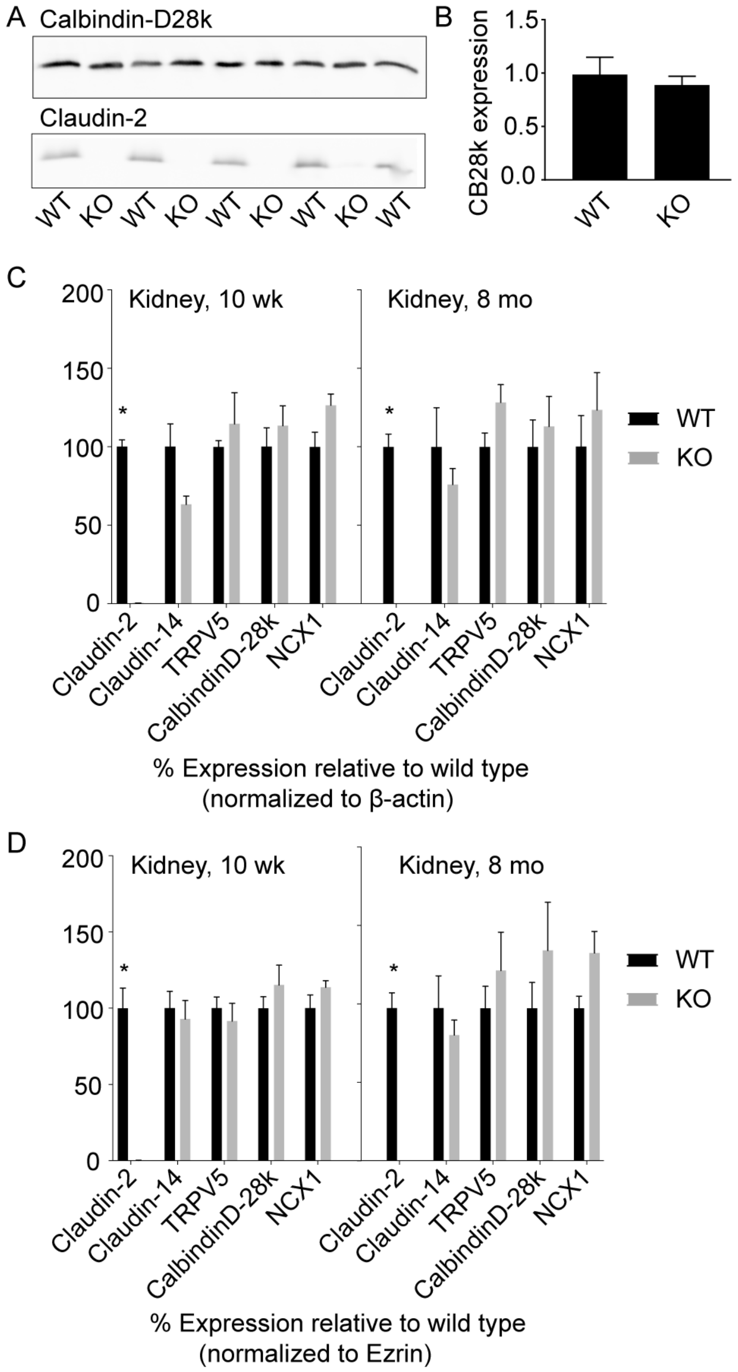
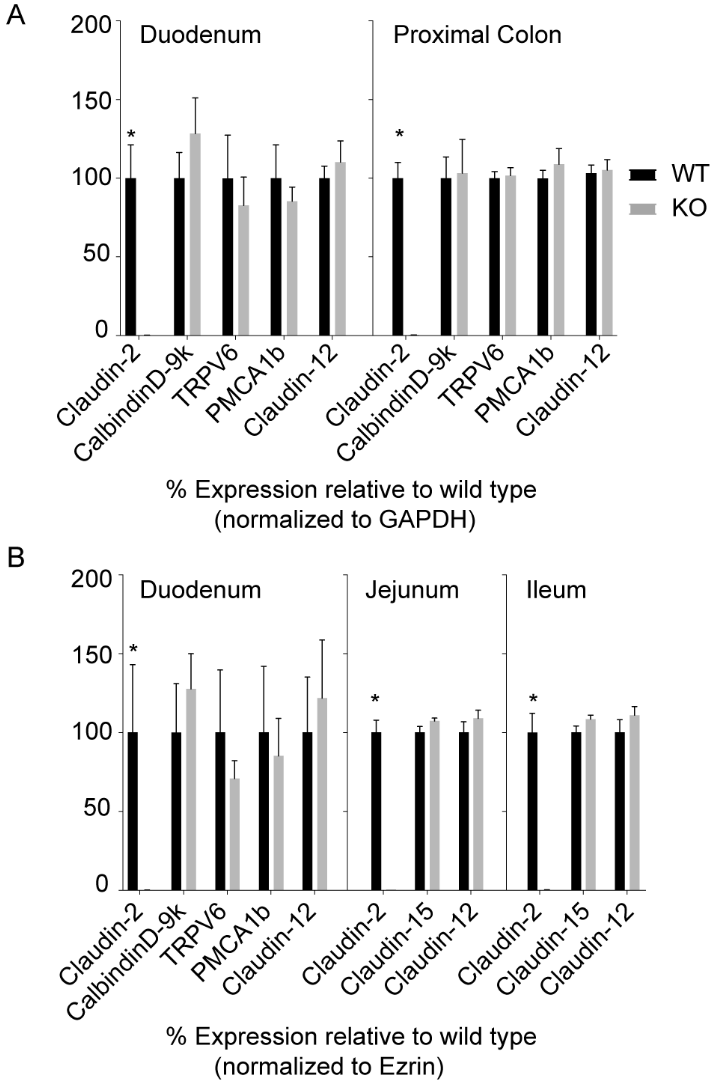
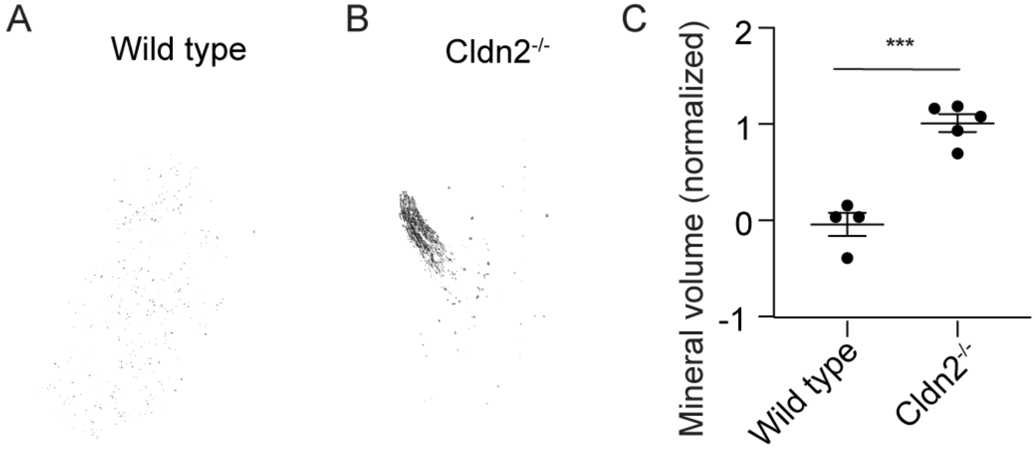


