Cloning and characterization of novel human *SLC4A8* gene products encoding Na⁺-driven Cl-HCO$_3$ exchanger variants NDCBE-A, -C and -D

1. PCR analysis of human NDCBE splice variants (page ii)

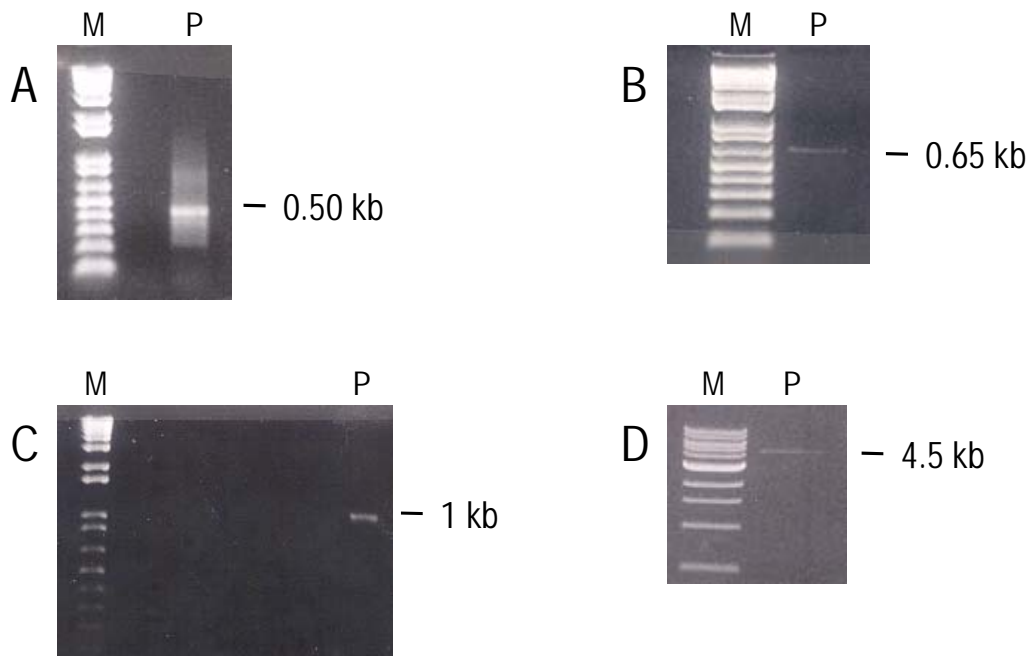**2. Data analysis (pages iii-ix)**

Supplementary Figure

FIGURE 1. **PCR analysis of human NDCBE splice variants.** PCR products (P) were separated by agarose gel electrophoresis and visualized on a UV transilluminator. *A:* Nested 5'–RACE product represented in manuscript-Figure 2*B*. *B:* Gene-specific PCR product corresponding to a fragment of SLC4A8 that includes exons 19–24, as represented in manuscript-Figure 2*C*. *C:* 3'–RACE product corresponding to a fragment of SLC4A8 including exons 24 and 25, as represented in manuscript-Figure 2*D*. *D:* Gene-specific PCR product corresponding to the extended fragment of exon 25, as represented in manuscript-Figure 2*E*. DNA ladders (M) were the 1-kb Plus Ladder from Invitrogen (panels *A–C*) and the 1-kb ladder from Sigma-Aldrich (panel *D*).

Data Analysis

Comparison of NDCBE-A, -B, -C, and -D (oocyte resting pH_i)

The table below shows the mean resting intracellular pH (pH_i) values for oocytes injected with H_2O or cRNAs encoding NDCBE-A, -B, -C or –D (see manuscript-Figure 7). Values are presented as means (column 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	Mean pH_i	**Standard Error**	**Number of Replicates**
NDCBE-A	7.35	0.04	14
NDCBE-B	7.33	0.03	13
NDCBE-C	7.39	0.03	15
NDCBE-D	7.35	0.03	14
H_2O	7.33	0.02	14

The results of a one-way ANOVA generated by Kaleidagraph for the above data set is shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-C ("C"), NDCBE-D ("D"), and H_2O-injected cells ("H_2O").

