ASL deficiency: pervasive genetic interactions between active site alleles restore near wildtype function

Michelle Tang[1], Gareth A. Cromie[1], Russell S. Lo[1], Martin S. Timour[1], Hiroki Morizono[2,3], Ljubica Caldovic[2,3], Nicholas Ah Mew[2,3], Andrea Gropman[2,3,4,5], Aimée M. Dudley[1]

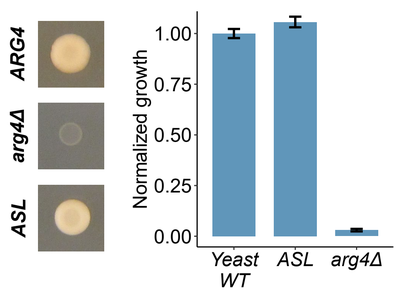
[1]Pacific Northwest Research Institute, Seattle, WA, USA, [2]Center for Genetic Medicine Research, Children's National Research Institute, Children's National Hospital, Washington, DC, USA, [3]Department of Genomics and Precision Medicine, School of Medicine and Health Sciences, The George Washington University, Washington, DC, USA, [4]Department of Neurology, Division of Neurogenetics and Neurodevelopmental Disabilities, Children's National Hospital, Washington, DC, USA, [5]Center for Neuroscience Research, Children's National Research Institute, Children's National Hospital, Washington, DC, USA

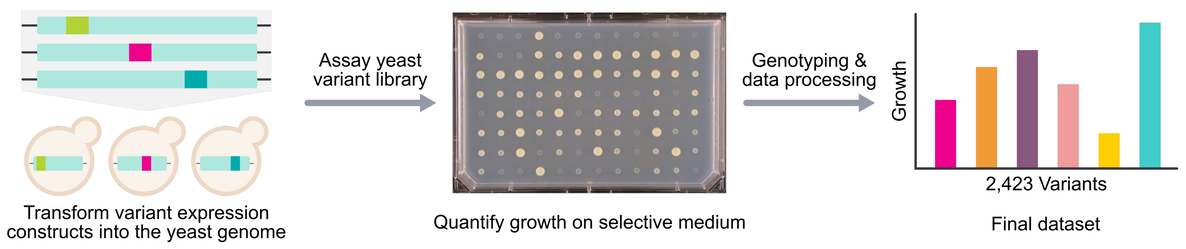
Argininosuccinate lyase deficiency

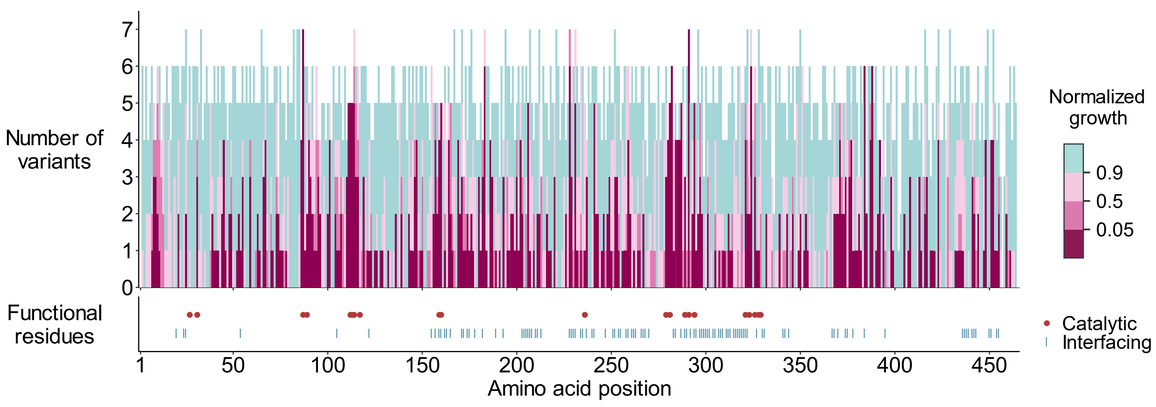
Argininosuccinate lyase (ASL) deficiency is the second most common urea cycle disorder (UCD), affecting ~1 in 70,000 live births and accounting for up to 20% of UCD cases. Deficiency in the ASL enzyme results in hyperammonemia, the buildup of blood ammonia to levels toxic to the brain, which can lead to lethargy, seizures, coma and even death. Complications may include neurocognitive deficiency and progressive liver damage. Disease severity ranges from mild to severe depending on level of enzyme activity, and the onset of ASL deficiency can occur in the neonatal period or at later stages of life. Clinical and genetic heterogeneity is further confounded by the phenomenon of intragenic complementation, in which the combination of two variants of the same protein have activity significantly greater than what is expected based on each allele's individual activity. To gain a better understanding of disease manifestation and genotype-phenotype correlation in ASL deficiency, we developed and performed high throughput yeast assays to assess the functional impact of all single nucleotide variant (SNV)-accessible *ASL* missense variants.

