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SLC6A1 – Characterization of variant interpretation tolerant and intolerant sites

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Cleveland Clinic

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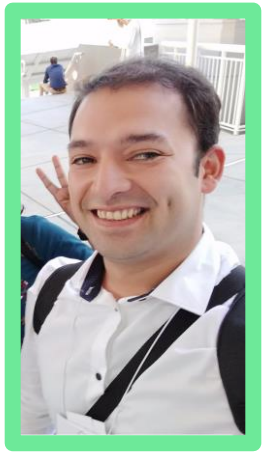
Analytic and Translational Genetic Unit, *Massachusetts General Hospital,* ***Harvard University, Boston, US***
Cologne Center for Genomics, ***University of Cologne, Cologne, Germany***

Team members who generated results which will be presented today

Sumaiya Iqbal
Postdoc



Eduardo Perez-Palma
Postdoc

Additional support
e.g. Nord Report

All results are unpublished and we are happy to share these

Variant interpretation is in its infancy

Important to acknowledge!

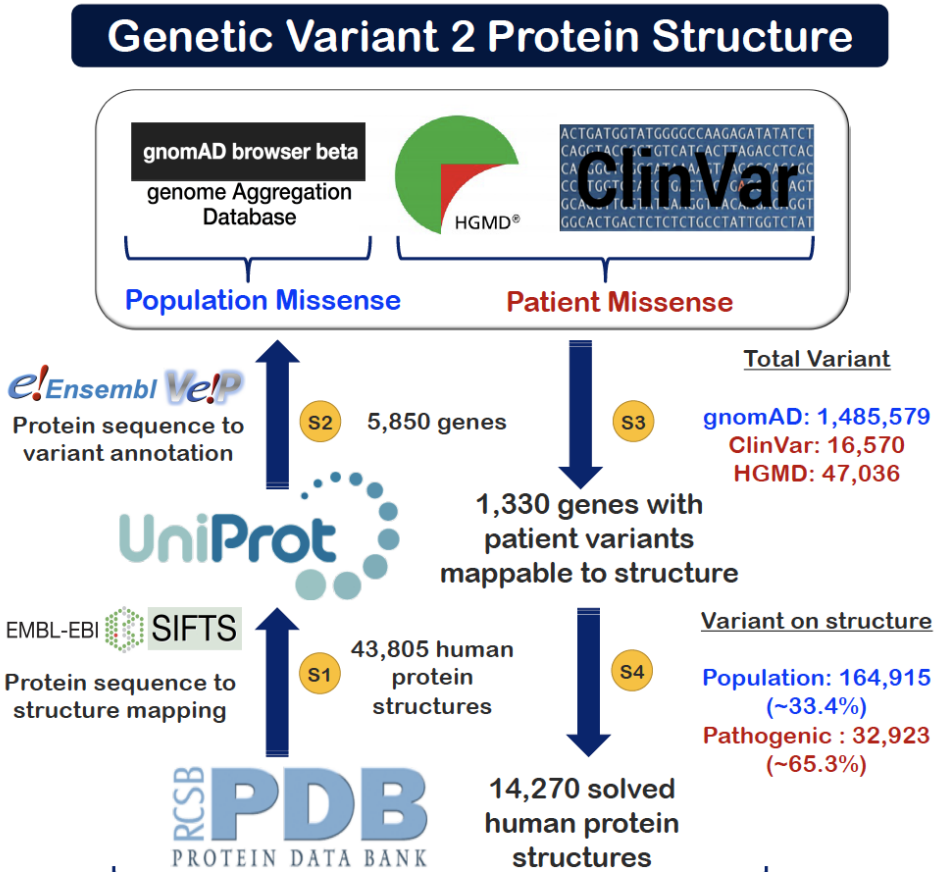
- Rare missense variants are not rare as an aggregate
- The chance to find in a genetic test a benign variant in a epilepsy gene that 'fits the broader phenotype' is high
- A large amount of previous pathogenic classified variants found in disease databases are becoming now reclassified as benign

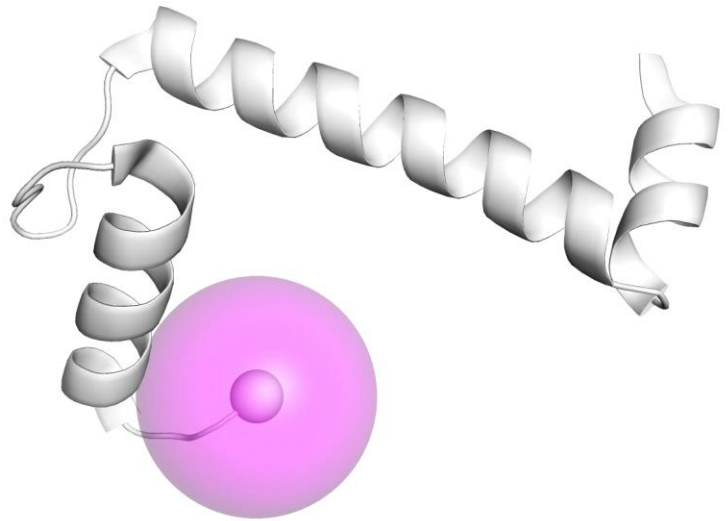
Variant interpretation is in its infancy

Not for every epilepsy/autism patient the variant in SLC6A1 is pathogenic!

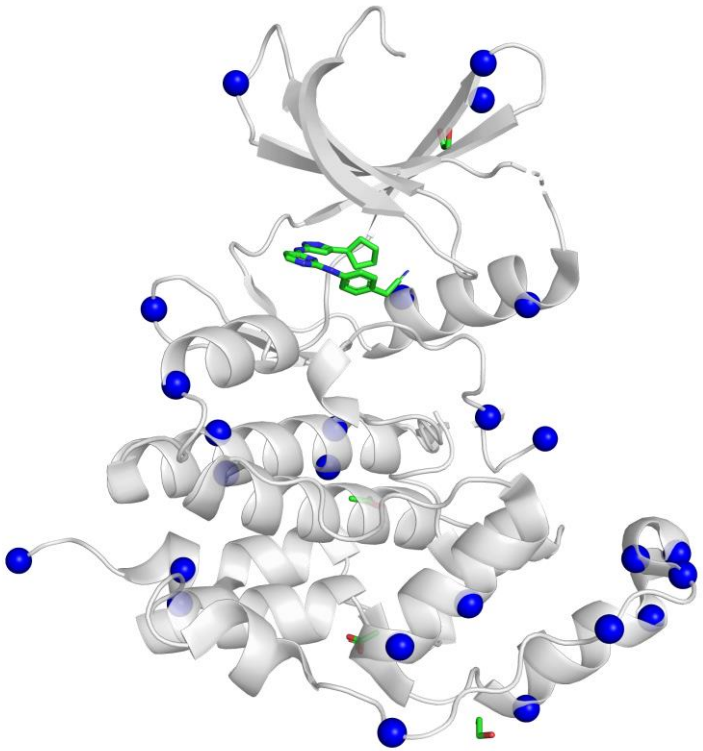
Essential: To test drugs and therapies using truly pathogenic variants

