Deep Mutational Scan of TYK2 Reveals Protein-Drug Interactions and Novel Therapeutic Sites of Action

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Abstract

Autoimmune disorders represent a complex and increasingly prevalent set of diseases for which many current treatments are associated with severe side-effects, including high risk of infection, blood clots, and cardiovascular disease. In recent years, Tyrosine Kinase 2 (TYK2) has emerged as a novel and safer target for therapeutic modulation of the immune system, and in 2022 the FDA approved the first small-molecule inhibitor of TYK2. A detailed understanding of TYK2 structure-function relationships and protein-ligand interactions would empower the design of new drugs to this important clinical target. To this end, we leveraged Deep Mutational Scanning (DMS) to map TYK2's protein-drug interactions and sites of allosteric regulation. In total, we measured the effect of >20,000 amino acid variants on TYK2's interferon alpha (IFN-α) signaling activity and protein expression. Our ability to quantify subtle differences in variant effects enabled mapping of core interactions responsible for drug binding, in addition to peripheral protein residues that contribute indirectly to inhibition. We can distinguish differences in binding interactions between two inhibitors that target the same site and capture variants that increase the potency of these compounds. Together, the drug-protein interactions define structure-activity relationships (SAR) for how the inhibitors functionally interact with TYK2 and point to where compounds could be optimized to increase potency. Finally, we identified allosteric sites of TYK2 to better understand how it can be regulated more generally. Altogether, our results demonstrate that DMS can be used to identify novel target sites and in the functional characterization and optimization of drug candidates.

Background

- TYK2 is a kinase protein related to Janus kinases with key roles in immune cytokine signaling
- Complete loss of TYK2 function results in immune deficiency
- A common human variant (P1104A) partially reduces TYK2 activity and is protective against a variety of autoimmune phenotypes
- Partial inhibition of TYK2 offers a potentially safer avenue for the treatment of autoimmune disease than previous therapies
- In 2022 the FDA approved the first TYK2 inhibitor for the treatment of moderate-to-severe plaque psoriasis
- Having a better understanding of the functional consequences of human variants, of how TYK2 structure relates to function, and how ligands interact with the protein would enable future drug discovery efforts for this critical target

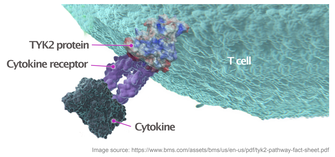
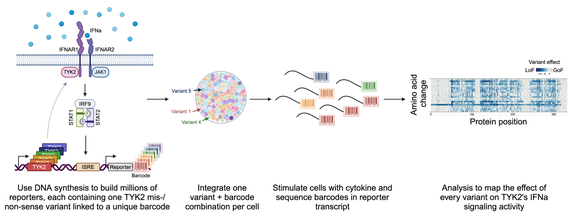


Image source: https://www.bms.com/assets/bms/us/en-us/pdf/tyk2-pathway-fact-sheet.pdf

Methods

- Deep mutational scanning (DMS) measures the effect of thousands of amino acid substitution on a protein's function
- We used DMS to measure the effects of all single amino acid substitutions and nonsense variants on two functions of TYK2: IFN-α signaling and TYK2 protein expression
- Every mutation is tagged by dozens of unique DNA barcodes that are read out by massively parallel sequencing, giving us the power to detect even small quantitative effects
- All told, we generated >1 million human cell lines each harboring a unique variant-barcode combination

IFN-α signaling



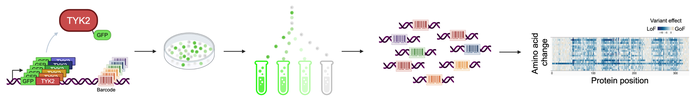
Use DNA synthesis to build millions of reporters, each containing one TYK2 mis-/non-sense variant linked to a unique barcode

Integrate one variant + barcode combination per cell

Stimulate cells with cytokine and sequence barcodes in reporter transcript

Analysis to map the effect of every variant on TYK2's IFNa signaling activity

TYK2 protein expression



Use DNA synthesis to build millions of reporters, each containing one TYK2 mis-/non-sense variant linked to a GFP tag and a unique barcode

Integrate one variant + barcode combination per cell

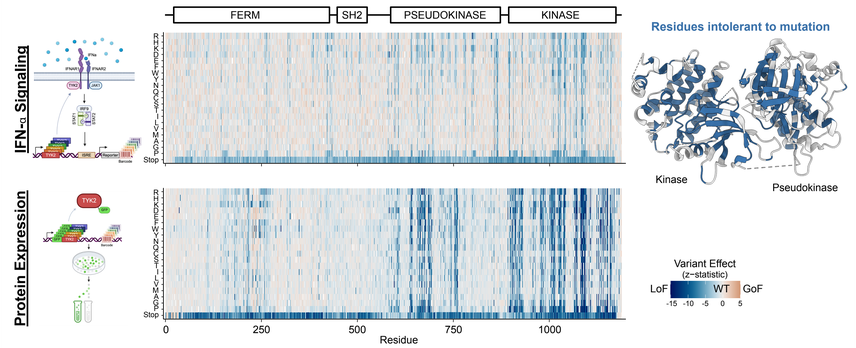
Flow sort cell library by GFP signal

Extract DNA and sequence barcodes in each pool

Analysis to map the effect of every variant on TYK2's protein expression

Results

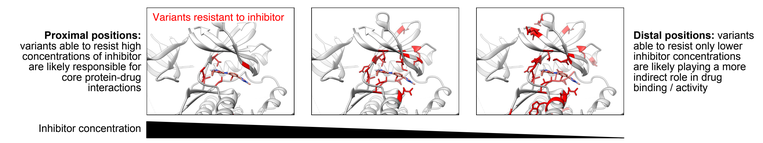
Functional effects of >20,000 TYK2 variants



