Supplementary Note

Case Reports

Subject 3-1, a female, was evaluated for primary aldosteronism at age 66 years, after a 30-year history of hypertension. Aldosterone/renin ratio (ARR) was 62.0 ng/dl:ng/ml/h (normal <20; plasma aldosterone 24.8 ng/dl, plasma renin activity (PRA) 0.4 ng/ml/h), and aldosterone failed to suppress during fludrocortisone suppression test (FST) (18.8 ng/dl at 10 AM on day 5). Dexamethasone produced complete suppression of recumbent, but not upright plasma aldosterone (<1 ng/dl at 8 AM and 22.7 ng/dl at 10 AM on day 5). Plasma aldosterone rose with upright posture (from 10.4 to 28.3 ng/dl). Adrenal CT was normal. Adrenal venous sampling was consistent with bilateral aldosterone production. The patient was treated with spironolactone.

Subject 3-2, a female, was diagnosed with hypertension at age 24 years and developed hypokalemia at 46 years. She was then found to have an elevated ARR with positive FST. Aldosterone did not suppress during dexamethasone suppression testing and was unresponsive to upright posture. CT showed a bulky left adrenal gland, but a 75 selenium-methyl-cholesterol scan was normal. Adrenal venous sampling was consistent with bilateral disease, and hypertension and hypokalemia responded to treatment with spironolactone.

Subject 3-4, a male, was evaluated at ages 24 years (BP 98/70 mmHg, aldosterone 14.5 ng/dl, PRA 1.8 ng/ml/h, K^+ 4.0 mmol/l (normal 3.5-5.5)), at age 29 years (aldosterone 17.3 ng/dl, PRA 1.7 ng/ml/h, K^+ 4.8 mmol/l) and at age 36 years (borderline elevated

ARR with aldosterone 24.5 ng/dl, PRA 1.2 ng/ml/h, K^+ 4.2 mmol/l). At age 49 years, his BP remained normal at 116/77 mmHg.

Subject 3-5, a female, was diagnosed with hypertension at the age of 19 years. At age 25 years, plasma K^+ was 3.3 mmol/L, aldosterone 27.4 ng/dl, PRA 0.1 ng/ml/h, and ARR 274 ng/dl:ng/ml/h. Plasma aldosterone did not suppress during FST (27.1 ng/dl at 10 AM on day 5) or dexamethasone suppression test (17.3 ng/dl at 8 AM and 28.3 ng/dl at 10 AM on day 5). Adrenal CT showed a small (8 mm) nodule in the left adrenal. Plasma aldosterone fell with upright posture (from 60.6 to 27.4 ng/dl). Aldosterone/cortisol ratios were higher in both adrenal veins when compared with peripheral ratios, consistent with bilateral production of aldosterone, and the patient was treated with spironolactone with correction of hypokalemia and hypertension.

Subject 3-7, a female, first developed mild hypertension at age 20 years and was later shown to have elevated ARR with positive FST. Aldosterone was only partially suppressible during dexamethasone suppression test, but fell with upright posture. CT showed mild bulkiness of the left adrenal gland. Adrenal venous sampling did not show lateralization. Hypertension was controlled on spironolactone.

Subject 3-9, a male, had normal BP (120/80 mmHg) at age 19 years, and repeated ARRs over a course of 12 years (starting at age 18 years) were normal.

Subject 3-10 had high-normal BP (130/85 mmHg) and was normokalemic when assessed at the age of 17 years. His ARR was elevated at 262 ng/dl:ng/ml/h (plasma aldosterone 26.2 ng/dl, PRA 0.1 ng/ml/h), but he declined further workup and deceased at age 29 years.

Subject 3-11, a female, had plasma aldosterone 21.2 ng/dl, PRA 0.1 ng/ml/h, and ARR 212 ng/dl:ng/ml/h at age 14 years. Her blood pressure was normal (94/62 mmHg), and CT was also normal. Aldosterone failed to suppress with fludrocortisone (11.5 ng/dl at 10 AM on day 4), consistent with primary aldosteronism, and aldosterone did not rise with upright posture (recumbent 16.8 ng/dl, upright 16.1 ng/dl).

Subject 3-19, a male, was assessed at age 41 years. His BP was 144/94 mmHg without medication, ARR was 38 ng/dl:ng/ml/h (plasma aldosterone 26.7 ng/dl, PRA 0.7 ng/ml/hr), and aldosterone suppressed during FST (4.6 ng/dl at 10 AM on day 5). Dexamethasone produced complete suppression of recumbent, but not upright plasma aldosterone (<1 ng/dl at 8 AM and 16.7 ng/dl at 10 AM on day 5). Plasma aldosterone rose with upright posture (from 16.3 to 28.1 ng/dl). Adrenal CT was normal.

