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Gene 814 (2022) 146134

Contents lists available at ScienceDirect

Gene

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

From delta to Omicron: S1-RBD/S2 mutation/deletion equilibrium in SARS-CoV-2 defined variants

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Variants
Mutations
RNA
Genome

ABSTRACT

Coronavirus-related Severe Acute Respiratory Syndrome (SARS-CoV) in 2002/2003, Middle-East Respiratory Syndrome (MERS-CoV) in 2012/2013, and especially the current 2019/2021 Severe Acute Respiratory Syndrome-2 (SARS-CoV-2) affected negatively the national health systems' endurance worldwide. SARS-Cov-2 virus belongs to lineage b of beta-CoVs demonstrating a strong phylogenetic similarity with BatCoVRaTG13 type. Spike (S) glycoprotein projections -consisting of two subunits S1/S2- provide a unique crown-like formation (corona) on virion's surface. Concerning their functional role, S1 represents the main receptor-binding domain (RBD), whereas S2 is involved in the virus-cell membrane fusion mechanism. On Nov 26th 2021, WHO designated the new SARS-CoV-2 strain – named Omicron, from letter ''*όμικρον*'' in the Greek alphabet - as a variant of concern (B.1.1529 variant). Potentially this new variant is associated with high transmissibility leading to elevated infectivity and probably increased re-infection rates. Its impact on morbidity/mortality remains under investigation. In the current paper, analyzing and comparing the alterations of SARS-CoV-2 S RNA sequences in the defined variants (Alpha to Omicron), we observed some interesting findings regarding the S1-RBD/S2 mutation/deletion equilibrium that maybe affect and modify its activity.

1. Introduction

Viruses-related pandemics are frequently characterized by different mutational levels that lead to specific variants (multiple viral strains) emergence (Burki, 2021; Sanjuán and Domingo-Calap, 2016). Concerning especially RNA genome - based viruses, they demonstrate significantly high mutational rates, as a result of proofreading activity absence in the corresponding RNA-dependent RNA polymerase enzymes (Fitzsimmons et al., 2018; Duffy, 2018; Wang et al., 2020a; 2020b). Although Severe Acute Respiratory Syndrome Coronavirus −2 (SARS-

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, coronavirus disease 2019; ACE, angiotensin-converting enzyme; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; RBD, receptor-binding domain; S, spike; TMPRSS2, transmembrane serine protease 2; World Health Organization (WHO) CDC, Centers for Disease control; Monoclonal antibodies mAbs, monoclonal antibodies; LNP, lipid nanoparticles; m RNA, messenger RNA; NSP, non-structural proteins.

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https://doi.org/10.1016/j.gene.2021.146134
Received 1 December 2021; Accepted 20 December 2021
Available online 4 January 2022

CoV-2) demonstrates a lower mutational rate compared with other RNA viruses -including influenza and Human I Virus (HIV)- a significant number of mutations (aprox 12,800) has been already identified worldwide (Callaway, 2020; Wu et al., 2020b; Winger and Caspari, 2021). This extensive genomic diversity pattern -characterized by a progressive accumulation of novel mutations – is focused predominantly on the spike (S) genomic sequence (Wang et al., 2020a; 2020b; Burki, 2021; Ozono et al., 2020; Wu et al., 2020a). A broad spectrum of specific point nucleotide changes -including missense, synonymous and non-synonymous deletions and single amino acid mutations – creates a SARS-CoV-2 genomic mosaic that critically influences basic pandemic clinic-molecular parameters (Sungnak et al., 2020; Lorenzo-Redondo et al., 2020). SARS-CoV-2 aggressive mutational landscape is associated with high affinity levels on the binding cell membrane sites (functional receptors), elevated intracellular viral replication rates, high transmissibility/infectivity, and finally severe disease phenotype (increased morbidity and mortality levels) in sub-group of infected patients with specific demographic, clinical and genetic/epigenetic signatures (V'kovski et al., 2021; Denison et al., 2020; Young et al., 2020; Tsiambas et al., 2020; Marchi et al., 2021). By this time, a continuously enriched spectrum of SARS-CoV-2 genomic variants has been detected and categorized by World Health Organization (WHO) using the Greek alphabet (Alpha to Omicron) discriminating them also as variants of interest and variants of concern. Understanding the molecular significance of these different viral strains is a crucial step for designing and implementing rational and effective anti-SARS-CoV-2 strategies. For this reason, many pharmaceutical companies worldwide invest in the development of monoclonal antibodies (mAbs) and vaccines based on different platforms including, DNA plasmid based and also innovative nucleoside-modified viral messenger RNA encapsulated within nanoparticles -specifically lipid ones (LNPs) (Lee et al., 2020; Tsiambas et al., 2021; Jackson et al., 2020). Efficient vaccines and mAbs are considered necessary in order normalization and restoration of disrupted global human social-economical activity to be established (Flanagan et al., 2020; Dai and Gao, 2020). Interestingly, a negative feedback loop between these agents and SARS-CoV-2 activity is under investigation. Some studies support the idea that the virus tries to increase its immune-escape from vaccines and mAbs by modifying its mutational landscape under the rules of an increased evolutionary pressure (Pachetti et al., 2020; Akkiz, 2021). Additionally, although B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), and B.1.617.2 (delta) represent the SARS-CoV-2 main variants -that have already spread to a broad population spectrum worldwide-, specific novel variants are characterized by new mutated nucleotides with unexplored influence regarding infectivity/morbidity/mortality and also antigenicity rates (Saha et al., 2020; Toyoshima et al., 2020; Li et al., 2020).

