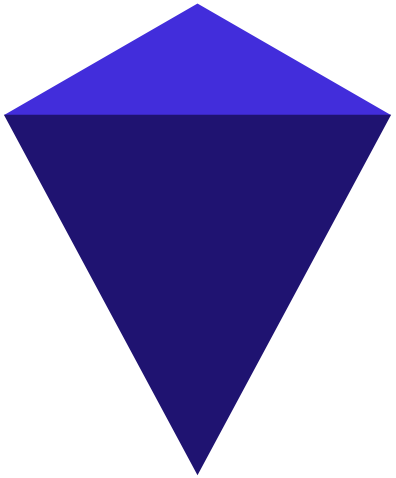


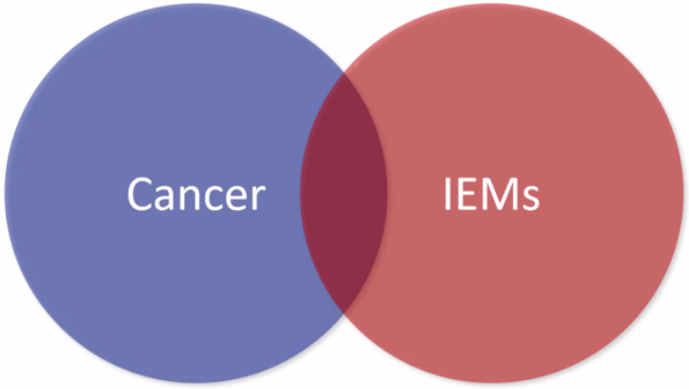
EmBold Therapeutics

(www.emboldtx.com)

Untangling genomics to treat mental health disorders metabolically

Human disease is a rich and relevant source of metabolic diversity

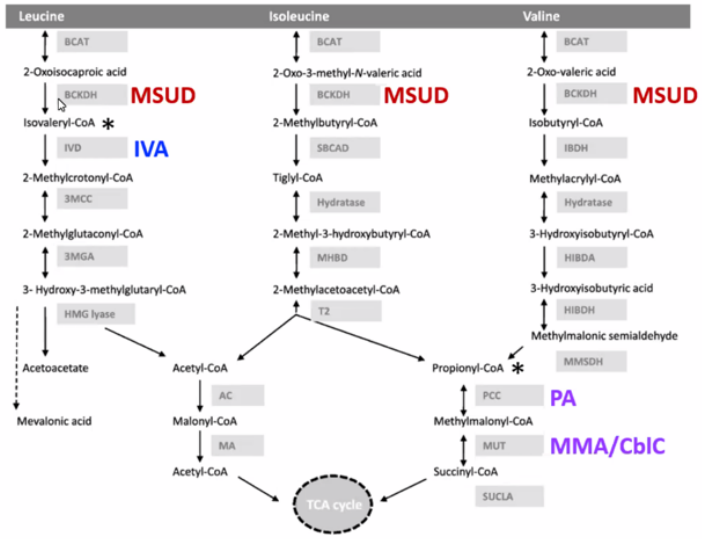
Why study both cancer and IEMs?

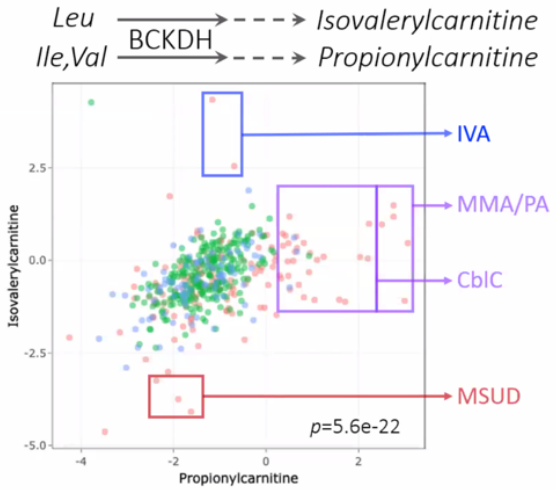


- Oncogenes and tumor suppressor genes regulate metabolism.
- Some metabolic enzymes are oncogenes or tumor suppressor genes (IDH1, IDH2, FH, SDH).
- Some IEMs increase cancer risk in children.

