SUPPORTING INFORMATION

Systematic quantification of the anion transport function of pendrin (SLC26A4) and its disease-associated variants

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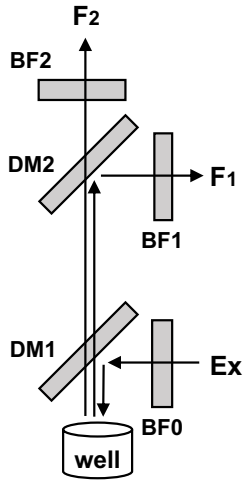
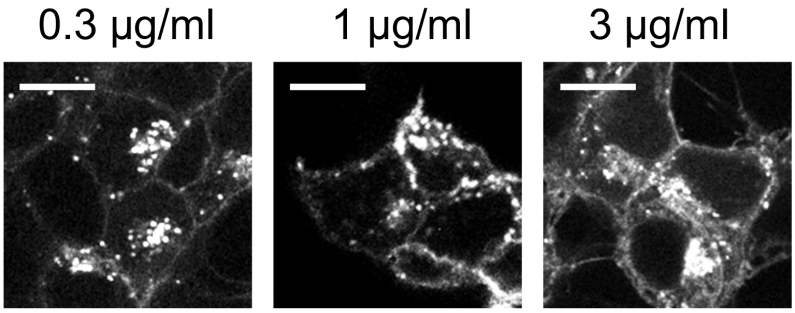
1. Supplemental Figures

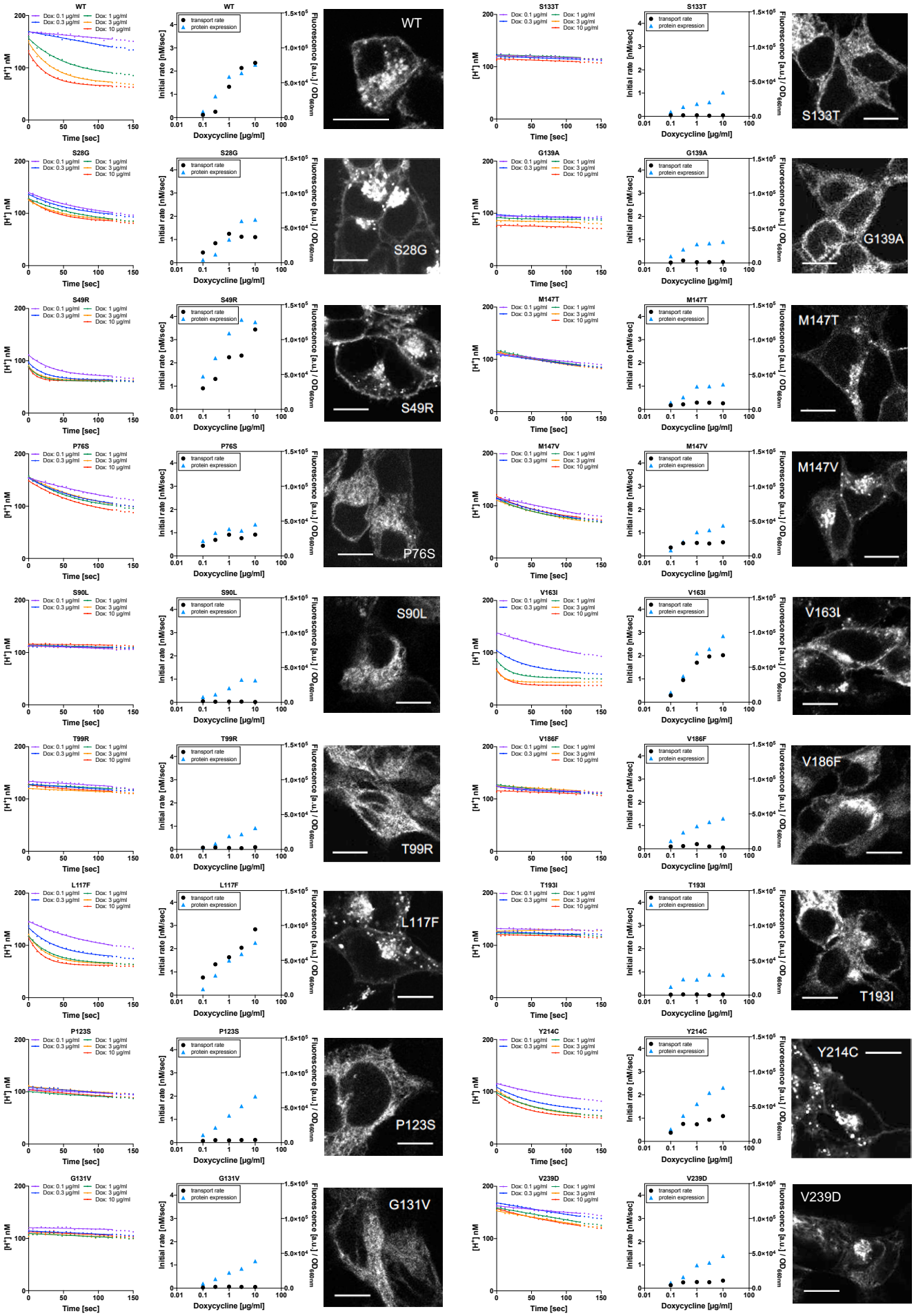


	HCO_3^-/Cl^- transport assay	I^-/Cl^- transport assay
BF0	**FF01-504/12 (Semrock)** (504/12 bandpass filter)	**59017x (Chroma)** (mTq2/mVenus dual excitation)
DM1	**T550lpxr (Chroma)** (550LP dichroic mirror)	**69008bs (Chroma)** (multi-edge dichroic mirror)
DM2	**T600lpxr (Chroma)** (600LP dichroic mirror)	**T495lpxr (Chroma)** (495LP dichroic mirror)
BF1	**FF01-572/28 (Semrock)** (572/28 bandpass filter)	**ET470/24m (Chroma)** (470/24 bandpass filter)
BF2	**FF01-709/167 (Semrock)** (709/167 bandpass filter)	**ET535/30m (Chroma)** (535/30 bandpass filter)

Supp. Figure S1. The optical configuration of the plate reader (Synergy Neo2) with a list of excitation/emission filters and dichroic mirrors used in this study.



Supp. Fig. S2. HEK293T cells heterologously expressing WT-pendrin-mTq2. Fluorescence of mTq2 was imaged. The doxycycline dosage used are shown atop each image. The scale bars indicate 10 µm.


