

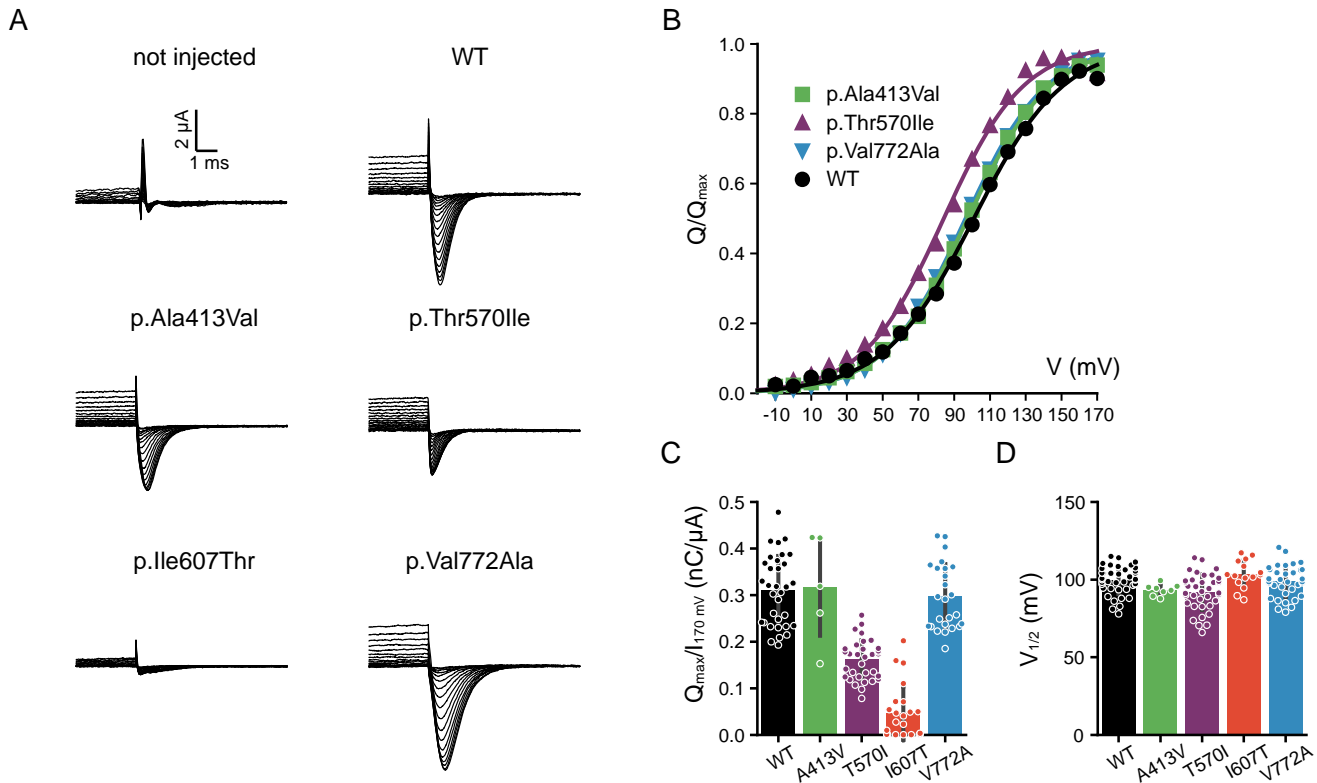
Supplemental information

Unique variants in *CLCN3*, encoding an endosomal anion/ proton exchanger, underlie a spectrum of neurodevelopmental disorders

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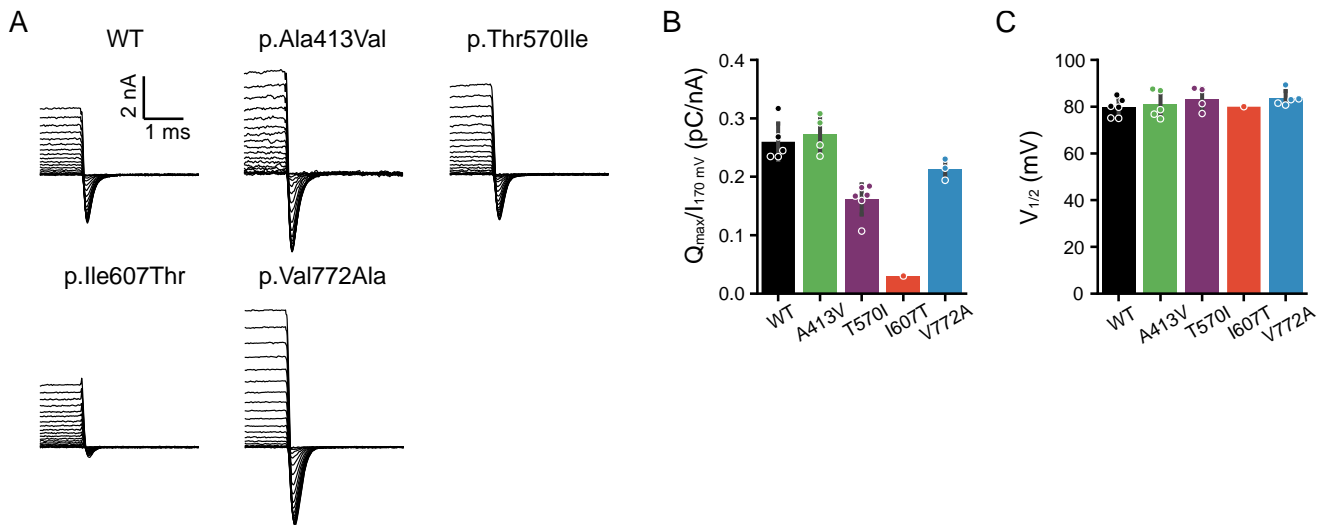
Supplemental Data