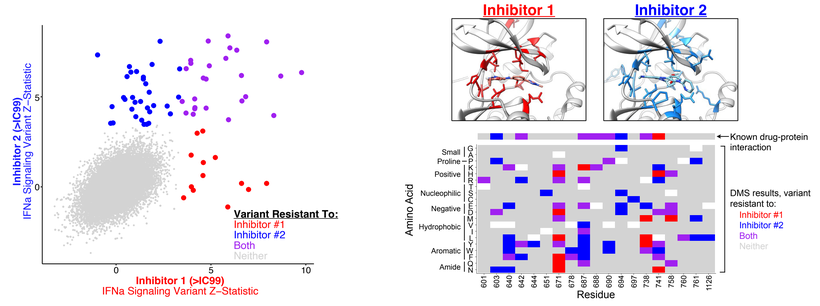
Protein-drug interactions for better chemical design

Variants that lead to drug resistance

- DMS in the presence of TYK2 inhibitors can identify protein variants that lead to drug resistance or potentiation
- Functional complement to structure: distinguish between direct binding and secondary drug-protein interactions

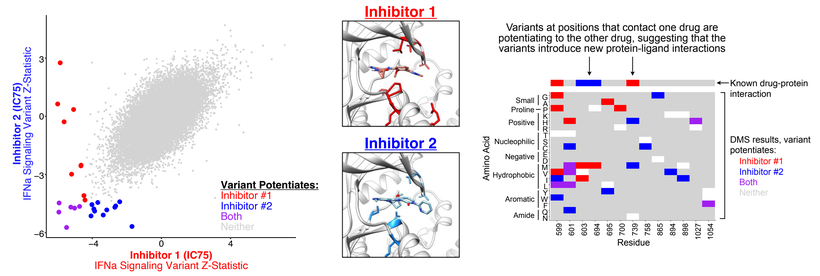


- Empower chemical decision making: prioritize compounds that are not susceptible to variants commonly found in the human population



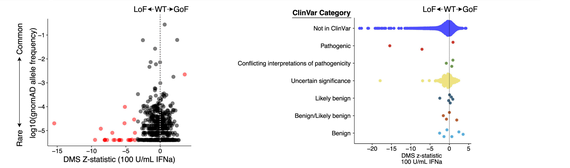
Variants that potentiate drug effects

- Speed up SAR-based chemical optimization: variants that potentiate drug effects can point to where the compound should be improved



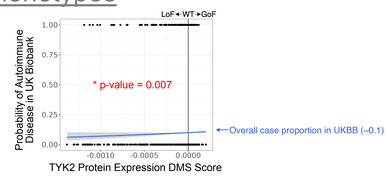
Human variant interpretation for target validation

Systematically tested all human mis-/non-sense alleles

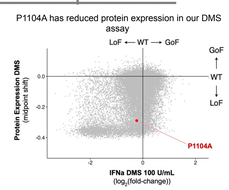


Rare alleles that reduce protein expression are protective for autoimmune phenotypes

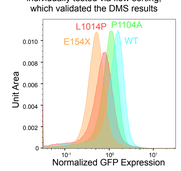
DMS-informed rare variant burden test replicates finding that loss of TYK2 activity is protective against autoimmune phenotypes



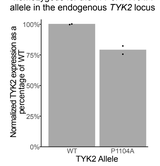
Common protective P1104A variant leads to reduced protein expression



Selected TYK2 alleles were cloned with GFP tags and individually tested via flow sorting, which validated the DMS results

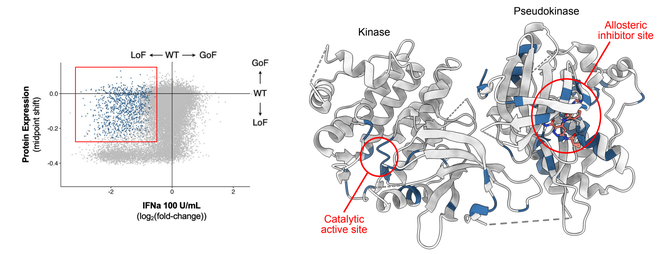


Western blot validation of TYK2 protein expression using kit225 cells homozygous for the WT or P1104A allele in the endogenous *TYK2* locus



Allosteric site identification

- Identifying variants that impact IFN-α signaling but not protein expression elucidates critical functional sites, including the catalytic site and allosteric regulatory domains



Conclusions

- We performed a comprehensive Deep Mutational Scan (DMS) of TYK2, an important target for the treatment of autoimmune disease
- Tested effects of all 20,000+ single amino acid substitutions and nonsense variants on two functions: IFN-α signaling and TYK2 protein expression
- DMS can empower multiple stages of drug discovery
- **Target validation:** we systematically tested the effects of all human TYK2 missense and nonsense variants to uncover a role for reduced TYK2 protein expression in protection against autoimmune phenotypes
- **Detailed protein structure-function for target site identification:** elucidated regions of TYK2 with key catalytic and allosteric activities
- **Compound optimization:** uncovered variants that lead to drug resistance and potentiation, which could facilitate the design of more efficacious therapies