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One Way ANOVA
Response: B
Factor A: 5 Groups
A, B, C, D, H₂O

Analysis of Variance Results
Source          DF           SS           MS             |    P
Total           69        0.791377     0.011469
A                4        0.038749     0.009687      0.836  0.5069
Error           65        0.752628     0.011579


Student-Newman-Keuls Multiple Comparison
Comparison        Mean            |q|            P
                  Difference
C vs H2O          0.065571        2.319         0.478
C vs B            0.059692        2.0703        0.4649
C vs D            0.047           1.6622        0.472
C vs A            0.044857        1.5864        0.2661
A vs H2O          0.020714        0.7203        0.9566
A vs B            0.014835        0.5062        0.9319
A vs D            0.002143        0.0745        0.9581
D vs H2O          0.018571        0.6458        0.8916
D vs B            0.012692        0.4331        0.7604
B vs H2O          0.005879        0.2006        0.8876
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Comparison of NDCBE-A, -B, -C, and -D (oocyte resting V_m)

The table below shows the mean resting membrane potential (V_m) values for oocytes injected with H_2O or cRNAs encoding NDCBE-A, -B, -C or –D (see manuscript-Figure 7). Values are presented as means (column 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	Mean V_m (mV)	Standard Error (mV)	Number of Replicates
NDCBE-A	–41	2	14
NDCBE-B	–39	2	13
NDCBE-C	–44	2	15
NDCBE-D	–45	2	14
H_2O	–53	3	14

The results of a one-way ANOVA generated by Kaleidagraph for the above data set is shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-C ("C"), NDCBE-D ("D"), and H_2O-injected cells ("H_2O").

One Way ANOVA
Response: B
Factor A: 5 Groups
A, B, C, D, H_2O

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	69	6240.986	90.44907		
A	4	1571.89	392.9724	5.470697	0.00074
Error	65	4669.096	71.83225		

Student-Newman-Keuls Multiple Comparison

Comparison	Mean Difference	|q|	P
B vs H2O	13.7582	5.9604	0.0007
B vs D	5.32967	2.3089	0.3678
B vs C	5.08205	2.2379	0.2605
B vs A	1.47253	0.6379	0.6534
A vs H2O	12.2857	5.4238	0.0016
A vs D	3.85714	1.7028	0.4551
A vs C	3.60952	1.6207	0.256
C vs H2O	8.67619	3.8958	0.0205
C vs D	0.247619	0.1112	0.9376
D vs H2O	8.42857	3.721	0.0106

Comparison of NDCBE-A, -B, -C, and -D (oocyte pH$_i$ recovery rate)

The table below shows the pH$_i$ recovery rates following a CO_2-induced acid-load for oocytes injected with H_2O or cRNAs encoding NDCBE-A, -B, -C or –D (see manuscript-Figures 7 and 8). Values are presented as means (columns 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	Mean pH$_i$ recovery rate ($\times 10^{-5}$ pH units/s)	Standard Error ($\times 10^{-5}$ pH units/s)	Number of Replicates
NDCBE-A	19	2	14
NDCBE-B	15	2	13
NDCBE-C	18	2	15
NDCBE-D	12	1	14
H_2O	2	1	14