Subject 3-35 was diagnosed with hypertension (blood pressure 170/110 mmHg) at age 16 years. Plasma potassium was 3.4 mmol/l, plasma aldosterone 25.5 ng/dl, PRA ≤0.2 ng/ml/h, and ARR was elevated at ≥127.5 ng/dl:ng/ml/h. Aldosterone failed to suppress during FST (37.5 ng/dl at 10 AM on day 4), consistent with primary aldosteronism. Aldosterone did not rise with upright posture. Both adrenal glands were normal by CT, and adrenal venous sampling indicated bilateral aldosterone production. Hypertension was controlled with amiloride.

Subject 318-1, a male, was diagnosed with migraine headaches and hypertension at age 7 years, with BP readings up to 170/140 mmHg. Workup for decreased renal function (serum creatinine 0.9-1.0 mg/dl (normal 0.2-0.6 mg/dl), endogenous creatinine clearance 75-90 ml/min), including renal ultrasound with Doppler, captopril nuclear renal scan and renal biopsy were insignificant. Persistent hypokalemia (serum K^+ 2.6 mmol/l) and mild

metabolic alkalosis (serum CO_2 29-30 mmol/l) were noted. He was diagnosed with primary aldosteronism (serum aldosterone 9.5 ng/dl, PRA 0.21 ng/ml/h, urinary aldosterone 15 μg/24 hours (normal 4-22 μg/24 hours)). His family history was significant for a mother who had been diagnosed with hypertension at age 18 years, a maternal grandmother who died in her 40s from cerebrovascular accident and maternal grandaunts with early-onset hypertension.

Subject 531-1, a female, presented with hypertension at age 6 years. Evaluation was consistent with primary aldosteronism (aldosterone 100 ng/dl; plasma renin activity (PRA) <3 ng/ml/h). At age 20 years, she was admitted with a diagnosis of hypertensive crisis (blood pressure (BP) 280/188 mmHg). Weight loss was attributed to anorexia nervosa, and chronic leukocytosis was found. Urine analysis was positive for blood. Her family history was significant for a mother who died at age 30 years from a cerebrovascular accident.

Subject 537-1, a female, presented with hypertension at age 11 years, with peak BPs of about 160/120 mmHg. Laboratory evaluation revealed persistent hypokalemia (serum K^+ 3.0-3.4 mmol/l), mild hypernatremia (Na^+ 146 mmol/l), and metabolic alkalosis (CO_2 33 mmol/l). Serum aldosterone was 26 ng/dl, and PRA was suppressed at 0.3 ng/ml/h. Urinary aldosterone was elevated at 28 μg/24 h, and serum 18-hydroxycorticosterone was normal at 19 ng/dl (normal 6-85 ng/dl). Captopril suppression test was positive, and adrenal computed tomography was negative for tumors or enlargement. The patient was treated with doxazosin, KCl and amiloride, with difficulties achieving normokalemia and normotension. The family history was positive for hypertension in both parents and a paternal grandmother. The patient was lost to follow up and deceased at age 21 years.

Subject 840-1, a male, was diagnosed with hypertension during a routine examination at age 15 years (BP 130/100 mmHg), but treatment was not initiated. At age 17 years, sustained BP elevations to 180/120 mmHg for one- to two-hour periods were noted. Urinary catecholamines were unremarkable, as were cranial and abdominal MRI, a renal arteriogram and renal ultrasound. Adrenal CT scan was normal. After initiation of treatment with atenolol and amlodipine, hypotensive episodes were observed, with the subject being near-syncopal or BP not measurable. Serum potassium was 2.6 mmol/l during one of these episodes, and potassium supplementation was started. Further evaluation at age 20 years revealed an aldosterone of 37 ng/dl, and PRA of 0.2 ng/ml/h without medication. Treatment with 25 mg spironolactone was then initiated. The family history was positive for hypertension in the maternal grandfather.

Subject 1281-1, a female, presented at age 1 year with hypertension (BP 117/71 mmHg) and transient hypokalemia and was diagnosed with primary aldosteronism (aldosterone 17 ng/dl, PRA <0.5 ng/dl/h). She was treated with amlodipine.

Subject 1492-1, a male, was admitted to the pediatric intensive care unit after an apneic episode at age 11 weeks. Severe hypertension with BP readings up to 150/90 mmHg was noted. Evaluation revealed normokalemia (4.0 mmol/l), elevated aldosterone (63.8 ng/dl) and suppressed PRA (<0.15 ng/ml/h). His perinatal history was significant for a twin pregnancy with mild pre-eclampsia in his mother, birth at 37 weeks gestational age and transient hypokalemia as a newborn. Renal ultrasound, evaluation for congenital adrenal hyperplasia, thyroid function, cardiac echocardiogram and cranial computed tomography were normal. After the blood draw for aldosterone and PRA, treatment with captopril 5 mg three times per day was started, with partial improvement of BP levels. Hypertension

resolved by age 2 years. The family history was positive for hypertension in the mother during adolescence, which was attributed to obesity and improved with weight loss, and essential hypertension in the maternal grandmother.

