Supplementary information

Criteria for inclusion of patient variants in the analysis

All included variants were missense, except that deletions of single amino acids were included without prejudice because several were recurrent in *ATP1A2* and *ATP1A3*. The few more complicated genetic variants, splice site variants, and premature stops were not considered. Variants were considered pathogenic if: 1) identical variants arose independently in two or more unrelated patients/families with similar symptoms; 2) variants caused alternative amino acid changes in a single codon, with symptoms on the spectrum; 3) variants occurred at the equivalent position in more than one paralog, with symptoms typical of that paralog; or 4) there was laboratory evidence of impairment of Na,K-ATPase activity or biosynthesis. Single-family variants not meeting those criteria were considered probably pathogenic if 1) variants were proven to be *de novo* with DNA from both parents, or 2) pedigree data showed segregation with only affected members of the family, and 3) were considered possibly pathogenic when there was minimal or ambiguous pedigree data. Variants that had no other supporting evidence were included if the amino acid was not at the aqueous surface of the protein where substitutions are likely to be tolerated, and if the variant did not appear more than once in ExAC or gnomAD. The criteria were applied for all three genes.

Because there are upwards of 700 known patients or families with variants in the Na,K-ATPase alpha subunits, but fewer than 230 reported variants, independent recurrences of variants are strongly suggestive of pathogenicity. When independent recurrences are used as a criterion of likely pathogenicity, there is of course the problem that benign variants present at detectable levels in a population would be false positives if not excluded. There were 4 variants in *ATP1A2* and 2 in *ATP1A3* that were present once each in gnomAD yet had strong laboratory data for being pathogenic, and so these were not excluded. We considered that these genes are highly constrained,[1,2] and gnomAD may have some pathogenic variants because it contains sequence studies motivated by clinical indications. The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) had 11 novel single variants in *ATP1A3* that look possibly or probably pathogenic based on our analysis, but they were excluded because we lack access to the clinical data. Seven other *ATP1A3* variants found in ClinVar were included because they were an alternative variant (i.e. different amino acid) of a codon already considered pathogenic in either gene. This increased the number of reported distinct variants, but did not affect the structure or homology analyses because the relevant amino acids were already included. Finally, we excluded variants, even if published, if there were two or more of the same variant in gnomAD, and/or if there was laboratory evidence of no functional effect. These are listed in table e-4 below.

1. Petrovski, S, Wang, Q, Heinzen, EL et al. Genic intolerance to functional variation and the interpretation of personal genomes. PLOS Genet 2013;9**,**e1003709.
2. Lek, M, Karczewski, KJ, Minikel, EV et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536**,**285-291.

Figure e-1. High homology of the four human Na,K-ATPase alpha subunits

Clustal Omega alignment. The notations are as follows: *, sequence identity in all four genes; **:**, conservation between side chains with strongly similar properties; **.**, conservation between side chains with weakly similar properties; **-**, gap inserted for alignment; ▮, gap equivalent to an indel of one amino acid. These paralog indels are exposed at the distal point of the N domain where they have minimal impact on structure.

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ATP1A4      MGLWGKKGTVAPHDQSPRRRPKKGLIKKKMVKREKQKRNMEELKKEVVMDDHKLTLEELS   60
ATP1A1      MGKGVGRDKY-----EPAAVSE---QGDKKGKKGKKDRDMDELKKEVSMDDHKLSLDELH   52
ATP1A2      MGRGAGRE-Y-----SPAA-TT---AENGGGKKKQKEKELDELKKEVAMDDHKLSLDELG   50
ATP1A3      MGDKKDDK-D-----S------------PKKNKGKERRDLDDLKKEVAMTEHKMSVEEVC   42
            **             .              :: :: :::::***** * :**::::*:

ATP1A4      TKYSVDLTKGHSHQRAKEILTRGGPNTVTPPPTTPEWVKFCKQLFGGFSLLLWTGAILCF   120
ATP1A1      RKYGTDLSRGLTSARAAEILARDGPNALTPPPTTPEWIKFCRQLFGGFSMLLWIGAILCF   112
ATP1A2      RKYQVDLSKGLTNQRAQDVLARDGPNALTPPPTTPEWVKFCRQLFGGFSILLWIGAILCF   110
ATP1A3      RKYNTDCVQGLTHSKAQEILARDGPNALTPPPTTPEWVKFCRQLFGGFSILLWIGAILCF   102
             ** .*  :* :  :* ::*:* ***::*********:***:*******:*** ******

ATP1A4      VAYSIQIYFNEEPTKDNLYLSIVLSVVVIVTGCFSYYQEAKSSKIMESFKNMVPQQALVI   180
ATP1A1      LAYSIQAATEEEPQNDNLYLGVVLSAVVIITGCFSYYQEAKSSKIMESFKNMVPQQALVI   172
ATP1A2      LAYGIQAAMEDEPSNDNLYLGVVLAAVVIVTGCFSYYQEAKSSKIMDSFKNMVPQQALVI   170
ATP1A3      LAYGIQAGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIMESFKNMVPQQALVI   162
            :**.**   :::*  *****.:**:.***:****************:*************

ATP1A4      RGGEKMQINVQEVVLGDLVEIKGGDRVPADLRLISAQGCKVDNSSLTGESEPQSRSPDFT   240
ATP1A1      RNGEKMSINAEEVVVGDLVEVKGGDRIPADLRIISANGCKVDNSSLTGESEPQTRSPDFT   232
ATP1A2      REGEKMQINAEEVVVGDLVEVKGGDRVPADLRIISSHGCKVDNSSLTGESEPQTRSPEFT   230
ATP1A3      REGEKMQVNAEEVVVGDLVEIKGGDRVPADLRIISAHGCKVDNSSLTGESEPQTRSPDCT   222
            * ****.:*.:***:*****:*****:*****:**:.****************:***: *

ATP1A4      HENPLETRNICFFSTNCVEGTARGIVIATGDSTVMGRIASLTSGLAVGQTPIAAEIEHFI   300
ATP1A1      NENPLETRNIAFFSTNCVEGTARGIVVYTGDRTVMGRIATLASGLEGGQTPIAAEIEHFI   292
ATP1A2      HENPLETRNICFFSTNCVEGTARGIVIATGDRTVMGRIATLASGLEVGRTPIAMEIEHFI   290
ATP1A3      HDNPLETRNITFFSTNCVEGTARGVVVATGDRTVMGRIATLASGLEVGKTPIAIEIEHFI   282
            .:******** *************:*: *** *******:*:***  *:**** ******

