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Forum

Biosensing Detection of the SARS-CoV-2 D614G Mutation

Yang Zhang [1,*] Hui Xi,[1] and Mario Juhas[2]

The emergence of a mutant strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with an amino acid change from aspartate to a glycine residue at position 614 (D614G) has been reported and this mutant appears to be now dominant in the pandemic. Efficient detection of the SARS-CoV-2 D614G mutant by biosensing technologies is therefore crucial for the control of the pandemic.

The D614G Mutation Increases SARS-CoV-2 Infectivity

The coronavirus disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2, has rapidly escalated into the largest global public health emergency of the past decades. As of December 2020, more than 75 million people have been infected and more than 1.7 million have died as a result of the SARS-CoV-2 infection[i]. SARS-CoV-2 shares similarities with two other beta coronaviruses that cause serious and potentially deadly respiratory tract infections, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). The open reading frames (ORFs) of the approximately 30-kb SARS-CoV-2 genome encode 16 nonstructural and four structural proteins, namely envelope (E), membrane (M), nucleocapsid (N), and spike (S) (Figure 1A) [1].

The sequencing of thousands of SARS-CoV-2 genomes revealed a number of recurrent mutations, out of which an amino acid change from aspartate to a glycine residue at position 614 (D614G) in the S protein has gained most attention. D614G is a missense mutation where the amino acid change is caused by an A-to-G nucleotide mutation at genomic position 23 403 [2]. The D614G mutation has been identified by phylogenetic analysis of over a thousand SARS-CoV-2 genomes. While the D614G mutation was relatively rare at the onset of the pandemic, by June 2020 this mutation was found in the majority of COVID-19 patients [1–3]. The D614G point mutation is predominant amongst the SARS-CoV-2 genomes currently circulating in the population [2]. Furthermore, the D614G mutation is located in the S1 subunit of the S protein, which is crucial for the entry of the SARS-CoV-2 virus into the host cell (Figure 1B,C). The S protein of SARS-CoV-2 is cleaved into S1 and S2 subunits by host proteases, with the S1 domain is responsible for receptor binding and the S2 domain for mediating membrane fusion [1,4]. The receptor binding domain (RBD) within the S1 subunit of S protein interacts with the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of the host cells, thus mediating virus entry [1,2]. Although, the D614G point mutation is outside of the RBD (Figure 1C), it has been suggested to have an impact on the infectivity of the virus. The rapid spread of the D614G mutant version of the virus was hypothesized to correlate with the higher infectivity of SARS-CoV-2 harboring this mutation [2,4]. Clinical samples from patients infected with the D614G mutant were shown to have higher viral RNA levels and higher pseudovirus titers [2]. Due to the impact of the D614G mutation on SARS-CoV-2 biology, novel methods for its fast and reliable detection are of the highest importance.

Biosensing Technologies for the Detection of the SARS-CoV-2 D614G Mutation at the Protein Level

Development of biosensing technologies for the efficient and rapid detection of SARS-CoV-2 and the D614G mutation is crucial for the control of the pandemic. One such promising method, surface plasmon resonance (SPR), has been used to investigate the effect of the D614G mutation on the binding kinetics of the SARS-CoV-2 S protein with human ACE2 [5]. The binding rates of the S protein with and without the D614G mutation were similar, but the dissociation rate of the D614G mutant was faster, which contributed to the decrease in binding affinity [5]. This observation could be exploited for the engineering of an SPR-based biosensing platform employing ACE2 protein for SARS-CoV-2 D614G mutant protein detection (Figure 2A). Alternatively, biosensing based on aptamers, antibodies, or nanobodies can be designed to distinguish the S protein with or without the D614G mutation (Figure 2A). Aptamers are short single-stranded nucleic acid sequences that fold into tertiary structures to bind any desired target [6]. Similar to antibodies, aptamers have also been used in ELISA and colloidal gold immunochromatographic strips for the detection of the N protein of SARS-CoV-2 [7].

In addition to antibodies, nanobodies derived from the VHH domain of single-heavy-chain antibodies have also been raised with high affinity for the S protein of SARS-CoV-2 [8,9].

Aptamers, antibodies, and nanobodies specifically distinguishing D614G mutation within the S protein can be used as biorecognition elements and then incorporated into ELISA and lateral flow assays (Figure 2A).