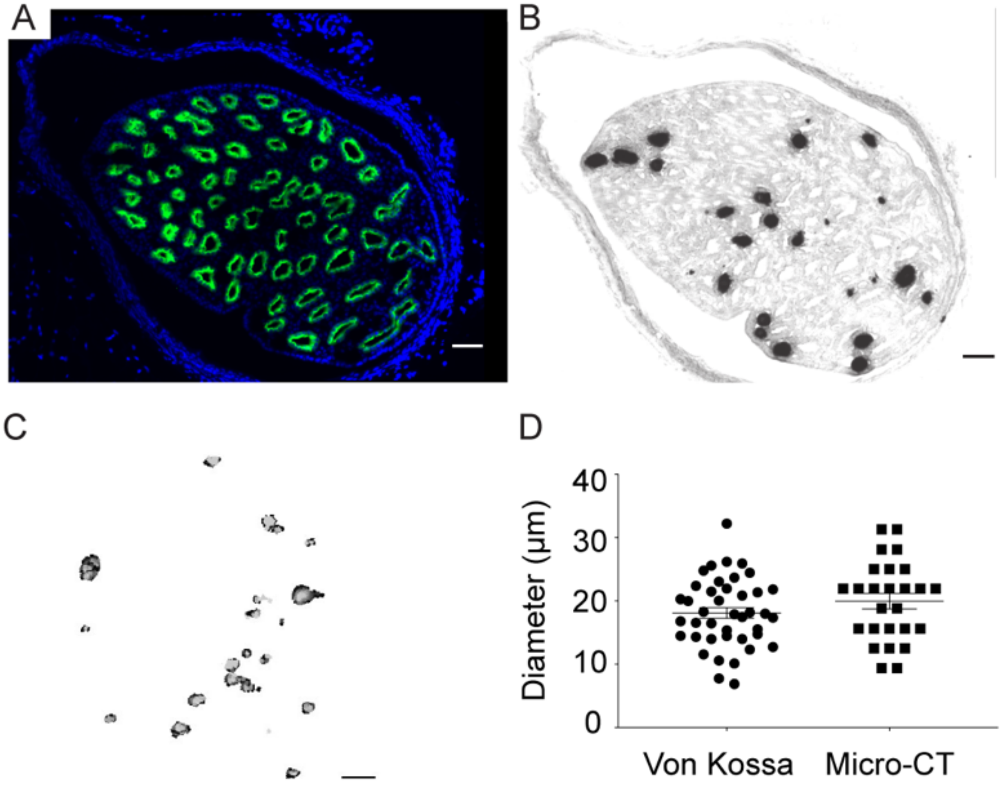
Supplementary Figure S1. Quantitation of renal calcium transporters in *Cldn2*$^{-/y}$ mice and wild-type controls. Quantitation of calcium transporters in kidneys of wild type (WT) and *Cldn2*$^{-/y}$ (KO) mice. (**A**) Western blotting for calbindinD-28k and claudin-2 and (**B**) quantitation of calbindin-D28k after normalization to β-actin protein expression. Quantitative PCR measurements of renal calcium transporter expression relative to (**C**) β-actin and (**D**) ezrin in 10-week old mice (left, 4 per group) and 8-month old mice (right, 6-9 per group) shows no difference in mRNA expression. Fold change values were calculated relative to β-actin or ezrin expression, and then data was normalized to 100% for wild type levels. Bars are mean ± SEM; *P >0.05 using unpaired two-tailed t-test.



