Group project 3: *Exploring Structural Variants of NR3C2*

Background

Ruzzo et al (2024) employed whole genome sequence analysis to identify over a dozen new genes that are significantly associated with Pseudohypoaldosteronism, the majority of which exhibit a contribution from rare inherited mutations.

The authors identified structural variants (SVs) that implicated recurrent deletions in the promoters of DLG2 and NR3C2.

The human gene NR3C2 (nuclear receptor subfamily 3 group C member 2) encodes the mineralocorticoid receptor and spans over chromosome 4 (4:148,078,762-148,444,698 reverse strand).

Tasks

ProtVar - www.ebi.ac.uk/protvar

ProtVar allows you to paste a list of variants from any protein and retrieve annotations or to retrieve all annotations and predictions for a protein.

- Enter accession P08235 in the Browse ProtVar search bar and click search

You should now see the results table displaying positional information for every variant. For each position ProtVar has three types of annotations, accessible by clicking on the buttons to the right of each row:

The first shows functional annotations from literature and predictions.
The second shows variant details including where it has been reported as well as co-located variants and disease information.
The third shows experimental and Alphafold structures and interface and pocket models.
Let's explore some specific variants to learn more about what their consequences might be.

- Go back to the ProtVar home screen and paste each of the below variant coordinates.

4 148194827 A G

- Click on the functional annotations button

Q. Is the position involved in an interaction with another molecule? Which?

- Click on population observation button

Q. Has a variant of this amino acid been implicated in a disease? Which?

- Click on the Structure annotation button. Click a row in the Predicted Interacting structures table:

Q. Which protein interaction might the variant affect?

Now investigate the following with fewer prompts:

4 148154569 A G

Q. Which domain is this variant in and what is its role?

- In the structure tab investigate the AlphaFill models. You can press the “zoom to variant” or “highlight pocket” buttons.

Q. Which ligands might the variant affect?

4 148081433 A G

Q. What is the experimental finding for the effect of this variant?

- Look at the AlphaFold structures and then to the right to see the Predicted align error plot (green box). It should be clear from this and from the previous variants that there are two functional domains. To investigate this further, follow the link to AlphaFold and investigate the TED domains and their boundaries. 

The variants above had high pathogenicity scores for both CADD and AlphaMissense, however the predictors do not always agree with each other.

- Have a look at this variant with “pathogenic” Alphamissense scores but “likely benign” CADD scores.

4 148120195 T G

Q. Using the available annotations and predictions can you evaluate whether this variant may have a molecular consequence?

Now look at the opposite where the AlphaMissense score is “benign” but CADD considers the variant to be “highly likely deleterious”

4 148152550 G A

Q. What is your assessment of this variant and why?

Q. Should researchers rely entirely on one or even multiple VEPs? Why?

PDB

PDB task 1:

Jupyter / Colab Notebook for this task:

01_structures_availability
(https://github.com/PDBeurope/pdbe-notebooks/blob/main/variants_embl-ebi_july2025/01_structures_availability.ipynb)

- Fill-in information on predicted and experimentally-determined structures availability.
  HINT: Use UniProt ID to help find structures.

Table 1: General Structure Availability	
No of predicted & exp-determined structures	
No of predicted structures	
No of exp-determined structures	
No of exp-determined structures from PDB	
No of exp-determined structures with resolution better than 3 Ångstrom	
PDB id(s) with best resolution	
PDB id(s) with most coverage	
PDB id(s) without any mutations	

PDB task 2:
Jupyter / Colab Notebook for this task:
01_structures_availability
(https://github.com/PDBeurope/pdbe-notebooks/blob/main/variants_embl-ebi_july2025/01_structures_availability.ipynb)

The following structures may help give insight into the genetic variant:

1. Experimentally-determined structure with an amino acid change at the position
2. Experimentally-determined structure with information at the variant position and no other mutations or variants
3. Experimentally-determined structure with information at the variant position but with additional mutations or variants
4. Predicted structure with information at the variant position

The usefulness of the structures for insight into genetic variants is as follows:
(1) is best, BUT if (1) does not exist, should consider (2), and if (2) does not exist should consider (3) or (4).

List 4-10 single amino acid changes from previous analysis (included 1 example)	645 (Cys/Arg),

- Identify the corresponding data from predicted and experimentally-determined structures for a specific set of variants.
  HINT: Use UniProt numbering for variant position.

Table 2: Find PDB ids / AFDB ids with coverage for understanding the variant		
Variant position	Best structure type available	PDB or AFDB id(s)
645	(2) Exp-det (no mut)	

PDB task 3:
Jupyter / Colab Notebook for this task:
02_structural_visuallisation
(https://github.com/PDBeurope/pdbe-notebooks/blob/main/variants_embl-ebi_july2025/02_structures_visuallisation.ipynb)

Visualising is a key part of understanding protein structures. We will use some of the structures previously identified for visualisation. Mutations that are clustered together spatially in a structure may be impacting in a similar manner. This visualisation will be useful for PDB task 5.