High throughput yeast growth assays for determining variant effects in *ASL*

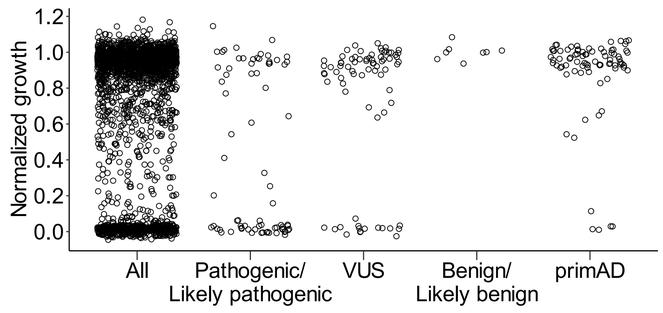
The assay leverages the ability of human ASL to functionally replace deletion of its yeast ortholog, *ARG4*, allowing yeast strains to grow on arginine-deficient medium. The growth of yeast cells harboring ASL variants as the sole source of the enzyme provides a quantitative readout of variant enzyme function expressed on an intuitive scale.





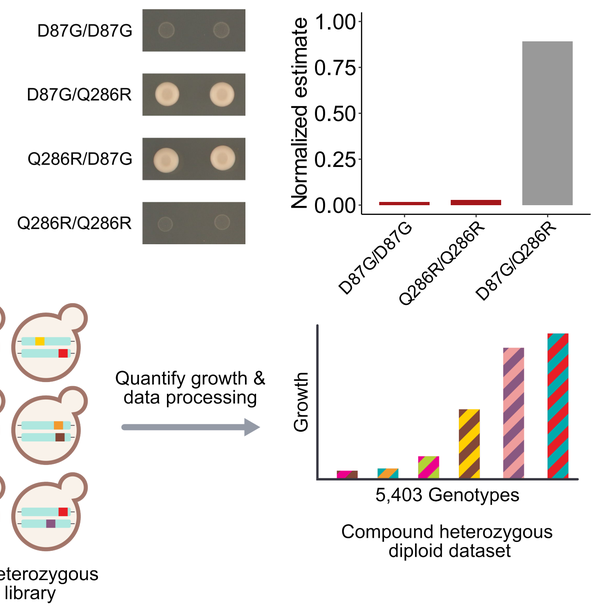


We first assessed the individual functional impact of ~2,400 *ASL* missense variants (88% of all possible SNV-derived missense variants) across the length of the protein.

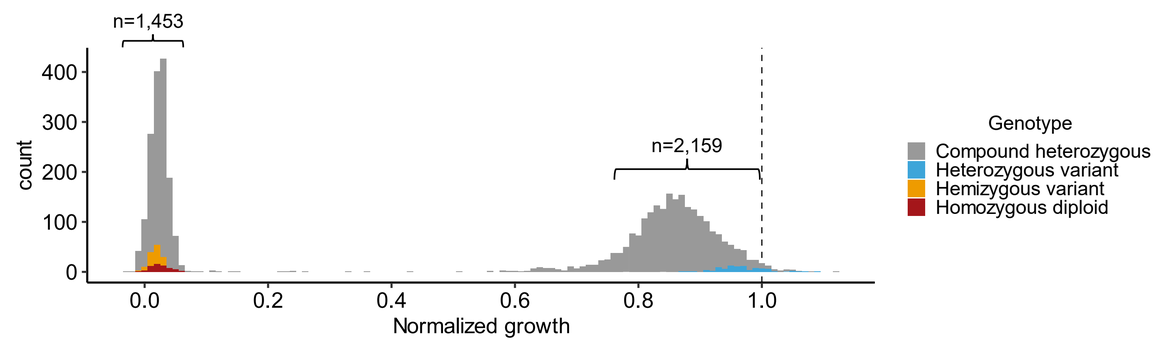


Genetic interactions of compound heterozygotes

Yeast mating of variant strains permits the detection of genetic interactions between different *ASL* alleles. We tested ~5,400 unique combinations of amorphic missense variants that individually exhibited no ASL activity.