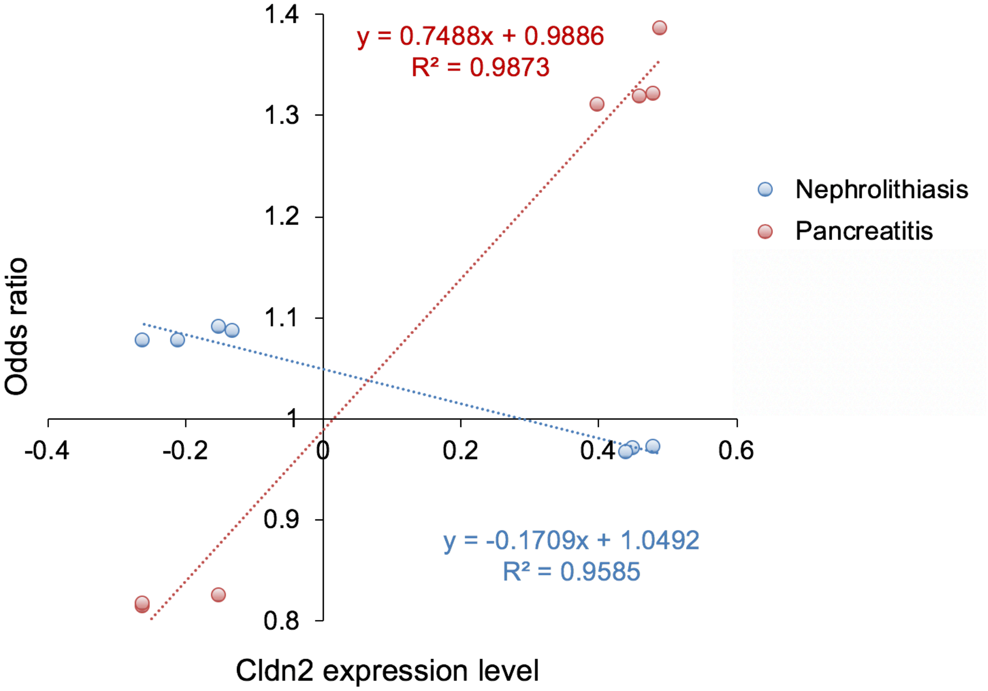
Supplementary Figure S2. Expression of intestinal calcium transporters in *Cldn2*$^{-/y}$ mice and wild-type controls. (A) Quantitative RT-PCR was used to measure expression of calcium transporters in the duodenum (left, 5 per group) and proximal colon (right, 6-13 per group) of wild type (WT) and *Cldn2*$^{-/y}$ (KO) mice relative to GAPDH. (**B**) Quantitative RT-PCR was used to measure expression of calcium transport proteins and claudins in the duodenum (left, 5 per group), jejunum (middle, 11-13 per group), and ileum (right, 11-13 per group) relative to ezrin. Fold change values were calculated relative to GAPDH or ezrin expression, and then data was normalized to 100% for wild type levels. No significant differences were detected other than for claudin-2. Bars are mean ± SEM; *P<0.05 using unpaired two-tailed t-test.