Although the serological assay is rapid and requires minimal equipment, its efficacy is limited due to the presence of low amounts of the viral protein or antigen in the blood of patients. Furthermore, current studies suggest that the D614G mutation

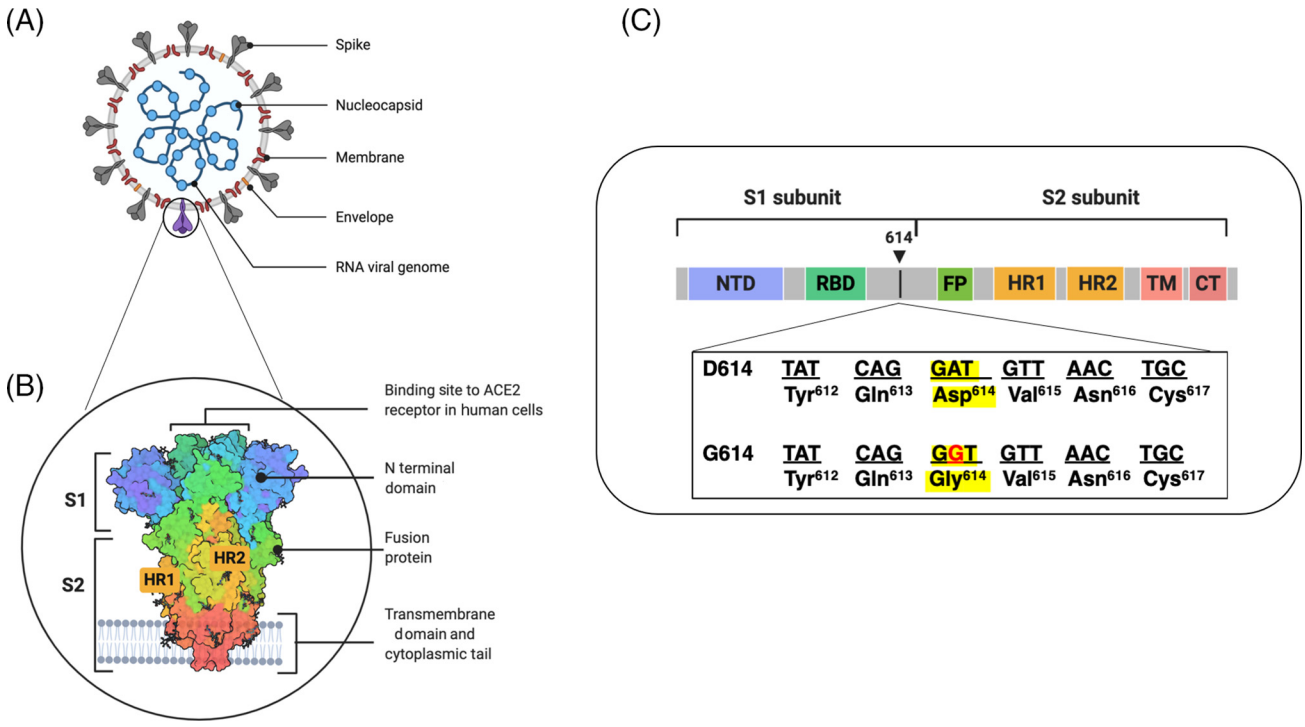

Figure 1. The D614G Mutation in Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) Spike Protein. (A) The structure of SARS-CoV-2 includes the surface spike (S) protein, the nucleocapsid (N) protein, the membrane (M) protein, the envelope (E) protein, and the RNA genome. (B) Crystal structure of SARS-CoV-2 S protein. Protein Data Bank ID: 6VXX. (C) Schematic representation of the SARS-CoV-2 S protein. Figure was created in part with BioRender. Abbreviations: ACE2, angiotensin-converting enzyme 2; CT, cytoplasmic tail; FP; fusion protein; HR1/2, heptad repeat 1/2; NTD, N terminal domain; RBD, receptor-binding domain; S1, S1 subunit; S2, S2 subunit; TM, transmembrane domain.

retains similar sensitivity to antibodies that target the RBD [5]. Thus, the possibility of reliable detection of mutation-specific antibodies by D614G and D614 antigens will require further investigation. Overall, despite the need for the conversion of an RNA single-stranded genome of SARS-CoV-2 into cDNA for detection purposes, biosensing technologies aimed at the detection of the D614G mutation at the gene level show much greater promise.

Biosensing Technologies for the Detection of the SARS-CoV-2 D614G Mutation at Gene Level

DNA sequencing and PCR are current standard tests for detecting the presence of SARS-CoV-2. In conjunction with DNA sequencing and different types of PCR-based techniques, capillary electrophoresis, surface-enhanced Raman spectroscopy (SERS), mass spectrometry (MS), and denaturing high performance liquid chromatography (dHPLC) [10–12] can also be used to screen for the presence of the D614G mutation. However, these conventional methods are often complex, tedious, and time consuming. Novel biosensing technologies for rapid monitoring of D614G are therefore urgently needed.

