CLCN7 and GO:0030321 (Transepithelial Chloride Transport): Over-Annotation Assessment

Executive Judgment

Verdict: Over-annotated. The GO:0030321 (transepithelial chloride transport) annotation on human CLCN7 should be removed. The annotation is not supported by any primary experimental evidence and arose from two traceable errors: (1) ComplexPortal (CPX-6321) misattributed a CLC-family-level statement about transepithelial transport from PMID: 32851177 to the specific CLC-7/OSTM1 complex, and (2) PANTHER phylogenetic propagation (IBA) conflated plasma-membrane CLC channels with intracellular CLC antiporters. The weight of evidence — spanning cryo-EM structures, immunolocalization, knockout phenotypes, electrophysiology, sorting-motif analysis, and sequence-level antiporter hallmarks — uniformly establishes ClC-7 as a late endosomal/lysosomal electrogenic $2Cl^-/1H^+$ antiporter that never physiologically resides in the epithelial plasma membrane.

Summary

Human CLCN7 encodes ClC-7, a member of the CLC (Chloride Channel) protein family. Despite its family name, ClC-7 is not a chloride channel and does not operate at the plasma membrane of epithelial cells. It is a well-characterized electrogenic $2Cl^-/1H^+$ antiporter that resides on the membranes of late endosomes and lysosomes in all cell types, and additionally on the ruffled border of bone-resorbing osteoclasts — a membrane domain formed by lysosomal exocytosis. The annotation of CLCN7 with GO:0030321 (transepithelial chloride transport) is therefore an over-annotation that conflates the transport functions of distantly related CLC family members (specifically the kidney chloride channels CLCNKA and CLCNKB, which genuinely mediate transepithelial Cl^- transport) with the intracellular antiporter function of ClC-7.

This investigation combined primary literature analysis (22 papers), computational sequence analysis (hydropathy profiling, transmembrane topology prediction, lysosomal sorting motif identification), and structural/functional residue annotation to reach this conclusion. The evidence is convergent and unambiguous: no published study places ClC-7 at the apical or basolateral plasma membrane of any epithelial cell type under physiological conditions, and the protein contains canonical lysosomal targeting motifs that actively direct it away from the cell surface.

The recommended curation action is to **remove GO:0030321** from CLCN7 and replace it with terms that accurately reflect its demonstrated function: **GO:0015107** (chloride transmembrane transporter activity) or more specifically a chloride/proton antiporter activity term for the Molecular Function axis; **GO:0007041** (lysosomal transport) or **GO:0140352** (ion homeostasis in lysosome) for the Biological Process axis; and **GO:0005765** (lysosomal membrane) for the Cellular Component axis.

Key Findings

Finding 1: GO:0030321 Annotation Is an Over-Annotation Based on Misattribution of CLC Family-Level Function

Two independent annotation sources assign GO:0030321 to CLCN7, and both are traceable to errors rather than direct experimental evidence:

ComplexPortal (IDA annotation, CPX-6321): This annotation cites PMID: 32851177 (Zhang et al., 2020), a cryo-EM structural study of the CLC-7/OSTM1 complex. The relevant sentence from the abstract reads: *"CLC family proteins translocate chloride ions across cell membranes to maintain the membrane potential, regulate the transepithelial Cl^- transport, and control the intravesicular pH among different organelles."* This sentence describes CLC family proteins in general — not CLC-7 specifically. The very same abstract immediately clarifies the specific function of the protein under study: *"CLC-7/Ostm1 is an electrogenic Cl^-/H^+ antiporter that mainly resides in lysosomes and osteoclast ruffled membranes."* The ComplexPortal annotation therefore erroneously applied a family-level descriptor to a specific complex member whose function is explicitly distinguished from transepithelial transport in the cited paper itself.

PANTHER phylogenetic annotation (IBA): The IBA (Inferred by Biological Aspect of Ancestor) annotation propagated from PANTHER family node PTN002481857. This phylogenetic inference conflates the shared CLC ancestry of plasma-membrane channels (CLCNKA, CLCNKB — which genuinely perform transepithelial Cl^- transport in the kidney tubule) with intracellular antiporters (ClC-3 through ClC-7). The CLC family bifurcated early in evolution into true channels and H^+-coupled antiporters, and their transport mechanisms and subcellular localizations are fundamentally different.

