Precision diagnostics for glucose transporter deficiency disorders using deep mutational scanning

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Introduction

Glucose transporter1(GLUT1) facilitates transport of glucose across the blood-brain barrier. Mutations in *SLC2A1*, the gene that encodes GLUT1, causes GLUT1 deficiency syndrome (DeVivo Syndrome) which has a wide phenotypic spectrum including epilepsy, cognitive impairment, dystonia, and microcephaly. Symptoms of GLUT1 deficiency syndrome can be treated with the ketogenic diet, therefore it is crucial to accurately identify pathogenic variants. However, genetic testing often identifies variants that have not been previously reported and that have insufficient evidence to label them as benign or pathogenic. Most *SLC2A1* missense variants deposited into ClinVar database are designated as variants of uncertain significance. Without functional evidence for these variants, precision medicine for these patients will remain elusive.

Goal

The goal of this study is to quantitatively determine the functional effect of every possible variant in SLC2A1 using large-scale functional genomics.

Method

SLC2A1 gene is essential for the growth of the haploid cell line, HAP1. As a proof of principle and in order to establish the growth assay, we first focused on 15 single nucleotide variants in exon 10 of *SLC2A1* gene.

- Use CRISPR to replace exon 10 with a DNA donor library containing 15 variants.
- Collect cells and extract DNA at days 5, and 11.
- Sequence cells to identify which variants drop out of the population over time.
- Quantify functional effects and build pathogenicity models

Results

According to the average of three biological replicates of cells containing 15 variants in exon 10, the nonsense variant and known pathogenic mutations dropped out of the population at early time points, while known benign mutations were never depleted .

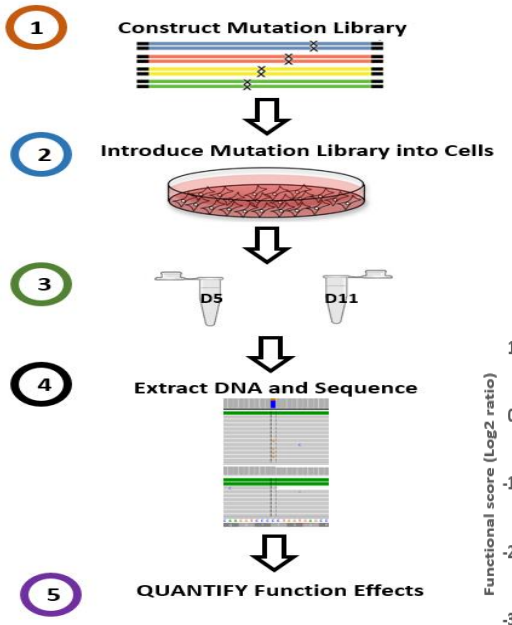
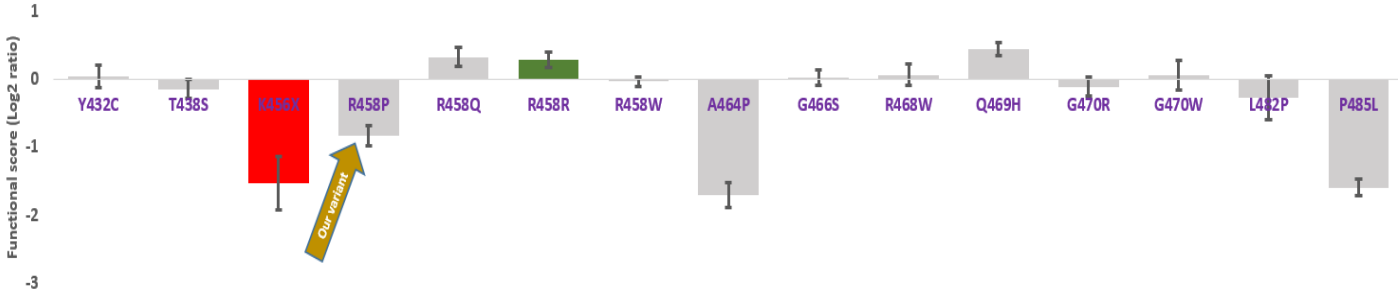


Fig.1. Schematic diagram of Knock-in experiment

Amino Acid Change	Inheritance	Clinical Phenotype	CSF:Serum	Glucose Uptake (RBC)	CADD score	gnomAD Frequency	Prediction (ClinVar)
Y432C	Unknown		0.37	56%	26.1	N/A	N/A
T438S	Unknown		N/A	N/A	21.7	N/A	N/A
K456X	De novo	infantile epilepsy	low	50%	39	N/A	Pathogenic
R458P	Inherited	childhood epilepsy, mild ID	0.65	N/A	26.2	N/A	N/A
R458Q	Unknown	Ataxia	N/A	N/A	27	1.20E-05	Likely pathogenic
R458R	Unknown	Dystonia		N/A	11.82	2.69E-03	Benign
R458W	De novo, AR (compound het), unknown	Multiple reports with variable phenotypes, including childhood PED (de novo), epilepsy (AR)	0.52	N/A	25	0.0063	Pathogenic/Likely pathogenic
A464P	Unknown		N/A	N/A	25.6	N/A	N/A
G466S	AR		N/A	N/A	19	4.96E-05	VUS
R468W	AR	Refractory epilepsy	0.46, 0.43,0.44	50%, 43%,46%	34	N/A	Likely pathogenic
Q469H	Unknown		N/A	N/A	17	3.98E-06	VUS
G470R	AR	Epilepsy worsened on KGD	N/A	N/A	20	8.60E-05	Conflicting
G470W	AR		N/A	N/A	24	2.83E-05	VUS
L482P	AR		N/A	N/A	23	1.59E-05	VUS
P485L	De novo	Infantile epilepsy, mild ID	0.45, 0.25	46%	19.84	N/A	Pathogenic



Conclusion

- Quantitative functional data can be generated on individual missense variants in a growth assay
- Intermediate functional effect occurred in a patient with missense variant R458P and a milder phenotype
- Quantitative functional scores are needed to improve diagnostic accuracy of glucose transporter deficiency disorders

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