Spatial scoring of patient vs. control variants to identify ”hot” and ”cold” zones

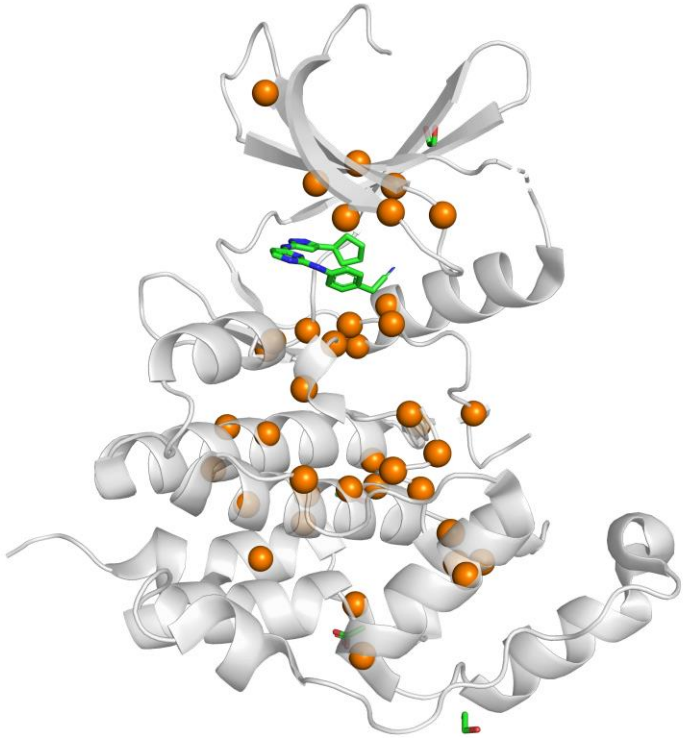




V2Pmapper: Output and Analysis – example: CDKL5

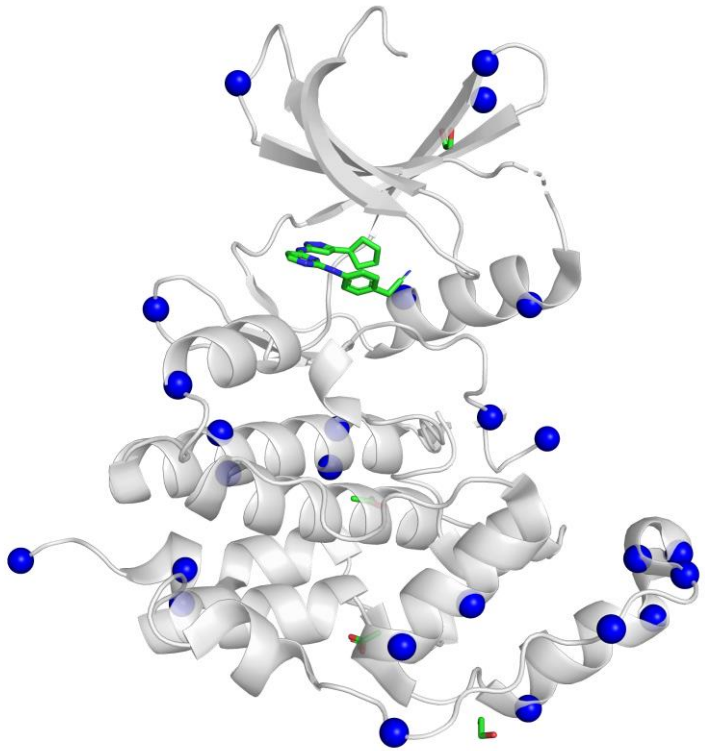


Control (gnomAD) variant positions

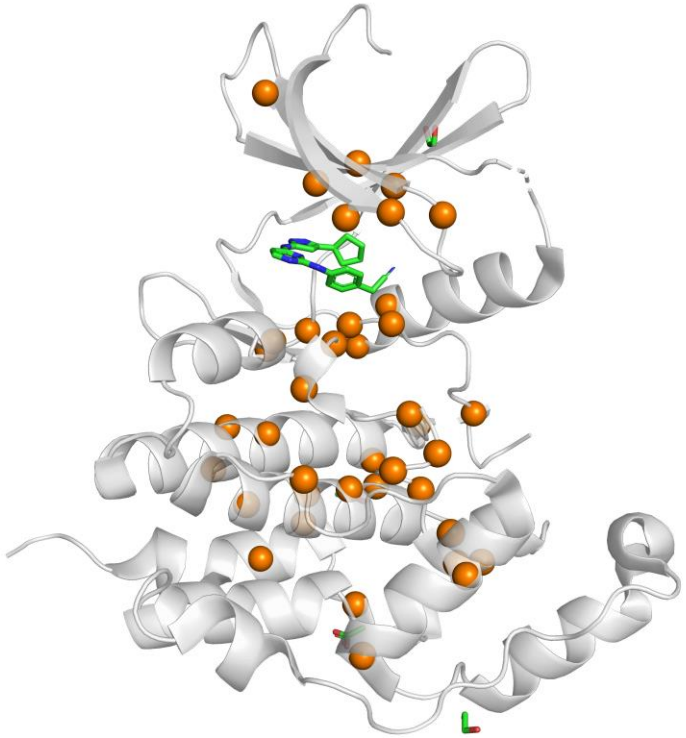


Patient (ClinVar) variant positions

V2Pmapper: Output and Analysis – example: CDKL5