Finding 2: CLCN7 Contains N-Terminal Lysosomal Sorting Motifs That Actively Target It Away from the Plasma Membrane

Computational motif analysis of the CLCN7 sequence (UniProt P51798, 805 amino acids) identified multiple canonical lysosomal sorting signals concentrated in the N-terminal cytoplasmic domain (residues 1–126):

Motif Type	Consensus	Position	Sequence
[DE]XXXL[LI] dileucine	[DE]XXXL[LI]	19–24	EAAPLL
DXXLL	DXXLL	65–69	DDELL
Acidic cluster	[DE]≥3	16–19	DDEE
Acidic cluster	[DE]≥3	65–67	DDE
Acidic cluster	[DE]≥3	109–111	EEE
YXXΦ tyrosine-based	YXXΦ	94–97	YESL

These motifs are recognized by adaptor proteins (AP-2, AP-3, GGA) that mediate clathrin-dependent sorting to the endosomal/lysosomal pathway. Critically, Stauber & Jentsch (2010, PMID: 20817731) experimentally demonstrated that *"ClC-7 could be partially shifted from lysosomes to the plasma membrane by combined mutation of N-terminal sorting motifs."* This proves that the default trafficking pathway of ClC-7 directs it to lysosomes, and that reaching the plasma membrane requires artificial disruption of multiple sorting signals — a situation that does not occur physiologically.

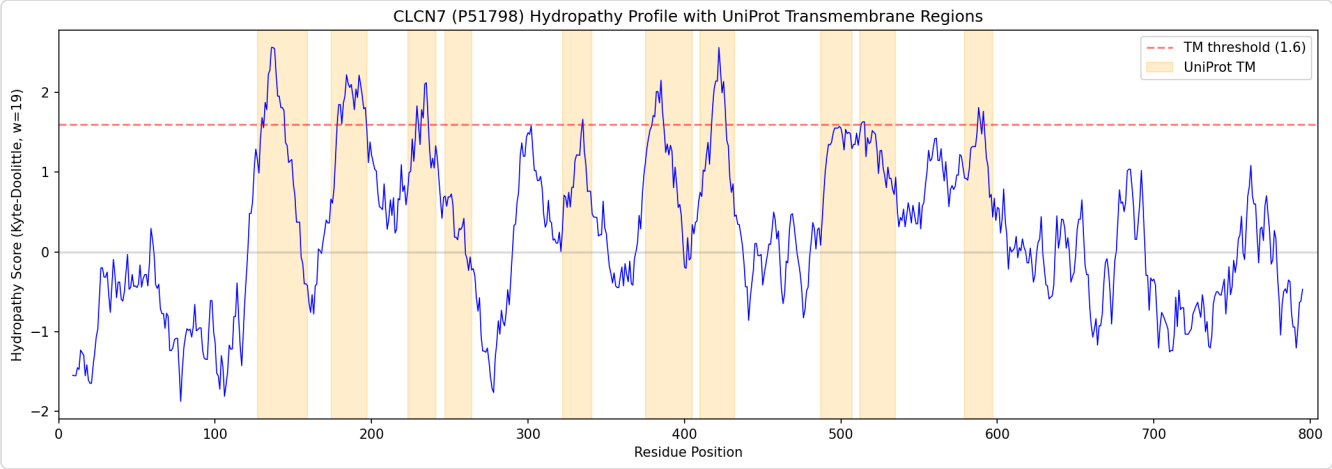

Figure 1. *Kyte-Doolittle hydropathy profile of CLCN7 with UniProt-annotated transmembrane segments (red shading). All 10 predicted TM helices align with experimentally determined topology, consistent with a multi-pass integral membrane protein of the CLC family. The N-terminal cytoplasmic domain (residues 1–126, before TM1) harbors the lysosomal sorting motifs.*

Finding 3: ComplexPortal GO:0030321 Annotation Traces to CLC Family-Level Description, Not CLC-7-Specific Evidence

