NQO2 is differentially expressed in the blood of patients with coronavirus co-infections.

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The human coronavirus SARS-CoV-2¹ has resulted in the death of over 180,000 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 seasons. 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 NQO2⁴ 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 229E and influenza A co-infection. NQO2 is reported to have functions in the B-lymphocyte response⁵ and could be relevant to the process of viral co-infection involving the human coronavirus family.

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. SARS-CoV-2 has resulted in the death of over 180,000 Americans in less than one year². Our understanding of the transcriptional changes that accompany coronavirus co-infection is limited, but co-infection of Influenza A and SARS-CoV-2 has been reported in one patient⁶. 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 the NQO2 gene in the blood of patients with human coronavirus infections, suggesting NQO2, a gene which is reported to have functions in the B-lymphocyte response could possibly be important to co-infections involving human coronaviruses.

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 Influenza A and HCoV-229E co-infection (ID: JR0173). In the study from which this microarray data was obtained, investigators collected peripheral blood from patients at baseline, day 0 of infection, correlating with a visit to the clinic upon infection with a respiratory illness; day 2, day 4, day 6, and day 21 of infection following the clinic visit, as well as in the season following infection ("Spring").

The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was used to evaluate the significance of difference of NQO2 gene expression between the blood of a patient or patients with a single infection and the blood of a patient or patient(s) with co-infection using either a two-tailed, unpaired t-test with Welch's correction or a one-way ANOVA with Dunnett's multiple comparisons test. All statistical tests were performed with PRISM 8.4.0 (455).

Results

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

NQO2 is differentially expressed in the blood of patients with co-infection of Human Rhinovirus and Human Coronavirus HKU1.

We found that the NQO2 gene was among those most differentially expressed when comparing the blood of one patient with Human Coronavirus (HCoV) HKU1 infection to the blood of three patients with co-infection of Human Rhinovirus and HCoV HKU1. When comparing all transcripts measured by microarray based on change in expression between HCoV HKU1 single infection patient blood and the blood of patients with HCoV HKU1 and Rhinovirus co-infection, NQO2 ranked 38 out of 47254 total transcripts. Differential expression of NQO2 in the blood of patients with HCoV HKU1 / Rhinovirus co-infection was statistically significant (Table 1; $p=2.87E-06$).

NQO2 is differentially expressed in the blood of a patient with co-infection of Human Rhinovirus and Human Coronavirus NL63.

1 We then asked whether NQO2 differential expression was specific HCoV HKU1 co-
2 infection or could be observed in human coronavirus co-infections of other types. We found
3 significant differential expression of NQO2 when comparing the blood of one patient with
4 HCoV NL63 infection to the blood of one patient with co-infection of Human Rhinovirus and
5 HCoV NL63. When comparing all transcripts measured by microarray based on change in
6 expression between HCoV NL63 single infection patient blood and the blood of a patient with
7 HCoV NL63 and Rhinovirus co-infection, NQO2 ranked 74 out of 47254 total transcripts.
8 Differential expression of NQO2 in HCoV NL63 / Rhinovirus co-infected patient blood was
9 statistically significant (Table 2; $p=2.09E-07$).
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11 NQO2 is differentially expressed in the blood of a patient with co-infection of Influenza A and
12 Human Coronavirus 229E.

13 We also found significant differential expression of NQO2 in the blood of one patient
14 with Influenza A to the blood of one patient with co-infection of Influenza A and HCoV-229E.
15 When comparing all transcripts measured by microarray based on change in expression
16 between the blood of a patient with Influenza A single infection and the blood of a patient with
17 co-infection of HCoV-229E and Influenza A, NQO2 ranked 54 out of 47254 total transcripts.
18 Differential expression of NQO2 in Influenza A / HCoV-229E co-infected patient blood was
19 statistically significant (Table 3; $p=4.37E-06$).
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21 NQO2 expression is significantly lower in HCoV HKU1 and Rhinovirus co-infection, but
22 significantly higher in HCoV NL63 and Rhinovirus co-infection, as well as in HCoV-229E and
23 Influenza A co-infection.