ATP1A4      HLITVVAVFLGVTFFALSLLLGYGWLEAIIFLIGIIVANVPEGLLATVTVCLTLTAKRMA   360
ATP1A1      HIITGVAVFLGVSFFILSLILEYTWLEAVIFLIGIIVANVPEGLLATVTVCLTLTAKRMA   352
ATP1A2      QLITGVAVFLGVSFFVLSLILGYSWLEAVIFLIGIIVANVPEGLLATVTVCLTLTAKRMA   350
ATP1A3      QLITGVAVFLGVSFFILSLILGYTWLEAVIFLIGIIVANVPEGLLATVTVCLTLTAKRMA   342
            ::** *******:** ***:* * ****:******************************

ATP1A4      RKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMWFDMTVYEADTTEEQTGKTFT   420
ATP1A1      RKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMWFDNQIHEADTTENQSGVSFD   412
ATP1A2      RKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMWFDNQIHEADTTEDQSGATFD   410
ATP1A3      RKNCLVKNLEAVETLGSTSTICSDKTGTLTQNRMTVAHMWFDNQIHEADTTEDQSGTSFD   402
            ****************************************  ::******:*:* :*

ATP1A4      KSSDTWFMLARIAGLCNRADFKANQEILPIAKRATTGDASESALLKFIEQSYSSVAEMRE   480
ATP1A1      KTSATWLALSRIAGLCNRAVFQANQENLPILKRAVAGDASESALLKCIELCCGSVKEMRE   472
ATP1A2      KRSPTWTALSRIAGLCNRAVFKAGQENISVSKRDTAGDASESALLKCIELSCGSVRKMRD   470
ATP1A3      KSSHTWVALSHIAGLCNRAVFKGGQDNIPVLKRDVAGDASESALLKCIELSSGSVKLMRE   462
            * * **  *::******** *:. *: : : ** .:********** ** . .**  **:

ATP1A4      KNPKVAEIPFNSTNKYQMSIHLREDSSQT▮HVLMMKGAPERILEFCSTFLLNGQEYSMND   539
ATP1A1      RYAKIVEIPFNSTNKYQLSIHKNPNTSEPQHLLVMKGAPERILDRCSSILLHGKEQPLDE   532
ATP1A2      RNPKVAEIPFNSTNKYQLSIHEREDSPQS▮HVLVMKGAPERILDRCSTILVQGKEIPLDK   529
ATP1A3      RNKKVAEIPFNSTNKYQLSIHETEDPNDNRYLLVMKGAPERILDRCSTILLQGKEQPLDE   522
            :  *:.***********:***   :  :  ::*:*********: **::*:.*:*  ::.

ATP1A4      EMKEAFQNAYLELGGLGERVLGFCFLNLPS▮SFSKGFPFNTDEINFPMDNLCFVGLISMI   598
ATP1A1      ELKDAFQNAYLELGGLGERVLGFCHLFLPDEQFPEGFQFDTDDVNFPIDNLCFVGLISMI   592
ATP1A2      EMQDAFQNAYMELGGLGERVLGFCQLNLPSGKFPRGFKFDTDELNFPTEKLCFVGLMSMI   589
ATP1A3      EMKEAFQNAYLELGGLGERVLGFCHYYLPEEQFPKGFAFDCDDVNFTTDNLCFVGLMSMI   582
            *:::******:************   **. .* .** *: *::**  ::******:***

ATP1A4      DPPRAAVPDAVSKCRSAGIKVIMVTGDHPITAKAIAKGVGIISEGTETAEEVAARLKIPI   658
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ATP1A1      DPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKGVGIISEGNETVEDIAARLNIPV   652
ATP1A2      DPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKGVGIISEGNETVEDIAARLNIPM   649
ATP1A3      DPPRAAVPDAVGKCRSAGIKVIMVTGDHPITAKAIAKGVGIISEGNETVEDIAARLNIPV   642
            ***********.*********************************.**.*::****:**:

ATP1A4      SKVDASAAKAIVVHGAELKDIQSKQLDQILQNHPEIVFARTSPQQKLIIVEGCQRLGAVV   718
ATP1A1      SQVNPRDAKACVVHGSDLKDMTSEQLDDILKYHTEIVFARTSPQQKLIIVEGCQRQGAIV   712
ATP1A2      SQVNPREAKACVVHGSDLKDMTSEQLDEILKNHTEIVFARTSPQQKLIIVEGCQRQGAIV   709
ATP1A3      SQVNPRDAKACVIHGTDLKDFTSEQIDEILQNHTEIVFARTSPQQKLIIVEGCQRQGAIV   702
            *:*:   *** *:**::***: *:*:*:**: * ******************** **:*

ATP1A4      AVTGDGVNDSPALKKADIGIAMGISGSDVSKQAADMILLDDNFASIVTGVEEGRLIFDNL   778
ATP1A1      AVTGDGVNDSPALKKADIGVAMGIAGSDVSKQAADMILLDDNFASIVTGVEEGRLIFDNL   772
ATP1A2      AVTGDGVNDSPALKKADIGIAMGISGSDVSKQAADMILLDDNFASIVTGVEEGRLIFDNL   769
ATP1A3      AVTGDGVNDSPALKKADIGVAMGIAGSDVSKQAADMILLDDNFASIVTGVEEGRLIFDNL   762
            ******************:****:**********************************

ATP1A4      KKSIMYTLTSNIPEITPFLMFIILGIPLPLGTITILCIDLGTDMVPAISLAYESAESDIM   838
ATP1A1      KKSIAYTLTSNIPEITPFLIFIIANIPLPLGTVTILCIDLGTDMVPAISLAYEQAESDIM   832
ATP1A2      KKSIAYTLTSNIPEITPFLLFIIANIPLPLGTVTILCIDLGTDMVPAISLAYEAAESDIM   829
ATP1A3      KKSIAYTLTSNIPEITPFLLFIMANIPLPLGTITILCIDLGTDMVPAISLAYEAAESDIM   822
            **** ***************:**:  *******:********************* ******

ATP1A4      KRLPRNPKTDNLVNHRLIGMAYGQIGMIQALAGFFTYFVILAENGFRPVDLLGIRLHWED   898
ATP1A1      KRQPRNPKTDKLVNERLISMAYGQIGMIQALGGFFTYFVILAENGFLPIHLLGLRVDWDD   892
ATP1A2      KRQPRNSQTDKLVNERLISMAYGQIGMIQALGGFFTYFVILAENGFLPSRLLGIRLDWDD   889
ATP1A3      KRQPRNPRTDKLVNERLISMAYGQIGMIQALGGFFSYFVILAENGFLPGNLVGIRLNWDD   882
            ** *** :**:***.***.************.***:********** *  *:*:*:.*:*