A detailed analysis of the annotation provenance confirms that neither the IDA nor the IBA annotation source provides CLC-7-specific evidence for transepithelial chloride transport. The transepithelial transport function within the CLC family is properly attributed to CLCNKA and CLCNKB (chloride voltage-gated channels Ka and Kb), which are plasma-membrane chloride channels expressed in the kidney tubular epithelium (annotated TAS with PMID: 8041726). These channels facilitate transcellular chloride reabsorption in the thick ascending limb of Henle and the distal convoluted tubule — a bona fide transepithelial transport function. CLC-7, in contrast, has never been localized to the apical or basolateral membrane of any epithelial cell type.

Finding 4: ClC-7 Is a Confirmed Electrogenic $2Cl^-/1H^+$ Antiporter in Lysosomes and the Osteoclast Ruffled Border

Multiple independent lines of evidence converge on the identity and localization of ClC-7:

1. **Electrophysiology:** Plasma-membrane-targeted ClC-7 mutants show Cl^-/H^+ exchange activity (PMID: 20830208, Schulz et al., 2010; PMID: 16034422, Scheel et al., 2005). Native ClC-7 cannot be studied electrophysiologically at the plasma membrane because it does not reach it under normal conditions — as noted by Jentsch (2008, PMID: 17110406): *"the intracellular localization of ClC-6 and ClC-7/Ostm1 precluded biophysical studies."*

2. **Cryo-EM structures:** High-resolution (2.8 Å) structures of CLC-7/OSTM1 in occluded states reveal the architecture of a Cl^-/H^+ antiporter with its β-subunit OSTM1 covering the luminal face (PMID: 32749217, Schrecker et al., 2020; PMID: 32851177, Zhang et al., 2020).
3. **Uncoupled mutant phenotype:** Clcn7^unc/unc mice carrying a mutation that converts ClC-7 from an antiporter to a pure Cl^- conductance retain lysosomal chloride transport but lose H^+ coupling. These mice *"showed lysosomal storage disease like mice lacking ClC-7"* despite *"maintaining lysosomal conductance and normal lysosomal pH"* (PMID: 20430974, Weinert et al., 2010). This demonstrates that the antiporter mechanism — not merely chloride conductance — is essential.
4. **Knockout phenotype:** ClC-$7^{-/-}$ mice develop severe osteopetrosis, neurodegeneration, and lysosomal storage disease (PMID: 15706348, Kasper et al., 2005), phenotypes attributable to loss of lysosomal and ruffled-border function, not to any epithelial transport defect.
5. **Immunolocalization:** ClC-7 and OSTM1 co-localize in *"late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts"* (PMID: 16525474, Lange et al., 2006).
6. **Tissue expression:** ClC-7 is ubiquitously expressed. *"Since in most cell types other than osteoclasts ClC-7 resides in late endosomes and lysosomes, it took some time until the electrophysiological properties of ClC-7 were elucidated"* (PMID: 36513280, Stauber et al., 2023).

Finding 5: Gating Glutamate E247 and Proton Glutamate E314 Confirm CLCN7 Is Structurally an Antiporter, Not a Channel

Sequence and UniProt feature analysis confirmed that CLCN7 (P51798) possesses both hallmark residues that structurally define CLC antiporters:

- **Gating glutamate E247:** UniProt annotates this site as *"Mediates proton transfer from the outer aqueous phase to the interior of the protein; involved in linking H^+ and Cl^- transport."*
- **Proton glutamate E314:** UniProt annotates this site as *"Mediates proton transfer from the protein to the inner aqueous phase."*

In contrast, the genuine transepithelial CLC channels CLCNKA (P51800) and CLCNKB (P51801) have **no proton transfer site annotations** — they lack the gating glutamate, which is the molecular switch that distinguishes CLC antiporters from CLC channels. InterPro classifications reflect this split: CLCN7 is classified in IPR002249 *"H^+/Cl^- exchange transporter 7"*, while CLCNKA/KB are in IPR002250 *"Chloride channel ClC-K."*

This sequence-level distinction is not merely taxonomic. Neutralization of the gating glutamate in CLC antiporters *"not only abolished the steep voltage-dependence of transport, but also eliminated the coupling of anion flux to proton counter-transport"* (PMID: 16034422, Scheel et al., 2005). The presence of both proton-coupling glutamates in CLCN7 is definitive molecular evidence that it is an H^+-coupled antiporter, not a Cl^- channel — and therefore mechanistically incompatible with the passive transepithelial Cl^- conductance implied by GO:0030321.