WT
Dox: 0.1 µg/ml
Dox: 0.3 µg/ml
Dox: 1 µg/ml
Dox: 3 µg/ml
Dox: 10 µg/ml
[H+] nM
Time [sec]
transport rate
protein expression
Initial rate [nM/sec]
Fluorescence [a.u.] / OD660nm
Doxycycline [µg/ml]
S133T
S28G
G139A
S49R
M147T
P76S
M147V
S90L
V163I
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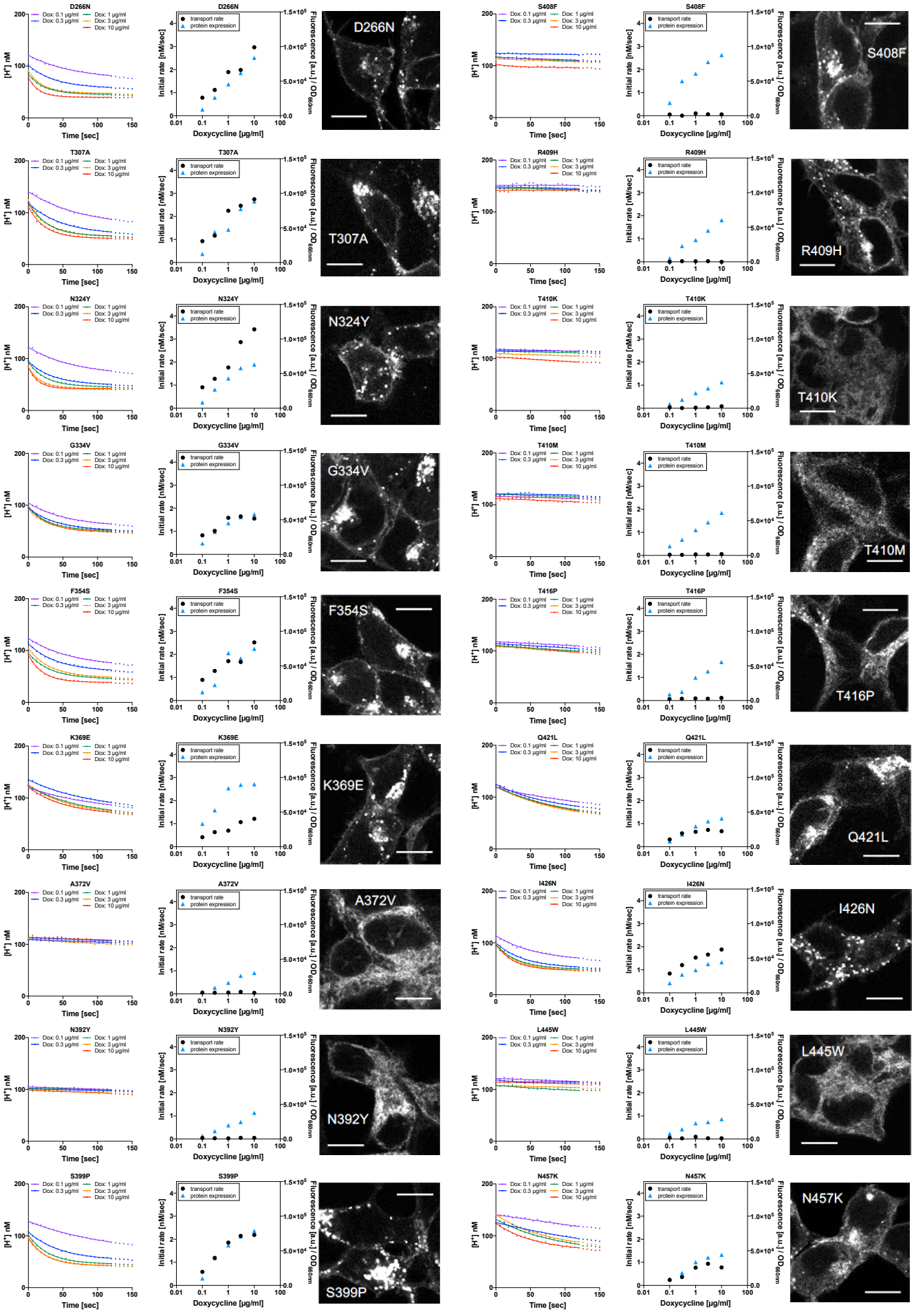
V186F
L117F
T193I
P123S
Y214C
G131V
V239D