The results of a one-way ANOVA generated by Kaleidagraph for the above data set are shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-C ("C"), NDCBE-D ("D"), and H_2O-injected cells ("H_2O").

```
One Way ANOVA
Response: B
Factor A: 5 Groups
A,  B,  C,  D,  H₂O

Analysis of Variance Results
Source      DF          SS          MS        F         P
Total       69      4473.943    64.83975
A            4      2726.645    681.6613   25.358    < .0001
Error       65      1747.298    26.88151

Student-Newman-Keuls Multiple Comparison
Comparison          Mean            |q|        P
                  Difference
A vs H2O            17.5          12.6292    < .0001
A vs D            7.35714         5.3094     0.0021
A vs B            3.90659         2.7666     0.1314
A vs C            1.61429         1.1849     0.4052
C vs H2O          15.8857        11.6602     < .0001
C vs D            5.74286         4.2153     0.0111
C vs B            2.29231         1.6501     0.2476
B vs H2O          13.5934         9.6265     < .0001
B vs D            3.45055         2.4436     0.0888
D vs H2O          10.1429         7.3198     < .0001
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The results of a two-way ANOVA generated by Kaleidagraph for the above data set are shown below. In order to make all groups equal size (i.e., n = 13), the one or two most recent experiments in each case were disregarded as necessary to equalize the size of the data groups. Groups are defined as Clones with the long Nt common to NDCBE-A/B ("NAB"), clones with a short Nt common to NDCBE-C/D ("NCD"), clones with a long Ct common to NDCBE-A/C ("CAC"), and clones with a short Ct common to NDCBE-B/D ("CBD").

Two Way ANOVA
Response: C
Factor A: 2 Groups
       NAB, NCD (long vs short N terminus)
Factor B: 2 Groups
       CAC, CBD (long vs short C terminus)

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	51	2077.75	40.7402		
A	1	81.25	81.25	2.399432	0.12795
B	1	360.9423	360.9423	10.65916	0.00202
Interaction	1	10.17308	10.17308	0.300426	0.58616
Error	48	1625.385	33.86218		

Similar results were obtained when the analysis was performed on a data set omitting the one or two oldest experiments to equalize the size of the data groups. The results of this analysis are reproduced below.

Two Way ANOVA
Response: C
Factor A: 2 Groups
NAB, NCD (long vs short N terminus)
Factor B: 2 Groups
CAC, CBD (long vs short C terminus)

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	51	2024.827	39.70249		
A	1	76.32692	76.32692	2.207044	0.14392
B	1	281.5577	281.5577	8.141427	0.00637
Interaction	1	6.942308	6.942308	0.200741	0.65614
Error	48	1660	34.58333		

Comparison of NDCBE-A, -B, and -X (oocyte resting pH$_i$)

The table below shows the resting intracellular pH (pH$_i$) values for oocytes injected with H$_2$O or cRNAs encoding NDCBE-A, -B, -X (see manuscript-Figure 9). Values are presented as means (column 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	Mean pH$_i$	**Standard Error**	**Number of Replicates**
NDCBE-A	7.31	0.01	6
NDCBE-B	7.37	0.03	6
NDCBE-X	7.33	0.03	7
H$_2$O	7.30	0.04	4

The results of a one-way ANOVA generated by Kaleidagraph for the above data set are shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-X ("X"), and H$_2$O-injected cells ("H$_2$O").

One Way ANOVA
Response: B
Factor A: 4 Groups
A, B, H$_2$O, X

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	22	0.090782	0.004126		
A	3	0.018869	0.00629	1.661824	0.20884
Error	19	0.071913	0.003785		

Student-Newman-Keuls Multiple Comparison

Comparison	Mean Difference	|q|	P
B vs H2O	0.075833	2.7006	0.2573
B vs A	0.066667	2.6543	0.1726
B vs X	0.039048	1.6134	0.2681
X vs H2O	0.036786	1.3491	0.6139
X vs A	0.027619	1.1412	0.4297
A vs H2O	0.009167	0.3264	0.8199

Comparison of NDCBE-A, -B, and -X (oocyte resting V_m)

The table below shows the resting membrane potential (V_m) values for oocytes injected with H_2O or cRNAs encoding NDCBE-A, -B, -X (see manuscript-Figure 9). Values are presented as means (column 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	V_m (mV)	Standard Error (mV)	Number of Replicates
NDCBE-A	–47	3	6
NDCBE-B	–44	4	6
NDCBE-X	–46	2	7
H_2O	–55	4	4

The results of a one-way ANOVA generated by Kaleidagraph for the above data set are shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-X ("X"), and H_2O-injected cells ("H_2O").

One Way ANOVA
Response: B
Factor A: 4 Groups
A, B, H₂O, X

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	22	1254.957	57.04348		
A	3	304.2661	101.422	2.026967	0.14422
Error	19	950.6905	50.03634		

Student-Newman-Keuls Multiple Comparison

Comparison	Mean Difference	|q|	P
B vs H2O	11	3.407	0.1092
B vs A	3.16667	1.0966	0.7222
B vs X	2.64286	0.9497	0.5099
X vs H2O	8.35714	2.6657	0.1703
X vs A	0.52381	0.1882	0.8955
A vs H2O	7.83333	2.4262	0.1025

Comparison of NDCBE-A, -B, -X (oocyte pH_i recovery rate)

The table below shows the pH_i recovery rates following a CO_2-induced acid-load for oocytes injected with H_2O or cRNAs encoding NDCBE-A, -B, or -X (see manuscript-Figure 9). Values are presented as means (columns 2) ± S.E (column 3) together with the number of replicate experiments (column 4).

	Mean pH_i recovery rate ($\times 10^{-5}$ pH units/s)	**Standard Error** ($\times 10^{-5}$ pH units/s)	**Number of Replicates**
NDCBE-A	25	1	6
NDCBE-B	16	1	6
NDCBE-X	25	1	7
H_2O	2	1	4

The results of a one-way ANOVA generated by Kaleidagraph for the above data set are shown below. Groups are defined as NDCBE-A ("A"), NDCBE-B ("B"), NDCBE-X ("X"), and H_2O-injected cells ("H_2O").

One Way ANOVA
Response: B
Factor A: 4 Groups
 A, B, X, H_2O

Analysis of Variance Results

Source	DF	SS	MS	F	P
Total	22	2.09E-07	9.52E-09		
A	3	1.67E-07	5.57E-08	25.01413	< .0001
Error	19	4.23E-08	2.23E-09		

Student-Newman-Keuls Multiple Comparison

Comparison	Mean Difference	|q|	P
A vs H_2O	0.00023	10.7181	< .0001
A vs B	0.00009	4.5856	0.0114
A vs X	0.00000	0.0641	0.9643
X vs H_2O	0.00023	10.9812	< .0001
X vs B	0.00009	4.6946	0.0036
B vs H_2O	0.00014	6.6166	0.0002