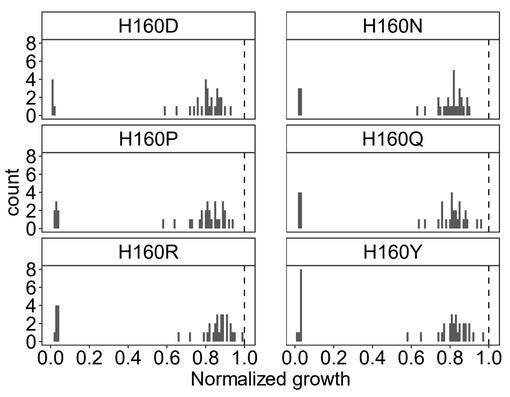


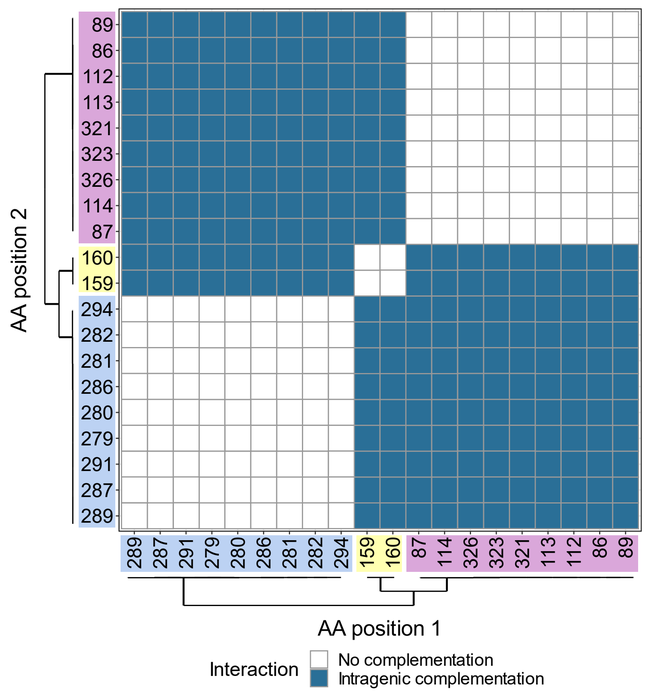
Of the ~3,600 unique combinations that involve variants of active site residues, over half (~2,200) exhibited strong intragenic complementation, in which the combination of two amorphic variants produced near wildtype levels of activity.



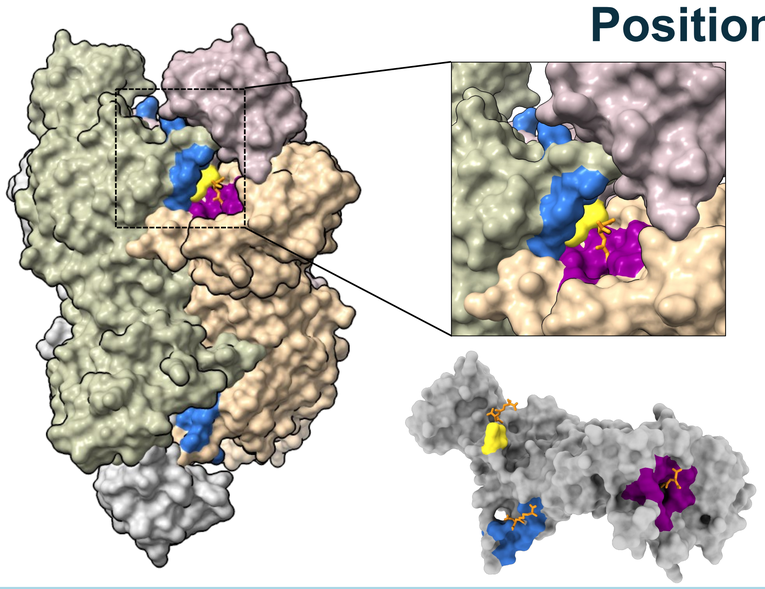
Intragenic complementation occurs frequently between amino acid positions within the active sites and is largely independent of the amino acid substitution.

A representative example with His160, a residue in the catalytic center of ASL, is shown below. Intragenic complementation was observed between every SNV-accessible variant of His160 and the same set of active site variants in our library.

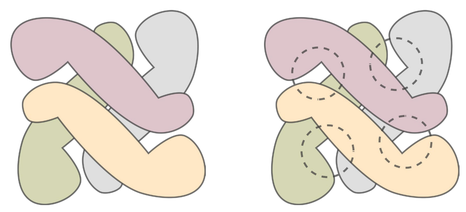




Position in active sites determine intragenic complementation



Schematic of the ASL tetramer assembly
Wildtype active sites are indicated by dashed circles

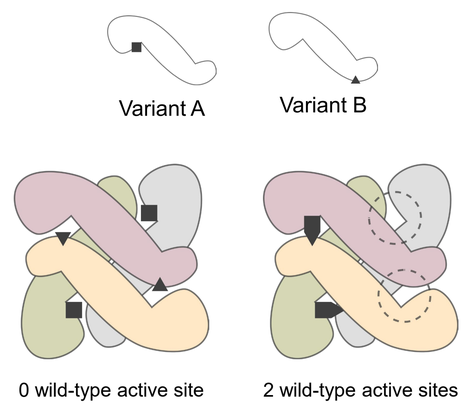


PDB=1AOS, ligand=AS (orange stick)
Tested amino acid positions
colored based on interaction clusters

Clustering of amino acid positions corresponds to the three interfaces that make up the active site. Intragenic complementation occurs between variants of amino acid positions from different interfaces of the monomer. Depending on the configuration of the ASL variant monomers in a compound heterozygote, the hetero-tetramer may have zero or two wild-type active sites.

Scan to view a video of the two tetramer configurations on the right.

Mechanism of intragenic complementation in ASL



Acknowledgements

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