ATP1A4      KYLNDLEDSYGQQWTYEQRKVVEFTCQTAFFVTIVVVQWADLIISKTRRNSLFQQGMRNK   958
ATP1A1      RWINDVEDSYGQQWTYEQRKIVEFTCHTAFFVSIVVVQWADLVICKTRRNSVFQQGMKNK   952
ATP1A2      RTMNDLEDSYGQEWTYEQRKVVEFTCHTAFFASIVVVQWADLIICKTRRNSVFQQGMKNK   949
ATP1A3      RTVNDLEDSYGQQWTYEQRKVVEFTCHTAFFVSIVVVQWADLIICKTRRNSVFQQGMKNK   942
            : :**:******:*******:*****:****.:*********:*.******:*****:**

ATP1A4      VLIFGILEETLLAAFLSYTPGMDVALRMYPLKITWWLCAIPYSILIFVYDEIRKLLIRQH  1018
ATP1A1      ILIFGLFEETALAAFLSYCPGMGVALRMYPLKPTWWFCAFPYSLLIFVYDEVRKLIIRRR  1012
ATP1A2      ILIFGLLEETALAAFLSYCPGMGVALRMYPLKVTWWFCAFPYSLLIFIYDEVRKLILRRY  1009
ATP1A3      ILIFGLFEETALAAFLSYCPGMDVALRMYPLKPSWWFCAFPYSFLIFVYDEIRKLILRRN  1002
            :****::*** ******* *** ********* :**:**:***:***:***:***::*:

ATP1A4      PDGWVERETYY 1029
ATP1A1      PGGWVEKETYY 1023
ATP1A2      PGGWVEKETYY 1020
ATP1A3      PGGWVEKETYY 1013
            * ****:****
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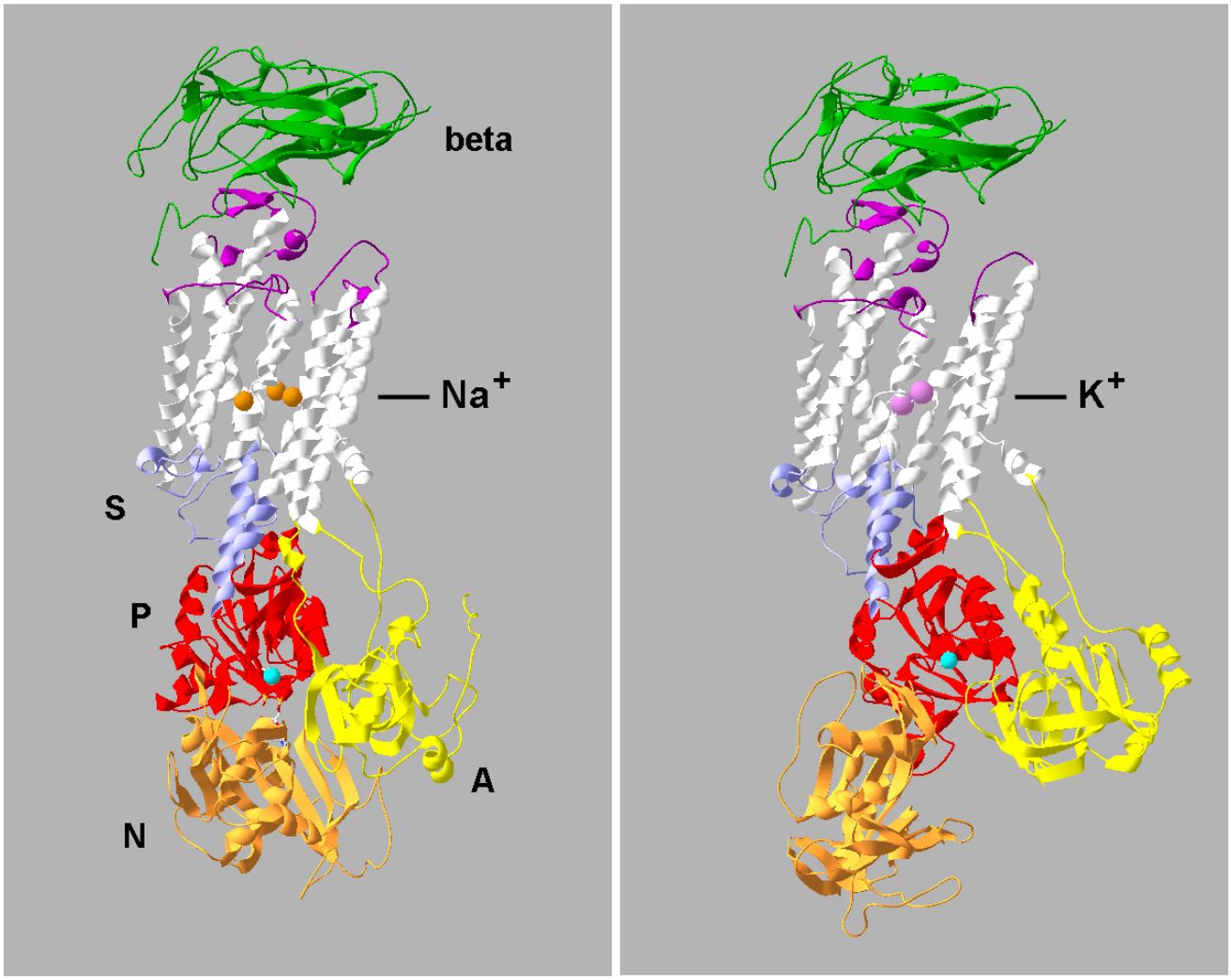
Figure e-2. Conformation differences when Na^+ or K^+ ions are bound

