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Mid-density Gene Expression profiling of SARS-COV-2 infected samples using Applied Biosystems™ TaqMan™ Flexible Array Panels

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INTRODUCTION

The broad spectrum of clinical manifestations from SARS-COV-2 infection, as well as the observed risk factors for severe disease, highlight the importance of understanding molecular mechanisms underlying disease development and progression. Research studies have identified a large number of host proteins that play roles in viral entry, innate immune response, or immune signaling during infection. For example, researchers have shown that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a receptor [1,2] while a number of host cofactors including cell-surface serine proteases TMPRSS2 and TMPRSS4 [3] help facilitate entry into the host cell [1,4]. In addition, a variety of immune pathways involved in inflammation, oxidative stress, and antiviral T cell responses are dysregulated In response to infection. In severe cases, a proinflammatory cytokine storm that includes upregulation of IL-1, IL-6, IL-12, IFN-γ, and TNF-α, is an indicator of poor prognosis [5-6].

The ability to interrogate subsets of these genes simultaneously within SARS-COV-2 infected samples is critical to understanding how their expression contribute to phenotypic variability of disease caused by the virus. To bridge this gap, we will use flexible TaqMan array panels designed by Applied Biosystems specifically for targeting the most cited genes related to entry and restriction factors as well as cytokines, chemokines, and growth factors. Each array features a curated list of predesigned TaqMan Gene Expression Assays that can be modified to meet research objectives. In this study, these arrays will be used to highlight gene expression patterns that exist within confirmed SARS-COV-2 positive and negative nasopharyngeal swab samples and demonstrate the utility of these panels for gene expression profiling of SARS-COV-2 infected samples at medium throughput and scale.

MATERIALS AND METHODS

A curated selection of predesigned Applied Biosystems™ TaqMan™ Gene Expression assays addressing signature genes identified in targeted host response were used. These FAM™ dye-labeled assays were preloaded on a flexible content, TaqMan® Array Plate (TAP). The TAP platform offers 384-well real-time PCR accommodating up to 381 targets, plus controls. Three preconfigured, flexible array plates were used to target 1) Viral entry factors, 2) Viral restriction factors and 3) Immune signaling genes (Table 1). A robust protocol was developed using Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG (Catalog number: A15299), for RT-qPCR step then ran on Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. Manufacturer recommended conditions were followed. The process and individual steps involved in setting up the qPCR experiment are outlined in Figure 1.

RNA materials were extracted from individual nasopharyngeal samples by using MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Catalog number: A48383R) on KingFisher Flex, following the workflow from the IFU for TaqPath™ COVID-19 Combo Kit (400uL sample input, 2 wash steps), Figure 2.

Table 1. Preconfigured, flexible TaqMan Arrays for SARS-CoV-2 research

Viral entry factors	Restriction factors	Immune signaling: cytokines, chemokines & growth factors		
ACE2	LY6E	IL1B	IFNB1	CCL2
TMPRSS2	IFITM1	IL2	IFNG	CCL3/MIP1a
TMPRSS4	IFITM2	IL4	TNF	CCL5
TMPRSS11A	IFITM3	IL5	TGFB1	CCL11
TMPRSS11B	ZAP / ZC3HAV1	IL6	M-CSF/CSF1R	CCL27
BSG	BST2	IL7	MIF	CXCL1
ANPEP	CLEC4D	IL10	VEGF	CXCL10
CLEC4G	ELF1	IL12	SCGF	CXCL12
FURIN	REC8	IL13	HGF	
CTSL	IFIT3	IL16	TNFSF10	
CTSB	DNAJC6	IL18		
DPP4	ZBP1			
NRP1	CH25H			

Figure 1. Real-time PCR workflow using TaqMan Array Plates

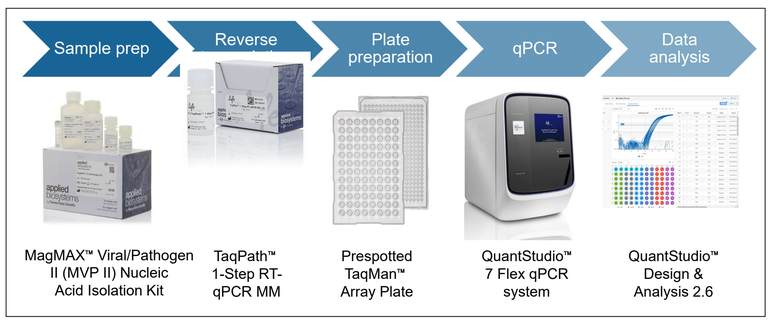
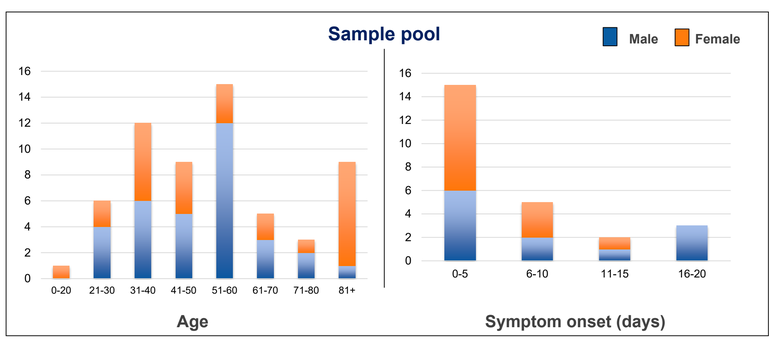