Suppl. Figure 1. Transient currents in *Xenopus* oocytes



A, typical current traces of WT and mutants in Cl^- free extracellular solution. Small differences in the kinetics of transient currents cannot be interpreted because of the limited time-resolution of the two-electrode voltage clamp technique. B, typical charge-voltage relationships (symbols) superimposed with fits of a Boltzmann distribution (lines). C, average ratio of maximal charge and current measured in high chloride at 170 mV. For variants p.Thr570Ile and p.Ile607Thr, values are significantly different from WT ($p < 0.01$). For variants p.Ala413Val and p.Val772Ala, values are not significantly different from WT ($p > 0.05$, Student's t-test). D, average voltage of half-maximal charge displacement. For none of the variants, values are significantly different from WT ($p > 0.05$, Student's t-test).

Suppl. Figure 2. Transient currents in HEK cells



A, typical transient current traces for the indicated constructs. The transient inward currents recorded at 0 mV after prepulses to voltages ranging from 200 mV to 0 mV were integrated and fitted with Boltzmann distributions as described in Methods. In these recording capacitive and leak currents were subtracted with a P/4 protocol as described in methods. Scale bars: 2 nA and 0.5 ms, respectively. B, average ratio of maximal charge and current measured at 170 mV (both in high chloride solution). For variants p.Thr570I and p.Ile607Thr, values are significantly different from WT ($p < 0.01$). For variants p.Ala413Val and p.Val772Ala, values are not significantly different from WT ($p > 0.05$, Student's t-test). C, average voltage of half-maximal charge displacement. For none of the variants, values are significantly different from WT ($p > 0.05$, Student's t-test). For variant p.Ile607Thr only in one case was the charge-voltage relationship of sufficient magnitude to allow the reliable fit of a Boltzmann distribution.

Suppl. Table 1: MRI findings for individuals with variants in *CLCN3*

Individual	Agenesis of the corpus callosum	Partial Agenesis of the corpus callosum	Thin corpus callosum	Enlarged perivascular space	Increased gyral folding	Simplified gyral folding	hyperintensity within the deep frontal white matter bilaterally	Prominent lateral ventricles	Hydrocephalus	Aqueductal stenosis	Pons hypoplasia	Disorganized cerebellar folia	Cerebellar hypoplasia	Prominent superior cerebellar peduncles	Delayed myelination	Decreased white matter volume	Small Hippocampi	Small Amygdala	Incomplete rotation of the hippocampi	Dysmorphic dentate nuclei	Prominent extra-axial spaces	Microcephaly	Plagiocephaly	
1																								
2		+		+							+													
3	+							+			+			+								+	+	
4																								
5							+																	
6					+																			
7			+		+							+												
8								+	+	+	+		+											
9		+	+												+	+			+	+				
10.1		+	+			+		+				+			+	+	+	+	+	+	+	+		
10.2		+	+					+				+			+	+	+	+	+	+	+	+		

MRI images 3, 5, 6, 7, 8, 9, 10.1 and 10.2 were all reviewed by a single pediatric radiologist at Boston Children's Hospital. No MRI was performed for individual 1, and the MRI from individual 2 and 3 were read by a radiologist at the clinical site.

Suppl. Table 2: Dysmorphic features for individuals with variants in *CLCN3*

Individual #	1	2	3	4	5	6	7	8	9	10. 1	10. 2
Microcephaly			+						+	+	+
Macrocephaly								+			
Brachycephaly						+					
Large fontanelle								+			
Metopic prominence									+		
Prominent forehead				+	+						
Long facies				+							
Midface retrusion									+		
Flat midface						+					
Full cheeks				+					+		
Low anterior hairline	+										
High anterior hairline					+						
Large ears	+										
Posteriorly rotated ears				+							
Low set ears								+			
Synophoris	+										
Bushy eyebrows	+			+							
Almond shaped eyes		+									
Hypertelorism					+			+			
Hypotelorism	+										
Epicanthic folds						+					
Down slanting palpebral fissures	+			+		+					
Bilateral ptosis							+				
Short base of nose											+
Bulbous nasal tip	+			+							
Pinched nares							+				
Prominent columella	+						+				
Short philtrum	+	+									
Long philtrum							+				
High arched palate	+			+							
Small mouth		+									
Arched everted upper lip		+									
Cupids bow upper lip	+										
Tented upper lip										+	+
Thin upper lip										+	+
Micrognathia		+				+					
Prognathism									+		
Wide spaced nipples									+		
Fifth finger clinodactyly					+						
Long digits						+					