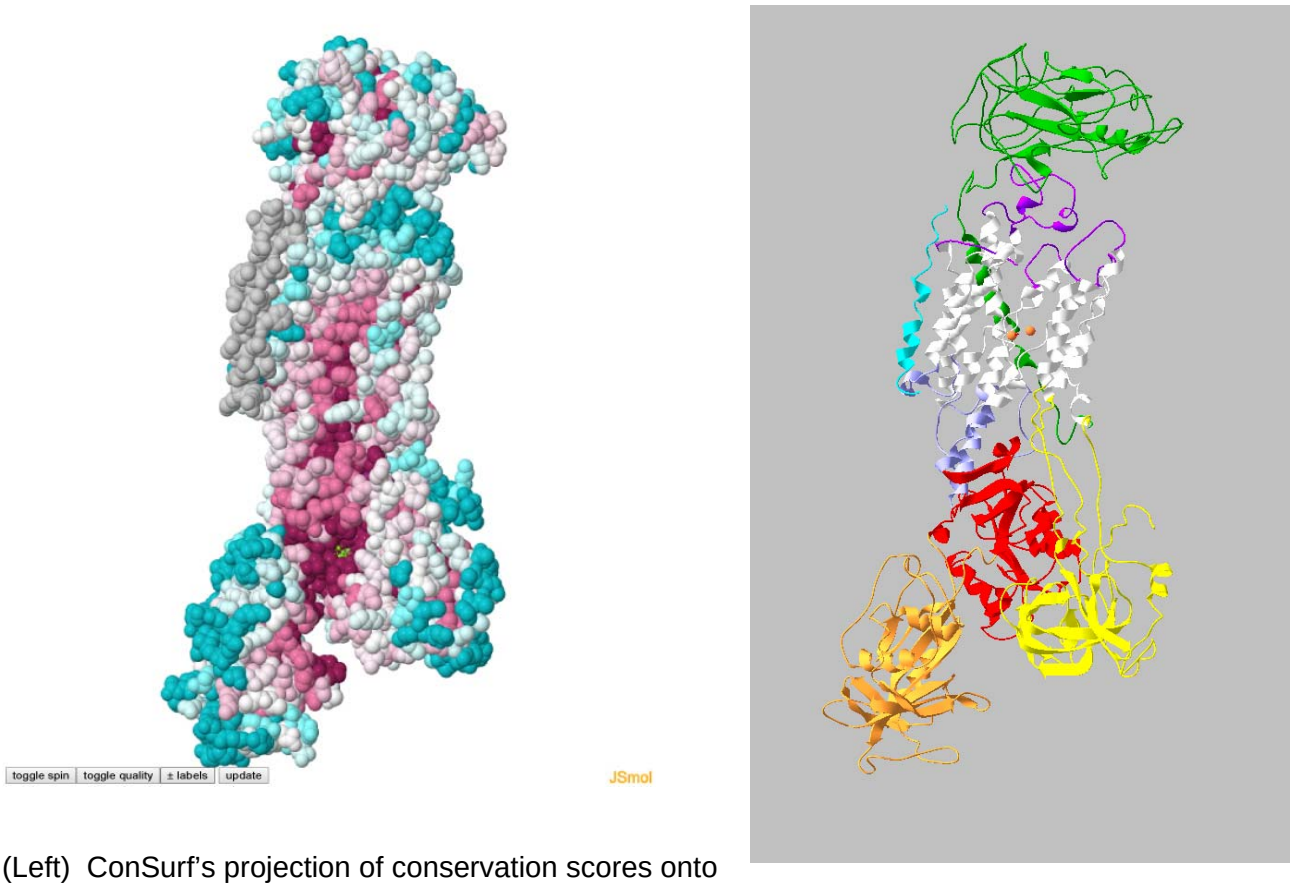
Ribbon views of Na,K-ATPase in two conformations. The transmembrane portion of the beta subunit and all of the FXYD subunit were omitted for clarity. The white α-helices span the lipid bilayer. Orange and pink spheres are sodium and potassium ions, respectively. The aqua sphere is magnesium ion at the active site. The structures are aligned on the beta subunits and transmembrane spans 7-10 on the left side of the images. The P, N, and A domains all undergo large movements: tilting of P, and rotation of both N and A so that N and A come into contact in the sodium-bound conformation. Movements of the transmembrane spans accommodate the binding of different-sized ions and generate the pathways for ion movement. It is relevant to the interpretation of variants that some internal domain-domain contact points change with conformation, and that variants at such surfaces can be pathogenic by altering enzyme kinetics without inhibiting activity.



Homology scoring by ConSurf

The ConSurf server grew from efforts to enhance the identification of functionally important areas of proteins by projecting phylogenetic conservation information onto known protein structures.[3] (http://consurf.tau.ac.il/overview.html) Conservation scores are calculated for all residues by searching for homologs using BLAST, eliminating highly-similar sequences, and constructing a phylogenetic tree. Amino acid (or DNA) position-specific conservation scores are computed using a Bayesian algorithm and a codon substitution matrix. For pig ATP1A1 and its PDB structure, 3KDP-A, the default parameters found 94 amino acid sequences: a wide array of P-type ATPases including mammalian, avian, and lower vertebrate calcium, manganese, and proton ATPases; cation and proton ATPases in *Drosophila*, *Neurospora*, yeast, *Trypanosoma*, bacteria, plants *Arabidopsis* and *Oryza*; and vertebrate lipid-transporting P-type ATPases. Because representatives of *ATP1A1*, *ATP1A2*, and *ATP1A3* were included, variability at isoform-specific residues is incorporated. The conservation score at each residue is related to the site's evolutionary rate, and the scores are normalized so that the average of all scores is zero to facilitate comparisons. A negative score means more conserved, i.e. more slowly-evolving, and a positive score means more variable or more rapidly evolving. If a different family of input sequences were used that was confined to one limb of a phylogenetic tree, such as vertebrates-only, or calcium, sodium-potassium, and proton-potassium ATPases-only, a chart such as in figure 5 would more narrowly highlight recent evolutionary differences.

Figure e-3. Color-coded conservation scores for ATP1A1 generated by ConSurf.



(Left) ConSurf's projection of conservation scores onto the crystal structure, as produced in Proteopedia (http://proteopedia.org/wiki/index.php/3kdp). The color scale is dark aqua (least-conserved) to dark magenta (most conserved). The active site for ATP hydrolysis is the large magenta patch between the N and A domains. The FXYD subunit was not scored due to insufficient data, and is gray. (Right) Ribbon view of the same structure. Beta subunit is green, FXYD subunit is aqua, the membrane domain is white, P domain is red, N domain is gold, and A domain is yellow. Two Rb^+ ions (orange, substituting for K^+ in this crystal, 3KDP-A) are in the ion binding site.

3. Landau, M, Mayrose, I, Rosenberg, Y et al. ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. Nucleic Acids Res. 2005;33,W299-W302.

Figure e-4. Milder and more severe *ATP1A3* pathogenic variants in the P domain are similar

Ribbon views of the beta-sheet and alpha-helix portions of the P domain to compare the distributions of variants with ATP1A3 severe (dark red) and milder (yellow) phenotypes. In spacefill, only the backbone atoms are shown for clarity. Strict differences in distribution are not seen. The aqua sphere is Mg^{2+} at the active site.