D266N
Dox: 0.1 µg/ml
Dox: 0.3 µg/ml
Dox: 1 µg/ml
Dox: 3 µg/ml
Dox: 10 µg/ml
[H+] nM
Time [sec]
transport rate
protein expression
Initial rate [nM/sec]
Fluorescence [a.u.] / OD660nm
Doxycycline [µg/ml]
T307A
N324Y
G334V
F354S
K369E
A372V
N392Y
S399P
S408F
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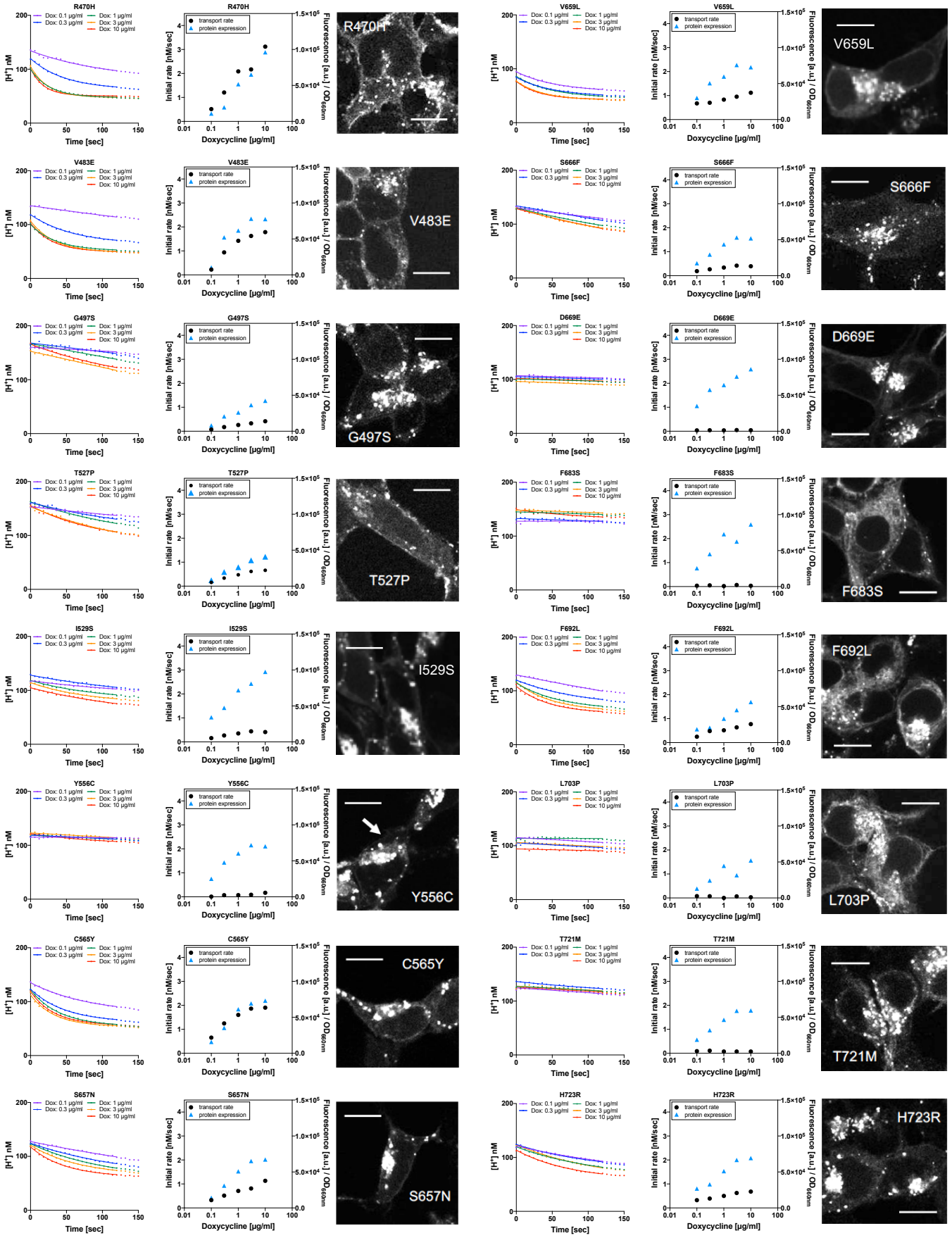
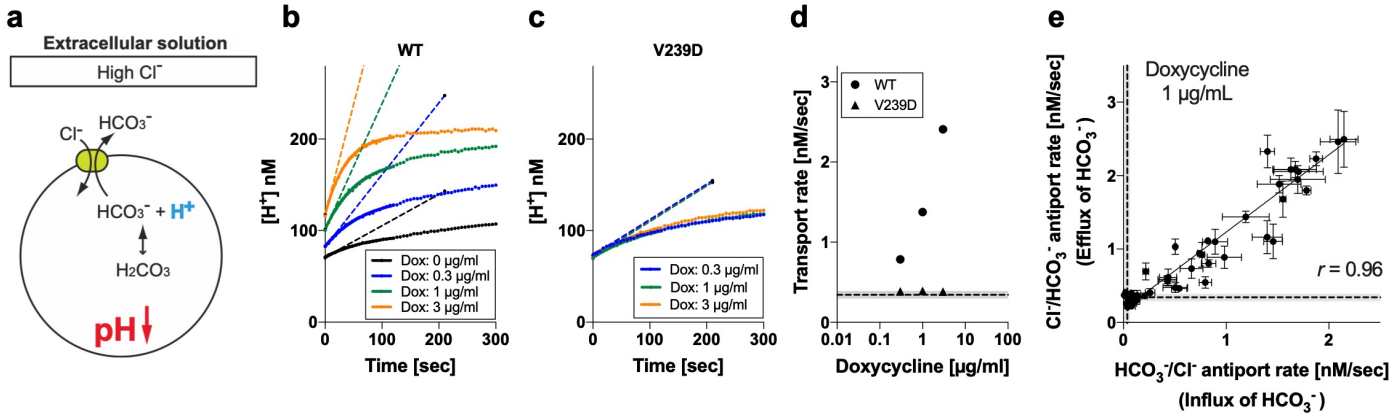
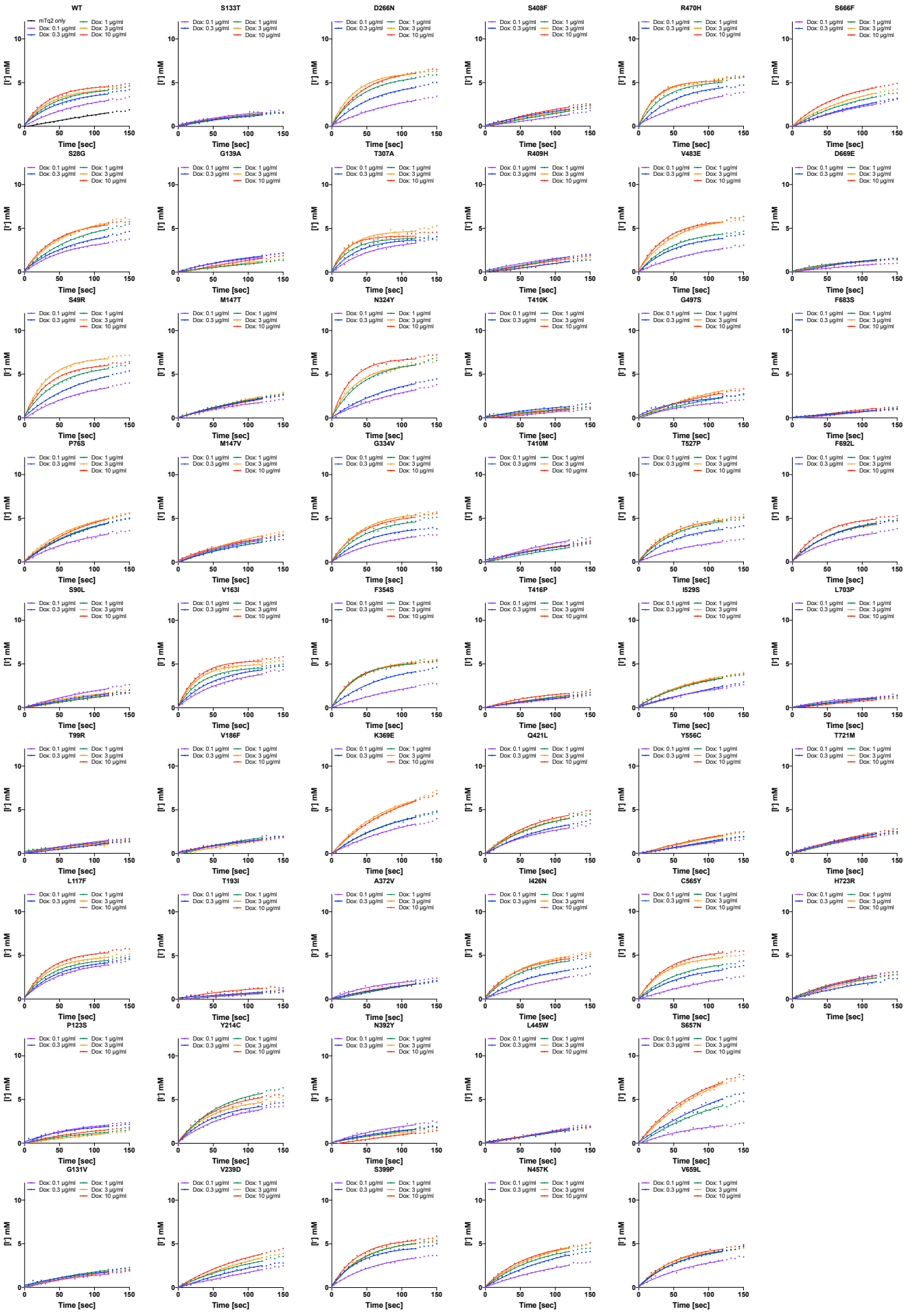
T410M
T416P
Q421L
I426N
L445W
N457K


R470H
Dox: 0.1 µg/ml
Dox: 0.3 µg/ml
Dox: 1 µg/ml
Dox: 3 µg/ml
Dox: 10 µg/ml
[H+] nM
Time [sec]
transport rate
protein expression
Initial rate [nM/sec]
Fluorescence [a.u.] / OD660nm
Doxycycline [µg/ml]
V659L
V483E
S666F
G497S
D669E
T527P
F683S
I529S
F692L
Y556C
L703P
C565Y
T721M
S657N
H723R