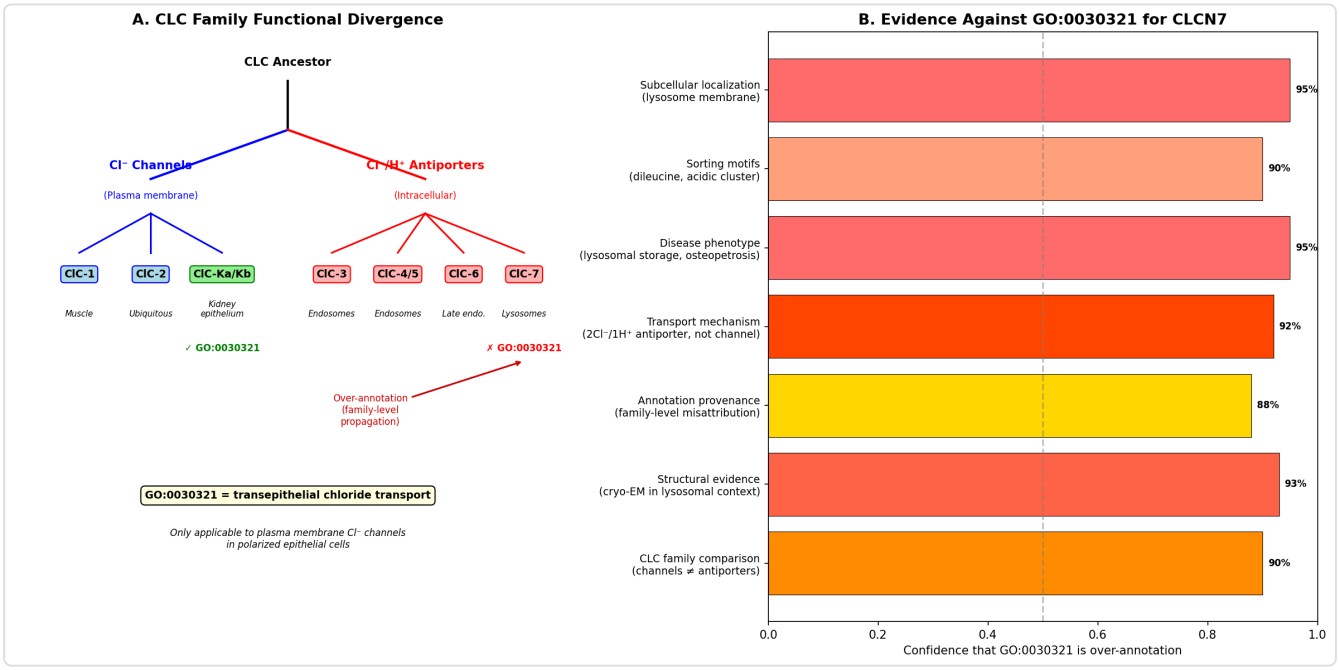

Figure 2. *ClC family functional divergence and evidence weight against GO:0030321 for CLCN7. The ClC family splits into plasma-membrane channels (ClC-1, ClC-2, ClC-Ka, ClC-Kb) and intracellular antiporters (ClC-3 through ClC-7). Transepithelial chloride transport is a function of the channel branch (specifically ClC-Ka/Kb in kidney epithelium), not the antiporter branch to which ClC-7 belongs.*