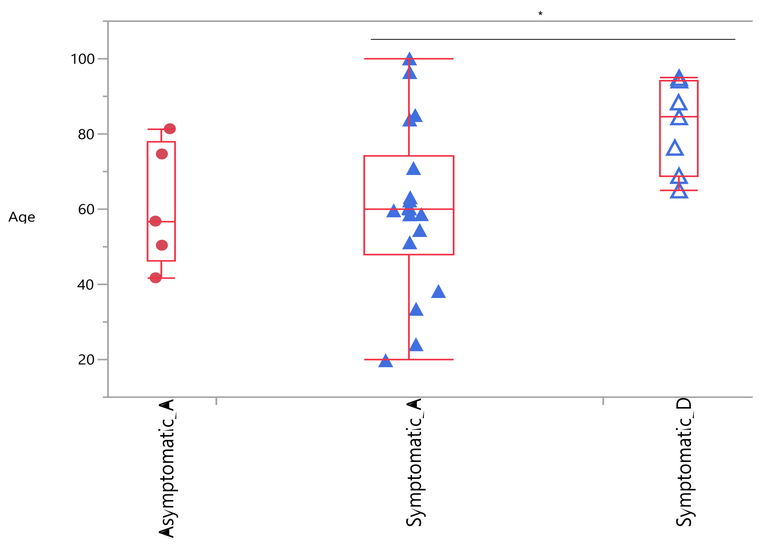
Figure 2. Demographics of study samples



Distribution of age, sex, and days of symptom onset for the 30 SARS-COV-2-positive samples used in this study. Nasopharyngeal swabs were collected in BD Viral Transport Medium and verified to be positive using the Roche cobas® Liat® molecular diagnostic test. Samples that were verified to be positive were subjected to further gene expression analysis using TaqMan Array panels.

RESULTS

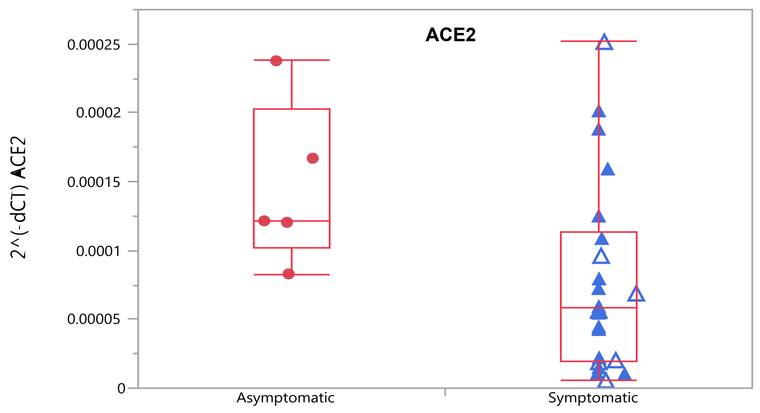
Figure 3. Clinical characteristics of study samples



Influence of age on SARS-CoV-2 disease outcome: SARS-CoV-2-infected study samples were categorized based on the disease severity and outcome of the subjects against age. Symptomatic study samples with fatal outcome were significantly older compared to symptomatic study samples that were alive ($p<0.05$). No significant difference was observed between study samples obtained from asymptomatic alive and symptomatic alive subjects. Tukey-Kramer HSD test was used to evaluate differences between the study groups.