Subject 1786-1, a female, was diagnosed with hypertension at age 15 years during a routine examination, with BP readings of 140-150/90-100 mmHg. She had been asymptomatic with the exception of shortness of breath while running. Laboratory evaluation revealed hypokalemia (K^+ 2.9 mmol/l), elevated aldosterone (47.9 ng/dl) and suppressed PRA (<1.0 ng/ml/h). Urinary aldosterone was 22.4 μg/24 h.

Subject 1786-2, her mother, was first diagnosed with hypertension at age 32 years. Her past medical history was significant for grand mal and petit mal seizures that were diagnosed at age 18 months and temporarily treated with phenobarbital, and a seizure-free interval after discontinuation of treatment from teenage to early adult years. After a fall, she developed myoclonic seizures that were attributed to traumatic brain injury and treated with clonazepam. There was an allergic reaction to lamotrigine. Seizures did not recur after discontinuation of treatment. Past medical history further included endometriosis and a gall bladder cyst, and her family history was significant for a father, paternal grandmother and paternal grandfather with hypertension. A blood pressure increase was associated with hypokalemia, and serum aldosterone was found to be elevated at 59.4 ng/dl. Urinary 18-hydroxycortisol was normal (90.9 μg/24h, reference range 43-295 μg/24 h), and plasma free metanephrine and normetanephrine were also normal. Saline suppression test was positive. Computed tomography did not reveal adrenal abnormalities, and there was no lateralization on adrenal venous sampling. Repeat aldosterone was 46.3 ng/dl, and renin was <1.0 ng/ml/h, with serum potassium 3.0

mmol/l. She was treated with 10 mg amlodipine, 75 mg eplerenone and 20 mmol KCl daily, but continued to have suppressed renin levels (<0.6 ng/ml/h).

Subject 1786-3, her 13-year old son, had an aldosterone of 12 ng/dl, with PRA <0.6 ng/ml/h and K^+ 4.4 mmol/l. 24h ambulatory BP monitoring revealed average values of 121/75 mmHg, with 123/77 mmHg during the wake period and 114/64 mmHg during the sleep period, which was classified as prehypertension.

Additional Online Methods

Confocal microscopy. H295R cells were transfected with 3 μg plasmid DNA (2 μg pRcCMV CLCN2 (WT or mutant) and 1 μg pECFP-Mem (Clontech, Palo Alto, CA, USA)) as for real-time PCR (Online Methods). After recovering of the cells, they were plated in ibidi μ-dishes (35mm, low, ibidi, Madison, WI, USA). The cells were cultured for 48 h and subsequently investigated at 37°C using the confocal microscope LSM 710 (Zeiss) at the Center for Advanced Imaging (CAi) at Heinrich Heine University Düsseldorf. Analysis was performed using ZEN (black edition, Zeiss). After determining fluorescence intensity thresholds based on cells transfected with only one construct, individual cells were manually identified as regions of interest, and Pearson's correlation coefficient was determined in ZEN. Cells with fluorescence below the threshold were excluded from the analysis.

Immunoprecipitation. HAC15 cells stably expressing ClC-2^{WT} were lysed in homogenization buffer containing cOmplete Protease Inhibitor Cocktail (Roche, Basel, Switzerland), Phosphatase Inhibitor Cocktail 3, 10 mM NaF, 10 mM β-Glycerophosphate and 10 mM sodium pyrophosphate (all Sigma-Aldrich). After centrifugation (125,000 g, 4°C, 40 min), the cell pellet was resuspended in solubilization buffer containing 1% NP-40. The cell suspension was incubated on ice for 30 min and centrifuged as above. The protein concentration of the supernatant (membrane fraction) was determined using the Micro BCA Protein Assay Kit (Thermo Scientific). 40 μl (run 1) or 100 μl (run 2) Dynabeads Protein A (Novex by Life Technologies) were labeled with 2 μg (run 1) or 10 μg (run 2) ClC-2 antibody (HPA014545, Sigma-Aldrich) and incubated with 1 mg

membrane fraction for 2.5 h at 4°C on an orbital shaker. Beads were washed 3x in homogenization buffer and 3x in PBS (5 min each, 4°C, orbital shaker), separated on a DynaMag-2 (Novex by Life Technologies) magnet and kept at -80°C.

Mass spectrometry.

