of different mutations was significantly higher at 0.1% than at higher cut-off levels.

The higher number of mutations in virus from mild patients is maintained at different depth levels.

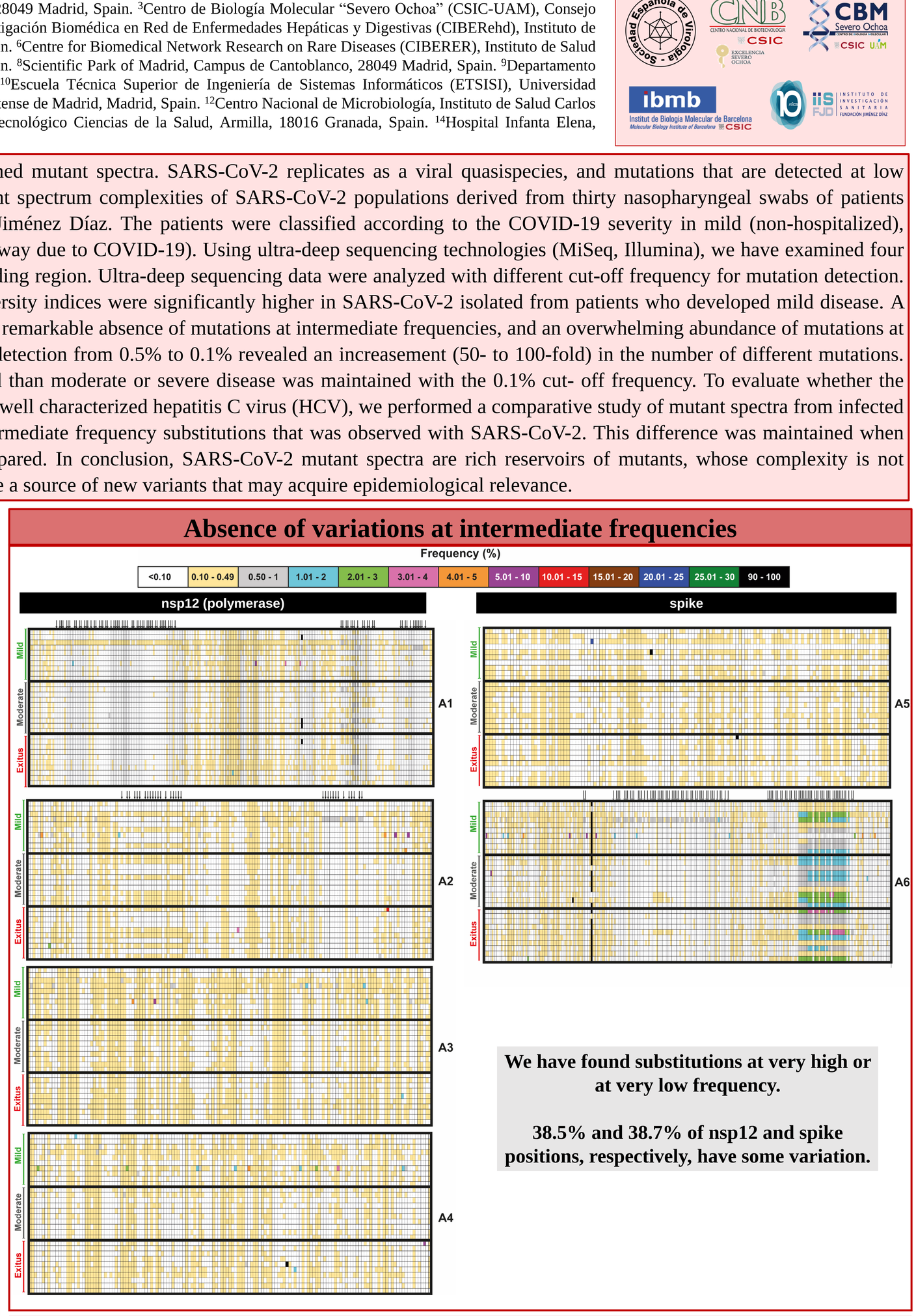
Higher frequency of mutations in virus from mild patients



A significantly higher number of different mutations was found in virus isolated from patients who developed mild COVID-19.

This tendency was observed at 0.5% cut-off level and at 0.1% cut-off level.

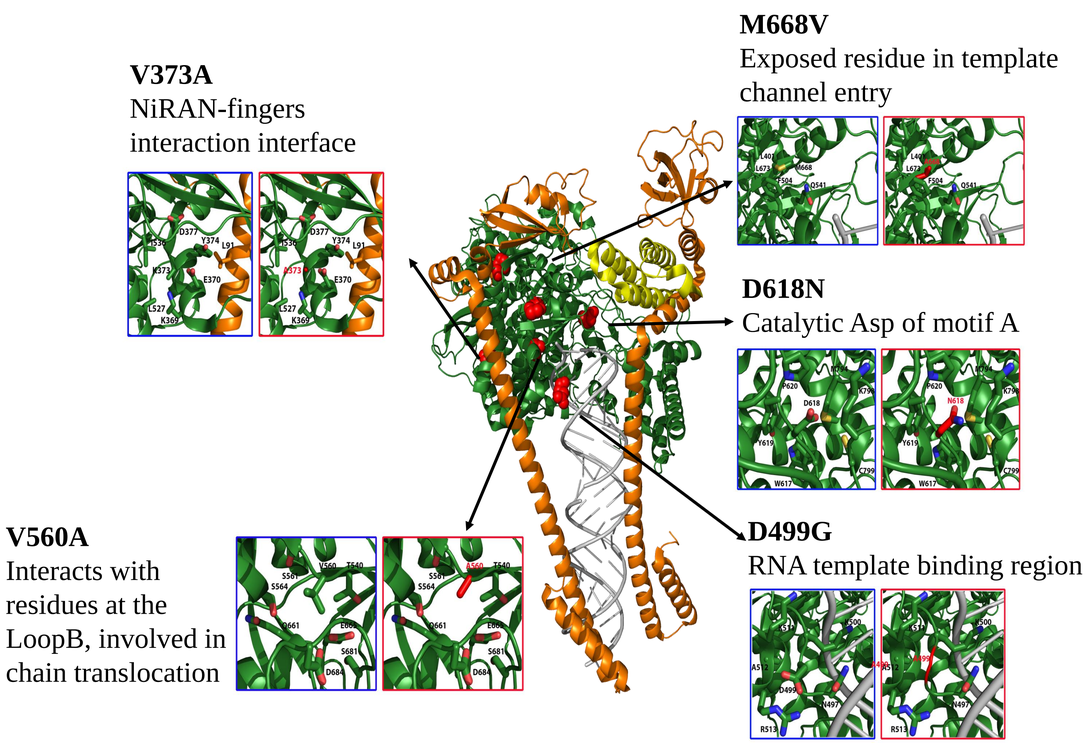
Absence of variations at intermediate frequencies