Rapid identification of outliers from correlated metabolite pairs

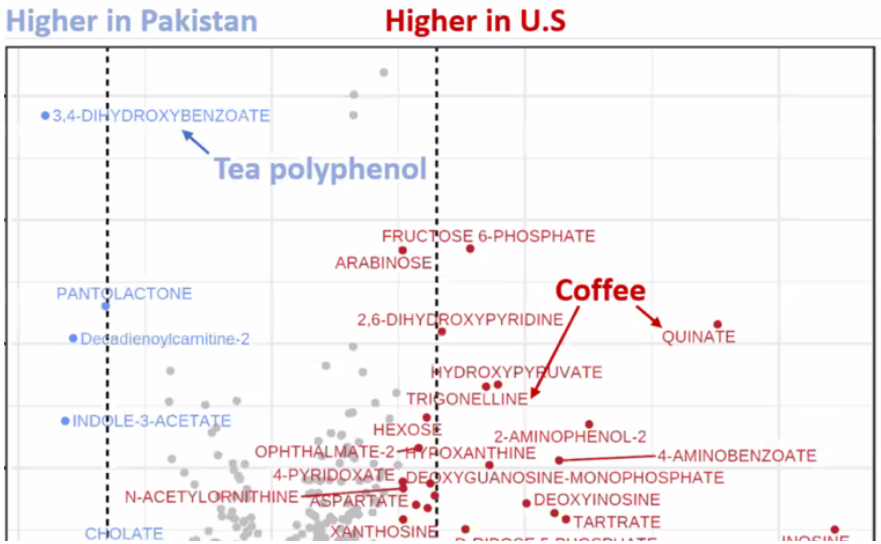
Branched-chain amino acid degradation





Manoli and Venditti, 2016

Metabolomic analysis of adult subjects from the U.S. and Pakistan



BBC June 14, 2022

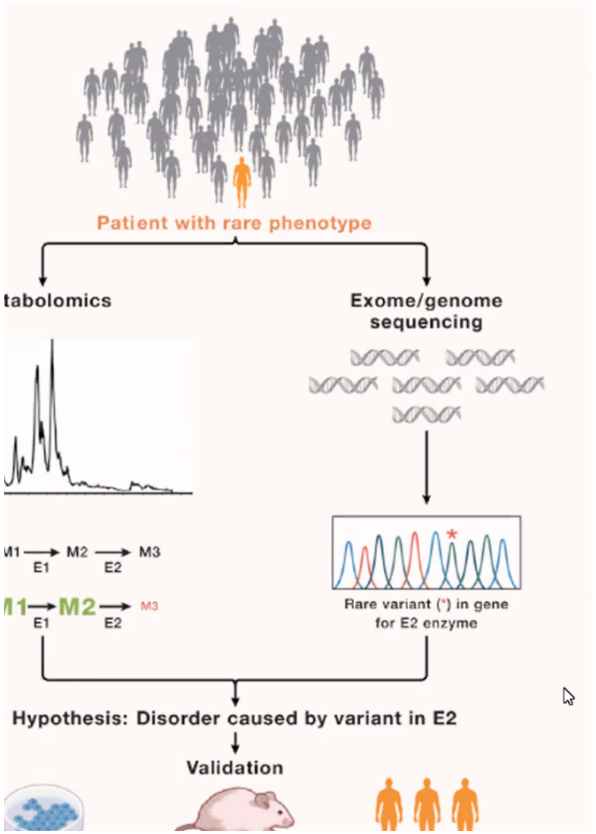
People in Pakistan urged to drink fewer cups of tea