After final washing in PBS, beads were resuspended in 100 µl 50 mM ammonium bicarbonate (ABC) buffer. Proteins were reduced for 30 min at room temperature in 10 mM DL-Dithiothreitol (DTT, Sigma), followed by alkylation with 55 mM chloroacetamide (Merck, Darmstadt, Germany) for 20 min in the dark at room temperature. The sample was digested with 1 µg endopeptidase LysC (Wako, Osaka, Japan) for 4 hours at 30°C, followed by an over-night digestion with 1 µg sequence-grade modified trypsin (Promega, Fitchburg, WI, USA) at 30°C. The supernatant (containing the peptides) was transferred to a fresh tube, and the beads were further incubated with 100 µl ABC buffer and 100 µl water (LC-MS grade). All fractions were pooled and acidified with formic acid (2% final concentration). Peptides were extracted and desalted using the standard StageTip protocol[1]. For mass spectrometric measurements, the peptide mixture was separated by reversed-phase chromatography using an EasyLC 1200 system (Thermo Fisher Scientific) on in-house-manufactured 20 cm fritless silica microcolumns (packed with ReproSil-Pur C18-AQ 3 µm resin) with an inner diameter of 75 µm. Peptides were separated using an 8–60% acetonitrile gradient (94 min length) at a nanoflow rate of 200 nl/min. Eluting peptides were directly ionized by electrospray ionization and analyzed on a Thermo Orbitrap Fusion instrument (Thermo). Mass spectrometry was performed in data-dependent positive mode with one full scan (m/z

range = 300-2000; R = 60,000; target value: 5×10^5; maximum injection time = 50 ms). The ten most intense ions with a charge state between 2 and 7 were selected (R = 15,000, target value = 5×10^4; isolation window = 0.7 m/z; maximum injection time = 500 ms). Dynamic exclusion for selected precursor ions was set to 30 s. Two replicates were measured. Data analysis was performed using MaxQuant software package (version 1.5.1.2). The internal Andromeda search engine was used to search MS^2 spectra against a decoy human UniProt database (HUMAN.2017-01) containing forward and reverse sequences. The search included variable modifications of methionine oxidation, N-terminal acetylation, deamidation (N and Q), phosphorylation (S, T and Y) and the fixed modification of carbamidomethyl cysteine. The minimal peptide length was set to seven amino acids, and a maximum of 3 missed cleavages were allowed. The FDR was set to 0.01 for peptide, protein and site identifications. To filter for confidently identified peptides, the MaxQuant score was set to a minimum of 40. Annotated MS2 spectra were extracted using MaxQuant Viewer application.

Splicing assay. A fragment encoding exons 9 to 11 of *CLCN2* was amplified by PCR from blood DNA of an unaffected individual using primers CLCN2E9-11_2Fs and CLCN2_E9-11_2R (Supplementary Table 10), cloned into the pCR2.1 TOPO vector (Invitrogen) and verified by Sanger sequencing. After subcloning into pcDNA3.1(+) using the HindIII and XhoI enzymes (NEB), the variant chr3:184074782T>A (hg19) was introduced by site-directed mutagenesis (QuikChange) using primers K362gF and K362gR (Supplementary Table 10) and again verified by Sanger sequencing. HEK293T cells (American Type Culture Collection, cultured as described[13] and authenticated

(Eurofins Genomics, Ebersberg, Germany)) were transfected with two independent clones of wildtype and mutant constructs of pcDNA3.1(+) *CLCN2* using Lipofectamine 2000 (Invitrogen). RNA was isolated using the RNeasy Mini Kit (Qiagen) and cDNA prepared using the QuantiTect Reverse Transcription Kit (Qiagen). After PCR amplification of the spliced region using primers C2_E9-11SF and C2_E9-11SR (Supplementary Table 10), the products were resolved on an agarose gel, an approximately 250 bp fragment was excised, purified and subjected to bidirectional Sanger sequencing.

Gene expression analysis

Gene expression data for *HEPACAM* were obtained from the GTEx Portal on 03/27/2017 (see URLs).

Orthologs. Proteins encoded by orthologs of ClC-2 in vertebrate and invertebrate species were identified by a BLAST search. GenBank accessions included NP_004357.3 (Homo sapiens), NP_034030.2 (*Mus musculus*), XP_015147031.1 (*Gallus gallus*), XP_002935192.2 (*Xenopus tropicalis*), NP_001303244.1 (*Danio rerio*), XP_018667478.1 (*Ciona intestinalis*), XP_015021958.1 (*Drosophila mojavensis*) and NP_001300530.1 (*Caenorhabditis elegans*).

Animal studies. Animals were housed under standard conditions in the animal facility of the Forschungszentrum Jülich, Germany, according to institutional guidelines under a 12-h light/dark cycle. All experiments were in compliance with the German Law for

Protection of Animals and were approved by the regulatory authorities, the Forschungszentrum Jülich, and the Landesamt für Natur, Umwelt und Verbraucherschutz of Nordrhein-Westfalen, Germany.

Supplementary Note Reference

1. Rappsilber, J., Ishihama, Y. & Mann, M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal Chem* **75**, 663-70 (2003).