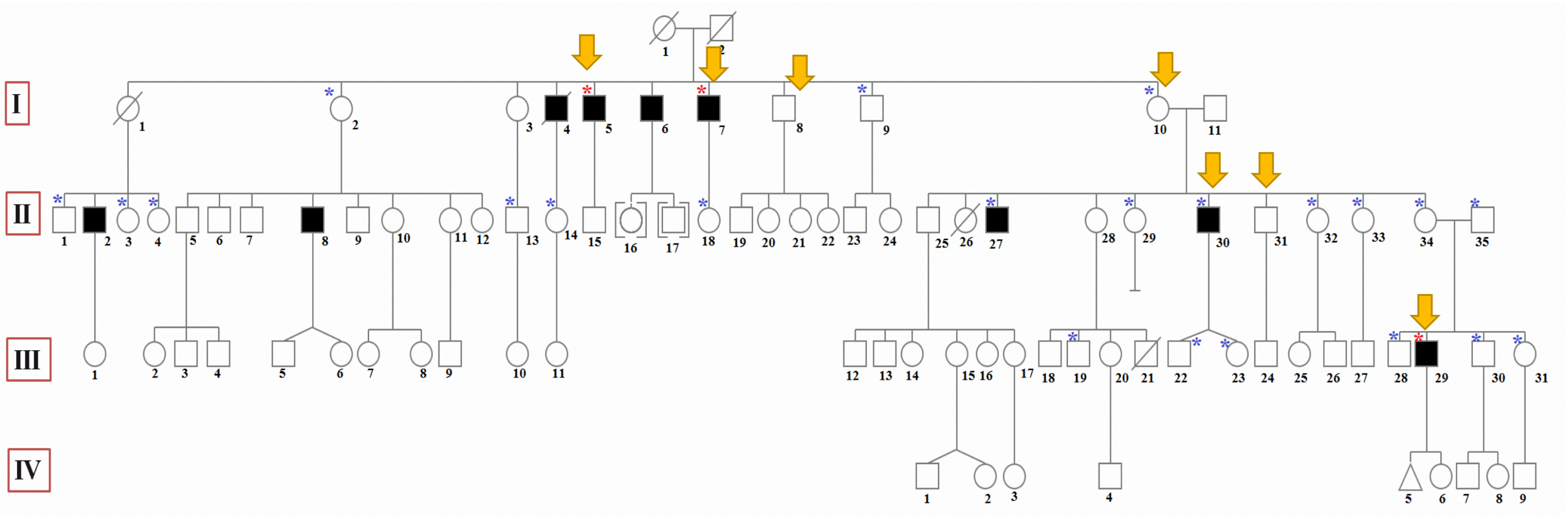
Supplementary Figure S3. Female Cldn2$^{-/-}$ mice develop nephrocalcinosis at 1 year. Representative 3D reconstructions of micro-CT analysis on kidneys from 1-year old female (**A**) wild type and (**B**) *Cldn2*$^{-/-}$ mice and (**C**) quantitation of the mineral volume in reconstruction analysis (n=4-5 per group). Data were normalized to wild type levels and log transformed prior to plotting for analysis. Bars are mean ± SEM; **** $P<0.001$ using unpaired two-tailed t-test.