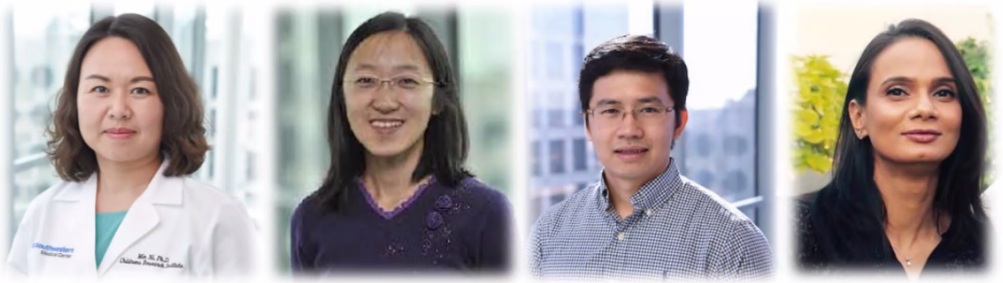
People in Pakistan have been asked to reduce the amount of tea they drink to keep the country's economy afloat.

Pakistan is the world's largest importer of tea, buying in more than $600m (£501m) worth last year.

"I appeal to the nation to cut down the consumption of tea by one to two cups

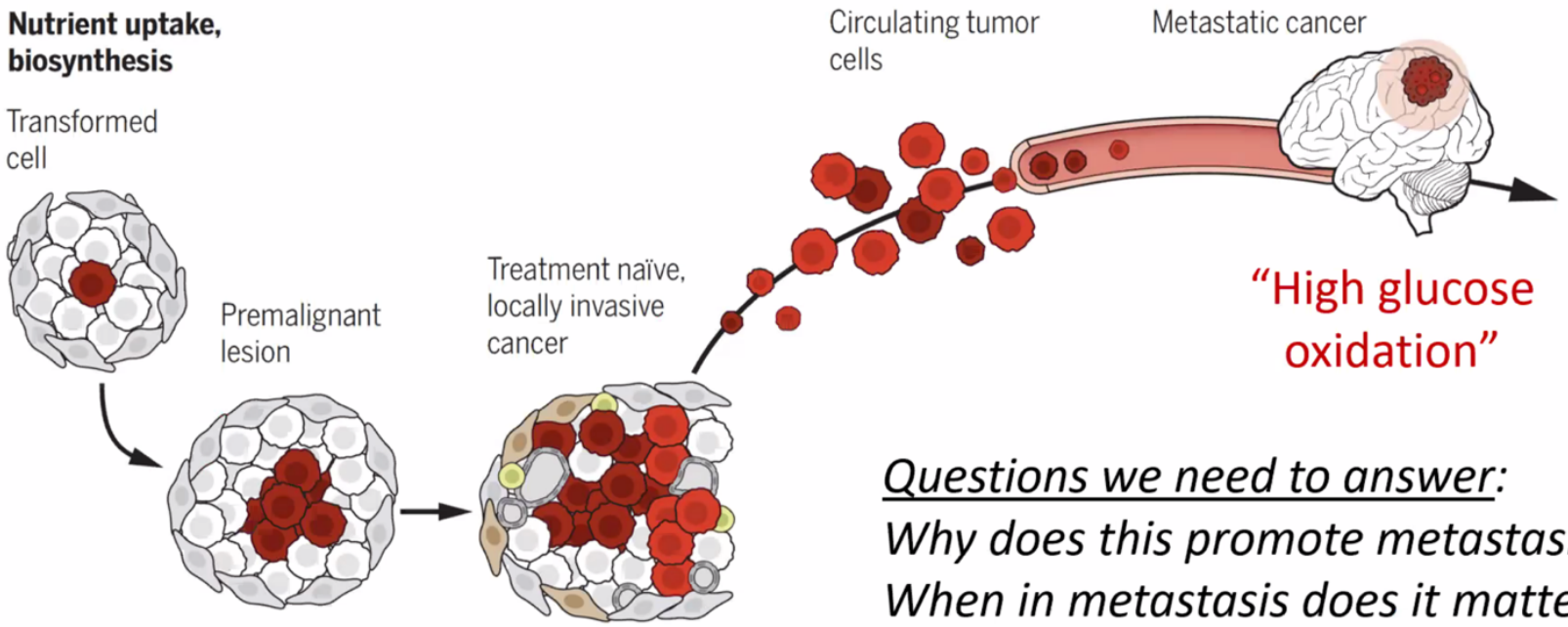
netic and Metabolic Disease Program at UT Southwestern