Supplementary Tables

Supplementary Table 1. Novel Variants

Variants Shared Among Three Affected Individuals in Family 3

Chr	Position (hg19)	Gene	Ref base	Nonref base	Het / Hom var	mRNA	ExAC	gnomAD	AA change
chr15	77906476	*LINGO1*	G	C	Het	NM_032808	Novel	Novel	p.His591Gln
chr3	184075850	*CLCN2*	C	T	Het	NM_004366	Novel	Novel	p.Arg172Gln

***CLCN2* Variants in Primary Aldosteronism**

Kindred	Chr	Position (hg19)	Ref base	Nonref base	Het / Hom var	ExAC	gnomAD	AA change
531	chr3	184076907	A	T	Het	Novel	Novel	p.Tyr26Asn
1281	chr3	184076918	A	T	Het	Novel	Novel	p.Met22Lys
3, 318, 1786, 537	chr3	184075850	C	T	Het	Novel	Novel	p.Arg172Gln
1492	chr3	184074782	T	A	Het	Novel	Novel	p.Lys362del
840	chr3	184064498	T	G	Het	Novel	Novel	p.Ser865Arg

Upper panel, novel variants shared among subjects 3-5, 3-11, and 3-35; lower panel, *CLCN2* variants in subjects with primary aldosteronism. Chr, chromosome; hg19, human genome version 19; Ref base, reference base; Nonref base, non-reference base; Het/ Hom var, heterozygous versus homozygous variant; ExAC, frequency in the ExAC database; gnomAD, frequency in the gnomAD database; AA change, amino acid change (corresponding to the proteins encoded by indicated mRNAs); *LINGO1*, leucine rich repeat and Ig domain containing 1; *CLCN2*, chloride channel, voltage-sensitive 2.

Supplementary Table 2. Sanger Sequencing of Candidate Variants in Kindred 3

Subject ID	Clinical diagnosis of primary aldosteronism	*CLCN2* p.Arg172Gln	*LINGO1* p.His591Gln
1	Yes	+/+	+/+
2	Yes	+/M	+/+
4	Possible	+/M	+/+
5	Yes	+/M	+/M
6	No	+/+	+/+
7	Yes	+/M	+/M
9	No	+/M	+/+
10	Yes	+/M	+/M
11	Yes	+/M	+/M
12	No	+/+	+/M
19	No	+/+	+/+
24	No	+/+	+/+
35	Yes	+/M	+/M

+/M, heterozygous for the indicated variant; +/+, homozygous reference sequence at the position of the indicated variant. See Supplementary Table 1 for the genomic positions of variants. *LINGO1* shows homozygous wildtype sequence at position His591 in affected individual 3-2.

Supplementary Table 3. Clinical Features of 45 Patients with Primary Aldosteronism and Onset by Age 20 Years

ID	Gen-der	Age at Dx	BP at Dx	K at Dx	Aldo at Dx	PRA at Dx	ARR at Dx	Age at Referral	BP at Referral	K at Referral	Aldo at Referral	PRA at Referral	ARR at Referral
195-1	F	13	NA	NA	NA	NA	NA	41	136/78	4.1	43.0	1.0	43.00
201-1	M	19	NA	NA	NA	NA	NA	24	130/78	2.6	35.0	0.2	175.00
360-1	F	12	240/90	2.9	62.0	1.5	41.33	13	128-140/88-96	3.6	98.0	0.8	122.50
370-1	F	20	185/110	NA	NA	NA	NA	45	178/110	3.1	16.0	0.35	45.71
424-1	F	15	150/100	NA	NA	NA	NA	15	150/100	2.9	19.0	0.1	190.00
463-1	M	20	NA	NA	NA	NA	NA	NA	105-157/85-112	3.4	35.0	0.4	87.50
510-1	M	16	140/90	NA	NA	NA	NA	19	142/94	4.6	18.3	0.04	45.75
526-1	F	17	NA	NA	NA	NA	NA	68	250/90	4.0	286.0	0.2	1430.00
537-1	F	11	NA	3.0	26.0	0.3	86.67	15	152/102	NA	18.0	0.4	45.00
642-1	M	19	NA	NA	NA	NA	NA	30	170/115-120	3.3	38.0	0.2	190.00
705-1	F	teens	NA	NA	24[a]	0.1[a]	240.00[a]	41	NA	NA	NA	NA	NA
722-1	M	16	160/90	NA	NA	NA	NA	15	148/100	2.8	107.0	0.2	535.00
753-1	M	20	NA	NA	NA	NA	NA	56	160/90	4.1	58.0	0.56	103.57
840-1	M	15	130/100	NA	NA	NA	NA	20	120/70	3.2	37.0	0.2	185.00
850-1	F	12	NA	NA	NA	NA	NA	32	153/97	3.3[b]	36.4[b]	0.4[b]	91.00[b]
855-1	M	16	160/110	NA	NA	NA	NA	55	140/95	3.0	56.0	0.6	93.33
949-1	M	18	NA	NA	NA	NA	NA	51	143/73	3.7	67.0	0.5	134.00
1038-1	F	16	199/102	NA	NA	NA	NA	52	160/95	3.1	29.9	<0.15	>199.33
1074-1	M	13	NA	NA	NA	NA	NA	42	172/102	3.1	36.2	0.1	362.00
1075-1	M	12	153-163/92-99	2.6	20.9	0.19	110.00	12	105/77	NA	NA	NA	NA
1101-1	F	13	NA	3.2-3.4	16.8	<0.15	>112	55	158/106	3.3	NA	NA	NA
1117-1	M	12	NA	NA	NA	NA	NA	45	186/94	3.5	117.5	<0.15	>783.33
1163-1	F	18	220/100	NA	NA	NA	NA	36	117/68	4.9	36.7	0.7	52.43
1191-1	F	12	210/140	2.8	66	8	8.25	12	125/66	4.3	108.0	<0.1	>1080.00