2. SARS-CoV-2 genomic profile

According to comparative genomic analyses combined with evolutionary tree synthesis, the initial SARS-Cov-2 virus strain belongs to lineage b of beta-CoV genus. It also shares a significant phylogenetic homology with BatCoVRaTG13 type (Li et al., 2005; Wu et al., 2020a). SARS-CoV-2 genome is consisted of 29903 nucleotide based non-segmented positive-sense RNA molecule combined with RNA-dependent RNA-polymerase (Rd-Rp) (Nie et al., 2020; Wu et al., 2020a). The enzyme is responsible for intracellular virus replication in the target epithelial cells. SARS-CoV-2 virion's spherical structure (diam ~ 100 nm), consists of four main proteins. Spike surface glycoprotein (S), main or matrix protein (M), envelope protein (E), and nucleocapsid protein (NC) have been already recognized. Additionally, sixteen non-structural proteins (NSP1–NSP16) have been also detected. They encode for virus' critical molecules such as helicase and RNA-directed RNA polymerase, responsible for virus replication and translation in intracellular ribosome machinery (Ahmad Abu et al., 2020). Furthermore, seven accessory proteins (ORF3a–ORF8) have been detected, but its functional role is under investigation (Finkel et al., 2021; da Silvaet al., 2020). Virion's surface consists of S glycoprotein projections -which demonstrate two subunits: S1/S2- creating a unique crown-like formation (corona). S1 acts as the main receptor-binding domain (RBD). In contrast, S2 is implicated in fusion mechanism between cell membrane and virus particles. Specific proteases, such as furin, thrypsin, cathepsin or serino-protease (transmembrane serine protease 2-TMPRSS2) are involved in the progression of virus cell entry stabilizing the intracellular infection signal (Lukassen et al., 2020; Chen and Zhong, 2020; Coutard et al., 2020). Human angiotensin-converting enzyme 2 (hACE2) has been found to be the main target-functional receptor for SARS-CoV-2 cell attack. It provides the substrate for a successive cell membranous attachment and entry activating the S1 and S2 subunits complex (Ge et al., 2013). h ACE2 - SARS-CoV-2 cell entry triggers a cataract of intracellular signaling transduction affecting hypoxia regulatory molecules (Tsiambas et al, 2020a). Furthermore, the role of chromosome X that hosts not only the hACE2 gene (band Xp22.2), but also other critical molecules involved in immune response seems to be crucial. Aggressive clinic-pathological phenotypes in subsets of infected patients -especially males- should be partially explained by chromosome X-linked genes modifications (Tsiambas et al., 2020b; Wabalo et al., 2021). Interestingly, other studies have already revealed interesting correlations between specific genetic signatures –such as ACE1 I/D genotype- and differences in prevalence and clinical outcome of COVID-19 patients (Yamamoto et al., 2020).

