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Nucleotide Sequence Sharing between the Human Genome and Primers for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Detection

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Abstract

This study shows that oligonucleotide sequences are shared between the human genome and primers that have been proposed/used for SARS-CoV-2 detection by polymerase chain reaction (PCR). The high level of sharing (namely, up to 19mer with a maximum number of gaps equal to 2) might bear implications for the diagnostic validity of SARS-CoV-2 detection by PCR.

Keywords

- PCR primers
- SARS-CoV-2 detection
- false positives

Introduction

Defining the relationship(s) between infectious agents and the human host is a crucial topic in immunology, microbiology, and infectious medicine. Although it has been proposed that genetic factors might play a role,[1,2] the exact mechanisms of chronic infections and occasional (re)activation of pathogens in the human host are largely misunderstood and poorly studied. The issue became even more relevant in light of the recent Ebola virus, Dengue virus, and SARS outbreaks associated with high morbidity and mortality.[3–5] In this context, there is a need not only for knowing the molecular basis of infections to define effective and safe preventive and therapeutic interventions but also for sensitive and specific diagnostic tools. Indeed, accurate screening of asymptomatic, presymptomatic, and symptomatic subjects might be key to effective epidemiological measures during pandemics. However, especially in analyzing SARS-CoV-2 as a paradigmatic example, contrasting data have been reported on the analytical performance of SARS-CoV-2 detection methods and claims about the rates of false negatives and false positives have been published.[6–11]

On the basis of all these, this study focused on the possible genetic basis of potential false polymerase chain reaction (PCR) results by comparing the nucleotide sequence of proposed/used SARS-CoV-2 primers versus the human genome. The scientific rationale is that—given the high level of amino acid sequence sharing between SARS-CoV-2 proteins and the human proteome[12–15]—parallel sequence matching at the nucleotide level might exist between the SARS-CoV-2 primer sequences and the human genome, in this way possibly explaining the generation of false-positive SARS-CoV-2 detection results. Data are reported here that confirm the likelihood of the research hypothesis.

De facto, using the nucleotide Basic Local Alignment Search Tool (BLASTn) program from NCBI (http://blast.ncbi.nlm.nih.gov,[16,17] a sample of 12 primers retrieved from literature,[18,19] proposed/used even by government health institutions[19] to detect SARS-CoV-2, and described here in ►**Table 1**, was analyzed for nucleotide sequence sharing with the human genome. BLASTn analyses documented a relevant viral versus human oligonucleotide overlap, with shared primer sequences repeatedly present in the human genome, disseminated among different chromosomes, and located in plus strands, minus strands, mRNAs, pseudogenes, etc. Due to space constraints, an *in extenso* description of the complete nucleotide sequence sharing is practically not possible, and only a synthetic snapshot is shown in ►**Table 2**.

In conclusion, this communication highlights the likelihood that viral versus human nucleotide sequence overlap

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Table 1 Nucleotide sequence of primers used/proposed for PCR detection of SARS-CoV-2[a]

Primer no.	Target gene[b]	Primer direction	Primer nucleotide sequence
1	S 2	F	CCACTAGTCTCTAGTCAGTGTGTTAAT
2	S 2	R	AAACTGAGGATCTGAAAACTTTGTC
3	8	F	GGAGCTAGAAAATCAGCACCTTTAA
4	8	R	TCGATGTACTGAATGGGTGATTTAG
5	E	F	ACAGGTACGTTAATAGTTAATAGCGT
6	E	R	ATATTGCAGCAGTACGCACAGA
7	N	F	GACCCCAAAATCAGCGAAAT
8	N	R	TCTGGTTACTGCCAGTTGAATCTG
9	N	F	GGGGAACTTCTCCTGCTAGAAT
10	N	R	CAGACATTTTGCTCTCAAGCTG
11	N	R	TAATCAGACAAGGAACTGATTA
12	N	F	TGGCAGCTGTGTAGGTCAAC

Abbreviations: F, forward; PCR, polymerase chain reaction; R, reverse.
[a]Primers retrieved from Gadkar et al[18] and Qasem et al,[19] and further details and references therein.
[b]Gene names given according to Uniprot.[20]

Table 2 Oligonucleotide sharing between the human genome and polymerase chain reaction (PCR) primers proposed/used to detect SARS-CoV-2: a few examples[a]

1. CCACTAGTCTCTAGTCAGTGTGTTAAT
   Glypican 5 (GPC5), Chromosome 13, Strand: Plus/Plus
   864805 TCTAGTCAGTGTGTTAAT 864822
2. AAACTGAGGATCTGAAAACTTTGTC
   DEP domain containing 5, Chromosome 22, Strand: Plus/Minus
   132374 CTGAGGATCTGAAAACTTT 132356
3. GGAGCTAGAAAATCAGCACCTTTAA
   DNA damage regulated autophagy modulator 2 (DRAM2), Chromosome 1, Strand: Plus/Plus
   3702 AGAACATCAGCACCTTTAA 3720
4. TCGATGTACTGAATGGGTGATTTAG
   Isolate CHM13 chromosome 17, Strand: Plus/Plus
   5169199 GATGTACTGAAAGGCTGATTTA 5169220
5. ACAGGTACGTTAATAGTTAATAGCGT
   Chromosome 18, SeqID:AP023478.1,Strand: Plus/Minus
   34259565 GTACGTTAATAGTAAATA 34259548
6. ATATTGCAGCAGTACGCACAGA
   Hemicentin 1, HMCN1, Chromosome 1, Strand: Plus/Plus
   379167 ATTGCAGCAGTAAGCACAG 379185
7. GACCCCAAAATCAGCGAAAT
   SLAM family member 8, SLAMF8, transcript variant 2, mRNA, SeqID: NM_001330741.2, Strand: Plus/Plus
   161 CCCCAACATCAGCGAAAT 178
8. TCTGGTTACTGCCAGTTGAATCTG
   Sciatic injury induced incRNA upregulator of SOX11, long non-coding RNA, SequID: NR_026832.1, Strand: Plus/Minus
   9779 TGGTTACTCCCAGTTGAAT 9761