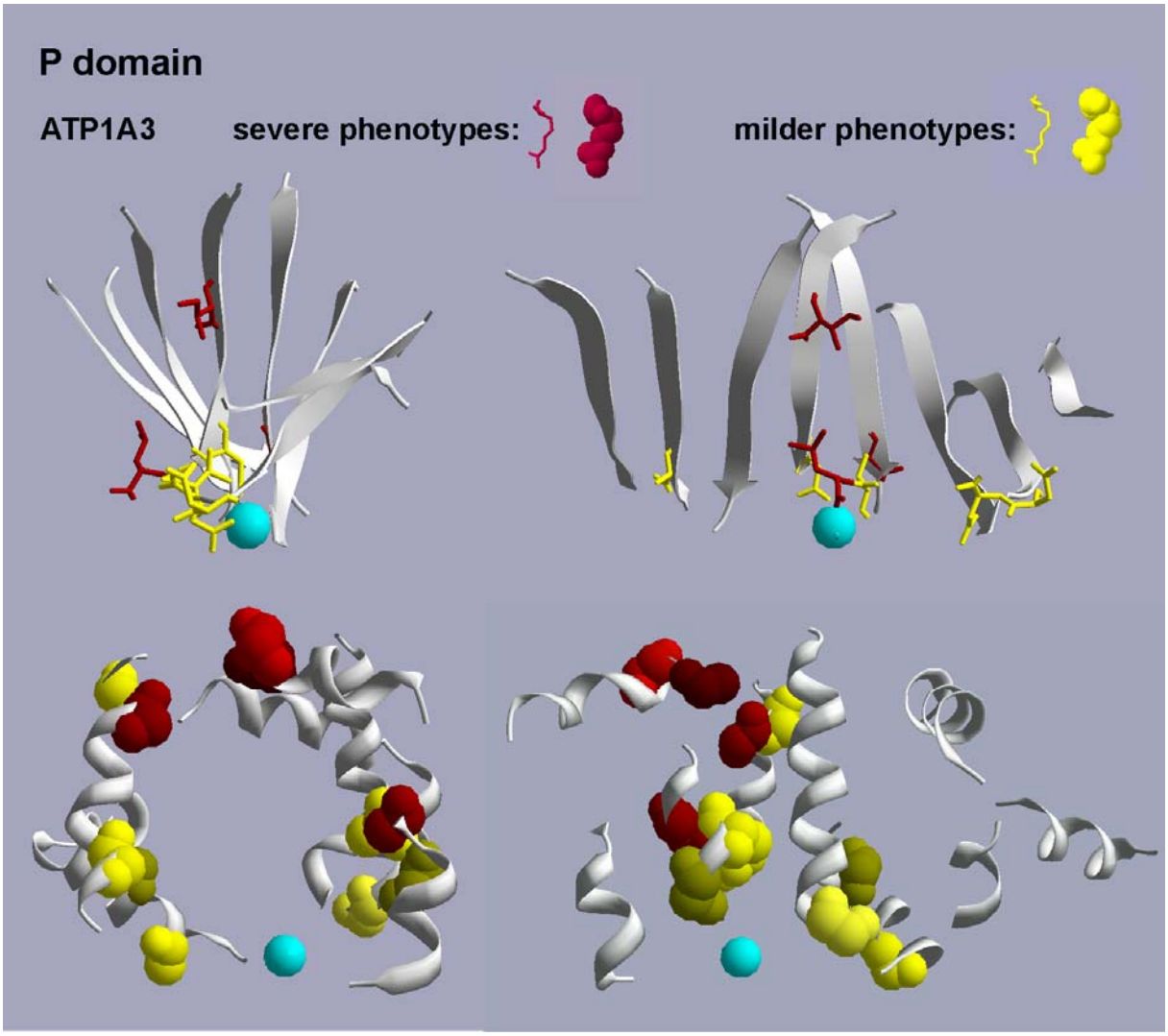


Figure e-5. *ATP1A2* and *ATP1A3* variants in the S domain are also similar

The S domain is comprised of five small segments that are well-separated in the linear structure (figure 1B). Color is used here to guide the eye to the intracellular portions of transmembrane helices M3, M4, and M5 (shades of lavender); the loops that connect helices M6 and M7 (magenta) and M8 and M9 (aqua); and the C-terminus (dark blue). The four white or pale gray amino acids have been found mutated in both *ATP1A2* and *ATP1A3*. Strict differences in distribution were not seen, nor were there differences between *ATP1A3* milder and more severe phenotypes (not shown).