Min Ni | Ling Cai | Danny Vu | Bushra Afroze

Summary



"High glucose oxidation"

"Variable glucose oxidation"

ensley et al., 2016 (PMID 26853473)
aubert et al., 2017 (PMID 28985563)
aubert et al., 2020 (PMID 32273439)

<u>Questions we need to answer</u>:

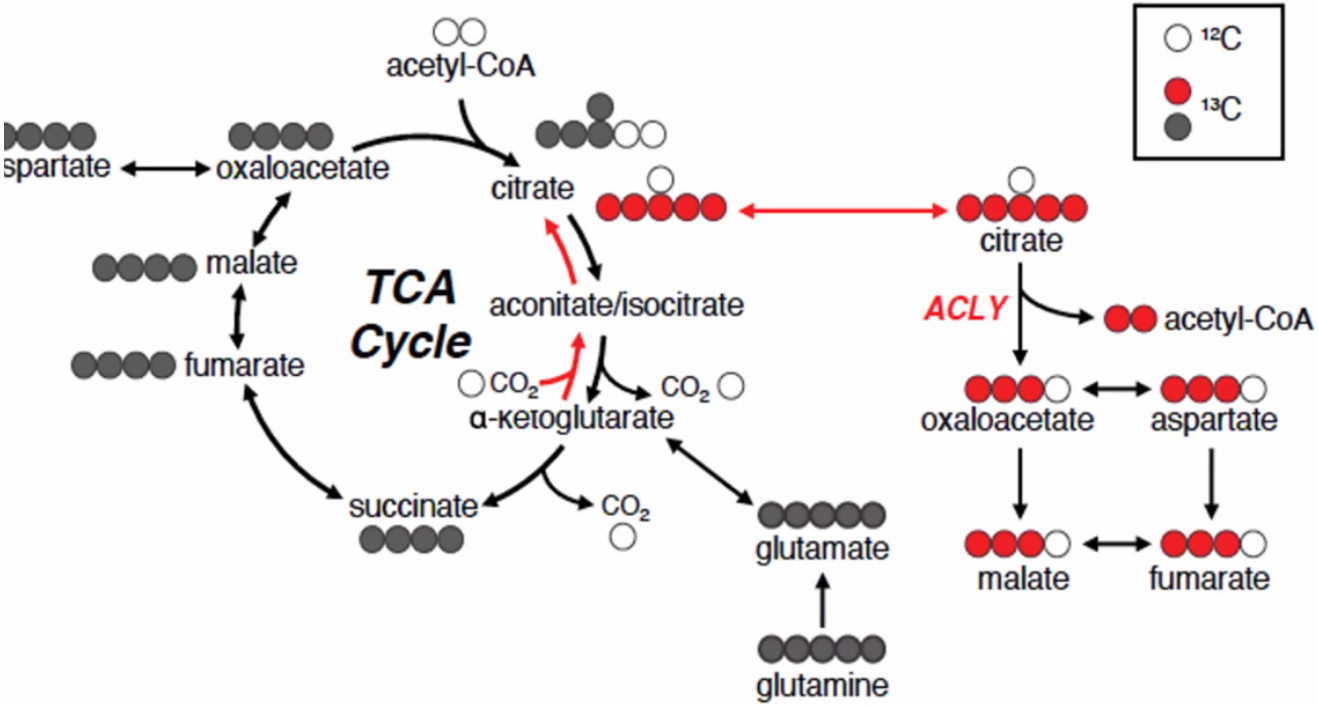
Why does this promote metastasis?

When in metastasis does it matter?