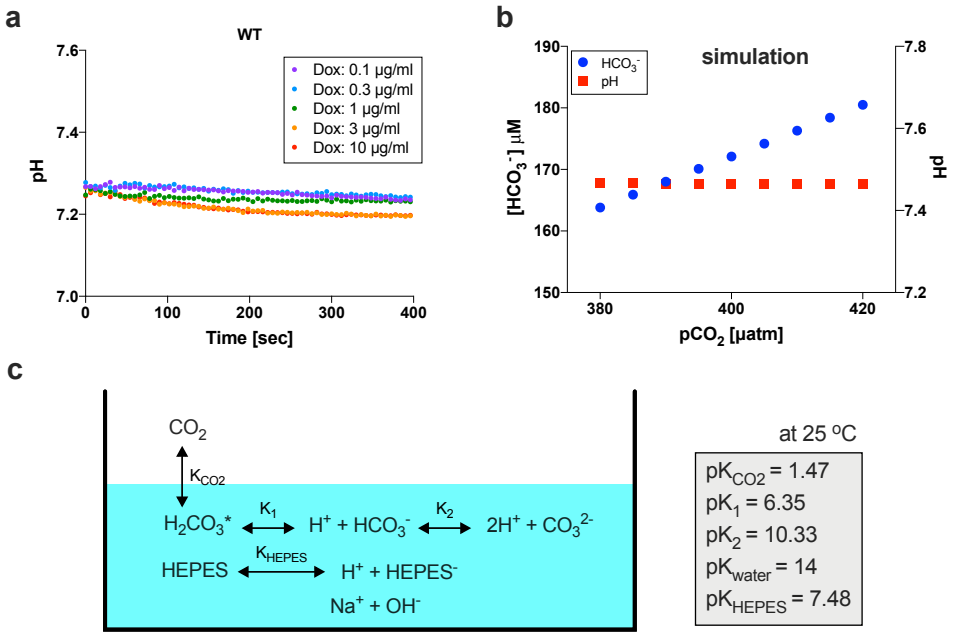
Supp. Figure S3. Representative examples of doxycycline dosage-dependent HCO_3^-/Cl^- antiport assay and fluorescence images of HEK293T cells expressing the mTq2-tagged pendrin constructs. Results of the transport assay are presented in the same way as in Figs. 2e and 2f. Scale bars indicate 10 μm.



Supp. Figure S4. HCO_3^-/Cl^- antiport assay conducted under a high extracellular Cl^- condition. (**a**) An inward Cl^- gradient across the cell membrane drives pendrin-mediated efflux of HCO_3^-, which concomitantly decreases intracellular pH. (**b** and **c**) Examples of HCO_3^-/Cl^- antiport assay for WT- and V239D-pendrin-expressing cells, respectively. The broken lines indicate the initial rates. (**d**) Summaries of doxycycline dosage-dependent HCO_3^-/Cl^- antiport assay shown in panels **b** and **c**. The broken line indicates the basal transport rate determined for non-induced cells (negative control). (**e**) A comparison of HCO_3^-/Cl^- antiport rates measured by opposite Cl^- gradients (inward vs. outward) at 1 µg/mL doxycycline. The error bars indicate the standard deviations. The solid line indicates Deming linear regression. The broken lines indicate the basal transport rates determined for non-induced cells (0.037 ± 0.024 nM/sec (n=5) and 0.34 ± 0.05 (n=9) nM/sec for "Efflux of HCO_3^-" and "Influx of HCO_3^-", respectively). This graph was generated using the numerical data shown in Table 1 (HCO_3^-/Cl^- antiport rates at 1 µg/mL doxycycline) and Supp. Table S3.


WT
S133T
D266N
S408F
R470H
S666F
S28G
G139A
T307A
R409H
V483E
D669E
S49R
M147T
N324Y
T410K
G497S
F683S
P76S
M147V
G334V
T410M
T527P
F692L
S90L
V163I
F354S
T416P
I529S
L703P
T99R
V186F
K369E
Q421L
Y556C
T721M
L117F
T193I
A372V
I426N
C565Y
H723R
P123S
Y214C
N392Y
L445W
S657N
G131V
V239D
S399P
N457K
V659L
mTq2 only
Dox: 0.1 µg/ml
Dox: 0.3 µg/ml
Dox: 1 µg/ml
Dox: 3 µg/ml
Dox: 10 µg/ml
[1'] mM
Time [sec]

Supp. Figure S5. Representative examples of doxycycline dosage-dependent I^-/Cl^- antiport assay.



$$[H^+]+[Na^+]=[OH^-]+[HCO_3^-]+2\times[CO_3^{2-}]+[HEPES^-] \quad \text{- - -} \quad \textbf{(Eq. 1)}$$

$$K_{CO2}=\frac{[H_2CO_3{*}]}{pCO_2} \qquad K_1=\frac{[H^+][HCO_3^-]}{[H_2CO_3{*}]} \qquad K_2=\frac{[H^+][CO_3^{2-}]}{[HCO_3^-]}$$

$$K_{water}=[H^+][OH^-] \qquad K_{HEPES}=\frac{[H^+][HEPES^-]}{[HEPES]}$$

Supp. Figure S6. Change of pH during I^-/Cl^- antiport assay. (**a**) Measurement of intracellular pH of WT-pendrin-expressing cells during I^-/Cl^- antiport assay. (**b**) Estimation of HCO_3^- concentration in the solutions used for I^-/Cl^- antiport assay under various atmospheric CO_2 conditions. The HCO_3^- concentration, $[HCO_3^-]$, was determined by numerically solving Eq. 1 shown in panel **c** using the equilibrium constants also shown in the panel. (**c**) A schematic representation of a solution containing carbonic acid, bicarbonate, and carbonate, which are in equilibrium with the atmospheric carbon dioxide. $[H_2CO_3{*}]$ denotes the equilibrium mixture of the aqueous carbon dioxide and carbonic acid. The solution also contains HEPES (20 mM) and NaOH (10 mM), which were added to adjust and maintain the solutions' pH at 7.5. This exercise shows that inclusion of pH buffer (20 mM HEPES-NaOH, pH 7.5) allows atmospheric CO_2-equilibrated HCO_3^- to reach ~170 μM, which would be sufficiently high to support the possibility raised in the main text that the small pH changes shown in panel **A** ($\Delta[H^+] \approx 10\ nM$) was due to efflux of endogenous HCO_3^- during I^-/Cl^- antiport assay.

2. The DNA sequences of portions of the pendrin gene cloned in a pET01 vector (splicing assay)

PCR primers used
5′-CCTGGCCTGCCCAGGCTTTTGTCAACA-3′
5′-CCACCTCCAGTGCCAAGGTCTGAAGGTCA-3′

Gray highlights indicate the annealing sites of the primers shown above. The region within the pET01 vector that is spliced out is shown by gray characters. Portions of the pendrin gene used for the splicing assay are indicated by pale blue (intron sequences) and bold blue with underlines (exon sequences).