We have found substitutions at very high or at very low frequency.

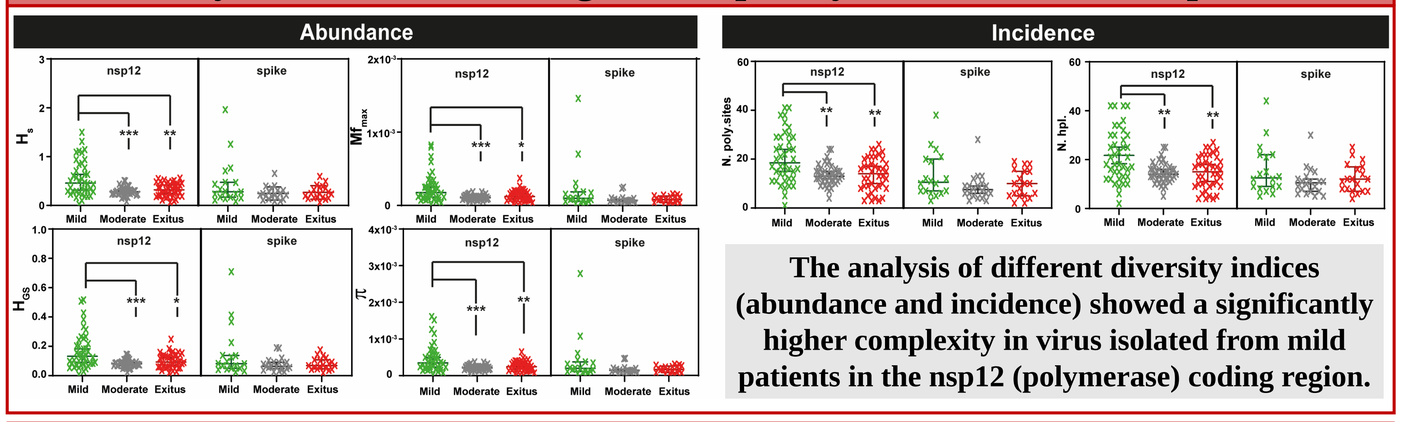
38.5% and 38.7% of nsp12 and spike positions, respectively, have some variation.

Prediction of possible structural and functional changes



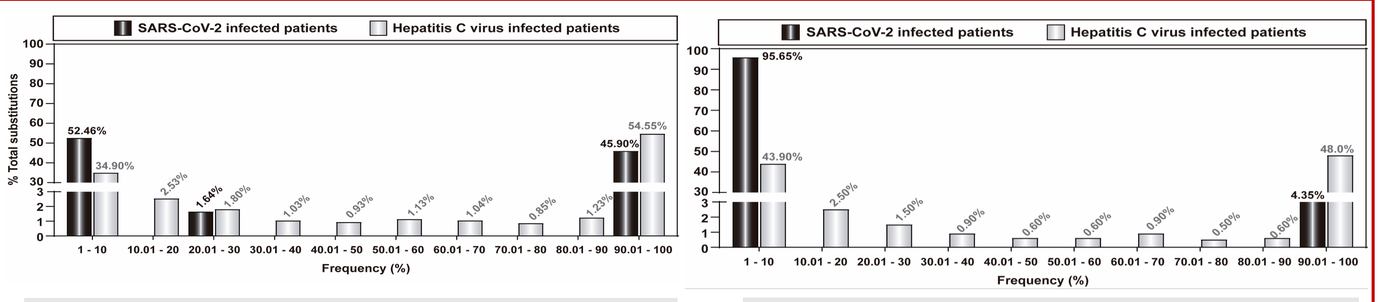
Some structural and functional changes in the nsp12 (polymerase) have been predicted

Diversity indices showed a higher complexity in virus from mild patients



The analysis of different diversity indices (abundance and incidence) showed a significantly higher complexity in virus isolated from mild patients in the nsp12 (polymerase) coding region.

Differences between SARS-CoV-2 and HCV



SARS-CoV-2 presents an absence of substitutions at intermediate frequencies compared to HCV.

The same tendency was observed comparing both polymerases.

- **A higher number of substitutions was observed in virus isolated from mild patients at different cut-off levels.**
- **This increased complexity of virus isolated from mild patients was confirmed studying different diversity indices.**
- **Some substitutions predicted structural and functional changes in the nsp12 protein.**
- **Most substitutions appeared at frequencies between 0.1% - 0.5%.**
- **A different distribution of mutant spectra was observed comparing SARS-CoV-2 with HCV.**

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