Supplementary Figure S4. Representative cross-section images used for quantitation of Ca^{2+} deposit diameter. Papillary tissue from a 6 month-old *Cldn2*$^{-/y}$ mouse stained with (**A**) AQP2 (green) and DAPI (blue) in addition to (**B**) von Kossa. AQP2 and von Kossa staining never overlap. Two sections from separate *Cldn2*$^{-/y}$ animals were used for quantitation. (**C**) Micro-CT reconstruction slice from a 1 year-old *Cldn2*$^{-/y}$ mouse papilla in cross-section. Scale bars = 50µm. (**D**) Density diameter, measured using ImageJ, for Von Kossa images and micro-CT cross-section.



Supplementary Figure S5. Relationship between CLDN2 tissue expression and disease risk. Odds ratios for nephrolithiasis (current study) and for alcohol-related pancreatitis (ref. (44)) are plotted against pancreatic CLDN2 expression (normalized effect size, as defined in Table 2) from GTEx. Linear regression analysis shows CLDN2 expression is highly and reciprocally correlated with disease risk for chronic pancreatitis ($p<0.0001$) and nephrolithiasis ($p<0.0001$).



Supplementary Figure S6. Pedigree of a family with obstructive azoospermia. Black and white squares represent infertile and fertile men, respectively. Adopted children are shown in brackets. Subjects that participated in the study and underwent genotyping are marked with an asterisk. Individuals that had urine collection for calcium determination are indicated by yellow arrows.

Supplementary Table S1. Comparison of urine calcium in male and female mice.

	Male		Female	
	Wild type	Cldn2$^{-/y}$	Wild type	Cldn2$^{-/-}$
N	5	7	6	6
Urine Ca (µmol/24 hr)	3.14 ± 0.48	6.65 ± 0.73*	2.21 ± 0.65	4.89 ± 0.99*
Urine creatinine (µmol/24 hr)	5.67 ± 1.15	7.63 ± 0.97	11.30 ± 1.29	11.11 ± 0.86
Ca/creatinine ratio (mol/mol)	0.27 ± 0.02	0.63 ± 0.11*	0.37 ± 0.05	0.64 ± 0.09*
FECa (%)[†]	2.12 ± 0.24	7.64 ± 1.60*	4.92 ± 1.00	7.06 ± 1.28

Values are mean ± SE.

[†] Fractional excretion of calcium. Serum creatinine value for this calculation was measured using the enzymatic method.

*p < 0.05 compared to wild type.

Supplementary Table S2. Quantitative micro-CT analysis of femurs from 10 week old animals