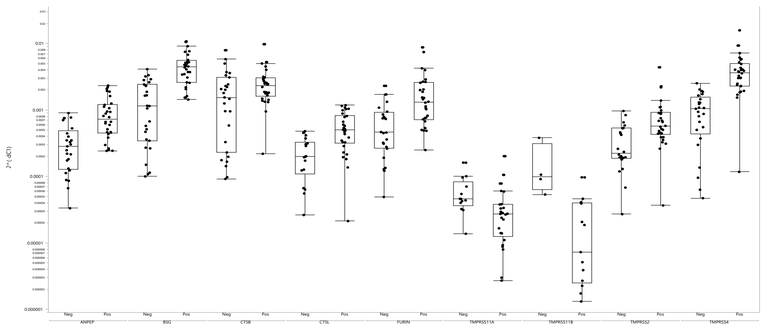
Asymptomatic_A: asymptomatic (alive)
Symptomatic_A: symptomatic (alive)
Symptomatic_D: symptomatic (deceased)

Figure 4. Significantly altered SARS-CoV-2-associated entry factors in SARS-Cov-2 infected study samples based on disease severity



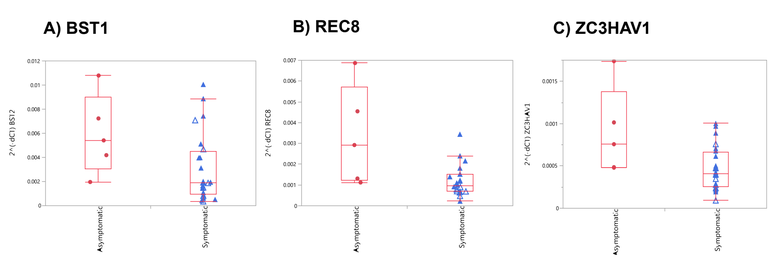
Relative gene expression comparison of ACE2, which was found to be significantly altered in symptomatic vs asymptomatic study samples ($p<0.05$). The expression levels of the gene was downregulated in symptomatic study samples compared to the asymptomatic SARS-CoV-2 infected study samples. Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between symptomatic and asymptomatic study samples were assessed utilizing Student's t-test.

Figure 5. Significantly altered SARS-CoV-2-associated entry factors in study samples based on infection status



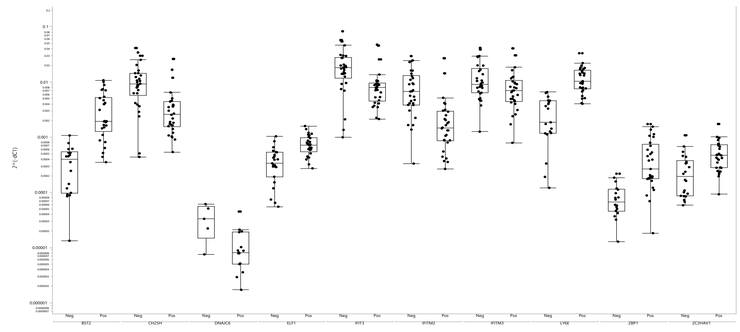
Relative gene expression comparison of significantly altered SARS-CoV-2 associated entry factors SARS-CoV-2 infected and healthy control study samples ($p<0.05$). Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between infected and control study samples were assessed utilizing Student's t-test. Neg= SARS-CoV-2 negative samples; Pos= SARS-Cov-2 positive samples.

Figure 6. Significantly altered SARS-CoV2- associated restriction factors in SARS-CoV-2 infected study samples based on disease severity



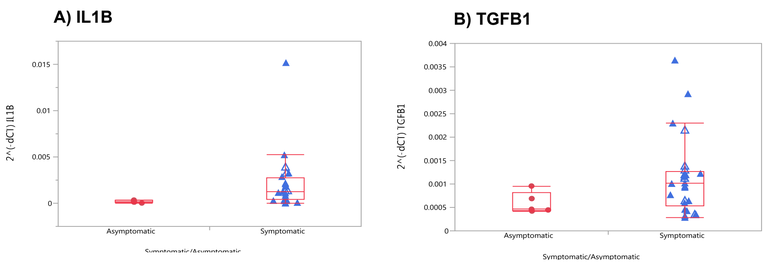
Relative gene expression comparisons of A) BST2, B) REC8, C) ZC3HAV1 which were found to be significantly altered in symptomatic vs asymptomatic study samples ($p<0.05$). The expression levels of the genes were decreased in symptomatic study samples compared to the asymptomatic SARS-CoV-2 infected study samples. Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between symptomatic and asymptomatic study samples were assessed utilizing Student's t-test.