9. GGGGAACTTCTCCTGCTAGAAT

CHM13 chromosome 20, SeqID: CP068258.2 Strand: Plus/Plus

11065407 AACTTCTCCAGCTAGAAT 11065424

10. CAGACATTTTGCTCTCAAGCTG

Rho GTPase activating protein 6, ARHGAP6), RefSeqGene on chromosome X, SeqID: NG_012494.2,Strand: Plus/Minus

488854 CAGACATTTTGCTTTCAAG 488836

11. TAATCAGACAAGGAACTGATTA

Chromosome 3 clone RP11-24C3, SeqID: AC104448.2, Strand: Plus/Minus

13048 TAATCAGACAAGGCACTGA 13030

12. TGGCAGCTGTGTAGGTCAAC

BAC clone RP11-150O15, SeqID: AC020591.7, Strand: Plus/Plus

24820 TGGCTGCTGTGTAGGTCAA 24838

[a]Twelve primers described in ►**Table 1** and derived from Gadkar et al[18] and Qasem et al[19] were analyzed for sharing of nucleotide sequences with the human genome. BLASTn[16,17] was used to find and localize regions of identity in the human nucleotide collection covering genomic and transcript sequences; further details are available at http://blast.ncbi.nlm.nih.gov. The 12 primers are listed with shared nucleotide sequences underlined.

can interfere with nucleic acid amplification testing and generate PCR false-positive results in SARS-CoV-2 detection, in this way affecting medical diagnoses.

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Conflict of Interest
None declared.

References

1 Kanduc D. Rare human codons and HCMV translational regulation. J Mol Microbiol Biotechnol 2017;27(04):213–216
2 Kanduc D. Human codon usage: the genetic basis of pathogen latency. Glob Med Genet 2021;8(03):109–115
3 Keita AK, Koundouno FR, Faye M, et al. Resurgence of Ebola virus in 2021 in guinea suggests a new paradigm for outbreaks. Nature 2021;597(7877):539–543
4 Dayama P, Sampath K. Dengue disease outbreak detection. Stud Health Technol Inform 2014;205:1105–1109
5 Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: what we know. Int J Infect Dis 2020;94:44–48
6 Patriquin G, Davidson RJ, Hatchette TF, et al. Generation of false-positive SARS-CoV-2 antigen results with testing conditions outside manufacturer recommendations: a scientific approach to pandemic misinformation. Microbiol Spectr 2021;9(02):e0068321
7 Benoit J, Benoit SW, Lippi G, Henry BM. False negative RT-PCR or false positive serological testing in SARS-CoV-2 diagnostics? Navigating between Scylla and Charybdis to prevent misclassification bias in COVID-19 clinical investigations. Diagnosis (Berl) 2020;7(04):405–407
8 Verna R, Alallon W, Murakami M, et al. Analytical performance of COVID-19 detection methods (RT-PCR): scientific and societal concerns. Life (Basel) 2021;11(07):660
9 Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(03):2000045
10 Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. Lancet Respir Med 2020;8(12):1167–1168
11 Eurosurveillance Editorial Team. Response to retraction request and allegations of misconduct and scientific flaws. Euro Surveill 2021;26(05):2102041
12 Kanduc D. From anti-severe acute respiratory syndrome coronavirus 2 immune response to cancer onset via molecular mimicry and cross-reactivity. Glob Med Genet 2021;8(04):176–182
13 Kanduc D. Thromboses and hemostasis disorders associated with coronavirus disease 2019: the possible causal role of cross-reactivity and immunological imprinting. Glob Med Genet 2021;8(04):162–170
14 Kanduc D. From anti-SARS-CoV-2 immune responses to COVID-19 via molecular mimicry. Antibodies (Basel) 2020;9(03):33
15 Kanduc D. From anti-SARS-CoV-2 immune response to the cytokine storm via molecular mimicry. Antibodies (Basel) 2021;10(04):36
16 Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25(17):3389–3402
17 Boratyn GM, Thierry-Mieg J, Thierry-Mieg D, Busby B, Madden TL. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. BMC Bioinformatics 2019;20(01):405
18 Gadkar VJ, Goldfarb DM, Young V, et al. Development and validation of a new triplex real-time quantitative reverse Transcriptase-PCR assay for the clinical detection of SARS-CoV-2. Mol Cell Probes 2021;58:101744
19 Qasem A, Shaw AM, Elkamel E, Naser SA. Coronavirus disease 2019 (COVID-19) diagnostic tools: a focus on detection technologies and limitations. Curr Issues Mol Biol 2021;43(02):728–748
20 Uniprot. Accessed November 2021 at: https://www.uniprot.org/uniprot/?query=proteome:UP000464024&sort=score