ID	Gen-der	Age at Dx	BP at Dx	K at Dx	Aldo at Dx	PRA at Dx	ARR at Dx	Age at Referral	BP at Referral	K at Referral	Aldo at Referral	PRA at Referral	ARR at Referral
1255-1	M	18	178/70	NA	NA	NA	NA	75	165/68	3.6	27.0	0.1	270.00
1270-1	F	36	NA	NA	NA	NA	NA	39	175/93	2.6	11.0	0.1	110.00
1291-1	F	16	NA	NA	NA	NA	NA	48	132/78	4.8	25.6	<0.15	≥170.67
1300-1	F	19	170/80	NA	NA	NA	NA	39	140-200/80-110	3.9-5.4	23.3	<0.15	>155.33
1317-1	F	5	NA	3.8	20.0	0.1	200.00	5	NA	NA	NA	NA	NA
1328-1	F	20	NA	3.4	19.3	0.1	193.00	36	140/100	NA	NA	NA	NA
1386-1	F	18	NA	3.2	19.3	<0.1	>193	18	130/90	4.6	NA	NA	NA
1415-1	M	18	NA	NA	NA	NA	NA	53	138/82	4.6	110.0	<0.6	>183.33
1417-1	F	15	NA	NA	NA	NA	NA	46	212/110	3.5	26.0	0.6	43.33
1426-1	M	17	NA	2.8[c]	22.0[c]	<0.6[c]	>36.67[c]	47	134/90	4.5	33.0	0.6	55.00
1447-1	F	17	200/100	NA	NA	NA	NA	24	150/100	4.5	17.0	0.2	85.00
1491-1	M	20	NA	NA	NA	NA	NA	54	203/113	3.9	28.4	<0.15	>189.33
1502-1	M	20	NA	NA	NA	NA	NA	54	138/96	NA	21.0	0.04	525.00
1504-1	M	20	NA	3.4	12.4	0.1	124.00	66	144/82	4.8	22.8	0.27	84.44
1562-1	M	11	NA	NA	NA	NA	NA	65	168/88	3.5	12.0	0.19	63.16
1566-1	M	16	NA	NA	NA	NA	NA	26	160/90	3.5	15.8	0.16	98.75
1572-1	F	16	180/110	NA	22[d]	<0.5[d]	>44.00[d]	21	155/85	3.3	13.0	0.28	46.43
1573-1	M	17	140/90	NA	NA	NA	NA	45	130/70	3.5	17.7	0.1	177.00
1590-1	F	15	NA	3.7	10.8	<0.15	>72.00	15	140/86	3.3	7.3	<0.15	>48.67
1645-1	M	34	208/104	NA	NA	NA	NA	43	138/88	2.7	16.0	0.22	72.73
1786-1	F	15	150/100	2.9	47.9	<1.0	>47.9	17	137/92	3.3	34.0	<0.6	>56.67

Subjects referred for genetic study of primary aldosteronism. ID, subject identification number; F, female; M, male; Dx, diagnosis; BP, blood pressure (mmHg); K, serum potassium (mmol/l, normal 3.5-5.5); Aldo, aldosterone (ng/dl); PRA, plasma renin activity

(ng/ml/h); ARR, aldosterone/renin ratio (ng/dl:ng/ml/h, values >20 with aldosterone >15 or borderline values in the presence of hypokalemia are considered indicative of primary aldosteronism); NA, not available. Subjects with *CLCN2* variants are shown in red. [a], at age 26 years; [b], at age 27 years; [c], before treatment at age 47 years; [d], at age 18 years.

Supplementary Table 4. Segregation of Very Rare Variants in Kindreds 318 and 1281 is Consistent with Paternity / Maternity

Chr	Position (hg19)	Ref base	Nonref base	Gene	ExAC	Genotypes		
						318-1 (index case)	318-2 (mother)	318-3 (father)
chr1	36933522	C	T	*CSF3R*	Novel	C**T**	CC	C**T**
chr10	49968457	C	T	*WDFY4*	Novel	C**T**	C**T**	CC
chr11	533586	G	C	*HRAS*	$8.3x10^{-6}$	G**C**	CC	G**C**
chr12	29649201	T	G	*OVCH1*	Novel	T**G**	T**G**	TT
chr12	95694096	T	C	*VEZT*	Novel	T**C**	TT	T**C**
chr15	81633821	T	A	*TMC3*	$6.5x10^{-5}$	T**A**	T**A**	AA
chr19	2808339	A	G	*THOP1*	Novel	A**G**	AA	A**G**
chr3	195594345	T	C	*TNK2*	Novel	T**C**	TT	T**C**
chr9	139890991	G	A	*CLIC3*	$4.7x10^{-5}$	G**A**	G**A**	GG