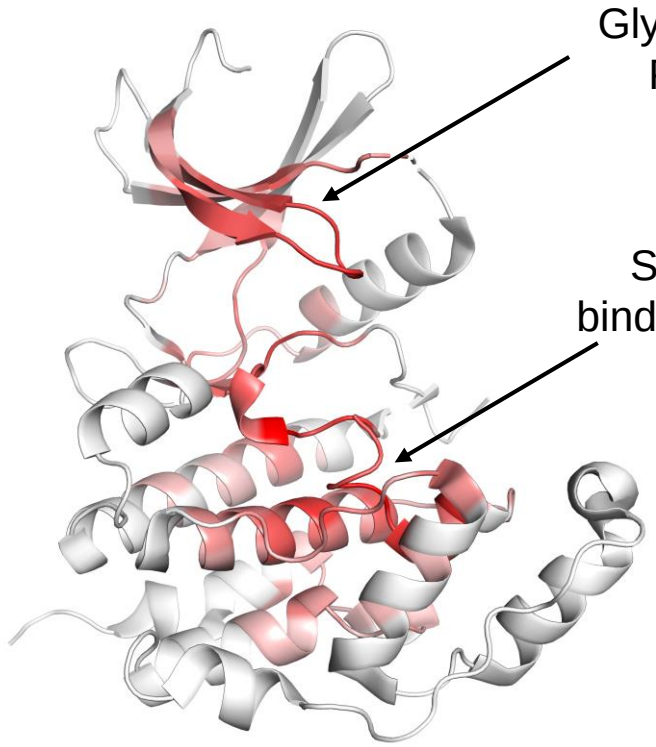


Control (gnomAD) variant positions



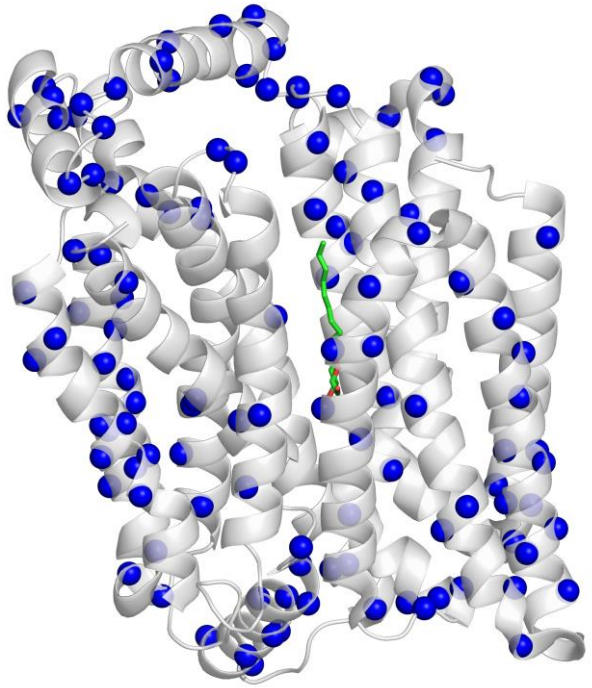
Patient (ClinVar) variant positions

Spatial scoring

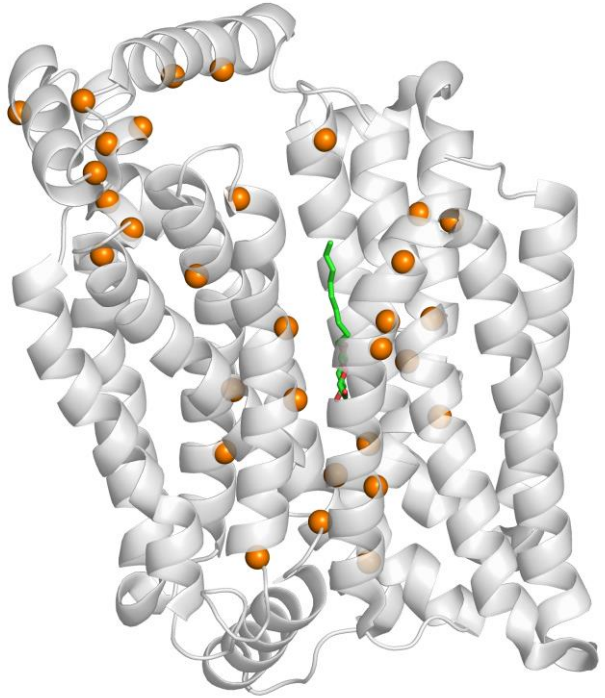


Hot-spots:
enriched with pathogenic (ClinVar)
and
Depleted of control (gnomAD)

V2Pmapper: Output and Analysis – example: SLC2A1 (GLUT1)

