DNA-binding consequences of human homeodomain missense variants

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Clinical significance of homeodomain missense variants

- Numerous homeodomain coding mutations, particularly within the DNA-binding domain (DBD), have been associated with an array of human Mendelian diseases[1].

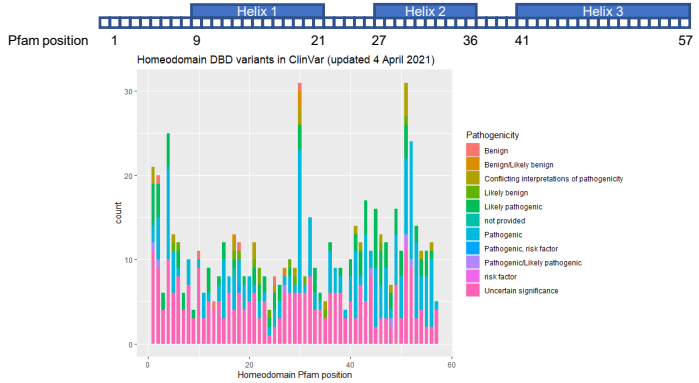
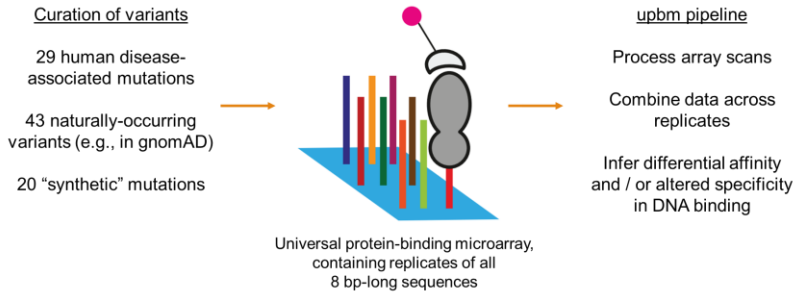


Figure 1: Every single position within the homeodomain DBD has been reported in ClinVar[2] to have pathogenic variants and variants of uncertain significance (VUS). (Top) Schematic of homeodomain DBD sequence, with the locations of the three α-helices depicted. (Bottom) Histogram of homeodomain DBD variants, per Pfam PF00046[3] amino acid residue position and differentiated by annotations, in ClinVar.

Study motivation and design

- We aim to more broadly and comprehensively understand the effects of homeodomain DBD variants on DNA binding, which is important for variant interpretation and insights into disease mechanisms.



DNA-binding properties of homeodomain missense variants

- We utilised a parametric model framework to make inferences about differential binding affinity and altered binding specificity by human homeodomain missense variants (including disease-associated variants) to sets of 8-mers.
- More than 75% of disease-associated variants we examined resulted in diminished binding affinity, particularly to 8-mers bound by the wild-type homeodomain with high affinity.

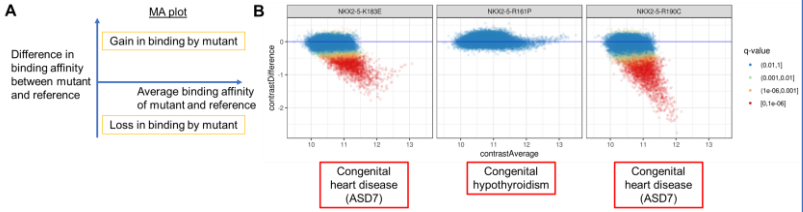


Figure 2: Diminished DNA-binding affinity in disease-associated variants. (**A**) Schematic of MA plots depicted in this poster. (**B**) MA plots of NKX2-5 variants annotated as "Pathogenic" in ClinVar. Each dot represents a single 8-bp sequence ("8-mer"). The K183E and R190C variants show drastic decreases in DNA-binding affinity, while the R161P variant, found in a patient with congenital hypothyroidism, does not result in a significant change in DNA-binding affinity or specificity.

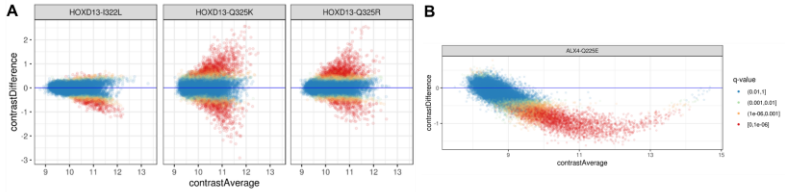


Figure 3: Altered DNA-binding specificity in disease-associated variants. (**A**) MA plots of ClinVar "Pathogenic" HOXD13 variants. (**B**) MA plot of ALX4 Q225E variant (also ClinVar "Pathogenic"); this variant, which is at a potentially novel specificity-determining position, appears to result in a subtler change in DNA-binding specificity than the HOXD13 variants.

Novel DNA-binding specificity-determining residues

- We identified 9 novel DNA-binding specificity-determining positions across our entire dataset, with several of these residues distal from homeodomain-DNA interfaces.

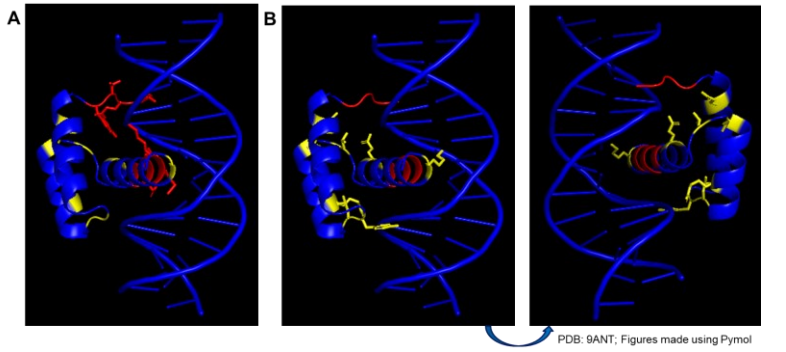
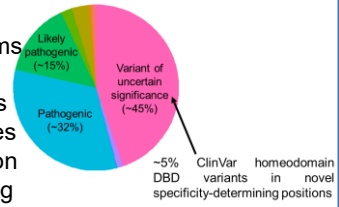


Figure 4: Possible mechanisms for alterations in DNA-binding specificity include changes in intra-homeodomain side-chain interactions, or introduction of contacts with DNA target sites. (**A, B**) Homeodomain-DNA complex (PDB: 9ANT); known specificity-determining residues[4] in red, novel specificity-determining residues in yellow. Side chains of known and novel residues shown in (**A**) and (**B**), respectively.

Implications

Our results suggest pathogenic mechanisms for several VUS and disease-associated variants and genes, and contribute towards clarification of VUS. Our study also provides insights into homeodomain-DNA recognition rules, beyond known specificity-determining positions and DNA-contacting positions.



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References

1) Chi, Hum Genet, 2005, PMID: 15726414
2) Landrum *et al.*, Nucleic Acids Res, 2018, PMID: 29165669
3) Mistry *et al.*, Nucleic Acids Res, 2021, PMID: 33125078
4) Noyes *et al.*, Cell, 2008, PMID: 18585360