How is it regulated?

Can we exploit it for therapy?

amine gives a complementary view of the TCA cycle



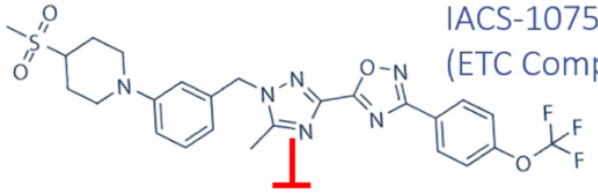
zoom.us Meeting View Edit Window Help — You are viewing UCLA Health IT's screen — View Options

Zoom Webinar

Is oxidative phosphorylation (OxPhos) required for TCA cycle labe

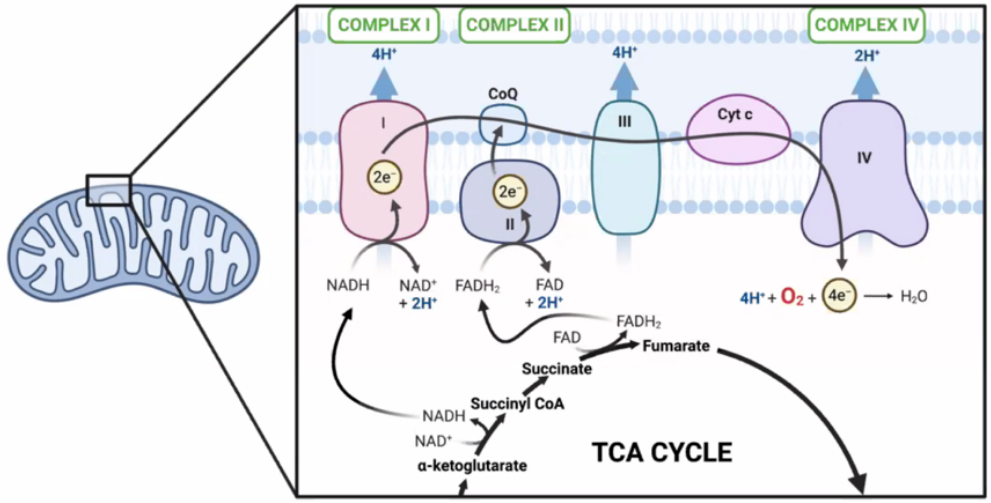
Gluco

○ ^{12}C
● ^{13}C

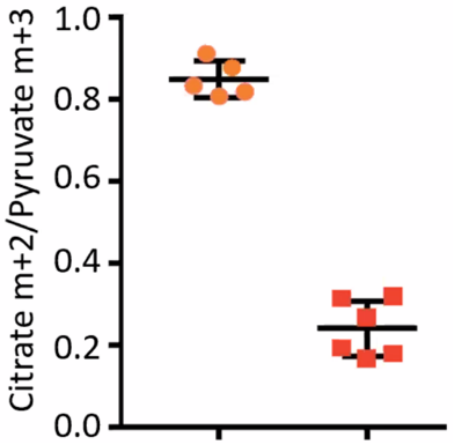


IACS-10759
(ETC Complex I inhibitor)