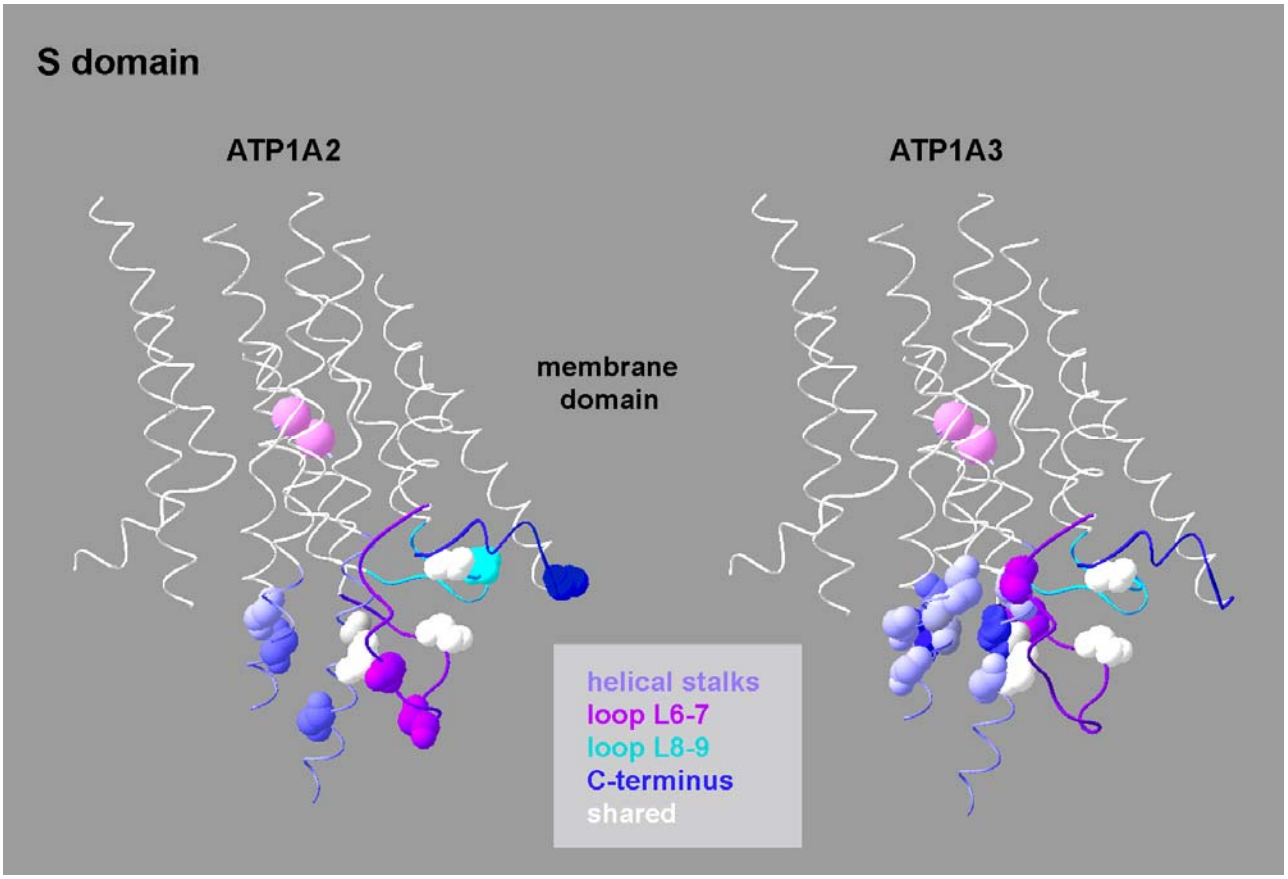


Table e-1. Variants in *ATP1A1*

1023 amino acid transcript	missense variants	in *ATP1A2* or *ATP1A3*	recurs	first report	Ref.
CMT2					
143T>G	L48R			Lassuthova 2018	1
1775T>C	I592T	A2 I589T			
1789G>A	A597T				
1798C>G	P600A				
1798C>A	P600T				
1801_1802delinsTT	D601P				
2432A>C	D811A	A3 D801N			
APA					
	G99R		√√	Williams 2014, Fernandes-Rosa 2014	2,3
	L104R		√√√	Azizan 2013	4
	L104V			Nishimoto 2015	5
	E123K			Nishimoto 2016	6
	L324F			Nishimoto 2016	6
	I327S			Nishimoto 2016	6
	V332G	A3 V322D	√√√	Beuschlein 2013	7
	L337M	A3 L327del		Nishimoto 2016	6

CMT2, Charcot-Marie-Tooth Type 2 (peripheral neuropathy, axonal type). APA, aldosterone-producing adenomas, which have somatic mutations in this or other genes that result in the clinical symptoms of acquired hyperaldosteronism. In the third column, when an identical or alternative substitution of the homologous amino acid has been found as a pathogenic variant in one of the other genes, it is listed here.

1. Lassuthova, P, Rebelo, AP, Ravenscroft, G et al. Mutations in ATP1A1 cause dominant Charcot-Marie-Tooth Type 2. Am. J. Hum. Genet. 2018;102**,**505-514.

2. Williams, TA, Monticone, S, Schack, VR et al. Somatic ATP1A1, ATP2B3, and KCNJ5 mutations in aldosterone-producing adenomas. Hypertension 2014;63**,**188-195.

3. Fernandes-Rosa, FL, Williams, TA, Riester, A et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. Hypertension 2014;64**,**354-361.

4. Azizan, EA, Poulsen, H, Tuluc, P et al. Somatic mutations in *ATP1A1* and *CACNA1D* underlie a common subtype of adrenal hypertension. Nat. Genet. 2013;45**,**1055-1060.

5. Nishimoto, K, Tomlins, SA, Kuick, R et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. Proc. Natl. Acad. Sci. U. S. A 2015;112**,**E4591-E4599.

6. Nishimoto, K, Seki, T, Kurihara, I et al. Case Report: Nodule development from subcapsular aldosterone-producing cell clusters causes hyperaldosteronism. J. Clin. Endocrinol. Metab 2016;101**,**6-9.

7. Beuschlein, F, Boulkroun, S, Osswald, A et al. Somatic mutations in *ATP1A1* and *ATP2B3* lead to aldosterone-producing adenomas and secondary hypertension. Nat. Genet. 2013;45**,**440-442.

Table e-2. Variants in *ATP1A2*.

1020 amino acid transcript	missense variants	in ATP1A1 or ATP1A3	recurs	first report	Ref.
103_105delAAG	K35del			Riant 2010	1
	E120A			de Vries 2007	2
	V138A			Thomsen 2007	3
520G>A	E174K			Todt 2006	4
571G>A	V191M			Oh 2015	5
	R202Q			Thomsen 2007	3
659C>T	S220L			Roth 2014	6
788C>T	T263M			Riant 2006	7
	I286T			Vanmolkot 2007	8
901G>A	G301R		√	Spadaro 2004	9
913_915delTTC	F305del			Riant 2010	1
1013T>C	V338A			Riant 2010	1
1025T>C	L342P			Asghar 2012	10
1028C>T	T343I cv	A3 T335P		ClinVar	
1033A>G	T345A			Kaunisto 2004	11
1043G>C	R348P			Pelzer 2017	12
	V362E			Castro 2008	13
1091T>C	T364M		√	Toldo 2011	14
1103C>A	T368K	A3 T360R		Riant 2010	1
1127C>T	T376M		√	Riant 2006	7
1127C>G	T376R cv		√	ClinVar	
	T378N	A3 T370N	√	Swoboda 2004, Bassi 2004	15,16
1148G>A	R383H	A3 R375H	√	Jurkat-Rott 2004,	17
	T415M			Vanmolkot 2007	8
1544G>A	C515Y			Todt 2006	4
1642T>C	R548C		√	Lebas 2008	18
1643G>A	R548H		√	Ambrosini 2006	19
1766T>C	I589T	A1 I592T		Al-Bulushi 2014	20
	R593W		√	Vanmolkot 2006a	21
1778G>A	R593Q cv		√	ClinVar	
1799T>C	V600A			De Cunto 2012	22
1811G>C	R604P	A3 R597P		Riant 2010	1
1816G>A	A606T	A3 A599T cv	√	Riant 202006	7
1817C>A	A606E			ClinVar	
	G615R		√	Vanmolkot 2006b	23
1844G>A	G615E		√	Riant 2010	1
	V628M			Vanmolkot 2006a	21
	R689Q			Vanmolkot 2003	24
2098G>A	E700K			Pierelli 2006	25
2105G>A	C702Y			Deprez 2008	26
2131G>C	V711L		√	Riant 2010	1
2131G>A	V711M		√	gnomAD	
2143G>A	G715R			De Sanctis 2011	27
	N717K			Jen 2007	28
2152G>A	D718N			Jurkat-Rott 2004	17
2161G>A	A721T	A3 A714T		Riant 2010	1
2295A>G	M731V		√	Pelzer 2014	29
	M731T		√	Vanmolkot 03	24
	M745I			Thomsen 2008	30
2285G>C	G762A cv	A3 G755A		ClinVar	
2288G>A	R763H	A3 R756H	√	Gardner 03, Jurkat-Rott 04	17,31
	R763C	A3 R756C	√	Thomsen 07	3
	L764P	A3 L757P		De Fusco 03	32
2324A>G	Y775C	A3 Y768C	√	Riant 2010,	1

