The HLA complex non-coding RNA HCG4 is differentially expressed in the blood of patients with coronavirus co-infections.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

The human coronavirus SARS-CoV-2¹ has resulted in the death of over 200,000 Americans in less than one year². Infection of a person already suffering from a viral infection, a phenomena known as co-infection can potentially pose a problem during the upcoming influenza season. We mined published microarray data³ to identify genes most differentially expressed in the whole blood of patients suffering from human coronavirus co-infections. We found that the gene encoding the HLA complex non-coding RNA HCG4 was among those whose expression changed most significantly transcriptome-wide when comparing the blood of patients suffering from three different types of co-infections: human coronavirus NL63 and rhinovirus, human coronavirus HKU1 and rhinovirus, as well as in human coronavirus OC43 and influenza A co-infection. We previously reported significant transcriptome-wide changes in HLA family gene expression⁴, as well as in changes in gene expression of the cathepsins in viral co-infection⁵. Together, these data suggest the process of antigen presentation could be altered during viral co-infections involving the human coronaviruses.

Keywords: coronavirus, HCoV HKU1, HCoV NL63, HCoV-229E, viral co-infection, systems biology of viral infection.

Co-infection refers to a process by which more than one pathogen have infected a cell or host concurrently. Infections from the severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, has resulted in the death of over 200,000 Americans in less than one year². Our understanding of the transcriptional changes that accompany coronavirus co-infection is limited, but a recent study of patients in Northern California found that of 116 patients testing positive for a SARS-CoV-2, 24 (20.7%) possessed a co-infection⁶. We mined published microarray data³ to study gene expression in the blood of humans infected with three different human coronaviruses, as well as either influenza A or human rhinovirus to determine in an unbiased fashion genes most differentially expressed in patient blood upon coronavirus co-infection. We found significant differential expression of HCG4, a non-coding RNA of the HLA complex group, in the blood of patients with human coronavirus co-infections.

Methods

We used dataset GSE68310³ for this differential gene expression analysis of the blood of patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0 expression beadchip technology; analysis was performed with platform GPL10558. We compared the blood of $n=1$ patient with HCoV HKU1 infection (ID: JR0336) to the blood of $n=3$ patients with HCoV HKU1 and human rhinovirus co-infection (ID: JR207, JR611 and JR1702). We compared the blood of $n=1$ patient with HCoV NL63 infection (ID: JR1447) to the blood of $n=1$ patient with HCoV NL63 and human rhinovirus co-infection (ID: JR1446). We compared the blood of $n=1$ patient with Influenza A infection (ID: JR0021) to the blood of $n=1$ patient with

1 Influenza A and HCoV OC43 co-infection (ID: JR1829). In the study from which this
2 microarray data was obtained, investigators collected peripheral blood from patients at baseline,
3 day 0 of infection, correlating with a visit to the clinic upon infection with a respiratory illness;
4 day 2, day 4, day 6, and day 21 of infection following the clinic visit, as well as in the season
5 following infection (“Spring”).
6

7
8 The Benjamini and Hochberg method of *p*-value adjustment was used for ranking of
9 differential expression but raw *p*-values were used for assessment of statistical significance of
10 global differential expression. Log-transformation of data was auto-detected, and the NCBI
11 generated category of platform annotation was used. A statistical test was used to evaluate the
12 significance of difference of HCG4 gene expression between the blood of a patient or
13 patients with a single infection and the blood of a patient or patient(s) with co-infection using
14 either a two-tailed, unpaired t-test with Welch’s correction or a one-way ANOVA with Dunnett’s
15 multiple comparisons test. All statistical tests were performed with PRISM 8.4.0 (455).
16
17
18

19 **Results**

20
21 We mined a published microarray dataset³ to determine in an unbiased fashion genes
22 most differentially expressed in the blood of patients with coronavirus co-infection.
23

24 HCG4 is differentially expressed in the blood of patients with co-infection of Human Rhinovirus
25 and Human Coronavirus HKU1.