➢ Empty pET01 vector

(Expected size of the PCR product: **190-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGAT
AAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACT
GACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTG
CTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGC
TGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon2 of wild-type human pendrin

(Expected size of the PCR product: 190+167 = **357-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCGAGGGCTGCAGGACGCGGA
CCAGACTCGCGGTGCAGGGGGGCCTGGCTGCAGCTAACAGGTGATCCCGTTCTTTCTGTTCCTCGCTCTTCCCCTCCGATCGTCCTCGCTTACCGCGTG
TCCTCCCTCCTCGCTGTCCTCTGGCTCGCAG**GTCATGGCAGCGCCAGGCGGCAGGTCGGAGCCGCCGCAGCTCCCCGAGTACAGCTGCAGCTACATGGT**
GTCGCGGCCGGTCTACAGCGAGCTCGCTTTCCAGCAACAGCACGAGCGGCGCCTGCAGGAGCGCAAGACGCTGCGGGAGAGCCTGGCCAAGTGCTGCAG
GTAGCGGCCGCGCGGGCCTGCGTAGAGAGAAGCGGAGCGGGGCGTCCACGCCTTGGGGAGGGAAGGGCGTCCCCAGCGGGCGAGAGTGGGGTGCGGGCG
GCGGAGCCCCTGGGCGCCAGCTGCTTCTCCCAGAGGCCCGACTTTCGGTCTTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCC
ACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAG
GTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGA
AAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATG
ACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon3 of wild-type human pendrin

(Expected size of the PCR product: 190+140 = **330-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCAGCACTTCAGGGTTATTATT
TTCCAGGAAATACTTATCCTTTTTCCAAATAGTTATAAACATCAGCAGAATCCAGTTCATAACTTTGTGATTTGCAAATTGGTTGTGACTGAGATTGGA
TTGAAAACCCAGTTTTCTTGCTTTTTGACAG**TTGTTCAAGAAAGAGAGCCTTTGGTGTGCTAAAGACTCTTGTGCCCATCTTGGAGTGGCTCCCCAAAT**
ACCGAGTCAAGGAATGGCTGCTTAGTGACGTCATTTCGGGAGTTAGTACTGGGCTAGTGGCCACGCTGCAAGGTAAGATGTTGGCAGATTGAGAGTTCT
GGTCTCCAGCAGGAGTTTAACACTTCTCCCCAGCTACCATAGGTCTGTGACAGATGGTTGCTTACCCTTCAAGGCCTGTATCTTTCCTGTAGAGCCCCT
TAGTGGAGAGAGTCACCTCTCTTCTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCG
GTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCAT
GACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTC
AGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAG
CTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon4 of wild-type human pendrin

(Expected size of the PCR product: 190+111 = **301-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG

ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCGGTTACTTAAAGTATGGTTT
CTACTGAATGCATATTGCTTTTGCATCATCATAAAGGCAAAGTCATAAGTGGAACCATTGTAAGTTGAGGACTTTCTGCATACTGTAACTTTGGTTTGT
GAATGTAATCACTTTGCATGTGCTTTCAG**GGATGGCATATGCCCTACTAGCTGCAGTTCCTGTCGGATATGGTCTCTACTCTGCTTTTTTCCCTATCCT**
GACATACTTTATCTTTGGAACATCAAGACATATCTCAGTTGGTAATTATAAGTATATTTTACAATTATATTTGCTCATGTTTAAAGTGTTTTGGCTATA
TTAAGTGCATTATACCTCTATTAGGTTGGTGCAAAAGTAATTGCGGTTTTCACAATTATACTTTTAATTGTGAAAACCGCAATTACTCGAGGTCGACGG
TATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCA
CTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCC
TCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCT
TCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTG
GAGGTGG

➢ Exon5 of wild-type human pendrin

(Expected size of the PCR product: 190+185 = **375-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCAGCCACTGGGTCCGGCTCAG
CTTCTTTCGTGAACAAACAATATTTTCCTAGTCACAGCTAAATCTTTTATACATTTTTTAAACCCTATGCAGACACATTGAACATTTGTGATTAATAAC
TGATTAATTGTTAGAGACTTTTTTTCCCCAG**GACCTTTTCCAGTGGTGAGTTTAATGGTGGGATCTGTTGTTCTGAGCATGGCCCCCGACGAACACTTT**
CTCGTATCCAGCAGCAATGGAACTGTATTAAATACTACTATGATAGACACTGCAGCTAGAGATACAGCTAGAGTCCTGATTGCCAGTGCCCTGACTCTG
CTGGTTGGAATTATACAGGTAATGAACTTACAAGTAAAATATAGATGGATGTAATTTTTATTTGAAATTAACTTTAAAGCATATAGACTTAAAGATTCT
ACTAAAAACAAAACAAAGTAATTTCCTGGAACCCAAAATTATTTTCTAAATTACGTTGTTTTAGGTCAGTCGAGGTCGACGGTATCGATAAGCTAATTC
CTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGA
TGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGC
AGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCT
CTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon6 of wild-type human pendrin

(Expected size of the PCR product: 190+165 = **355-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCAAGAAATTCATATTTTTTTC
TACCAGTATTTTTGTGCTATAGGCAGGCTACTAGTGTTTTCATTGGTATTAAGCTTGATGTAATATTTCCAGAGAGTAGGTTTCTATCTCAGGCAAACA
TTTAATTTTTCTTTCCTTTTCCTTATCGTAG**TTGATATTTGGTGGCTTGCAGATTGGATTCATAGTGAGGTACTTGGCAGATCCTTTGGTTGGTGGCTT**
CACAACAGCTGCTGCCTTCCAAGTGCTGGTCTCACAGCTAAAGATTGTCCTCAATGTTTCAACCAAAAACTACAATGGAGTTCTCTCTATTATCTATGT
AAGTGTTGCTTCTTGCTCCAGGGATGGGTCACTGTTCATTCCAGAAACAATTGTATTCATTCTCTGAGTCTGGGCCAGGCGTGGTGGCTCACACCTGTA
ATCCCAGCACTTTGGAAGGCCGAGGTGGGCAGATTGCTTGAGCCCAGGATCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCAC
TAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGT
TTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAA
GCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGAC
CTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon7 and exon8 of wild-type human pendrin

(Expected size of the PCR product: 190+153+83 = **426-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCTTACTGAAACTTTTGAGTGT
TGTTTGATGCTGATATCATGGTTTTTCATGTGGGAAGATTCATATGAGAATTGATTGTGTGTGTGTGCGTGTGTGTGTGCTCGTGTGCGTGTAGCAGCA
GGAAGTATATAAAATTATTTTCTTTTTATAG**ACGCTGGTTGAGATTTTTCAAAATATTGGTGATACCAATCTTGCTGATTTCACTGCTGGATTGCTCAC**
CATTGTCGTCTGTATGGCAGTTAAGGAATTAAATGATCGGTTTAGACACAAAATCCCAGTCCCTATTCCTATAGAAGTAATTGTGGTAAGTAGAATATG
TAGTTAGAAAGTTCAGCATTATTTGGTTGACAAACAAGGAATTATTAAAACCAATGGAGTTTTTAACATCTTTTGTTTTATTTCAG**ACGATAATTGCTA**
CTGCCATTTCATATGGAGCCAACCTGGAAAAAAATTACAATGCTGGCATTGTTAAATCCATCCCAAGGGGGTGAGTGTGGTGTTCCTCTTAGTACTAAT
ACATTAAGTCAGTAAGTCAGTCTTTTTTATTTAAATAAAACCTTTTATTACAAGCTTCATTTCACTGATACTCCTTCAATAGTCCTATTTGTGTGTGAT
CTGGAAGAAACAACCATAAGACTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGT
GGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGA
CCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAG
CTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCT
GGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon9 of wild-type human pendrin

