Characterising the Effects of Single Nucleotide Polymorphisms on the Structure and Function of AdipoR1

Adiponectin, AdipoR1 and Retinitis Pigmentosa:

AdipoR1 (figure 1) is a seven transmembrane receptor found predominantly in the skeletal muscle, adipocytes and the retina. Its ligand is the 30KDa protein adiponectin which, upon binding to AdipoR1, stimulates beta oxidation of fatty acids, ultimately resulting in the generation of ATP.

Retinitis Pigmentosa (RP) is a genetic condition with a broad spectrum of symptoms, but is primarily characterised by a loss of rod cells from the retina, resulting in blindness. It's caused by a mutation in one of over 50 genes, although many of the genes that cause the disease are still unknown (Figure 2).

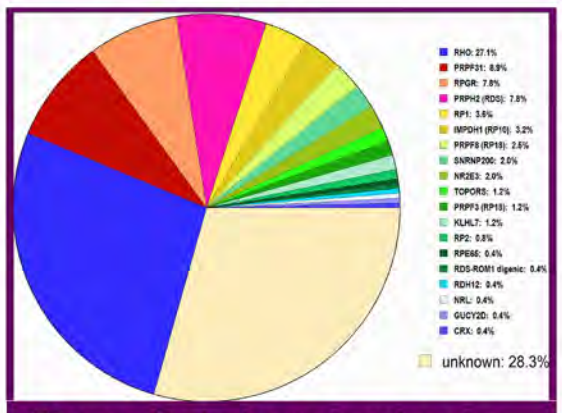


Figure 2: A chart showing the prevalence of genes causing dominant RP.

In a recent study, "A mutation in AdipoR1 causes nonsyndromic autosomal dominant retinitis pigmentosa" (Zhang, J. et al, 2016) it was found that a single nucleotide polymorphism (Y310C) in AdipoR1 caused development of nonsyndromic RP in a Zebrafish. In another paper, "AdipoR1 is mutated in syndromic Retinitis Pigmentosa" (Mingchu, X., et al, 2016), a homozygous frameshift mutation in AdipoR1 is described in a patient with syndromic RP; a severe variant of RP characterised by developmental delay, truncal obesity, and photoreceptor loss. This work provides the basis for our hypothesis: Deleterious homozygous mutations in AdipoR1 results in the development of syndromic RP. To test this, a series of AdipoR1 mutants needed to be generated and characterised; this poster describes the generation and preliminary characterisation of these mutants.

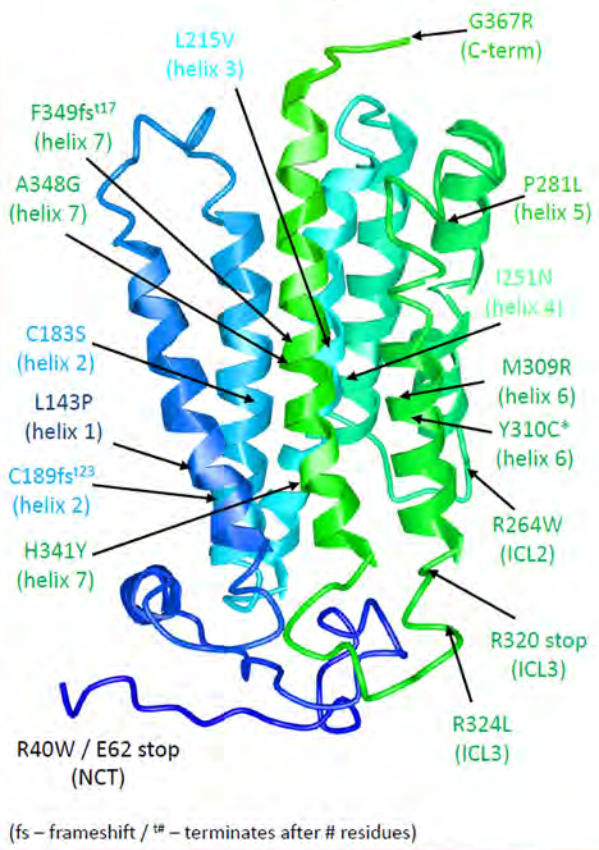
FABS: Zebrafish are commonly used in Molecular Biology as they share 70% of their genes with us. Their bodies are also transparent, allowing for observation of any genetic alterations to the body made by researchers.