	Wild type	*Cldn2*$^{-/y}$
Sample size (n)	5	4
Epiphyseal cancellous bone		
BS/BV	97.1 ± 2.4	92.6 ± 6.2
BV/TV trabecular	0.0426 ± 0.0049	0.0507 ± 0.0083
BV/TV cortical	0.992 ± 0.001	0.993 ± 0.000
Tb.N (1/mm)	3.65 ± 0.41	4.01 ± 0.06
Tb.Th (mm)	0.0305 ± 0.0025	0.0332 ± 0.0021
Tb.Sp (mm)	0.290 ± 0.043	0.247 ± 0.005
Connectivity (1/mm^3)	47.8 ± 13.7	74.6 ± 12.9
SMI (1)	3.08 ± 0.09	2.82 ± 0.12
Anisotropy (1)	1.55 ± 0.05	1.60 ± 0.08
MOI (mm^4)	0.532 ± 0.024	0.613 ± 0.021*
Ct.Th (mm)	0.154 ± 0.008	0.149 ± 0.011
Cortical bone		
MOI (mm^4)	0.337 ± 0.035	0.378 ± 0.058
BV (mm^3)	1.33 ± 0.09	1.51 ± 0.12
TV_{δ} (mm^3)	1.32 ± 0.09	1.50 ± 0.13
BV/TV_{δ}	0.988 ± 0.002	0.990 ± 0.001
TV_{α} (mm^3)	2.93 ± 0.15	3.36 ± 0.21
BV/TV_{α}	0.451 ± 0.008	0.447 ± 0.010
HAA		
Ct.Th (mm)	0.184 ± 0.009	0.188 ± 0.010
Ct.Ar		
BS/BV	11.5 ± 0.5	11.2 ± 0.5
BS (mm^2)	15.1 ± 0.4	16.8 ± 0.6
$MV_{\rho}=TV_{\alpha}-BV$ (mm^3)	1.60 ± 0.06	1.86 ± 0.09*
BA (mm^2)	0.758 ± 0.050	0.798 ± 0.068
TA (mm^2)	1.68 ± 0.08	1.78 ± 0.11
BA/TA	0.451 ± 0.008	0.447 ± 0.010
Periosteal circ. (mm)	4.58 ± 0.11	4.72 ± 0.15
Endosteal circ (mm)	3.39 ± 0.06	3.51 ± 0.09

BS/BV, bone surface/bone volume; BV/TV, bone volume/tissue volume; BMD, bone mineral density; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; SMI, structural model index;

Supplementary Table S3. Effect of stripping the seromuscular layer on colonic ion permeability.

	Unstripped	Stripped
N	7	10
TER ($\Omega \cdot cm^2$)	54.7 ± 2.27	51.9 ± 3.00
P_{Na}/P_{Cl}	0.918 ± 0.02	0.954 ± 0.02
P_{Na} (10^{-4} cm/s)	0.249 ± 0.01	0.264 ± 0.01
P_{Cl} (10^{-4} cm/s)	0.273 ± 0.01	0.278 ± 0.02
P_{Ca}/P_{Na}	7.31 ± 0.16	7.20 ± 0.14
P_{Ca} (10^{-4} cm/s)	1.82 ± 0.08	1.90 ± 0.10

Ion permeabilities were determined from biionic dilution potential measurements in Ussing chambers using proximal colon tissue from wild type male mice. Unstripped indicates full thickness intestinal tissue used and stripped group indicates experiments with seromuscular stripped off from the epithelial layer. Data presented as mean ± SEM. TER, transepithelial resistance; P_X, transepithelial permeability to X.

Supplementary Table S4. Calcium permeability in proximal colon of female mice**.**

	Wild type	Cldn2 $^{-/-}$
N	7	7
TER (Ω·cm^2)	46.1 ± 1.82	51.1 ± 3.23
P_{Na}/P_{Cl}	1.00 ± 0.03	0.948 ± 0.03
P_{Na} (10^{-4} cm/s)	0.292 ± 0.01	0.262 ± 0.02
P_{Cl} (10^{-4} cm/s)	0.296 ± 0.02	0.279 ± 0.02
P_{Ca}/P_{Na}	7.24 ± 0.12	6.94 ± 0.11
P_{Ca} (10^{-4} cm/s)	2.12 ± 0.09	1.73 ± 0.09 **

Ion permeabilities were determined from biionic dilution potential measurements in Ussing chambers using proximal colon tissue from female wild type and Cldn2$^{-/-}$ mice. All experiments were performed after stripping seromuscular layer from tissue. Data presented as mean ± SEM. **$P<0.01$ using unpaired two-tailed t-test. TER, transepithelial resistance; P_X, transepithelial permeability to X.