(Expected size of the PCR product: 190+148 = **338-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCTATCATGTATGTATTTCGTG
TGCTTTTTGTTCTTTTGGATCAAGTACTTTCATGTCTAATATGTGACTGAGCAGATATAGCATTTGATGAGATGGGGAAAAAGGATGGTGGTCAAATCT
TCACAGCATTTTTCACTTAAAAACTCACTAG**GTTTTTGCCTCCTGAACTTCCACCTGTGAGCTTGTTCTCGGAGATGCTGGCTGCATCATTTTCCATCG**
CTGTGGTGGCTTATGCTATTGCAGTGTCAGTAGGAAAAGTATATGCCACCAAGTATGATTACACCATCGATGGGAACCAGGTATGGGTGCCCTTTTGCT
GAACTGGTTTTATAGGGCTGGAAACAGGAAAAAAACATAAATGGAAAAGATTTTGGTGTCAGCTAAAGAAGGGGTTGGATTCTTTCACCAGACCTTATT
GGGTTGGTTTTCCTCTTGTGTTTGCTAATTAGTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCG
CCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGT
CCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGG
GGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCAC
AACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon10 of wild-type human pendrin

(Expected size of the PCR product: 190+114 = **304-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCCCCAAGTACCTATCACGGTA
AAAATTAAATTGGACCACCACGCAGAGTAGGCATGGGAGTTTTCATTCTTAATGTACTTCCTGAAATACTCAGCGAAGGTCTTGCAAAGATTCAATTTG
TAGGATCGTTGTCATCCAGTCTCTTCCTTAG**GAATTCATTGCCTTTGGGATCAGCAACATCTTCTCAGGATTCTTCTCTTGTTTTGTGGCCACCACTGC**
TCTTTCCCGCACGGCCGTCCAGGAGAGCACTGGAGGAAAGACACAGGTAGGAACAACAGCCTTATGATATCCATCTCAGAGAACAAGTCGAGGAATGGC
AACAGAGGAAGGCTCGCACCGAGCTTAGCAGGACAATTTGCCTTTCAGACTTGTACTTCCTAATCTGATTCACCTCAGGCCTATTCCTCTTGTTCCATC
GAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGAC
CCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATG
CAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTAT
GTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGA
CCTTGGCACTGGAGGTGG

➢ Exon11 + exon12 of wild-type human pendrin

(Expected size of the PCR product: 190+78+96 = **364-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCATCACTTTTCTCGACAGTAT
TGAGCAGAAGGGGGAGACAGGGAAGTATGAAGTGTGTCTGTGAACAGGCTGTCTCATACACACATCCAGTGAGCTGGAAGACACAAGGGAGAAGGACGA
ATCCTTTTCATAGGAGGTGTGTGTCTTCCAG**GTTGCTGGCATCATCTCTGCTGCGATTGTGATGATCGCCATTCTTGCCCTGGGGAAGCTTCTGGAACC**
CTTGCAGAAGGTATAACCCTGCTTCTCTGCATACCGATTGCATAATTTCCCTTCACTACTCTGCTACCAGATAAATAACAGGAGATTTAACAATCATCA
CATGGAAAACCATTCCCTGAATAACACAGCCTTCTCTGTCTCTCTTGGCAG**TCGGTCTTGGCAGCTGTTGTAATTGCCAACCTGAAAGGGATGTTTATG**
CAGCTGTGTGACATTCCTCGTCTGTGGAGACAGAATAAGATTGATGCTGTAAGTCACCTACCACCTATATTTATCTGAAATAAGATTTGGTTCTTATAT
GCTTCCTGCCATATCACTATATTCCCCCCATCCCCTAAGTCTCACTTGTGCTTTGGGAACTCCAGAGGAGAAATTAGAATTGTGGGGATAAATCAAAGC
TCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGG
ACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCA
TGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCT
ATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCA
GACCTTGGCACTGGAGGTGG

➢ Exon13 of wild-type human pendrin

(Expected size of the PCR product: 190+107 = **297-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCCAGCCTGGGCAATAGAGTGTG
ACCCTATCTCAAAAGAAAAAAAAAAAATGTAATTTGTTTGTGGATCATTGATCTTATTTTTATAGGTAGTTATCACATGATGGTACCTGATACATTAATA
TAATTCTTTTCATTTCTATTTTTTTCCCTAG**GTTATCTGGGTGTTTACGTGTATAGTGTCCATCATTCTGGGGCTGGATCTCGGTTTACTAGCTGGCCT**
TATATTTGGACTGTTGACTGTGGTCCTGAGAGTTCAGTTGTGAGTAACGTAAAACCCAGATTTCCTATAAACAGAACAACACACTCTGAGCTTCCTTAT
ACCATTTTGATAAATATAGTGAAGCCACTTTCTTTCGTTATAGTTACTGTATATTGAGTGCTTCTATGCATTAAGCAGACAGTGTTTTACTCGAGGTCG
ACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAG
AGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCT

TGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTT
TGCTTCTGTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGC
ACTGGAGGTGG

➢ Exon14 of wild-type human pendrin

(Expected size of the PCR product: 190+70 = **260-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCTCAGAGTTAGCTACAGGAAA
ATGTCATCTGCAATAAAGACAGAGTCCAAAACACCAGAATGATGGGCTCTTTAGTAGCTGTTGTTTTTAACTTTTTATTCCAAAATACGGCTGTTCCAA
AAAATCTTGACCTTGATATTTTTTCTTCTAG**TCCTTCTTGGAATGGCCTTGGAAGCATCCCTAGCACAGATATCTACAAAAGTACCAAGAATTACAAAA**
ACGTAAGTACCTTTGTGAGACATTTGCTGGACTTGGGTTTACTAGCCTGAAGTTTCAGCAGCTCCATTTTACGTACAAGGTAGCCAAAGGGAGAAAATG
CCTATTGGGAAAGTCTGTTAGTCCACAGGGAGTGTCATGAAAACTTTTGATCCTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGAT
CCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGT
AGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGA
GAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACA
TGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon15 of wild-type human pendrin