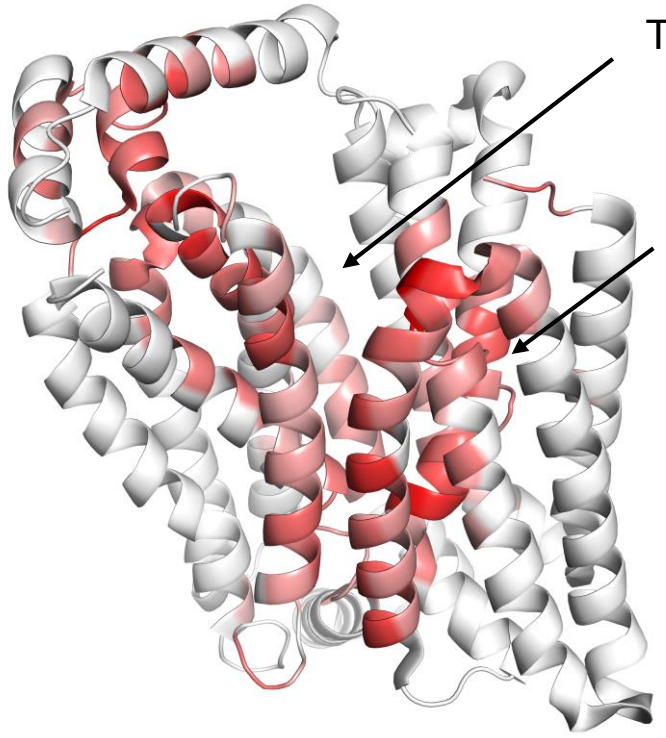


Control (gnomAD) variant positions



Patient (ClinVar) variant positions

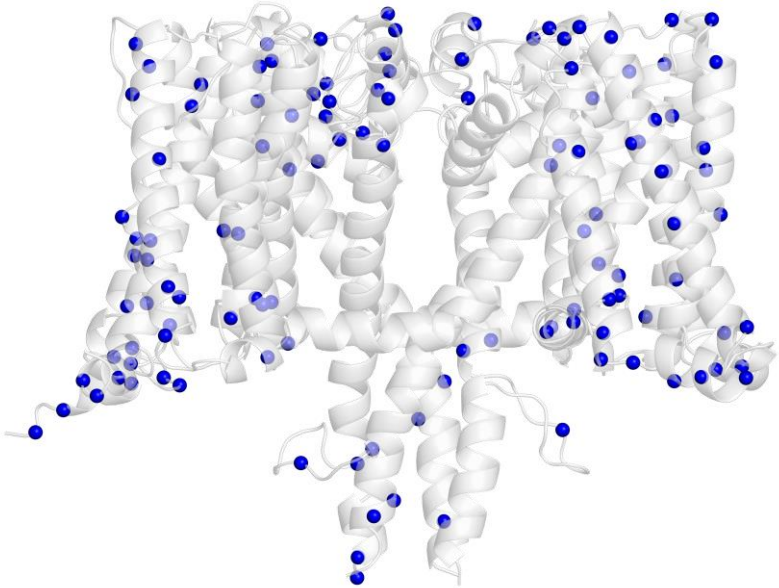
Spatial scoring



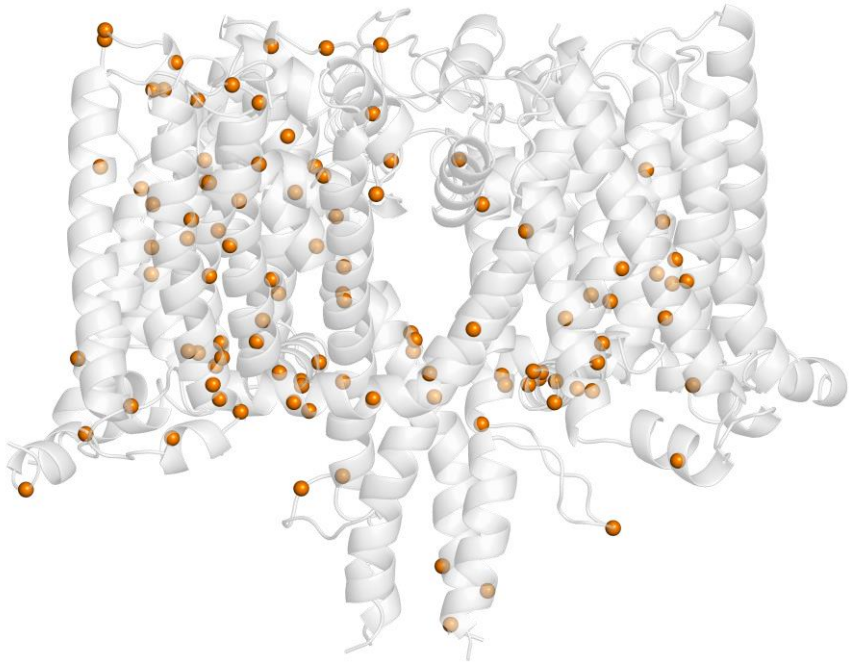
Hot-spots:
enriched with pathogenic (ClinVar)
and
Depleted of control (gnomAD)

V2Pmapper: Output and Analysis – example: SCN2A (homology model)

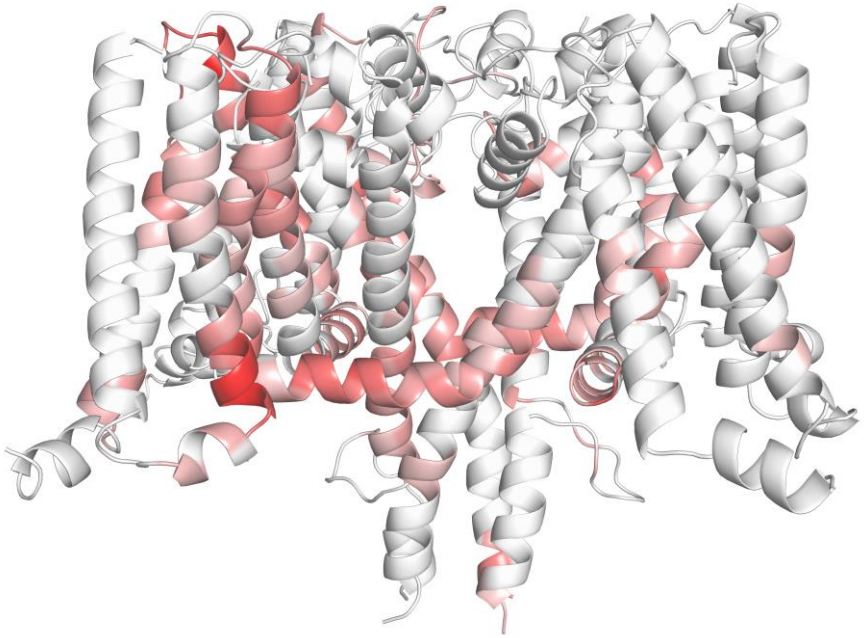
Spatial scoring



Control (gnomAD) variant positions



Patient (ClinVar) variant positions



Hot-spots:
enriched with pathogenic and
Depleted of control

SLC6A1

- Sodium- and chloride-dependent GABA transporter 1
  - Terminates the action of GABA by its high affinity sodium-dependent reuptake into presynaptic terminals.