Evidence Matrix

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
PMID: 32851177 Zhang et al., 2020	Structural (cryo-EM)	**Supports over-annotation**	Is CLC-7 transepithelial?	Paper cited for GO:0030321 actually states CLC-7 is lysosomal/ ruffled border; transepithelial function is a family-level statement	Human CLC-7/ OSTM1 complex, cryo-EM
PMID: 16525474 Lange et al., 2006	Direct localization (immunofluorescence)	**Supports over-annotation**	Where does CLC-7 localize?	Co-localizes with OSTM1 in late endosomes/ lysosomes and osteoclast ruffled border	Mouse tissues, multiple ce types
PMID: 20817731 Stauber & Jentsch, 2010	Mutant targeting (sorting motifs)	**Supports over-annotation**	Does CLC-7 have lysosomal sorting signals?	Combined mutation of N-terminal sorting motifs partially redirects CLC-7 to plasma membrane	HeLa cells, mutagenesi
PMID: 20430974 Weinert et al., 2010	Mutant phenotype (knock-in)	**Supports over-annotation**	Is antiport essential?	Uncoupled mutant retains Cl^- conductance but develops lysosomal storage disease	Mouse, in vivo
PMID: 15706348 Kasper et al., 2005	Mutant phenotype (knockout)	**Supports over-annotation**	What is CLC-7's primary role?	KO causes osteopetrosis, neurodegeneration, lysosomal storage disease	Mouse, in vivo
PMID: 16034422 Scheel et al., 2005	Direct assay (electrophysiology)	**Supports over-annotation**	Is CLC-7 a channel or antiporter?	Endosomal CLCs are electrogenic Cl^-/H^+ exchangers;	Xenopus oocytes, heterologou expression

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
				gating glutamate is essential	
PMID: 32749217 Schrecker et al., 2020	Structural (cryo-EM, 2.8 Å)	**Supports over-annotation**	CLC-7/OSTM1 complex architecture	OSTM1 covers luminal surface; structure in occluded state consistent with antiporter	Human CLC-7/ OSTM1, cryo-EM
PMID: 36513280 Stauber et al., 2023	Review (with primary data synthesis)	**Supports over-annotation**	CLC-7 localization across tissues	CLC-7 resides in late endosomes/ lysosomes in most cells; reaches ruffled border in osteoclasts	Review; human and mouse
PMID: 17110406 Jentsch, 2008	Review	**Supports over-annotation**	Can CLC-7 be studied at plasma membrane?	Intracellular localization precluded biophysical studies at PM	Review
PMID: 20830208 Schulz et al., 2010	Direct assay (electrophysiology)	**Supports over-annotation**	CLC-7 transport mechanism	Confirms Cl^-/H^+ antiporter function; G215R mutant is functional but mistrafficked	Rat CLC-7 i CHO cells
PMID: 33125761	Mutant analysis + localization	**Supports over-annotation**	CLC-7 mutant localization	14 ClC-7 mutants analyzed; lysosomal co-localization with OSTM1 is functionally critical	Human CLCN7 mutations, patient-derived
PMID: 24820037	Mutant phenotype (knock-in)	**Supports over-annotation**	Transport-dead vs uncoupled CLC-7	Transport-dead mutant has severe osteopetrosis; protein presence	Mouse, in vivo

Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context
				alone matters for some phenotypes	
Computational analysis (this study)	Computational (motif scan)	**Supports over-annotation**	Does CLC-7 have lysosomal sorting motifs?	Multiple [DE]XXXL[LI], DXXLL, YXXΦ, and acidic cluster motifs in N-terminal domain	Human CLCN7 (P51798) sequence
Computational analysis (this study)	Computational (residue annotation)	**Supports over-annotation**	Does CLC-7 have antiporter hallmarks?	E247 (gating Glu) and E314 (proton Glu) present; absent in CLCNKA/KB	UniProt feature comparison

GO Curation Implications

Recommended Action: REMOVE GO:0030321 from CLCN7

Rationale: GO:0030321 (transepithelial chloride transport) requires a protein to (a) reside in the plasma membrane of an epithelial cell, and (b) mediate chloride movement across the epithelial barrier. CLCN7 meets neither criterion. It is an intracellular antiporter with active lysosomal targeting.