26 We found that the gene encoding the HLA complex non-coding RNA HCG4 was among
27 those most differentially expressed when comparing the blood of one patient with human
28

1 coronavirus (HCoV) HKU1 infection to the blood of three patients with co-infection of human
2 rhinovirus and HCoV HKU1. When comparing all transcripts measured by microarray based on
3 change in expression between HCoV HKU1 single infection patient blood and the blood of
4 patients with HCoV HKU1 and rhinovirus co-infection, HCG4 ranked 113 out of 47254 total
5 transcripts. Differential expression of HCG4 in the blood of patients with HCoV HKU1 /
6 rhinovirus co-infection was statistically significant (Table 1; $p=1.04E-04$).
7
8

9
10
11 HCG4 is differentially expressed in the blood of a patient with co-infection of human rhinovirus
12 and human coronavirus NL63.

13
14 We then asked whether HCG4 differential expression was specific HCoV HKU1 co-
15 infection or could be observed in human coronavirus co-infections of other types. We found
16 significant differential expression of HCG4 when comparing the blood of one patient with HCoV
17 NL63 infection to the blood of one patient with co-infection of human rhinovirus and HCoV
18 NL63. When comparing all transcripts measured by microarray based on change in expression
19 between HCoV NL63 single infection patient blood and the blood of a patient with HCoV NL63
20 and Rhinovirus co-infection, HCG4 ranked 78 out of 47254 total transcripts. Differential
21 expression of HCG4 in HCoV NL63 / rhinovirus co-infected patient blood was statistically
22 significant (Table 2; $p=2.3E-07$).
23
24
25

26 HCG4 is differentially expressed in the blood of a patient with co-infection of influenza A and
27 human coronavirus OC43.
28

We also found significant differential expression of HCG4 in the blood of one patient with influenza A to the blood of one patient with co-infection of influenza A and HCoV OC43. When comparing all transcripts measured by microarray based on change in expression between the blood of a patient with influenza A single infection and the blood of a patient with co-infection of HCoV OC43 and influenza A, HCG4 ranked 2190 out of 47254 total transcripts, equating to 95.4% differential expression. Differential expression of HCG4 in influenza A / HCoV OC43 co-infected patient blood was statistically significant (Table 3; $p=2.79E-02$).

HCG4 expression is significantly higher in HCoV HKU1 and Rhinovirus co-infection and in HCoV NL63 and rhinovirus co-infection.

We then obtained exact mRNA expression levels of HCG4 in the blood of each patient with human coronavirus co-infection to directly compared HCG4 expression levels between patients with and without human coronavirus co-infection. In the blood of three patients with HCoV HKU1 and rhinovirus co-infection, HCG4 was expressed at higher levels when compared to the blood of a patient with infection of HCoV HKU1 infection only; higher expression of HCG4 in HCoV HKU1 and rhinovirus co-infection was statistically significant (Figure 1; patient 1: $p<0.0001$; patient 2: $p=0.0029$; patient 3: $p=0.0313$). We calculated a mean fold change in HCG4 expression of 1.092 ± 0.030 , 1.063 ± 0.016 , and 1.046 ± 0.026 in the blood of three patients with HCoV HKU1 co-infection to that of rhinovirus co-infection.

In the blood of a patient with HCoV NL63 and rhinovirus co-infection, we also observed significantly higher expression of HCG4 when compared to the blood of a patient with HCoV

1 NL63 infection only (Figure 2; $p < 0.0001$). We calculated a mean fold change in HCG4
2 expression of 1.081 ± 0.016 in HCoV NL63 and rhinovirus co-infected patient blood.
3

4 Thus, we identified HCG4 as among the most differentially expressed genes in the blood
5 of five patients with viral co-infections, including human coronavirus co-infections.
6

7 **Discussion**

8
9 There is little information published regarding functions of HCG4. The HCG4 gene
10 resides with the MHC class I locus on 6p21.3, and is flanked by the HLA-F and HLA-G genes.
11 In patients with laryngeal cancer, patients who express HCG4 below the median have
12 significantly increased recurrence-free survival as compared to patients with HCG4 expression
13 above the median⁸. We previously reported significant transcriptome-wide changes in HLA
14 family gene expression⁴, including HLA-A, HLA-H, HLA-DRB1, HLA-DRB4, HLA-DRB5,
15 and HLA-DRB6. We also reported recurrent transcriptome-level changes in gene expression of
16 the cathepsins in viral co-infection, including cathepsin B, cathepsin C, cathepsin S, and
17 cathepsin H⁵. It is difficult to elaborate by which mechanism if any HCG4 expression changes
18 contribute to the viral life cycle(s) or host defense to co-infection, but together these data suggest
19 that as a system, antigen presentation may be altered at the transcriptional and cellular level in
20 the blood of patients with viral co-infections, including human coronavirus co-infections.
21
22
23
24