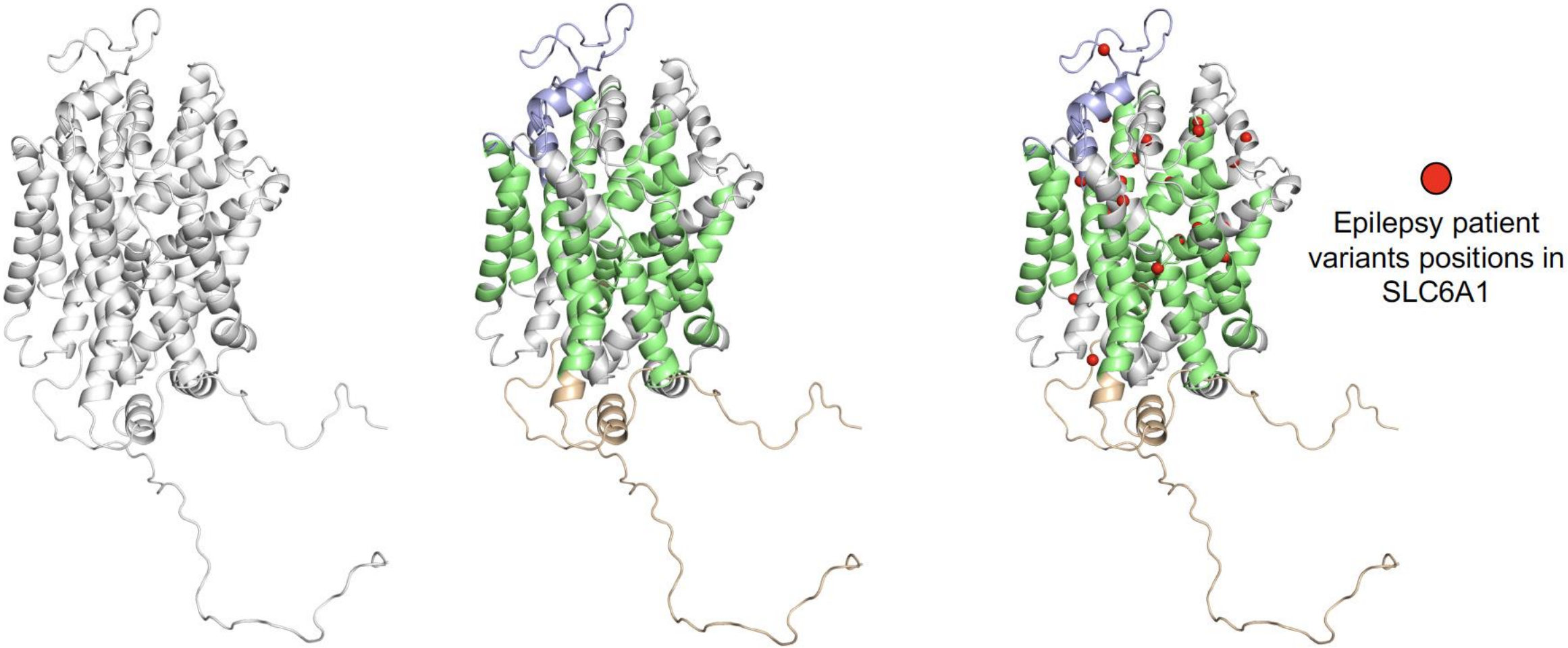
- Protein length: 599

SLC6A1

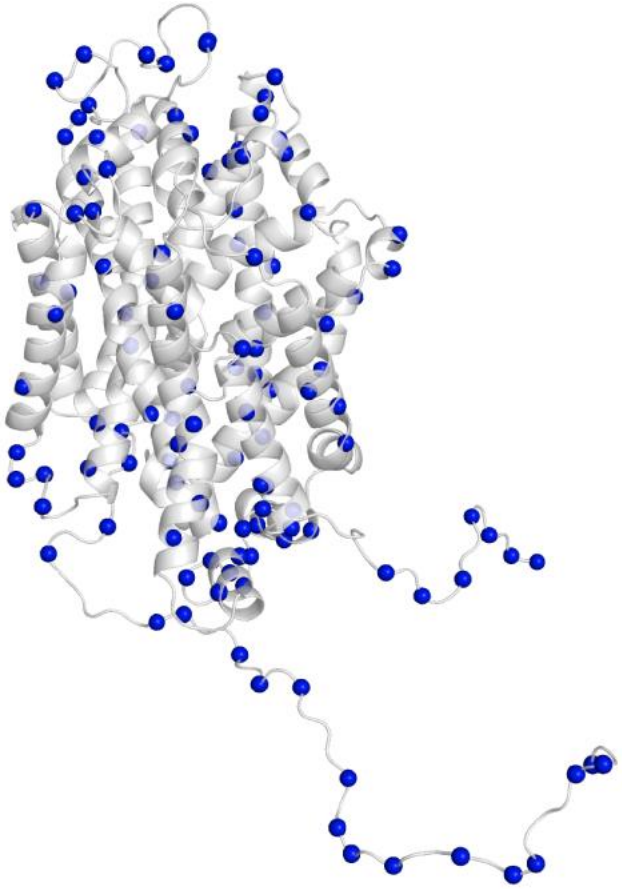
- No experimentally solved structure
- Structure computationally determined using an existing solved structure of 41% sequence identity
  - Using RaptorX software
  - P-value of the predicted structured = $2.79e^{-14}$ ($< e^{-14}$ is a good indicator)
- Domains: 12 Helical domains, 1 Extracellular and 2 cytoplasmic domains, and rest are the hinge regions in between helical segments

SLC6A1 and the GAT1 protein

12 **Helical domains**, 1 **Extracellular** and 2 **Cytoplasmic** domains, and rest are the **hinge regions** in between helical segments