3. SARS-CoV-2 genomic diversity: The deletion/mutation equilibrium riddle

SARS-CoV-2 is now characterized by a continually enriched mutational mosaic regarding the S amino acid sequence that triggers formation of specific novel variants (Boehm et al., 2021). Among them, some selective mutations seem to be highly conserved demonstrating a strong penetration in significant variants (Centers for Disease Control and Prevention. Coronovirus Disease, 2019; World Health Organisation, 2021; Li et al., 2021). The strongest S1 (non-RBD) protein region missense substitution is the D614G followed by the G142D, whereas major (non) synonymous mutations that affect the crucial RBD area include the N501Y, E484K, L452R, and K417N/T (Winger and Caspari, 2021; Rahimi et al., 2021). Additionally, P681H/R substitution influences S1/S2 furine cleavage site, whereas V1176F, A701V, T20N are recognized on S2 region. Interestingly, critical deletions affect exclusively the non-RBD S1 region (ΔH69/ΔV70/Δ156/Δ157/ΔΥ144/ΔL242/ΔΑ243/ΔL244) increasing virus transmission ability/infectivity affecting also negatively the immune response levels to neutralizing antibodies (decreased serum neutralization titles) (Bal et al., 2021; Leung et al., 2021; Supasa et al., 2021; Grubaugh et al., 2020). It is also well known that D614G/ N501Y/ E484K-Q/ K417N/T/ L452R mutations are associated significantly with strong h ACE2 binding affinity, elevated viral load production, increased human to human transmissibility/infectivity, disease phenotype severity and partially limited efficacy (immune escape) to vaccine and antibody therapeutic strategies due to low immune response rates (Gobeil et al., 2021; Daniloski et al., 2021; Luan et al., 2021; Nelson et al., 2021; Hoffmann et al., 2021).

Besides the broad landscape of point mutations (substitutions) that affect the S1-RBD and S2 SARS-CoV-2 RNA genomic sequences, specific deletions target critically the S1 domain. Interestingly, not all of the distinct variants demonstrate these modifications. They are identified as members of the mutational panel predominantly in alpha/beta/delta/eta/theta strains. Another important observation is the persistent, simultaneous presence of D614G in all of them, but the lack of E484K in delta variant. Instead of this, E484Q has been confirmed. This combination (del/ D614G/ E484Q) seems to be unique leading the virus to significantly elevated infectivity/transmissibility, and potentially to decreased response rates to mAbs and targeted vaccines (Wang et al., 2021; Liu et al., 2021). Additionally, in our view, this mutational panel