Supplementary Table S5. Characteristics of gene association study population

Stage	Sample type	Source[a]	Platform[b]	(Number of samples)	Number of samples	Female	(%)	Age[c]
GWAS study 1	Kidney stone	BBJ	OmniExpressExome_v10	(393)	6,246	1,531	(24.5%)	54.7 +/- 13.8
			OmniExpressExome_v12	(4,618)				
			OmniExpress + HumanExome_v10	(1,089)				
			OmniExpress + HumanExome_v11	(146)				
	Control	JPHC, J-MICC, ToMMo	OmniExpressExome_v12	(28,867)	28,867	17,490	(60.6%)	56.3 +/- 10.0
GWAS study 2	Kidney stone	BBJ	OmniExpressExome_v10	(912)	4,884	1,303	(26.7%)	62.5 +/- 12.0
			OmniExpressExome_v12	(3,227)				
			OmniExpress + HumanExome_v10	(257)				
			OmniExpress + HumanExome_v11	(488)				
	Control	BBJ	OmniExpressExome_v10	(29,757)	158,772	75,209	(47.4%)	63.0 +/- 14.4
			OmniExpressExome_v12	(99,491)				
			OmniExpress + HumanExome_v10	(10,583)				
			OmniExpress + HumanExome_v11	(18,941)				

[a] BBJ: Biobank Japan, JPHC: Japan Public Health Center-based Prospective Study, J-MICC: Japan Multi-Institutional Collaborative Cohort study, ToMMo: Tohoku Medical Megabank Organization, NCU: Nagoya City University.

[b] HumanExome_v10: 247,870 SNPs, HumanExome_v11: 242,901 SNPs, HumanOmniExpress: 730,525 SNPs, HumanOmniExpressExome_v10: 951,117 SNPs, HumanOmniExpressExome_v12: 964,193 SNPs.

[c] Mean +/- SD.

Supplementary Table S6. Meta-analysis of gene association data with all 12 SNPs

MarkerName	Allele1	Allele2	Effect	StdErr	P-value	Direction	HetISq	HetChiSq	HetDf	HetPVal
METAL_GWAS1										
rs7057398	t	c	0.0314	0.0172	0.06741	++	24.6	1.326	1	0.2495
rs55662019	a	t	-0.0519	0.0511	0.3095	--	0	0.682	1	0.4089
rs76106514	t	c	-0.0219	0.0702	0.7551	--	0	0.692	1	0.4056
rs187570171	c	g	0.0219	0.0702	0.755	++	0	0.696	1	0.4042
rs12837024	t	c	-0.0333	0.0183	0.06915	--	40.4	1.679	1	0.195
rs73247968	a	c	-0.0452	0.0248	0.06872	--	0	0.042	1	0.8382
rs12008279	a	g	0.0519	0.0502	0.3008	++	0	0.641	1	0.4232
rs5917027	t	c	0.0389	0.0421	0.356	++	0	0.109	1	0.7412
rs6523906	a	g	-0.0389	0.0421	0.356	--	0	0.109	1	0.7412
rs148778547	t	c	0.0144	0.0634	0.8204	++	0	0.767	1	0.3813
rs6622121	t	c	-0.0389	0.0421	0.356	--	0	0.109	1	0.7412
rs147829672	a	c	0.0674	0.0415	0.1045	-+	49.8	1.991	1	0.1582
METAL_GWAS2										
rs7057398	t	c	0.0266	0.0165	0.1066	++	9.2	1.102	1	0.2939
rs55662019	a	t	-0.1137	0.0457	0.0128	--	0	0.65	1	0.4201
rs76106514	t	c	0.0037	0.0684	0.9568	-+	0.1	1.001	1	0.317
rs187570171	c	g	-0.0037	0.0684	0.9563	+-	0	0.997	1	0.3179