- Using the tool developed by PDBe and RSCB and partners called MolStar we can view where the variants are in a structure.

PDB task 4:
- Use the Ligand subpage of a PDBe-KB page to assess whether a ligand binding site is applicable for the variant or not. This analysis will be useful for PDB task 5.
  HINT1: Use UniProt ID to find the appropriate PDBe-KB page.
  HINT2: If no experimental structures are available, skip this task.

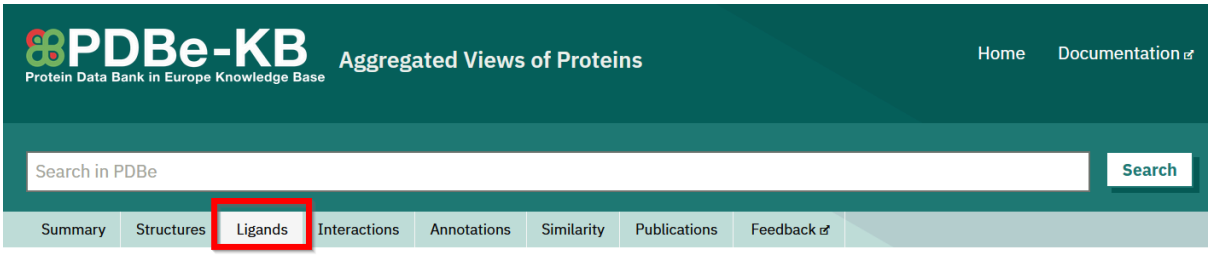

Table 3: Find PDB ids / AFDB ids if ligand is involved		
Variant position	Near ligand site? (CCD ID)	PDB id(s)
645	Zinc (ZN)	4tnt

PDB task 5:
Jupyter / Colab Notebook for this task:
03_structural_assessment
(https://github.com/PDBeurope/pdbe-notebooks/blob/main/variants_embl-ebi_july2025/03_structural_assessment.ipynb)

- If possible, propose an interpretation of why the genetic variant may impact on the protein's function and thus cause disease.
  HINT: Use the tools from PDB task 3 & 4 to aid answering structural assessment questions.

Table 4: Propose why variant might impact on protein function	
Variant	**Variant interpretation (hypothesis based on structure)**
645 (Cys/Arg)	Zinc binding site is disrupted by the genetic variant.

Table 4: Propose why variant might impact on protein function	

Ensembl

We identified 5 structural variants with the loss and deletion allele type with clinical assertions. (Note: You can also view DECIPHER structural variants on the location tab).These are: nsv533825, nsv3923320, nsv3919258, nsv4455223, nsv6311715 (deletion)) https://may2025.archive.ensembl.org/Homo_sapiens/Share/8520a71f607e2a4aeb1e4ad6c5a33a7c?redirect=no

Use Ensembl VEP to find out more information about these variants:

1. Are there any regulatory features affected by these variants?
2. Are any of them promoters that may affect NR3C2.
3. Which variants affect this promoter?

G2P

1. Are there any diseases associated with DLG2 or NR3C2 that you can identify in the Gene2Phenotype resource?
2. If there is no information in G2P, does the Gene Curation Coalition (GenCC) have curated disease associations for this gene? For what disease is there strong evidence of association?
3. If there is information in G2P, is this from the same paper mentioned above? Does the published information provide strong evidence for the disease association to this gene?

Open Targets

NR3C2 is a well-known receptor for both mineralocorticoids, such as aldosterone and glucocorticoids, which, after binding, regulates transcription of target genes involved in sodium and potassium homeostasis.

- Is NR3C2 causal for a particular type of pseudohypoaldosteronism?

NR3C2 represents an established clinical target with a well-characterised association with pseudohypoaldosteronism type 1. Given the clear genetic basis of this rare disease, NR3C2 theoretically represents a rational therapeutic target. However, the mechanism of action required for therapeutic intervention is a critical consideration that impacts drug development feasibility.

Multiple lines of evidence support the NR3C2-pseudohypoaldosteronism association. The direction of effect (loss-of-function vs. gain-of-function) has important implications for therapeutic strategy.

- Examine the ClinVar evidence for NR3C2 variants associated with pseudohypoaldosteronism. Describe how NR3C2 variants impact disease pathogenesis.
- Given the answer above, what type of pharmacological mechanism would be required to therapeutically target this pathway (e.g., agonism, antagonism, allosteric modulation)? Discuss whether this represents a straightforward drug development strategy compared to typical target classes.
- The mineralocorticoid receptor has physiological functions beyond the kidney and is involved in cardiovascular homeostasis. Are there any safety concerns associated with the modulation of this target?