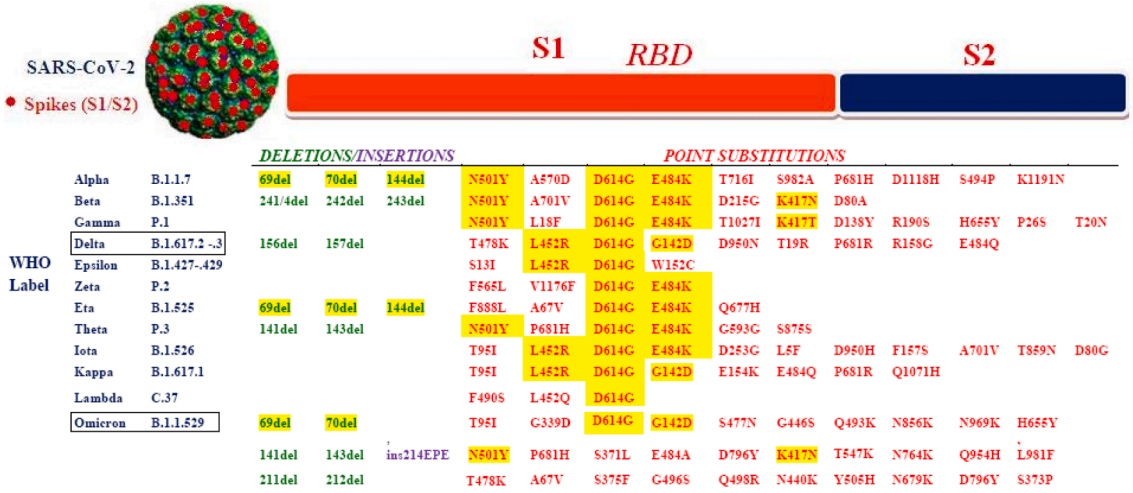

Fig. 1. Schematic, algorithmic representation of the SARS-CoV-2 variants landscape. Specific combinations of mutations and deletions are recognized. Critical deletions affect exclusively the non-RBD S1 region (ΔH69/ΔV70/Δ156/Δ157/ΔY144/ ΔL242/ΔA243/ΔL244) increasing virus transmission ability/infectivity affecting also negatively the immune response levels to neutralizing antibodies (decreased serum neutralization titles). D614G is the dominant and highly conserved substitution, whereas N501Y/ E484K-Q/ K417N/T/ L452R mutations are very important. del/ D614G/ E484Q combination in delta variant seems to be unique leading COVID-19 to a specific disease phenotype. A710V/V1176F/T716I/T1027/D1118H is the most important mutations in the S2 region. Omicron variant is characterized by an ''exotic'' combination of many mutations, deletions and one novel insertion.

could reflect a progressive virus self-adjustment process for responding to the continuously vaccination spreading. By this time, delta variant is characterized by increased infectivity/transmissibility rates combined with decreased or medium levels of morbidity and especially mortality in Europe/USA. It seems that the virus tries to survive modifying its strength in a low level of activity. For this reason, point deletions under the pressure of natural selection and evolution act sometimes negatively to the viral spreading (Akkiz, 2021; Korber et al., 2020). Furthermore, the mechanism of deletion is frequently observed in the SARS-CoV-2 viral genome in other regions including open reading frames (ORF) 7 and 8 correlated surprisingly to low replication load, but also strong response to mAbs (Su et al., 2020; Young et al., 2020). By definition, deletion is a main mechanism for suppressor genes deactivation in cell neoplastic to malignant transformation procedure. Although there is no exact parallelism regarding the intracellular activity of the virus, deletions in the critical S1 and ORF regions it seems to lead some variants to a progressively –temporary or not- self-deactivation profile.

4. Delta vs Omicron

On Nov 26th 2021, WHO designated the new SARS-CoV-2 strain –named Omicron, from letter ''*όμικρον*'' in the Greek alphabet - as a variant of concern (B.1.1529 variant). Potentially this new variant is associated with high transmissibility leading to elevated infectivity and probably increased re-infection rates. Its impact on morbidity/mortality remains under investigation. Delta variant -which is by now the prominent one worldwide- is characterized also by high infectivity. In comparison with Delta, Omicron variant demonstrates two main and 'exotic' molecular features: a combination of deletions and one insertion (Δ69-70, Δ143-145, Δ211-212, ins214EPE) and a significantly increased mutational landscape (A67V, T95I, **G142D**, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, **T478K**, E484A, Q493K, G496S, Q498R, **N501Y**, Y505H, T547K, **D614G**, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F) in S1/RBD and S2 spike regions, respectively. Interestingly, a fragment of highly conserved point substitutions (**in bold**) demonstrate a remarkable penetration in both of them. Besides them, many others have been already detected in interim variants including the Zeta, Eta, Theta, Iota, and Kappa, respectively (Fig. 1). The impact of this mutational algorithm regarding the Omicron-mediated infectivity/ morbidity/ mortality still remains unrevealed. Although the number and combination of mutations/deletions/insertion in Omicron is impressive, the whole molecular image of the spike demonstrates a partial re-or dis-organization of the corresponding RNA sequence. Increased rates of infectivity and re-infection of pure or vaccinated individuals do not mean that morbidity/ mortality levels could be dramatically increased also. Maybe this internal reprogramming is the next step for the virus to survive in a lower profile, stabilizing its presence in host cells.

5. Conclusions

SARS - Cov-2 related COVID-19 pandemic influences significantly the global socio-financial stability and national health systems' endurance. Among the most critical features of the virus is the ability to demonstrate rapidly developed S- related variants escaping partially from vaccine and mAbs based targeted strategies (Greaney et al., 2021; McCarthy et al., 2021). But, although systematic vaccination rates are increased –especially in western world- there is an urgent need for updating the commercially available vaccines. Enriching and reprogramming them is an urgent progressive procedure, essential for blocking virus' activity. Understanding the role of mutational modifications' balance (substitutions/deletions) in viral genome is a critical step for earning specific molecular knowledge in order efficient antiviral agents to be developed. Reading, matching and decoding the corresponding genomic transformation of the virus we observed that maybe S1-RBD/S2 mutation/deletion equilibrium inside its variants is a key for unlocking its infectivity/morbidity/mortality cataract predicting also its activity and biological behaviour.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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.

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