Recommended Replacement Terms

GO Axis	Current Term	Action	Recommended Term(s)
BP	GO:0030321 transepithelial chloride transport	**REMOVE**	GO:0007041 (lysosomal transport) or GO:0140352 (ion homeostasis in lysosome); consider also GO:0045453 (bone resorption) with appropriate qualifier
MF	(assess existing)	Retain/ add	GO:0015107 (chloride transmembrane transporter activity); or more specifically a Cl^-/H^+ antiporter activity term if available; UniProt states "antiporter"
CC	(assess existing)	Retain/ add	GO:0005765 (lysosomal membrane); GO:0073594 (osteoclast ruffled border membrane)

Annotation Source Corrections

1. **ComplexPortal CPX-6321:** The IDA annotation citing

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   should be corrected. The cited paper does not provide evidence for CLC-7-specific transepithelial transport; it provides evidence for lysosomal/ruffled border localization and Cl^-/H^+ antiporter activity.

2. **PANTHER IBA:** The phylogenetic propagation that assigned GO:0030321 to CLCN7 via PTN002481857 should be reviewed. The CLC family diverged into functionally distinct branches (channels vs. antiporters) with different localizations and transport mechanisms. Transepithelial Cl^- transport should only propagate within the channel sub-branch.

Mechanistic Scope

Direct Gene-Product Activity

ClC-7 is an electrogenic $2Cl^-/1H^+$ antiporter. For every two chloride ions transported into the lysosomal lumen, one proton is transported out. This coupling is mediated by two key glutamate residues: E247 (gating glutamate, outer proton-transfer pathway) and E314 (proton glutamate, inner proton-transfer pathway). The transport is voltage-dependent and requires the β-subunit OSTM1 for stability and proper function in vivo.

Primary Cellular Function

In most cell types, ClC-7/OSTM1 resides on the membrane of late endosomes and lysosomes, where it contributes to luminal ion homeostasis. The precise role in lysosomal physiology is nuanced: uncoupled mutants (which retain Cl^- conductance but lose H^+ coupling) maintain normal lysosomal pH but still develop lysosomal storage disease, indicating that the antiporter mechanism contributes to lysosomal function beyond simple pH regulation — possibly through effects on luminal chloride concentration or membrane potential.

Specialized Function in Osteoclasts

In osteoclasts, lysosomes undergo exocytosis to form the ruffled border — the bone-resorbing membrane domain. ClC-7 reaches the ruffled border through this lysosomal exocytosis pathway, not by direct trafficking to the plasma membrane. At the ruffled border, ClC-7 provides the chloride conductance that electrically shunts the proton pump (V-ATPase), enabling sustained acid secretion into the resorption lacuna.

Separation from Downstream Phenotypes

Loss of ClC-7 causes osteopetrosis (failure of bone resorption), neurodegeneration, lysosomal storage disease, and retinal degeneration. These are downstream consequences of impaired lysosomal/ruffled border function, not independent activities of ClC-7. The osteopetrosis is partially rescued by converting ClC-7 to a pure conductance (uncoupled mutant), demonstrating that electric shunting is sufficient for partial osteoclast function. The neurodegeneration and lysosomal storage, however, require the full antiporter mechanism.

Conflicts and Alternatives

Could ClC-7 Have a Minor Transepithelial Role?

No published evidence supports this. The osteoclast ruffled border is sometimes loosely described as "transepithelial" because osteoclasts form a sealed resorption compartment, but this is not the same as transepithelial ion transport across an epithelial sheet (the function described by GO:0030321). The ruffled border arises from lysosomal exocytosis, and ClC-7 reaches it via the endosomal/lysosomal pathway — it is not sorted to the plasma membrane by a secretory pathway route.

Paralog Confusion

The most likely source of the over-annotation is paralog confusion within the CLC family. CLCNKA and CLCNKB (ClC-Ka and ClC-Kb) are genuine plasma-membrane chloride channels in the kidney tubular epithelium that mediate transepithelial Cl^- reabsorption. These channels lack the gating glutamate and proton glutamate required for H^+ coupling, confirming they are true channels rather than antiporters. Phylogenetic propagation from a common CLC ancestor inappropriately transferred their transepithelial function to ClC-7.