FABS: For **A**spiring **B**iology **S**tudents

Aims of this Project:

- Generate variants of AdipoR1 with potentially deleterious SNPs
- Monitor the expression of mutant AdipoR1 variants to determine the effect of the SNP on protein structure and function.

AdipoR1



(fs – frameshift / $^{t\#}$ – terminates after # residues)

Figure 1: The structure of AdipoR1, including potentially deleterious SNPs.

Method:

Using bioinformatic databases, we were able to find 16 potentially deleterious SNPs in AdipoR1, shown in Figure 1. Of these, we were able to create 10 AdipoR1 variants containing one of these SNPs. These variants were transfected into human embryonic kidney (HEK) cells and assayed using immunofluorescence to determine the ratio of cell surface expression (CSE) of AdipoR1 against its total expression (TE).

Green Fluorescent Protein (Figure 3) was used as an indicator of transfection efficiency during the assay, whilst the wild type variant of AdipoR1 was used as a positive control and the 309 variant was used as a negative control.

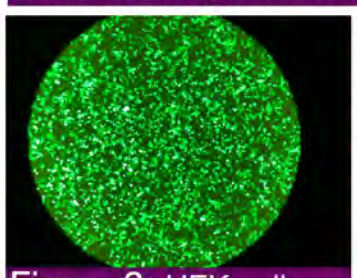


Figure 3: HEK cells containing transfected Green Fluorescent Protein.

FABS: Green Fluorescent Protein (GFP) is used to monitor transfection efficiency due to its distinct green glow. By transfecting a known quanity of a GFP gene into a batch of cells, you can calculate the efficiency of the transfection by measuring the amount of cells emitting a green fluorescence.

Procedures:

- **Site Directed Mutagenesis:**
A variant of PCR that introduces a specific change to the DNA sequence of AdipoR1.
- **Heat Shock Transformation:**
Movement of the mutated AdipoR1 gene into bacterial cells to allow the gene to multiply in the cell.
- **DNA Purification:**
Purification and removal of the AdipoR1 gene from the bacterial cells.
- **Transfection:**
Movement of the mutated AdipoR1 gene into HEK cells.
- **Immunofluorescent Assay:**
An assay which uses fluorescently labelled antibodies that bind to AdipoR1 in the HEK cells. Fluorescence is measured to determine the CSE:TE ratio.

FABS: Antibodies are commonly used in research due to their high specificity and affinity to a single molecule. By tagging antibodies with fluorescent labels, researchers are able to search for a single molecule in a cell without removing any other cellular components.

Results:

Name of Variant	Mutation	Generated
A348G	Alanine to Glycine	✓
H341Y	Histidine to Tyrosine	✓
R324L	Arginine to Leucine	✓
R320*	Arginine to STOP codon	✓
Y310C	Tyrosine to Cysteine	✗
M309R	Methionine to Arginine	✓
P281L	Proline to Leucine	✓
I251N	Isoleucine to Asparagine	✓
L215V	Leucine to Valine	✓
C183S	Cysteine to Serine	✓
L143P	Leucine to Proline	✓

Table 1: A list of AdipoR1 variants that have been generated during the experimental process.

Of all the mutants generated, one particular variant, M309R, showed the most deleterious effects on protein expression, as shown in figure 4. This shows that the CSE:TE of M309R is considerably lower than the CSE:TE of the wild type. This suggests that the mutation has similar effects on AdipoR1 structure as the Y310C mutation, which has been associated with RP pathogenesis.

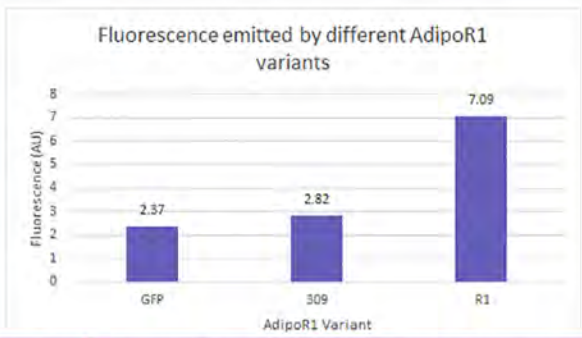


Figure 4: A graph displaying the fluorescence of different AdipoR1 variants and GFP.

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