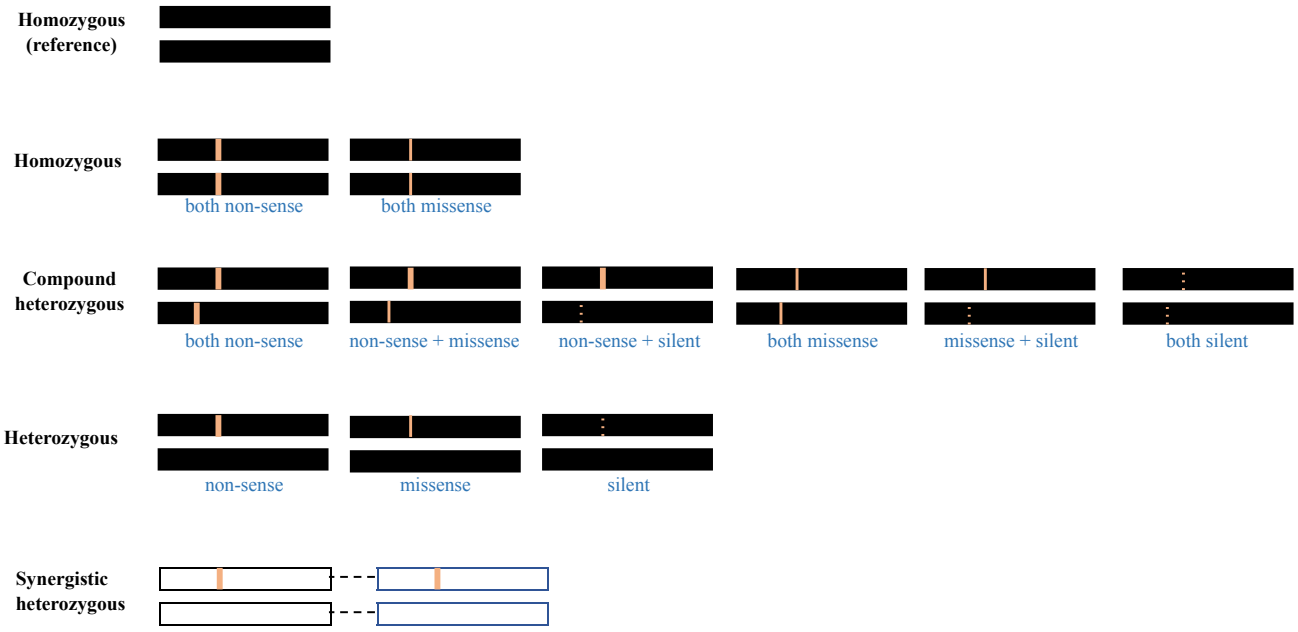
HIFα → PDK1



Infuse with [U-^{13}C]glucose
Examine labeling in tumor met



Possible alleles



Impact of Mutation on Activity of a Gene Product

	ref seq	non-sense LoF	missense LoF	missense GoF	silent LoF	silent GoF
ref seq	100	60-90	80-95	125-200	90-95	105-120
non-sense LoF		0	20-50	125-250	75-90	120-150
missense LoF			0-75	50-150	50-90	75-125
missense GoF				150-400	125-300	150-250
silent LoF					75-90	90-110
silent GoF						120-150

For instance, an individual with a normal and a null allele has 60-90% activity

An individual with a Reduced Function allele and a null allele has 20-50% activity

An individual with a GoF allele and silent LoF allele has an elevated activity

(using data from Gnomad and Exac databases, I looked at allele frequency for different types of mutations.
That data can then be used to calculate frequency of genotypes, and then number of individuals in the US with that genotype)
NEXT SLIDE

Frequency of Genotypes based on PAH Alleles (PKU)

The data from the right can then be used to calculate frequency of genotypes

using data from Gnomad and Exac databases for PAH, I looked at allele frequency for different types of mutations.

calculated using table on the right

	carrier freq	0.520	0.00041	0.00944	0.00236	0.37414	0.093535
carrier freq		ref seq	non-sense LoF	missense LoF	missense GoF	silent LoF	silent GoF
0.520	ref seq	0.27051961	0.0002	0.0049	0.0012	0.1946	0.0486
0.00041	non-sense LoF	0.0002	0.0000	0.0000	0.0000	0.0002	0.0000
0.00944	missense LoF	0.0049	0.0000	0.0001	0.0000	0.0035	0.0009
0.00236	missense GoF	0.0012	0.0000	0.0000	0.0000	0.0009	0.0002
0.37414	silent LoF	0.1946	0.0002	0.0035	0.0009	0.1400	0.0350
0.093535	silent GoF	0.0486	0.0000	0.0009	0.0002	0.0350	0.0087

	in gnomad 2.1/ 3.1/ exac	allele frequency	fraction	GH commentary
ref seq				
non-sense LoF (fs or stop gained)	40	0.00041	1.0	likely all fully pathogenic alleles
missense LoF	326	0.0118	0.80	estimate 90
missense GoF			0.20	
silent LoF	119	1.8707	0.20	estimate 20
silent GoF			0.05	
total	485			

I am not sure I did this right. Ultimately it fits, but I am not totally clear of what these numbers mean.