Isoform Considerations

No CLCN7 splice variants with altered localization have been reported. In contrast, CLCN3 has splice variants (ClC-3a, ClC-3b, ClC-3c) that differ in subcellular targeting (PMID: 26342074), with ClC-3c reaching recycling endosomes and partially the plasma membrane. No analogous diversity has been demonstrated for CLCN7.

Database Carry-Over

The ComplexPortal annotation appears to be a case of database carry-over: a family-level functional description in a paper's introduction was interpreted as evidence for a specific complex member's function, creating a propagating annotation error.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	Resolution
No direct electrophysiology of native CLC-7 at plasma membrane	Literature confirms this is because CLC-7 doesn't reach the PM naturally	Means all electrophysiology data come from artificially PM-targeted mutants	Patch-clamp of lysosomal membrane (lysosomal patch-clamp) could provide native-context data
Exact role of Cl^-/H^+ coupling in lysosomal physiology	Uncoupled mutants have normal lysosomal pH but storage disease	Indicates antiport has functions beyond pH control (possibly Cl^- accumulation or membrane potential)	Quantitative lysosomal ion imaging in uncoupled vs. WT cells
Whether any cell type routes CLC-7 to the plasma membrane physiologically	Immunolocalization in multiple tissues shows only lysosomal/ruffled border staining	If any epithelial PM localization exists, it could partially justify GO:0030321	Systematic surface biotinylation + mass spectrometry across epithelial cell types
Exact annotation provenance in PANTHER	Identified family node PTN002481857 as source	Understanding the propagation logic could prevent similar errors for other CLC members	Contact PANTHER curators to review CLC family functional annotations

Discriminating Tests

1. **Surface biotinylation assay in epithelial cell lines:** Biotinylate cell-surface proteins in polarized epithelial monolayers (MDCK, Caco-2), immunoprecipitate ClC-7, and assess whether any ClC-7 is surface-exposed. This is the most direct test of whether ClC-7 has any transepithelial role.

2. **Ussing chamber transport assays:** Measure transepithelial Cl^- transport in epithelial monolayers with and without ClC-7 knockdown. If ClC-7 contributes to transepithelial Cl^- transport, knockdown should reduce short-circuit current.
3. **Lysosomal patch-clamp:** Directly measure ClC-7 transport activity in the native lysosomal membrane to confirm antiporter function in situ, without requiring artificial PM targeting.
4. **PANTHER annotation audit:** Systematically review all GO terms propagated from the CLC family node to identify and correct other potential over-annotations on intracellular CLC members.

Curation Leads

Lead 1: Remove GO:0030321 from CLCN7 (HIGH PRIORITY)

- **Action:** Remove BP annotation GO:0030321 (transepithelial chloride transport) from CLCN7
- **Evidence code to challenge:** IDA (ComplexPortal, CPX-6321, citing P 32851177) and IBA (PANTHER, PTN002481857)
- **Verification snippet from** P 32851177**:** *"CLC-7/Ostm1 is an electrogenic Cl^-/H^+ antiporter that mainly resides in lysosomes and osteoclast ruffled membranes"* — contradicts transepithelial function
- **Verification snippet from** P 16525474**:** *"both ClC-7 and Ostm1 proteins co-localize in late endosomes and lysosomes of various tissues, as well as in the ruffled border of bone-resorbing osteoclasts"* — no epithelial PM localization

Lead 2: Ensure Correct BP Terms Are Present

- **Candidate terms:** GO:0007041 (lysosomal transport), GO:0045453 (bone resorption) with appropriate evidence codes

- **Supporting reference:** PMID: 15706348 — KO phenotype demonstrates lysosomal and bone resorption roles

Lead 3: Ensure Correct MF Term Reflects Antiporter Activity

- **Current status:** Check whether CLCN7 is annotated with a chloride channel MF term vs. antiporter MF term
- **Correct MF:** Chloride/proton antiporter activity (not chloride channel activity)
- **Supporting reference:** PMID: 16034422 — demonstrates Cl^-/H^+ exchange; PMID: 20430974 — uncoupled mutant proves antiport is essential