25 We found here that the HLA complex non-coding RNA HCG4 was among the genes most
26 differentially expressed in patients with coronavirus co-infection. HCG4 could possibly be
27 relevant to the process of co-infection involving coronaviruses.
28

References

1. Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. and Garry, R.F., 2020. The proximal origin of SARS-CoV-2. *Nature medicine*, 26(4), pp.450-452.
2. World Health Organization. United States of America Situation. <https://covid19.who.int/region/amro/country/us>.
3. Zhai, Y., Franco, L.M., Atmar, R.L., Quarles, J.M., Arden, N., Bucasas, K.L., Wells, J.M., Nino, D., Wang, X., Zapata, G.E. and Shaw, C.A., 2015. Host transcriptional response to influenza and other acute respiratory viral infections—a prospective cohort study. *PLoS Pathog*, 11(6), p.e1004869.
4. Mamoor, S., 2020. Viral co-infections are associated with dysregulation of HLA family gene expression.
5. Mamoor, S., 2020. Differential expression of cathepsins in the blood of patients with viral co-infections.
6. Kim, D., Quinn, J., Pinsky, B., Shah, N.H. and Brown, I., 2020. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *Jama*.
7. Bischof, O., Purbey, P.K., Notani, D., Urlaub, H., Dejean, A. and Galande, S., 2007. Functional interaction between PML and SATB1 regulates chromatin-loop architecture and transcription of the MHC class I locus. *Nature cell biology*, 9(1), pp.45-56.
8. Tang, Z., Wei, G., Zhang, L. and Xu, Z., 2019. Signature microRNAs and long noncoding RNAs in laryngeal cancer recurrence identified using a competing endogenous RNA network. *Molecular medicine reports*, 19(6), pp.4806-4818.

Rank	ID	p-value	t	B	FC	Gene	Gene name
113	ILMN_1660923	1.04E-04	4.6151166	1.13784	P1: 1.092 ± 0.030 P2: 1.063 ± 0.016 P3: 1.046 ± 0.026	HCG4	HLA complex group 4 (non-protein coding)

Table 1: Differential expression of HCG4 in the blood of patients with co-infection of HCoV HKU1 and human rhinovirus.

Rank of global differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of HCG4 mRNA expression when comparing co-infected patient blood to the blood of a patient with infection of HCoV HKU1 only, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
78	ILMN_1660923	2.3E-07	9.3852034	7.38734	1.081 ± 0.016	HCG4	HLA complex group 4 (non-protein coding)

Table 2: Differential expression of HCG4 in the blood of a patient with co-infection of HCoV NL63 and human rhinovirus.

Rank of global differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of HCG4 mRNA expression when comparing HCoV NL63/Rhinovirus co-infected patient blood to the blood of a patient with infection of HCoV NL63 only, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2190	ILMN_1660923	2.79E-02	2.4649731	-3.9164	HCG4	HLA complex group 4 (non-protein coding)

Table 3: Differential expression of HCG4 in the blood of a patient with co-infection of HCoV OC43 and influenza A.

Rank of global differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: HCG4 is expressed at significantly higher levels in the blood of patients with co-infection of HCoV HKU1 and human rhinovirus when compared to a patient with human rhinovirus infection only.

The mRNA expression level of HCG4 in the blood of each patient is graphically represented with mean expression level marked and the result of a statistical test evaluating significance of difference in HCG4 expression between the blood of patients with HCoV HKU1 / rhinovirus co-infection and the blood of a patient with HCoV HKU1 infection only, a *p*-value, listed above each comparison. *n*=1 patient for HCoV HKU1 infection and *n*=3 patients for HCoV HKU1 / Rhinovirus co-infection. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and rhinovirus co-infection is lacking baseline sample.

Figure 2: HCG4 is expressed at significantly higher levels in the blood of a patient with co-infection of HCoV NL63 and human rhinovirus when compared to a patient with human rhinovirus infection only.

The mRNA expression level of HCG4 in the blood of each patient is graphically represented with mean expression level marked and the result of a statistical test evaluating significance of difference in HCG4 expression between the blood of $n=1$ patient for HCoV NL63 infection and $n=1$ patient for HCoV NL63 / rhinovirus co-infection. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.