Loop-mediated isothermal amplification (LAMP) is a PCR-based method for the amplification of nucleic acids under isothermal conditions, which does not require a thermal cycler or expensive reagents. LAMP has been successfully applied for the rapid (as little as 30 min) and colorimetric detection of SARS-CoV-2 [13]. A set of primers can be designed and optimized to specially identify the D614G mutant genotype. LAMP is fast and its products can be detected with electrochemical and optical readouts, or even by the naked eye, making it suitable for point-of-care testing of the SARS-CoV-2 D614G mutation (Figure 2B).

The D614G mutation corresponds to the alteration of an adenine to a guanine at genomic position 23 403 (Figure 1C) [2]. Consequently, the D614G mutation can be detected using restriction endonucleases to enzymatically cleave deoxynucleotides at this specific site, following double-stranded cDNA synthesis. In this case, restriction endonucleases FokI, BtsCI,

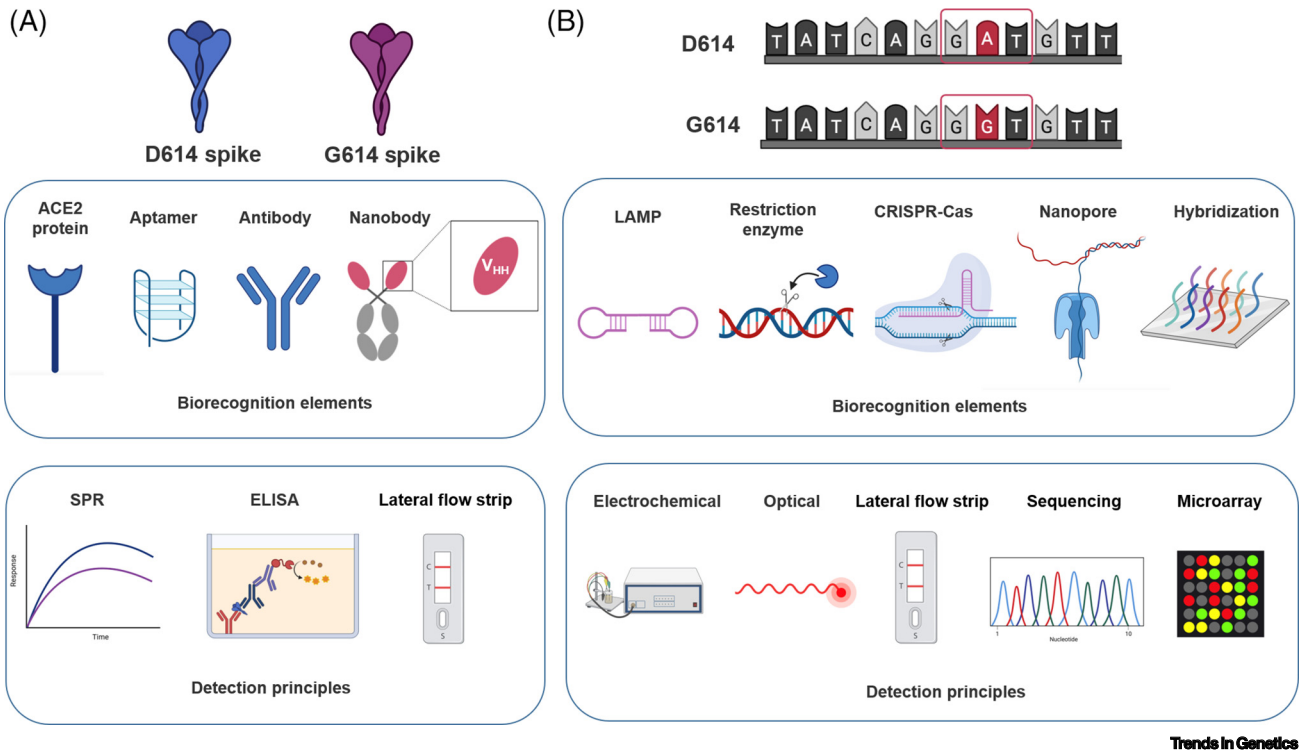

Figure 2. Biosensing Technologies for the Detection of the D614G Mutation. (A) Detection of the D614G mutation at the protein level. The D614G mutation at the protein level can be identified using angiotensin-converting enzyme 2 (ACE2) protein, aptamers, antibodies, or nanobodies. Signal generated by these biorecognition elements can be detected by different detection principles, such as surface plasmon resonance (SPR), ELISA, and lateral flow strips. (B) Detection of the D614G mutation at the gene level. The D614G mutation in severe acute respiratory coronavirus 2 (SARS-CoV-2) spike protein corresponds to the alteration of an adenine to a guanine at genomic position 23 403. Promising approaches for the recognition of the D614G mutation at the gene level include loop-mediated isothermal amplification (LAMP), restriction enzymes, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas, nanopore, and oligo probe hybridization. Specific signal generated by these biorecognition techniques can be then used for the detection of the D614G mutation using a variety of detection principles, such as electrochemical and optical techniques, lateral flow strips, sequencing, or microarrays. Figure was created in part with BioRender.