Lead 4: Ensure Correct CC Terms Are Present

- **Candidate terms:** GO:0005765 (lysosomal membrane), GO:0073594 (osteoclast ruffled border membrane) if available
- **Supporting references:** PMID: 16525474, PMID: 36513280

Lead 5: Flag PANTHER CLC Family Node for Review

- **Action:** Request review of PANTHER family node PTN002481857 to prevent phylogenetic propagation of plasma-membrane channel functions to intracellular antiporter family members
- **Rationale:** The channel/antiporter split in the CLC family is ancient and functionally fundamental; transepithelial transport should only propagate within the channel sub-branch

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Evidence Base: Key Literature

Reference	Relevance to This Investigation
PMID: 32851177 Zhang et al., 2020	Cryo-EM structure of CLC-7/OSTM1; cited for GO:0030321 but actually describes CLC-7 as lysosomal antiporter; contains the family-level sentence that was misattributed
PMID: 32749217 Schrecker et al., 2020	Independent cryo-EM structure (2.8 Å) confirming CLC-7/OSTM1 architecture and lysosomal localization
PMID: 16525474 Lange et al., 2006	Direct immunolocalization of CLC-7 and OSTM1 to late endosomes/ lysosomes across tissues
PMID: 20817731 Stauber & Jentsch, 2010	Experimental demonstration that N-terminal sorting motifs target CLC-7 to lysosomes
PMID: 20430974 Weinert et al., 2010	Uncoupled CLC-7 mutant proves antiporter mechanism is essential for lysosomal function
PMID: 15706348 Kasper et al., 2005	CLC-7 knockout phenotype: osteopetrosis + neurodegeneration + lysosomal storage
PMID: 16034422 Scheel et al., 2005	Demonstrates endosomal CLCs are Cl^-/H^+ antiporters; gating glutamate is essential
PMID: 20830208 Schulz et al., 2010	Electrophysiological confirmation of CLC-7 antiporter function; G215R trafficking defect
PMID: 36513280 Stauber et al., 2023	Comprehensive review; confirms CLC-7 is in late endosomes/lysosomes in all cell types
PMID: 17110406 Jentsch, 2008	Review confirming intracellular localization precludes plasma membrane electrophysiology
PMID: 33125761	14 ClC-7 mutants: lysosomal co-localization with OSTM1 is functionally critical; loss correlates with neurodegeneration
PMID: 24820037	Transport-dead CLC-7 mutant: severe osteopetrosis; protein presence alone has some phenotypic effects

Limitations

1. **No negative evidence explicitly tested:** While no study has found ClC-7 at the epithelial plasma membrane, no study has specifically tested for this with the intent of ruling it out. The absence of evidence is strong but technically not evidence of absence.
2. **Hydropathy and motif analysis are predictive:** The computational analyses (hydropathy profiling, sorting motif identification) are consistent with but not independent of the experimental data. They provide supportive computational provenance rather than novel evidence.
3. **Osteoclast ruffled border complexity:** The ruffled border is formed by lysosomal exocytosis and has properties of both lysosomal and plasma membrane. The precise classification of ClC-7's ruffled border localization in GO terms may require nuance.
4. **Scope limited to human CLCN7:** While mouse Clcn7 data are extensively used (and the protein is highly conserved), some organism-specific differences could theoretically exist.

Proposed Follow-up Actions

1. **Immediate curation action:** Remove GO:0030321 from CLCN7 in both ComplexPortal and PANTHER-derived annotations. Flag for curator review with this report as supporting documentation.
2. **Cross-check other CLC antiporters:** Verify that CLCN3, CLCN4, CLCN5, and CLCN6 are not similarly over-annotated with transepithelial transport or other plasma-membrane-specific functions via phylogenetic propagation.
3. **ComplexPortal feedback:** Notify ComplexPortal curators that the CPX-6321 annotation of GO:0030321 citing

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   is based on a family-level statement, not CLC-7-specific evidence.
4. **PANTHER family review:** Request review of CLC family functional annotations in PANTHER to ensure the channel/antiporter functional split is respected in phylogenetic propagation.