rs12837024	t	c	-0.0261	0.0177	0.1396	--	0	0.76	1	0.3834
rs73247968	a	c	-0.0243	0.0241	0.3146	--	0	0.735	1	0.3914
rs12008279	a	g	0.1188	0.0455	0.009086	++	0	0.617	1	0.4322
rs5917027	t	c	0.1067	0.037	0.003957	++	0	0.007	1	0.9322
rs6523906	a	g	-0.1067	0.037	0.003957	--	0	0.007	1	0.9322
rs148778547	t	c	-0.0072	0.0635	0.9094	+-	0	0.989	1	0.32
rs6622121	t	c	-0.1059	0.037	0.004258	--	0	0.006	1	0.9398
rs147829672	a	c	0.0824	0.039	0.03457	++	0	0.807	1	0.3691
METAL_GWAS1+GWAS2										
rs7057398	t	c	0.0289	0.0119	0.01522	++	0	0.041	1	0.8404
rs55662019	a	t	-0.0862	0.0341	0.01136	--	0	0.813	1	0.3673
rs76106514	t	c	-0.0088	0.049	0.858	-+	0	0.068	1	0.7939
rs187570171	c	g	0.0088	0.049	0.858	+-	0	0.068	1	0.7939
rs12837024	t	c	-0.0296	0.0127	0.02007	--	0	0.08	1	0.7773
rs73247968	a	c	-0.0345	0.0173	0.04623	--	0	0.365	1	0.5456
rs12008279	a	g	0.0886	0.0337	0.008566	++	0	0.975	1	0.3234
rs5917027	t	c	0.0772	0.0278	0.005502	++	31.7	1.463	1	0.2264
rs6523906	a	g	-0.0772	0.0278	0.005502	--	31.7	1.463	1	0.2264
rs148778547	t	c	0.0036	0.0449	0.9357	+-	0	0.058	1	0.8098
rs6622121	t	c	-0.0767	0.0278	0.005783	--	30	1.429	1	0.2319

rs147829672	a	c	0.0754	0.0284	0.008005	++	0	0.069	1	0.7922

METAL_GWAS1, meta-analysis of males and females in Study 1

METAL_GWAS2, meta-analysis of males and females in Study 2

METAL_GWAS1+GWAS2, meta-analysis of Study 1 and Study 2

Effect, overall estimated effect size for Allele1

StdErr, overall standard error for effect size estimate

P-value, meta-analysis p-value

Direction, summary of effect direction for each of the two studies. "+" indicates increased risk with Allele1

HetISq, I^2 in the heterogeneity test

HetChiSq, chi-squared statistic in simple test of heterogeneity

HetDf, degrees of freedom for heterogeneity statistic

HetPVal, P-value for heterogeneity statistic

Supplementary Table S7. Primers used for qRT-PCR

Gene	**5'- Forward sequence -3'**	**5'- Reverse sequence -3'**	**Product Size (bp)**
Calbindin-D28k	GTGTGTCCTCTGCTGGTTATT	CCTGACCCAAACCTGCATTA	127
Calbindin-D9k	TTCAGTCAGAGTTCCCCAGC	CCATCGCCATTCTTATCCAGC	82
Claudin-12	AGGTATTCCCGAGCGGAGCCA	CCCGGAGGCTTCAGGGAACCA	56
Claudin-14	GCAGCTGCGGCAAAGGAGTCT	ACGGCCGTCTAATGGGTCCCT	136
Claudin-15	CTTCCCTACAAGCCTTCTACG	AGACAGTGGGACAAGAAATGG	138
Claudin-2	TGGCGTCCAACTGGTGGGCT	ACCGCCGTCACAATGCTGGC	120
GAPDH	CCCAGCAAGGACACTGAGCAAGAG	GGCTCCCTAGGCCCCTCCTGTTATT	116
NCX1	AGTCTCCCACCCAATGTTTC	CTCCTGTTTCTGCCTCTGTATC	106
PMCA1	TTAGAGAAGCCAGAATCAAGAAGT	CAGCATCAGTGTCATCAATAAGG	109
TRPV5	GAGGAAGTACAAGAGCAGCTATC	CCTGGACAAAGGAGGTGTTT	102
TRPV6	ACACACACTGCAGAAAGTCCA	TCAGAGCCTGGACATCGTTT	96
β-actin	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA	104
Ezrin	TTCCTACCTGGCTGAAACTTG	TGTGATGTCCTGGATGAGTTC	131