Population by Genotype

Use frequency from previous slide and multiply by US population

US population	331000000					
genotypes in the US:						
	ref seq	**non-sense LoF**	**missense LoF**	**missense GoF**	**silent LoF**	**silent GoF**
ref seq	89,541,992	70,585	1,625,172	406,293	64,411,218	16,102,805
non-sense LoF	70,585	56	1,281	320	50,775	12,694
missense LoF	1,625,172	1,281	29,497	7,374	1,169,053	292,263
missense GoF	406,293	320	7,374	1,844	292,263	73,066
silent LoF	64,411,218	50,775	1,169,053	292,263	46,333,625	11,583,406
silent GoF	16,102,805	12,694	292,263	73,066	11,583,406	2,895,852

Patient Population Analysis suggests that there are about 30-x more GoF individuals

		population	Phe activty	0	25	50	75	100	125	150	200	250	300	350	400
ref seq	ref seq	89,541,992	100					89,541,992							
ref seq	non-sense LoF	141,170	60-90			14,117	112,936	14,117							
ref seq	missense LoF	3,250,344	80-95				325,034	2,925,310							
ref seq	missense GoF	812,586	125-200							406,293	406,293				
ref seq	silent LoF	128,822,437	90-95					128,822,437							
ref seq	silent GoF	32,205,609	105-120					25,764,487	6,441,122						
non-sense LoF	non-sense LoF	56	0	56											
non-sense LoF	missense LoF	2,562	20-50		1,537	1,025									
non-sense LoF	missense GoF	641	125-250						641						
non-sense LoF	silent LoF	101,549	75-90				50,775	50,775							
non-sense LoF	silent GoF	25,387	120-150						12,694	12,694					
missense LoF	missense LoF	29,497	0-75	8,849	14,748	5,899									
missense LoF	missense GoF	14,748	50-150			2,950	2,950	2,950	2,950	2,950					
missense LoF	silent LoF	2,338,106	50-90			935,242	1,402,863								
missense LoF	silent GoF	584,526	75-125				146,132	292,263	146,132						
missense GoF	missense GoF	1,844	150-400							461	369	369	277	184	184
missense GoF	silent LoF	584,526	125-300						233,811	116,905	116,905	58,453	58,453		
missense GoF	silent GoF	146,132	150-250							73,066	43,839	29,226			
silent LoF	silent LoF	46,333,625	75-90				23,166,812	23,166,812							
silent LoF	silent GoF	23,166,812	90-110					23,166,812							
silent GoF	silent GoF	2,895,852	120-150						1,447,926	1,447,926					
				8,905	16,286	959,233	25,207,502	293,747,955	8,285,274	2,060,294	567,407	88,048	58,729	184	184
				PKU patients		asymptomatic					2X elevated actvity				
				25,190		330,260,257.70562					714,552				

Summary (Thinking out loud)

- There are ~25,000 PKU patients in the US. This aligns with the accepted patient population.
- There are >700,000 people in the US with increased PAH activity. What could this lead to?
- Speculation 1: more PAH activity could lead to increased tyrosine levels. This may be shunted to dopamine and cause a phenotype.
- Speculation 2: more PAH activity would require more BH4 and could keep this away from other BH4 requiring enzymes. This may lead to reduced dopamine and serotonin synthesis.
- …
- There are 1M+ people with reduced PAH activity, however not quite at the PKU level. What could this lead to:
- More Phe in plasma and potential brain fog, similar to PKU patients
- Less Tyr and hence less dopamine
- Complications if other enzymes in the pathway are impacted.
- …