	P786L			de Vries 07	2
2387C>G	P796R			Jurkat-Rott 04	17
	P796S			Castro 2008	13
2426T>G	L809R cv	A3 L802P		ClinVar	
2473G>A	E825K	A3 E818K		Carreno 2013	33
2486T>G	M829R			Riant 2006	7
2501G>A	R834Q			Riant 2006	7
2563G>A	G855R		√	de Vries 2009	34
2564G>T	G855V		√	Riant 2010	1
2620G>A	G874S	A3 G867D		Costa 2014	35
	W887R			De Fusco 03	32
2698G>C	G900R	A3 G893R		Deprez 2008 sygq	26
2704G>A	E902K			Jurkat-Ratt 04	17
	R908Q	A3 R901T	√	de Vries 07	2
2722C>T	R908W			ClinVar	
2747A>T	H916L			Iizuka 2012	36
2780A>G	Q927P			Riant 2010	1
	W928R			Barrett 2011	37
2810G>A	R937H ¶	A3 R930W	√	Riant 2006, ClinVar	7
2819C>T	S940L			Montani 2014	38
2936C>T	P979L		√	Jurkat-Rott 04	17
2980_2982delCTC	L994del			Riant 2010	1
2995G>C	D999H	A3 D992H	√	Fernandez 2008	39
	R1002Q			Jen 07	28
3007A>G	K1003E			Riant 2010	1
3019C>T	R1007W			Pisano 2013	40
3022C>T	R1008W			Wilbur 2017	41

Some of the older references used an earlier version of a reference transcript that apparently did not start at ATG. Those published transcript numbers are omitted as confusing. ClinVar was used as a source only if the same residue was also mutated in ATP1A2 or ATP1A3 or both. Some substitutions are usually more damaging than others (gain or loss of a proline for example), and so pathogenicity is not guaranteed. The notation cv shows which mutation was from ClinVar.

¶ Published as R937P.

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Table e-3. Variants in *ATP1A3*

1013 amino acid transcript	missense variants	published as[§]	in *ATP1A1* or *ATP1A2*	recurs	severity	first report	Ref.
367G>C	G123R				M	Sampson 2016	1
385G>A	V129M	V142M			M	Smedemark-Margulies 2016	2
410C>A	S137Y			√	S	Heinzen 2012	3
410C>T	S137F			√	S	Heinzen 2012	3
409-411delGAG	S137del	S148del		√	M	Wilcox 2015	4
419A>T	Q140L			√	S	Heinzen 2012	3
420G>C or T	Q140H			√	S	Prange 2017	5
821T>A	I274N			√	S	Rosewich 2012, Heinzen 2012	3,6
821T>C	I274T			√	M	de Carvalho Aguiar 2004	7
829G>A	E277K			√	M	de Carvalho Aguiar 2004	7
946G>A	G316S				M	Sweadner 2016	8
958G>A	A320T	A333T[9]		√	S	Trump 2016	10
958G>C	A320P			√		ClinVar	
965T>A	V322D		A1 V332G	√	S	Rosewich 2012	6
967C>T	P323S	P336S		√	S	Masoud 2017	9
968C>T	P323L			√		ClinVar	
970G>C	E324Q			√	S	Panagiotakaki 2015, Viollet 2015	11,12
971A>G	E324G			√	S	Prange 2017	5
972G>C	E324D	Q324D		√	S	Viollet 2015	12
974G>A	G325D				S	Lee 2014	13
977T>G	L326R			√	S	Panagiotakaki 2015, Viollet 2015	11,12
976-978delCTG	L327del		A1 L337M		M	Kamm 2008	14
983C>A	A328D				S	K.J. Swoboda this study	
998G>T	C333F			√	S	Heinzen 2012	3
1003A>C	T335P		A2 T343I cv	√	S	Rosewich 2014	15
1004C>T	T335M			√		ClinVar	
1004C>A	T335K			√		ClinVar	
1012G>C	A338P				S	Gurrieri 2016	16
1072G>T	G358C			√	S	Sasaki 2014	17
1072G>A	G358S			√	S	Panagiotakaki 2015	11
1073G>T	G358V			√	S	Paciorkowski 2015	18
1073G>A	G358D			√	M	Pereira 2015	19
1079C>G	T360R		A2 T368K	√	S	Prange 2017	5
1088T>A	I363N				S	Paciorkowski 2015	18
1096G>C	D366H				M	A. Brashear, this study	
1108G>A	T370A			√	M	A. Brashear, this study	
1109C>A	T370N		A2 T378N	√	M	Yang 2014, Rosewich 2014	15,20
1112T>C	L371P				S	Rosewich 2012	6
1123C>T	R375C			√		ClinVar	
1124G>A	R375H		A2 R383H	√		ClinVar	
1144T>C	W382R				M	Rosewich 2014	15
1244C>A	A415D				M	A. Brashear, this study	
1250T>C	L417P			√	M	Rosewich 2014	15
1747G>T	D583Y				M	Nicita 2016	21
1765G>T	V589F			√	S	Richards 2018	22
1786T>C	C596R			√	S	Viollet 2015	11,12
1790G>C	R597P		A2 R604P	√	M	Wenzel 2017	23
1795G>A	A599T		A2 A606T	√		ClinVar	
1838C>T	T613M			√	M	de Carvalho Aguiar 2004	7
2041G>A	A681T				M	Torres 2018	24
2051C>T	S684F				M	Svetel 2010	25
2116G>A	G706R			√	S	Yang 2014	20
2140G>A	A714T		A2 A721T	√	M	Meijer 2016	26