Figure 7. Significantly altered SARS-CoV2- associated restriction factors in study samples based on infection status



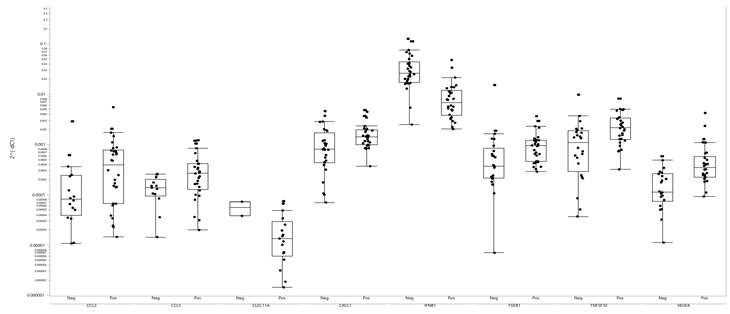
Relative gene expression comparison of significantly altered SARS-CoV-2 associated cellular restriction factors SARS-CoV-2 infected and healthy control study samples ($p<0.05$). Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between infected and control study samples were assessed utilizing Student's t-test. Neg= SARS-CoV-2 negative samples; Pos= SARS-Cov-2 positive samples.

Figure 8. Significantly altered immune signaling genes in SARS-CoV-2 infected study samples based on disease severity



Relative gene expression comparison of A) IL1B and B) TGFB1 genes, which were found to be significantly altered in symptomatic vs asymptomatic study samples ($p<0.05$). The expression levels of both the genes were elevated in symptomatic study samples compared to the asymptomatic SARS-CoV-2 infected study samples. Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between symptomatic and asymptomatic study samples were assessed utilizing Student's t-test.

Figure 9. Significantly altered immune signaling genes in study samples based on infection status



Relative gene expression comparison of significantly altered immune signaling genes between SARS-CoV-2 infected and healthy control study samples ($p<0.05$). Target gene expression was normalized to the geometric mean of 18S, *GAPDH*, and *PPIA* expression. Gene expression differences between infected and control study samples were assessed utilizing Student's t-test. Neg= SARS-CoV-2 negative samples; Pos= SARS-Cov-2 positive samples

CONCLUSIONS

The pathogenesis of SARS-CoV-2 infection is an active area of research investigation. Given the urgency to develop effective therapeutics against the virus and the disease caused by it, understanding of the host response as well as virus life cycle is imperative. Here, we presented data from 30 SARS-CoV-2 infected and 30 control samples to determine the expression levels of SARS-CoV-2-associated 1) entry receptors, 2) cellular restriction factors, and 3) immune mediators from nasopharyngeal samples, and compare the relative gene expression differences based on disease severity and infection status. Our data indicated that not only were the levels of several of these host factors differentially modulated between infected and control samples, but also varied in infected study samples based on the severity of the disease. For instance, ACE2 expression was found to be downregulated in symptomatic samples compared to asymptomatic in our study, a finding that has been previously observed in SARS-CoV-2-infected patients with or without respiratory distress [7]. Several host restriction factors such as BST1, REC8 and ZC3HAV1 that are known to exert anti-viral activities were also suppressed in symptomatic samples and may contribute at least in part to the disease severity. Studies such as ours may reveal novel insights into how the pathogenesis may also vary depending of gender, age etc. The 384-well flexible content, TaqMan array plates provide a fast, mid-throughput solution to determine the levels of several virus and host-associated factors in various cell and sample types, and can be used to widen our understanding of disease pathogenesis of SARS-CoV-2 and other organisms of interest.

REFERENCES

1. Shang J, Wan Y, Luo C, et al. "Cell entry mechanisms of SARS-CoV-2." *PNAS*. 2020;117(21):11727-11734. doi:10.1073/pnas.2003138117

2. Yang J, Petitjean SJL, Koehler M, et al. "Molecular interaction and inhibition of SARS-CoV-2 binding to the ACE2 receptor." *Nature Communications*.2020;11(1):4541. doi:10.1038/s41467-020-18319-6

3. Zang R, Gomez Castro MF, McCune BT, et al. "TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes." Sci Immunol. 2020;5(47):eabc3582. doi:10.1126/sciimmunol.abc3582

4. Singh M, Bansal V, Feschotte C. "A Single-Cell RNA Expression Map of Human Coronavirus Entry Factors." Cell Reports. 2020;32(12):108175. doi:10.1016/j. celrep.2020.108175

5. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. "COVID-19: consider cytokine storm syndromes and immunosuppression." The Lancet. 2020;395(10229):1033-1034. doi:10.1016/S0140-6736(20)30628-0

6. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. "SARS-CoV-2 infection: The role of cytokines in COVID-19 disease." Cytokine & Growth Factor Reviews. 2020;54:62-75. doi:10.1016/j.cytogfr.2020.06.001

7. Rossi ÁD, de Araújo JLF, de Almeida TB, et al. "Association between ACE2 and TMPRSS2 nasopharyngeal expression and COVID-19 respiratory distress". Sci Rep. 2021 May 6;11(1):9658. doi: 10.1038/s41598-021-88944-8

CONFLICT OF INTEREST

All authors on this study are employees of Thermo Fisher Scientific

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