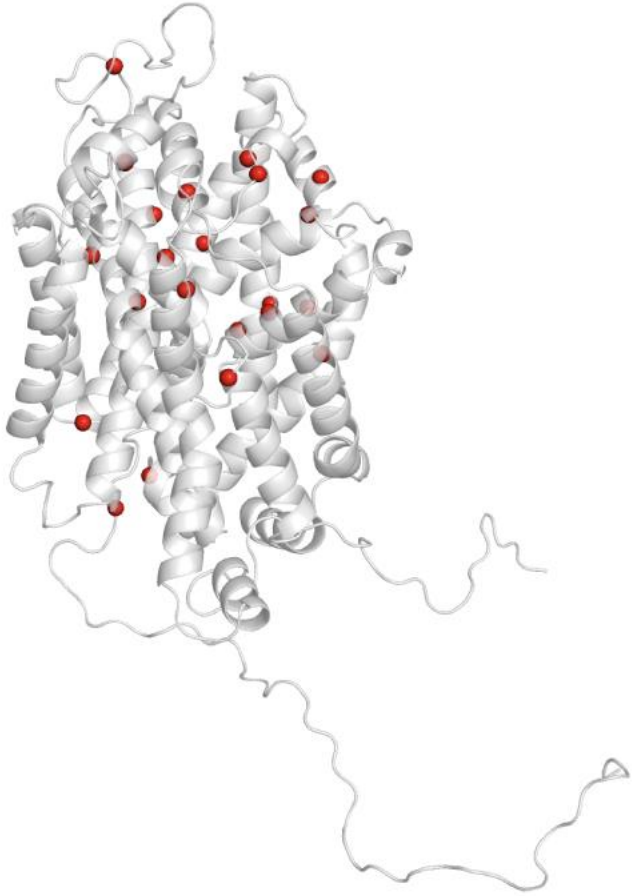


Genetic variants on *SLC6A1* and the GAT1 protein

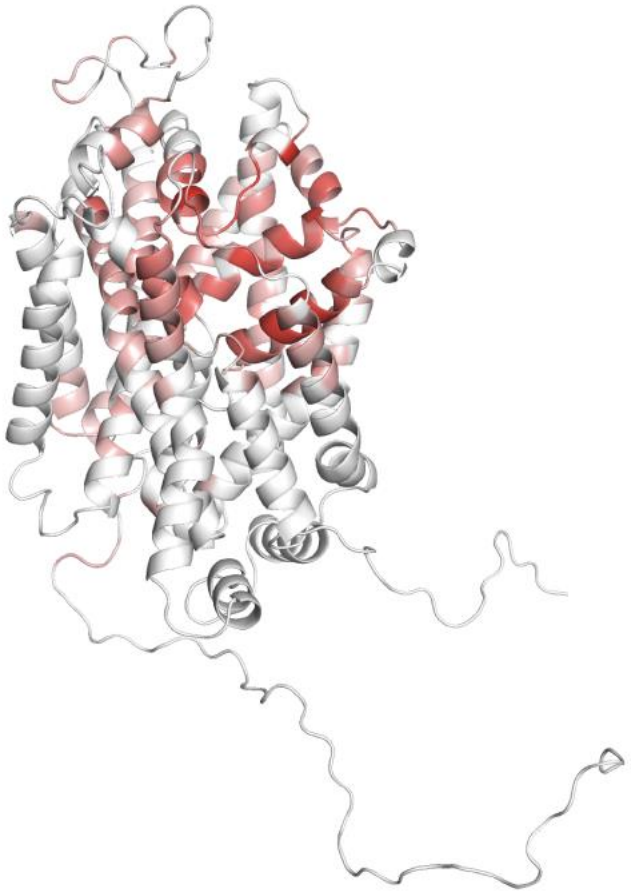


gnomAD variant positions in SLC6A1

(control)



Epilepsy patient variants positions in SLC6A1



Hot-spots: enriched with pathogenic and depleted of control

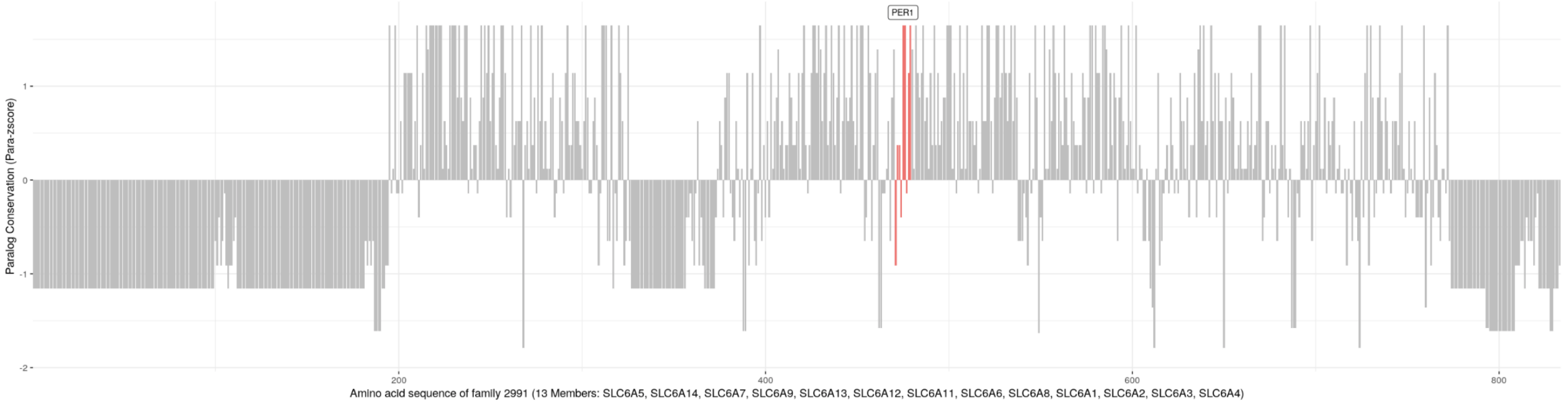
Related disorders: *SLC6A**

Amino Acids are conserved across 13 proteins

- *SLC6A8* - Creatine deficiency
- *SLC6A3* - Dopamine deficiency
- *SLC6A3* - Serotonin deficiency
- …
- …
- 13 more related SLC6A* proteins

Related disorders: *SLC6A**

Amino Acids are conserved across 13 *SLC6A** proteins



Related disorders: *SLC6A**

Patient variants hit conserved sites –
Likely similar molecular loss of protein function

PERs main Features | Browse sequence

Index	SLC6A5	SLC6A14	SLC6A7	SLC6A9	SLC6A13	SLC6A12	SLC6A11	SLC6A6	SLC6A8	SLC6A1	SLC6A2	SLC6A3	SLC6A4	Gene:Disease
All	All	All	All	All	All	All	All	All	All	All	All	All	All	All
473	A_464	A_309	S_285	A_358	P_276	P_281	P_296	P_389	P_303	S_282	A_305	A_308	T_323	N/A
474	T_465	E_310	K_286	K_359	Q_277	Q_282	Q_297	Q_390	Q_304	E_283	T_306	S_309	G_324	SLC6A8:Creatine deficiency,not provided
475	V_466	V_311	V_287	V_360	V_278	V_283	V_298	V_391	V_305	V_284	V_307	V_310	V_325	N/A
476	W_467	W_312	W_288	W_361	W_279	W_284	W_299	W_392	W_306	W_285	W_308	W_311	W_326	N/A
477	K_468	K_313	I_289	G_362	M_280	M_285	V_300	I_393	I_307	L_286	I_309	I_312	I_327	SLC6A3:Parkinson disease and ADHD
478	D_469	D_314	E_290	D_363	D_281	D_286	D_301	D_394	D_308	D_287	D_310	D_313	D_328	N/A
479	A_470	A_315	A_291	A_364	A_282	A_287	A_302	A_395	A_309	A_288	A_311	A_314	A_329	SLC6A8:SLC6A8 deficiency;SLC6A1:Myoclonic-atonic epilepsy|not provided;SLC6A3:Dopamine transporter deficiency syndrome
480	A_471	A_316	A_292	A_365	G_283	G_288	G_303	G_396	G_310	A_289	A_312	A_315	A_330	N/A