(Expected size of the PCR product: 190+93 = **283-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCAGAAAAGAAAGAAAAGTTGA
GTGCTGCTACCCAGCTCCTCTGAGCAACTGTGACTTGACTCCTTGCTAAGTAGCCAGAAATGTAATTAAATACTTGAGGCTTGAAATTATTTAATCCCA
GACAATTTCTTTTAATGCCAG**ATTGAAGAACCTCAAGGAGTGAAGATTCTTAGATTTTCCAGTCCTATTTTCTATGGCAATGTCGATGGTTTTAAAAAA**
TGTATCAAGTCCACAGTAAGTATTTTATCCCTAGAAATTTGTTTTCTAACCTCTTTTGAGACTTCATTCATTCTACAAGTATTTACTGGGGTCCAATCA
GGAATAGGCCCTAGACCCTCTTCCCTTTGTGTAGGGCAATGAGAATTAAAATATAACATCCTTGCCTCGAGGTCGACGGTATCGATAAGCTAATTCCTG
CAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGG
TAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGG
GGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTG
CCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon16 of wild-type human pendrin

(Expected size of the PCR product: 190+96 = **286-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCAGATCTACTCCATCAGACCT
TACAATTTCTTTTTTGGCAGGATAGCTCAAGGAATTATACCCTTTGAGAAATAGCCTTTCCAGATAACAGTTGCCATTAATAAGCTTTAGGTGCCAGGC
ATTTTAAGTAACTTGACATTTATTTCCAAAG**GTTGGATTTGATGCCATTAGAGTATATAATAAGAGGCTGAAAGCGCTGAGGAAAATACAGAAACTAAT**
AAAAAGTGGACAATTAAGAGCAACAAAGGTGAGATGACATCTTTCTTTTCCCCCTTAAATTATTTCCTTTCCCTGATGAGAGCAGTTAGAGGGTCTAAA
ATTAAATCTATCCTCTTTAGTATCCAGATGTGAATGAACAAATGACATGTACGTATCAAAGAACAACTGAGCTATTCTTTCGAGGTCGACGGTATCGAT
AAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACT
GACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTG
CTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGC
TGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon17 of wild-type human pendrin

(Expected size of the PCR product: 190+231 = **421-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCGGGCTGAGGTGAAACCCATC
CTTAAAAATTCATCTCCTTGATGTCTTGCTTACCAAGGAACAGTGTGTAGGTCTTTTGGATAATTTGATATGAATGGTTGAAAGATTTCAAATCTTTGA
CAATTAAGTTGACAGTGTTTTCTTCGTTTAG**AATGGCATCATAAGTGATGCTGTTTCAACAAATAATGCTTTTGAGCCTGATGAGGATATTGAAGATCT**
GGAGGAACTTGATATCCCAACCAAGGAAATAGAGATTCAAGTGGATTGGAACTCTGAGCTTCCAGTCAAAGTGAACGTTCCCAAAGTGCCAATCCATAG
CCTTGTGCTTGACTGTGGAGCTATATCTTTCCTGGACGTTGTTGGAGTGAGATCACTGCGGGTGGTAAGGTTCTGGTTTTCTGAATTATACATTTGGAG
CTTTGGCAATAGTAAAATGATGTGGGTTGTCCAGTATTGCAACAGGGCAAATACATGGGCTTTGTAATTTTTCTAGGTGAATGCTTTTGTAAAAAAGTG
TAATATTTTAAAGCATTCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCT

CGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCCATTCATGACCAGTG
ACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGGTCGCTCAGCTCCTC
CTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAACTGGAGCTGGGTGG
AGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon18 of wild-type human pendrin

(Expected size of the PCR product: 190+55 = **245-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCACCTCCATGGTTTTGCAATA
ATAACCTTTCCTTAAAGTCCTGATTAACCATGAAAGTAATAATGTTTCTCCTGAGCAAGTAACTGAATGCTACTGAATTATGGGCAGATAAGGTTGTTA
ATTGTTACAAACTCTCCTTTTTTATTTTTAG**ATTGTCAAAGAATTCCAAAGAATTGATGTGAATGTGTATTTTGCATCACTTCAAG**GTAAATACATATA
TCTACATATCTACCTGTAAGACTTTCCCGTAAGCCCTTTCTCCTATCTGGGACTGTGGTCACATTATGTCTGAAGGCCTTTTTTTTTTTTCTTTTAAAG
ATCTCAATTGTCATTATTTGCAGTTCTGGAATCTGGCATCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAG
CGGCCGCCACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCC
ATATGTCCATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAA
TCAGGGGGTCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAG
TGGCACAACTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

➢ Exon19 of wild-type human pendrin

(Expected size of the PCR product: 190+146 = **336-bp**)

CCTGGCCTGCCCAGGCTTTTGTCAACAGCACCTTTGTGGTTCTCACTTGGTGGAAGCTCTCTACCTGGTGTGTGGGGAGCGTGGATTCTTCTACACACC
CATGTCCCGCCGCGAAGTGGAGGACCCACAAGGTAAGCTCTGCTCCTGAATTAATTCTATCCCAAGTGCTAACTACCCTGTTTGTCTTTCACCCTTGAG
ACCTTGTAAATTGTGCCCTAGGTGTGGAGGGTCTCAGGCTAACCAGTGGGGGGCACATTTCTGTGGGCAGCTAGACATATGTAAACATGGTAGCTGCCA
GGAAGGAGTGAGAATCCTTCCTTAAGTCTCCTAGGTGGTGACGGGTGGCTAGGCCCCAGGATAGGTACCGGGCCCCCCCGGGGATCACTTGAACTTGGG
ACGCGGAGGTTGCAGTGAGCAATGATGCCACTGCACTCCAGCCTGGGCAATAGAATGAGACTCTGTCTCAAAAACAAACAAAAATTTCTTTTCCTAGGA
ACTAACAAAACATTGTGTCTTTCTTTTGAAG**ATTATGTGATAGAAAAGCTGGAGCAATGCGGGTTCTTTGACGACAACATTAGAAAGGACACATTCTTT**
TTGACGGTCCATGATGCTATACTCTATCTACAGAACCAAGTGAAATCTCAAGAGGGTCAAGGTTCCATTTTAGAAACGGTAAATATTCAACCTTTCTAC
AGATGTATCTTTTCTAAACTATCATGATTTCTATAAATGGCAAACATTACACAAGTCTAGTCTAGCTGTTGAATTTTAAGCTACCTATATAACTTCATG
GAGCCTCAGTTTTTTCATCAGTAAAATGGATCGAGGTCGACGGTATCGATAAGCTAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGAGCGGCCGCC
ACCGCGGTGGAGCTCGGTACCTATTTGGGGACCCCATAGAGCACTGCACTGACTGAGGGATGGTAACAGGATGTGTAGGTTTTGGAGGCCCATATGTCC
ATTCATGACCAGTGACTTGTCTCACAGCCATGCAACCCTTGCCTCCTGTGCTGACTTAGCAGGGGATAAAGTGAGAGAAAGCCTGGGCTAATCAGGGGG
TCGCTCAGCTCCTCCTAACTGGATTGTCCTATGTGTCTTTGCTTCTGTGCTGCTGATGCTCTGCCCTGTGCTGACATGACCTCCCTGGCAGTGGCACAA
CTGGAGCTGGGTGGAGGCCCGTGACCTTCAGACCTTGGCACTGGAGGTGG

Supp. Table S1. SLC26A4 genotype and clinical phenotype of subjects.