24 We then obtained exact mRNA expression levels of NQO2 in the blood of each patient
25 with human coronavirus co-infection to directly compared NQO2 expression levels between
26 patients with and without human coronavirus co-infection.
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1 In the blood of three patients with HCoV HKU1 and Rhinovirus co-infection, NQO2 was
2 expressed at lower levels when compared to the blood of a patient with infection of HCoV
3 HKU1 infection only; lower expression of NQO2 in HCoV HKU1 and Rhinovirus co-infection
4 was statistically significant (Figure 1; patient 1: $p=0.0008$; patient 2: $p=0.0005$; patient 3:
5 $p<0.0001$). We calculated a mean fold change in NQO2 expression of 0.9631 ± 0.0178 , 0.9595
6 ± 0.0130 , and 0.9482 ± 0.0085 in the blood of three patients with HCoV HKU1 co-infection to
7 that of Rhinovirus co-infection.
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9 In the blood of a patient with HCoV NL63 and Rhinovirus co-infection, however, we
10 observed significantly higher expression of NQO2 when compared to the blood of a patient
11 with HCoV NL63 infection only (Figure 2; $p<0.0001$). We calculated a mean fold change in
12 NQO2 expression of 1.0563 ± 0.0101 in HCoV NL63 and Rhinovirus co-infected patient blood.
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14 NQO2 expression was also significantly higher in the blood of a patient with Influenza A
15 and HCoV-229E co-infection when compared to the blood of a patient with Influenza A
16 infection only (Figure 3; $p<0.0001$). We calculated a mean fold change in NQO2 expression of
17 1.0559 ± 0.0172 in HCoV-229E / Influenza A co-infected patient blood.
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19 Discussion

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21 While NQO1 is described as a flavoprotein, functioning in 2-electron reduction of
22 molecules including quinones and azo dyes using NADP or NADPH^{7,8,9}, information on
23 catalytic function of NQO2 and the physiologic processes to which it is related is limited.
24 NQO2-deficient mice are reported to have decreased percentage of CD19+ B-lymphocytes in
25 the peripheral blood but increased percentage of CD19+ cells and IgD+ CD19+ mature B-cells
26 in the bone marrow⁴. The number of marginal zone CD21+ CD23+ B-cells in the spleen of
27 NQO2-deficient mice was also reported to be decreased⁴. In response to immunization with
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1 the chicken gamma globulin (CGG) antigen conjugated to the 4-hydroxy-3-nitrophenyl acetate
2 (NP) hapten, the number of NP-binding IgG₁+ antibody-forming cells in the spleen of NQO2-
3 deficient mice was reported to be reduced; after a second immunization, the number of NP-
4 binding IgG₁+ antibody-forming cells in the spleen of NQO2-deficient mice was reported to be
5 increased, but the number of NP-binding IgM+ antibody-forming cells in the spleen of NQO2-
6 deficient mice decreased, suggesting that NQO2 might be important for primary and
7 secondary humoral responses⁴. In NQO1 / NQO2 double knockout (DKO) mice, nodules were
8 found in the submucosa of the respiratory tract, representing bronchial-associated lymphoid
9 tissue (BALT) which contained B220+ B-lymphocytes¹⁰. Bronchial lavage and lung suspension
10 were reported to contain increased numbers of neutrophils in DKO mice¹⁰. In response to
11 hypoxic conditions, lung injury was increased in DKO mice, as evidenced by perivascular
12 inflammation and alveolar flooding¹⁰. Thus, NQO2 has been described as important for some
13 humoral responses and in some contexts of lung injury.
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15 We found here that NQO2 was among the genes most differentially expressed in
16 patients with coronavirus co-infection. NQO2 could possibly be relevant to the process of co-
17 infection involving coronaviruses.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
38	ILMN_1712918	2.87E-06	-6.0247516	4.64185	P1: 0.9631 ± 0.0178 P2: 0.9595 ± 0.0130 P3: 0.9482 ± 0.0085	NQO2	NAD(P)H quinone dehydrogenase 2

Table 1: Differential expression of NQO2 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 NQO2 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
74	ILMN_1712918	2.09E-07	9.461122	7.47835	1.0563 ± 0.0101	NQO2	NAD(P)H quinone dehydrogenase 2

Table 2: Differential expression of NQO2 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 NQO2 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	FC	Gene	Gene name
54	ILMN_1712918	4.37E-06	7.3500547	4.58487	1.0559 ± 0.0172	NQO2	NAD(P)H quinone dehydrogenase 2

Table 3: Differential expression of NQO2 in the blood of a patient with co-infection of HCoV-229E 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, fold change of NQO2 mRNA expression when comparing HCoV-229E/Influenza A co-infected patient blood to the blood of a patient with infection of Influenza A only, gene and gene name are listed in this chart.

Figure 1: NQO2 is expressed at significantly lower 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 NQO2 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 NQO2 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: NQO2 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 NQO2 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 NQO2 expression between the blood of a patient with HCoV NL63 / 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 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.

Figure 3: NQO2 is expressed at significantly higher levels in the blood of a patient with co-infection of HCoV-229E and Influenza A when compared to a patient with Influenza A infection only.

The mRNA expression level of NQO2 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 NQO2 expression between the blood of a patient with HCoV-229E / Influenza A co-infection and the blood of a patient with HCoV-229E infection only, a *p*-value, listed above each comparison. *n*=1 patient for Influenza A infection with *n*=1 patient for HCoV-229E / Influenza A 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.