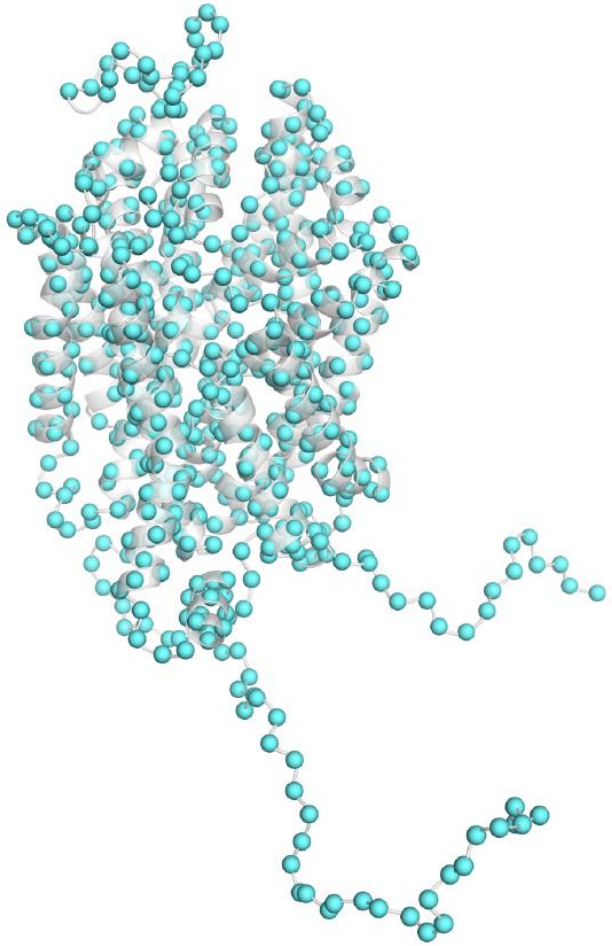
Example: *To evaluate missense variant "NM_000833.4(GRIN2A):c.1895C>T (p.Val632Phe)" search for "GRIN2A" gene and then look for "V_632" in its corresponding column*

Download

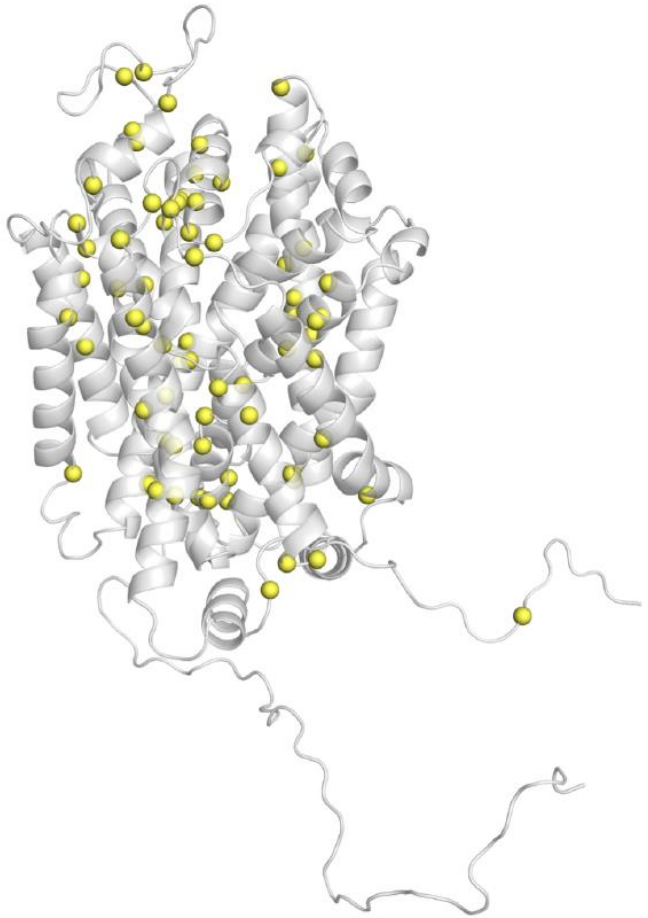
http://per.broadinstitute.org

Perez-Palma et al., *in review*

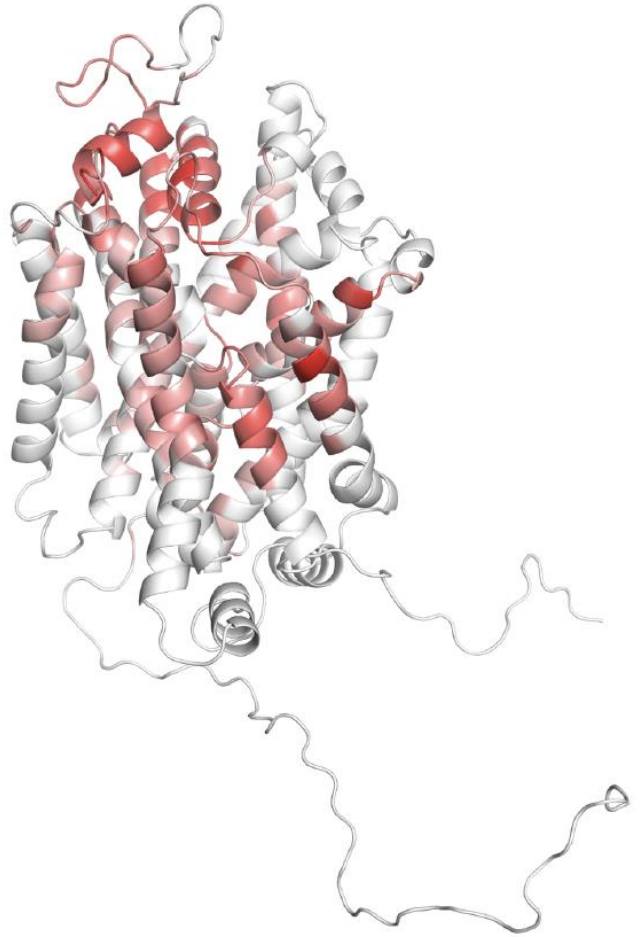
Genetic variants from the whole gene family mapped on the GAT1 protein