Pedigree No.	individual ID	Age at genetic test	Relationship	Amino acid change on Allele 1	Amino acid change on Allele 2	Hearing loss	Onset age of hearing loss (y)	EVA	PTA	DP-OAE	ABR	ASSR	COR	Goiter
867	1	16	Proband	Y214C	H723R	Yes	0	Yes	Moderate-to-severe	No response	-	-	-	No
	2	-	Father	=	H723R	No	-	-	-	-	-	-	-	-
	3	-	Mother	Y214C	=	No	-	-	-	-	-	-	-	-
2467	4	24	Proband	Y214C	H723R	Yes	0	Yes	Profound	-	-	-	-	No
	5	61	Father	=	H723R	No	-	-	-	-	-	-	-	-
	6	57	Mother	Y214C	=	No	-	-	-	-	-	-	-	-
2518	7	6	Proband	Y214C	H723R	Yes	0	Yes	Moderate	-	-	-	-	No
1936	8	1	Poband	T410K	L703P	Yes	0	Yes	-	-	No response to 100dB click	-	Moderate to severe	No
	9	-	Father	T410K	=	No	-	-	-	-	-	-	-	-
	10	-	Mother	=	L703P	No	-	-	-	-	-	-	-	-
1130	11	1	Proband	L703P	S551Ffs*13	Yes	0	Yes	-	-	Mild~moderate	-	Moderate	No
	12	4	Brother	L703P	H723R	Yes	0	Yes	Severe	-	-	-	-	No
	13	-	Father	L703P	H723R	Yes	0	-	Profound	-	-	-	-	Yes, found at 25y.o.
	14	-	Mother	H723R	S551Ffs*13	Yes	0	-	Profound	-	-	-	-	Yes, found at 12y.o.
	15	36	Father's sister	L703P	H723R	Yes	0	Yes	Profound	-	-	-	-	Yes, found at 31y.o.
	16	36	(11 and 12 are identical twins.)	L703P	H723R	Yes	0	Yes	Profound	-	-	-	-	Yes, found at 31y.o.
2345	17	27	Proband	L703P	H723R	Yes	0	Yes	Profound	No response	No response to 90dB click	-	-	Yes, found at 22y.o.
2208	18	41	Proband	L703P	H723R	Yes	0	Yes	Severe	-	-	-	-	No
873	19	7	Proband	V483E	=	Yes	7	Borderline	Mild	Normal	Response to 30dB click	40~50dB	-	Yes (mild)

EVA, Enlarged Vestibular Aqueduct
PTA, Pure Tone Audiometry
DP-OAE, Distortion Product Otoacoustic Emission
ABR, Auditory Brainstem Response
ASSR, Auditory Steady State Response
COR, Conditioned Orientation Reflex audiometry
=, not detected
-, not available

Supp. Table S2. A summary of novel pendrin missense variants.

Genome position (hg19)	Exon	Nucleotide change (NM_000441)	Amino acid change	dbSNP150	1000genomes _east_asia	gnomAD_exome_ALL	HGVD	iJGVD3.5K	CADD	REVEL
chr7: 107315430	Exon 6	c.641A>G	p.Y214C	none	none	none	0.000826	none	28.6	0.969
chr7: 107330648	Exon 10	c.1229C>A	p.T410K	none	none	none	none	none	27.1	0.901
chr7: 107336388	Exon 13	c.1448T>A	p.V483E	none	none	none	none	none	26.7	0.932
chr7: 107350517	Exon 19	c.2108T>C	p.L703P	none	none	none	none	none	31	0.925

dbSNP, The Single Nucleotide Polymorphism Database
1000genomes, The 1000 genomes project
gnomAD, The Genome Aggregation Database
HGVD, Human Genetic Variation Database
iJGVD, Integrative Japanese Genome Variation Database
CADD, Combined Annotation Dependent Depletion （v1.4)
REVEL, Rare Exome Variant Ensemble Learner

Supp. Table S3. A summary of HCO_3^-/Cl^- antiport activities measured under a high Cl^- condition (1 μg/mL doxycycline).

	mean ± SD (n)		mean ± SD (n)		mean ± SD (n)		mean ± SD (n)
WT	2.58 ± 0.79 (15)	**p.V163I**	1.88 ± 0.21 (3)	**p.S399P**	2.08 ± 0.19 (3)	**p.T527P**	0.73 ± 0.26 (4)
p.S28G	0.89 ± 0.31 (4)	**p.V186F**	0.37 ± 0.11 (3)	**p.S408F**	0.31 ± 0.11 (3)	**p.I529S**	0.61 ± 0.26 (5)
p.S49R	2.46 ± 0.86 (4)	**p.T193I**	0.37 ± 0.08 (3)	**p.R409H**	0.34 ± 0.03 (3)	**p.Y556C**	0.40 ± 0.02 (3)
p.P76S	0.54 ± 0.16 (4)	**p.Y214C**	1.10 ± 0.38 (5)	**p.T410K**	0.26 ± 0.02 (3)	**p.C565Y**	1.68 ± 0.55 (5)
p.S90L	0.26 ± 0.05 (3)	**p.V239D**	0.36 ± 0.05 (4)	**p.T410M**	0.30 ± 0.04 (3)	**p.S657N**	0.80 ± 0.10 (4)
p.T99R	0.23 ± 0.03 (3)	**p.D266N**	2.49 ± 0.66 (3)	**p.T416P**	0.35 ±0.14 (3)	**p.V659L**	1.11 ± 0.07 (5)
p.L117F	2.08 ± 0.44 (8)	**p.T307A**	2.05 ± 0.08 (4)	**p.Q421L**	0.46 ± 0.02 (3)	**p.S666F**	0.56 ± 0.12 (4)
p.P123S	0.25 ± 0.01 (3)	**p.N324Y**	2.23 ± 0.16 (3)	**p.I426N**	1.10 ± 0.41 (3)	**p.D669E**	0.37 ± 0.02 (3)
p.G131V	0.26 ± 0.05 (3)	**p.G334V**	1.44 ± 0.14 (3)	**p.L445W**	0.30 ± 0.09 (3)	**p.F683S**	0.41 ± 0.06 (5)
p.S133T	0.22 ± 0.06 (3)	**p.F354S**	1.80 ± 0.11 (3)	**p.N457K**	0.94 ± 0.05 (3)	**p.F692L**	1.03 ± 0.24 (5)
p.G139A	0.31 ± 0.09 (3)	**p.K369E**	0.92 ± 0.34 (4)	**p.R470H**	1.95 ± 0.38 (4)	**p.L703P**	0.37 ± 0.02 (3)
p.M147T	0.41 ± 0.12 (4)	**p.A372V**	0.28 ± 0.04 (3)	**p.V483E**	1.16 ± 0.50 (5)	**p.T721M**	0.36 ± 0.02 (3)
p.M147V	0.48 ± 0.11 (3)	**p.N392Y**	0.21 ± 0.02 (3)	**p.G497S**	0.70 ± 0.23 (4)	**p.H723R**	0.58 ± 0.11 (4)