Chr	Position (hg19)	Ref base	Nonref base	Gene	ExAC	Genotypes		
						1281-1 (index case)	1281-2 (father)	1281-3 (mother)
chr15	90333774	T	C	*ANPEP*	Novel	T**C**	TT	T**C**
chr12	1967782	C	T	*CACNA2D4*	$1.7x10^{-5}$	C**T**	C**T**	CC
chr17	78899186	A	G	*RPTOR*	$9.2x10^{-5}$	A**G**	A**G**	AA
chr14	94942473	C	G	*SERPINA9*	Novel	C**G**	CC	C**G**
chr1	53555521	C	T	*SLC1A7*	$8.2x10^{-6}$	C**T**	C**T**	CC

Chr, chromosome; hg19, human genome version 19; Ref base, reference base; Nonref base, non-reference base; Het/ Hom var, heterozygous versus homozygous variant; ExAC, frequency in the ExAC database; AA change, amino acid change.

All variants were heterozygous in the index case and were genotyped by PCR and Sanger sequencing in both parents. The nonreference allele is marked in bold. Segregation was consistent with paternity / maternity for all variants tested.

Supplementary Table 5. Haplotypes flanking *CLCN2*^{Arg172Gln} in three kindreds support no recent shared ancestry.

Chr 3 Pos.	rs #	ExAC Freq	537 inferred haplotype	1786 inferred haplotype	3 inferred haplotype	537-1 Gntp	1786-1 Gntp	1786-2 Gntp	1786-3 Gntp	3-1 Gntp	3-4 Gntp	3-7 Gntp
184071017	rs41266265	0.265	**G**	**A**	**A**	G/G	G/A	A/A	G/A	G/A/	A/A	G/A
184071019	rs11920716	0.265	**T**	**T**	**C**	T/T	C/T	T/T	T/C	T/C	C/C	T/C
184071063	rs9820367	0.537	**G**	**C**	**C**	G/G	C/C	C/C	C/C	G/C	C/C	G/C
184075047	rs2228291	0.243	**A**	**A**	**A**	A/A	A/G	A/A	A/G	A/A	NA	A/A
184075850	***p.Arg172Gln***	Novel	**T**	**T**	**T**	C/T	C/T	C/T	C/T	C/T	C/T	C/T
184075958	rs41266271	0.285	**C**	**T**	**T**	C/C	C/T	T/T	T/C	C/T	T/T	C/T
184090266	rs6141	0.532	**C**	**T**	**T**	C/C	T/T	T/T	T/T	C/T	T/T	C/T
184099378	rs35929225	0.315	**C**	**A**	**A**	C/C	C/A	A/A	C/A	C/A	A/A	C/A
184101408	Novel	Novel	**G**	**G**	**G**	G/G	G/G	G/G	G/G	G/G	G/G	G/T
184289152	rs13069661	0.216	**A**	**A**	**A**	A/A	A/A	A/A	A/A	A/A	A/G	A/A
184293610	rs9823034	0.154	**T**	**T**	**T/C**	T/T	T/T	T/T	T/T	NA	T/C	T/C
184293769	rs7652597	0.329	**T**	**C/T**	**T/C**	T/T	C/T	C/T	C/T	T/C	T/C	T/C
184299068	rs9881589	0.181	**G**	**G**	**G/A**	G/G	G/G	G/G	G/G	G/A	G/A	G/A
184299167	rs1138510	0.316	**T**	**T**	**T/C**	T/T	T/T	T/T	T/T	T/C	T/C	T/C
184299414	rs2230596	0.224	**C**	**C**	**C/T**	C/C	C/C	C/C	C/C	NA	C/T	C/T
184428903	rs9872799	0.737	**G/T**	**G**	**T**	G/T	G/T	G/T	G/A	T/T	T/T	T/G

The inferred haplotypes for SNPs flanking the *CLCN2*^{Arg172Gln} mutation in 3 kindreds (537, 1786 and Family 3) are shown. The maximum haplotype shared by kindred 537 with either of the other two kindreds is indicated in peach, and the maximum haplotype shared by kindreds 1786 and Family 3 is indicated in gray. The maximum haplotype shared among all three kindreds is enclosed by the black box. To the right of the columns showing inferred haplotypes, the genotypes (Gntp) of SNPs at each position in each family member are indicated. Chr 3 Pos., position on chromosome 3 in hg19; rs#, SNP identifier in dbSNP database (the novel mutation

shared by all kindreds is denoted *p.Arg172Gln*); ExAC Freq, frequency of minor allele in ExAC database; NA, insufficient data to make a genotype call.