gnomAD variant positions in all paralogs

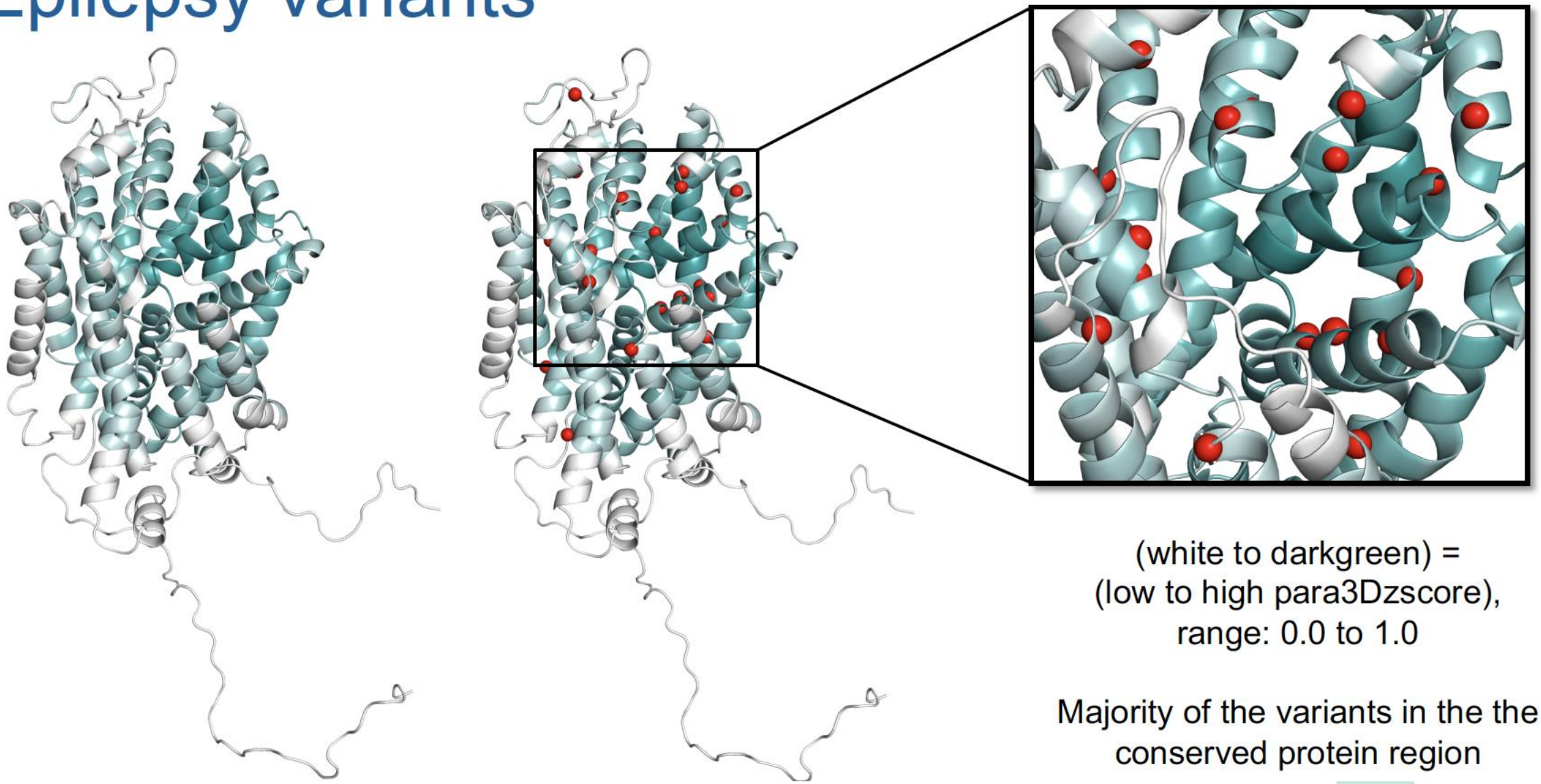


ClinVar and HGMD variant positions in all paralogs



Hot-spots: enriched with pathogenic and depleted of control

SLC6A1 3D Paralog conservation and Epilepsy variants



(white to darkgreen) =
(low to high para3Dzscore),
range: 0.0 to 1.0

Majority of the variants in the the conserved protein region

All scores can be combined to inform variant interpretation

position	Amino acid	Epilepsy variants	Patient variant enrichment hotspot score	3D paralog score	domain
44	R	1	0	0	cytoplasmic
75	G	1	0.495371	0.675033	hinge
111	G	1	0	0.522668	hinge
140	Y	1	0.531703	0.466251	helical3
145	S	1	0.329921	0.499699	extracellular
164	C	1	0	0	extracellular
193	W	1	0.461669	0.325806	extracellular
232	G	3	0.275969	0.481668	hinge
251	L	1	0.299785	0.811604	helical5
270	F	1	0.727513	0.483781	hinge
288	A	2	0.340289	0.70013	hinge
291	Q	1	0.764189	0.842191	hinge
295	S	1	0.399117	0.768916	helical6
329	C	1	0	0.606696	helical7
342	V	2	0.230762	0.648798	helical7
357	A	1	0.925919	0.508167	hinge
362	G	2	1	0.382076	hinge
385	F	1	0.516775	0.680148	helical8
448	K	1	0.327281	0.411297	hinge
459	S	1	0	0.264743	helical10
511	V	1	0	0.266792	helical11
534	Q	1	0.217067	0.203813	hinge

All scores can be combined to inform variant interpretation

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Not all pathogenic?
Some ‘mild’ pathogenic?

Next steps

- Correlate scores with clinical data of patients
- Compare results and functional data across related disorders
- We are happy to share all results
- Further support the SLC6A1 Connect team

Next steps

- Correlate scores with clinical data of patients
- Compare results and functional data across related disorders
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Acknowledgement

Cleveland Clinic, US
Charis Eng
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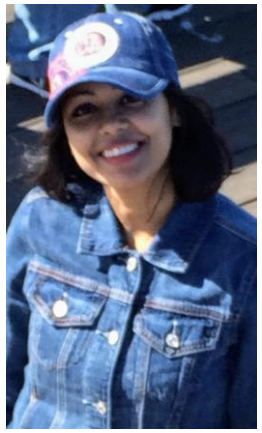
Peter Nuernberg

Danish Epilepsy Center
Katrine Johannson
Rikke Moller

SLC6A1 Connect
SLC6A1 Research & Support



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