BseGI, and BstF5I can be used for specific recognition and cleaving of the GGATG sequence in the wild-type D614, while leaving the mutant G614 sequence intact. The specific cleaving of the point mutation can be combined with suitable signal amplification strategies, such as rolling circle amplification (RCA), strand displacement amplification (SDA), and isothermal exponential amplification (EXPAR) [14], for the optical and electrochemical detection of the D614G mutant version (Figure 2B).

Besides restriction endonucleases, RNA-guided Cas endonucleases that catalyze site-specific cleavage of DNA can also be used to detect point mutations [15]. For example, a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas12-based assay was combined with LAMP for rapid (less than 40 min) and accurate detection of SARS-CoV-2 in clinical samples. Followed by Cas12 detection of viral sequences, visualization was achieved using FAM-biotin reporter molecule and lateral flow strips [16]. Similar CRISPR/Cas systems can be exploited for the accurate and scalable detection of the SARS-CoV-2 D614G mutation on a lateral flow strip using different signal readout based on electrochemical and optical techniques (Figure 2B).

Besides using endonucleases for mutation site recognition, engineered pore-forming proteins could be applied directly to gene sequencing and D614G mutant detection. Nanopore sequencing technologies constitute a versatile platform for DNA/RNA sequencing and have been also used for real-time sequencing of the SARS-CoV-2 genome [17,18]. Nanopore sequencing directly measures changes in currents generated when DNA/RNA strands pass through the nanopore. The electrical signal corresponding to each nucleic acid can be recorded in real-time and used for subsequent sequence analysis. Although nanopore sequencing offers high throughput and accurate

genotyping of SARS-CoV-2 [14], it is slower, taking hours to obtain testing results (Figure 2B).

Hybridization-based biosensing platforms are highly specific and represent a promising tool for nucleic acid and single-nucleotide mutation detection. SARS-CoV-2 RNA was detected on formalin-fixed paraffin-embedded tissue from COVID-19 autopsies by *in situ* hybridization [19]. Moreover, an amplification-free immunoassay was developed for the fluorescence detection of SARS-CoV-2 RNA in the throat swab or sputum sample. The assay is implemented on a lateral flow strip based on hybridization of viral RNA with DNA probes and selective capture of RNA–DNA hybrids by monoclonal antibodies [20]. Hybridization techniques using point-mutated probes and corresponding perfect matching probes can be also confidently employed for the D614G mutation detection. To scale, from small sample numbers to thousands, microarray hybridization analysis is the most appropriate technology, employing tens to hundreds of probes for single base mismatch discrimination (Figure 2B).

Considering the incredible demand for SARS-CoV-2 genotyping, a number of different biosensing techniques with various biorecognition elements and detection principles from diverse industry sources are needed to address the immediate shortage of reliable and efficient tests in the face of the global COVID-19 pandemic.

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Resources

[i]www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/

[1]College of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong, 518055, China
[2]Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland

*Correspondence:
zhangyang07@hit.edu.cn (Y. Zhang).

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Forum

Feralization: Confronting the Complexity of Domestication and Evolution

Makenzie E. Mabry,[1,2,8,@] Troy N. Rowan,[3,4,5,6,8,@] J. Chris Pires,[1,4,7,*,@] and Jared E. Decker[3,4,7,*,@]

Feral populations, those which successfully persist outside of cultivation or husbandry, provide unique opportunities to study the genomic impacts of domestication and local adaptation. We argue that by leveraging genomic resources designed for domestic counterparts, powerful phylogenetic and population genomic data collection and analyses can be designed to disentangle complex demographic processes.

Emerging Interest in Feral Populations

Surveys of feral populations in a wide range of organisms from animals to plants reflects growing interest in **feralization** (see Glossary) [1–3]. Although feral populations have fascinated researchers since Darwin, the demographic and selective processes that accompany feralization are not well characterized. Further, defining feral populations is difficult due to each population's unique life history (see Table 2 in [1]). However, feral populations can be used to improve domesticated populations (Box 1) and offer opportunities to understand important concepts applicable to many different fields of study. These opportunities come with many potential confounders; temporal, geographic, and demographic in nature. In this forum, we showcase opportunities