Supplementary Table 6. Enrichment of Rare *CLCN2* Variants with Large Effect in a Cohort of 35 Patients with PA Diagnosed by Age 10 years

	PA		Controls			
Gene	**Number of Variant Alleles**	**Number of Reference Alleles**	**Number of Variant Alleles**	**Number of Reference Alleles**	**OR (95% CI)**	**Fisher's Exact P-value**
CLCN2	8	154	6	7150	61.7 (18.5, 219.4)	$1.3x10^{-10}$

Heterozygous variants were filtered for rarity (allele frequency $\leq 10^{-5}$ across all samples in 1000 Genomes, EVS, and ExAC), and high-quality heterozygotes (pass GATK VQSR, minimum 8 total reads, GQ score ≥ 20). Only MetaSVM[17]-deleterious missense variants and loss-of-function (nonsense, canonical splice-site, frameshift indels) were included in the analysis. All variants were confirmed by *in silico* visualization of aligned reads. OR, odds radio; 95% CI, 95% confidence interval. A two-tailed Fisher's exact test was conducted to compare the frequency of rare heterozygotes among index cases to 3,578 independent autism parental controls.

Supplementary Table 7. Clinical Characteristics of Individuals from Family 3 without *CLCN2* p.Arg172Gln Variant

Subject ID	Gender	Age dx/blood draw	BP (mmHg)	K^+ (mM)	Aldo (ng/dl)	PRA (ng/ml/h)	ARR (ng/dl: ng/ml/h)	FST	CT Adrenals
Normal range			<140/90	3.5-5.5			< 20	neg	normal
1	F	66	168/98**	3.7	24.8	0.4	62.0	pos	normal
6	F	49	Normal*	4.2	4.9	10.2	0.5	N/A	NA
12	F	20	110/70	4.2	10.1	2.9	3.5	N/A	NA
19	M	41	144/94**	3.9	26.7	0.7	38.1	neg	normal
24	M	35	Normal*	4.7	8.9	2.2	4.0	N/A	NA

Age dx, age at diagnosis; BP, blood pressure; K^+, serum potassium level; Aldo, serum aldosterone level; PRA, plasma renin activity; ARR, aldosterone/renin ratio; FST, fludrocortisone suppression test; F, female; M, male; NA, not available; pos, positive; neg, negative; reference values are given below each parameter.

*Self reported by patient and not on any antihypertensive medications

**On no medications

Supplementary Table 10. Primer Sequences.

	Forward		Reverse
CLCN172F	5'-GGACCCCTGAAATGGGTGCATCG-3'	CLCN172R	5'-GTGAGTCGGGAGGGGGCCCGCCCTG-3'
CLCN865F	5'-GAGGAGTCTGACATCTGGGTCCAGAC-3'	CLCN865R	5'-CATTCTGGGCTGACGGGCATGGCTAGC-3'
CLCN2_4_5_F	5'-ACAGCCTGTCGTATCAGCG-3'	CLCN2_4_5_R	5'-GCCTGGCCTCCTCTTCC-3'
CLCK362SF	5'-CAGAGGACCTGAGGAGGCCTTAGTC-3'	CLCK362SR	5'-CTGCAGGAGCTGCCAGCCTTTGCTG-3'
clcm22Sf	5'-CTGGGAGAAGAGGAGTGGAGGCTC-3'	clcm22SR	5'-CAGGTCCCCTGCCCCCACCCCAG-3'
M22K_F	5'-GAGCAGACCCTGAAGTATGGCCGGTAC-3’	M22K_R	5'-GTACCGGCCATACTTCAGGGTCTGCTC-3’
Y26N_F	5'-GATGTATGGCCGGAACACTCAGGACC-3’	Y26N_R	5'-GGTCCTGAGTGTTCCGGCCATACATC-3’
R172Q_F	5'-GAAGACCATCTTGCAGGGAGTGGTGCTG-3’	R172Q_R	5'-CAGCACCACTCCCTGCAAGATGGTCTT C-3’
K362 del new_F	5’-CTTCCTCATGAGACGCCTGCTCTTC-3’	K362 del new_R	5’-GAAGAGCAGGCGTCTCATGAGGAAG-3’
CLCN2E9-11_2Fs	5’-TCCTGTTCTGCCTGTGGTTA-3’	CLCN2E9-11_2R	5’-CCTCTGCTGTTCTTCCCTTTAG-3’
K362gF	5'–CTTCCTCATGAGGTAGTGAGTGGCTCTC-3'	K362gR	5'-GAGAGCCACTCACTACCTCATGAGGAAG-3'
C2_E9-11SF	5’-CCCTCTTCAAAACCCGATTC-3’	C2_E9-11SR	5’-ATGAACTGTCCAAAGCCAGG-3’

Sequences of indicated primers (see also Online Methods and Supplementary Note).