2144T>C	L715P				S	Panagiotakaki 2015	11
2224G>T	D742Y			√	S	Marzin 2018	27
2227G>C	D743H	D742H			M	Meijer 2016	26
2263G>A	G755S			√	S	Heinzen 2012	3
2263G>T	G755C	G775C[9]		√	S	Rosewich 2012	6
2264G>T	G755V			√	S	Viollet 2015	12
2264G>C	G755A		A2 G762A cv	√	S	Sasaki 2014	17
2266C>T	R756C		A2 R763C	√	M	Dard 2015	28
2267G>A	R756H		A2 R763H	√	M	Morrison 2012	29
2267G>T	R756L			√	M	Yano 2017	30
2270T>C	L757P		A2 L764P	√	S	Rosewich 2014	15
2272A>T	I758F			√	M	A. Brashear, this study	
2273T>G	I758S			√	M	de Carvalho Aguiar 2004	7
2281A>C	N761H				S	Viollet 2015	12
2302T>C	Y768H			√	S	Viollet 2015	12
2303T>C	Y768C		A2 Y775C	√	S	Viollet 2015	12
2305A>C	T769P				S	Viollet 2015	12
2309T>G	L770R				S	Yang 2014	20
2312C>A	T771N			√	S	Sasaki 2014	17
2312C>T	T771I			√	S	Yang 2014	20
2314A>C	S772R			√	S	Panagiotakaki 2015, Viollet 2015	11,12
2316C>A	S772R			√	S	Rosewich 2012	6
2316C>G	S772R			√	S	Yang 2014	20
2317A>C	N773H			√	S	Viollet 2015	12
2318A>G	N773S			√	S	Heinzen 2012	3
2318A>T	N773I			√	S	Rosewich 2012	6
2318A>C	N773T			√	S	Yang 2015	31
2323C>A	P775L			√		ClinVar (2)	32,33
2324C>T	P775T			√		ClinVar	32,33
2338T>C	F780L				M	de Carvalho Aguiar 2004	7
2401G>A	D801N			√	S	Heinzen 2012 (>300)	3
2401G>T	D801Y			√	M-S	de Carvalho Aguiar 2004	7
2401G>C	D801H			√		ClinVar	
2402A>T	D801V		A1 D811A	√	S	Panagiotakaki 2015	12
2403T>A	D801E			√	S	Hoei-Hansen 2014	36
2405T>C	L802P		A2 L809R cv	√	S	Yang 2014	20
2408G>A	G803D			√	S	Gall 2017	34
2411C>T	T804I			√	S	Ulate-Campos 2014, Rosewich 2014	15,35
2413G>A	D805N			√	S	Viollet 2015	12
2413G>C	D805H			√	S	Yang 2014	20
2415C>G	D805E			√	S	Rosewich 2014	15,36
2417T>G	M806R			√	S	Heinzen 2012	3
2417T>A	M806K			√	S	Yang 2014	20
2423C>T	P808L			√	S	Yang 2014	20
2428A>T	I810F			√	S	Rosewich 2014	15
2429T>G	I810S			√	S	Heinzen 2012	3
2429T>A	I810N			√	S	Yang 2014	20
2431T>C	S811P			√	S	Heinzen 2012	3
2438C>T	A813V	A826V		√	M	Kubota 2017	37
2443G>A	E815K			√	S	Heinzen 2012 (>200)	3
2452G>A	E818K		A2 E825K	√	M	Demos 2014	38
2501T>C	L834S				S	Yang 2015	31
2516T>C	L839P			√	S	Yang 2014	20
2552A>G	Q851R			√	S	Masoud 2017	9
2552A>C	Q851P			√	S	Yang 2015	31
2558T>G	L853R	L866R			M	Rodriguez-Quiroga 2016	39
2600G>A	G867D		A2 G874S	√	M	Rosewich 2014b	40

2663T>C	L888P			√	S	Panagiotakaki 2015, Viollet 2015	11,12
2677G>A	G893R		A2 G900R	√	S	Yang 2014	20
2702G>C	R901T		A2 R908Q	√	S	Viollet 2015	12
2755-2757delGTC	V919del			√	S	Heinzen 2012	3
2767G>T	D923Y			√	S	Rosewich 2012	6
2767G>A	D923N			√	M	Zanotti 2008	41
2780G>A	C927Y			√	S	Iishi 2013	42
2780G>T	C927F			√	S	Sasaki 2014	17
2781C>G	C927W			√	S	Ulate-Campos 2014	43
2788C>T	R930W		A2 R937H	√	M	Meijer 2016	26
2839G>A	G947R			√	S	Heinzen 2012 (>30)	3
2839G>C	G947R			√	S	Heinzen 2012 (> 50)	3
2851G>A	E951K			√	S	Panagiotakaki 2015, Viollet 2015, Termserasab 2015	12
2864C>A	A955D			√	S	Heinzen 2012	3
2974G>C	D992H		A2 D999H	√	S	K.J. Swoboda, this study	
2974G>T	D992Y			√	S	Heinzen 2012	3

§Mutations in ATP1A3 are occasionally reported with numbering according to an alternative transcript, usually one one that has retained intron near the N-terminus. This transcript is currently used by GeneDx and by gnomAD as the reference standard, but the scientific literature generally uses the 1013 amino acid transcript. Severity is mild (M) or severe (S).

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Table e-4. Missense variants excluded from the analysis.

variant	# in gnomAD	structure aspects	first report	Ref.
ATP1A2				
Y9N	411	not conserved, unstructured	Tonelli 2007, Thomsen 2008, Gallanti 2011	1-3
R51H	30	at the surface	Castro 2008	4
R65W	18	at the surface	Tonelli 2007, Gallanti 2011	1,3
R196C	5	at surface in K^+ form	Choi 2017	5
E492K	122	at the surface	De Vries 2007	6
T570M	7	at the surface	Maksemous 2016	7
R879Q	16	at the surface	Thomsen 2008	2
R879W	4	at the surface	Thomsen 2008	2
ATP1A3				
D9N	0	not conserved, unstructured	Wenzel 2017	8
D220N	1	at the surface; see [12]	Heinzen 2012, Yang, 2014, Rosewich 2014	9-11
R463C	130	at the surface	Heinzen, 2014, Yang, 2014	10,12
R507H	23	at the surface	ClinVar	
V885I	46	not conserved, at the surface	Yang 2014	10

Varients were excluded from the analysis because of their frequency as random variants or their position in protein structure and lack of supporting evidence.

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