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Regulatory variants in *LDLR* and *PCSK9* promoters and 5'UTRs: investigating the impact in Familial Hypercholesterolaemia

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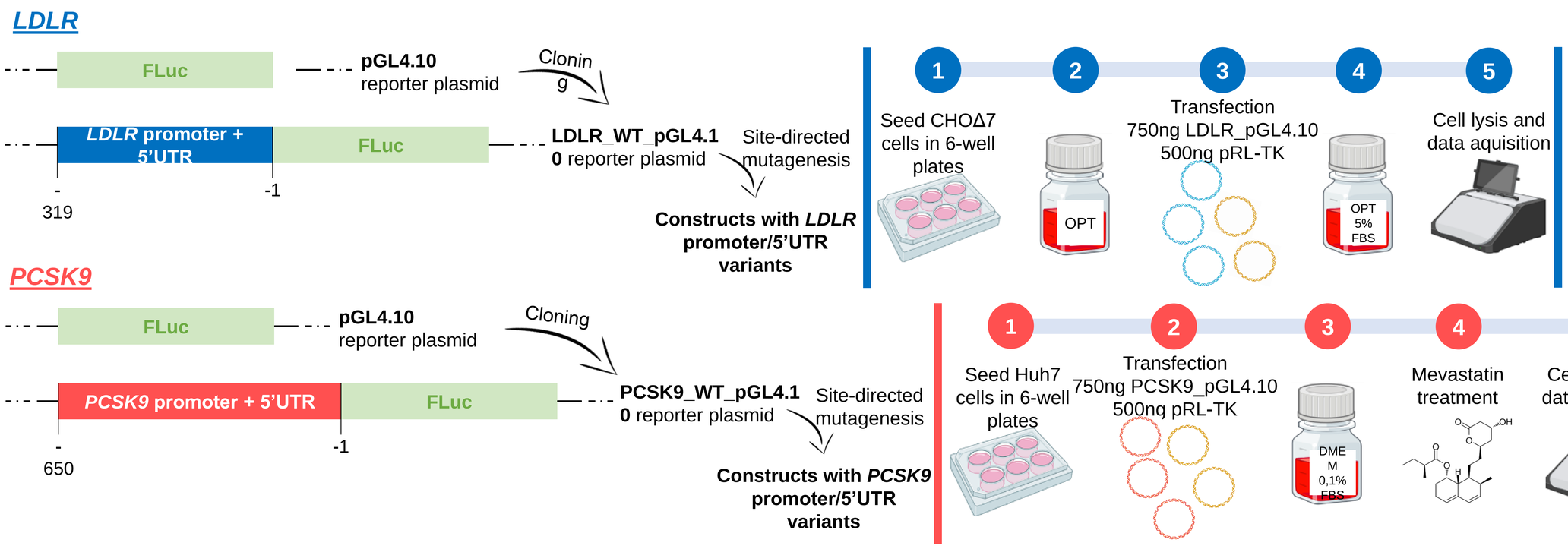
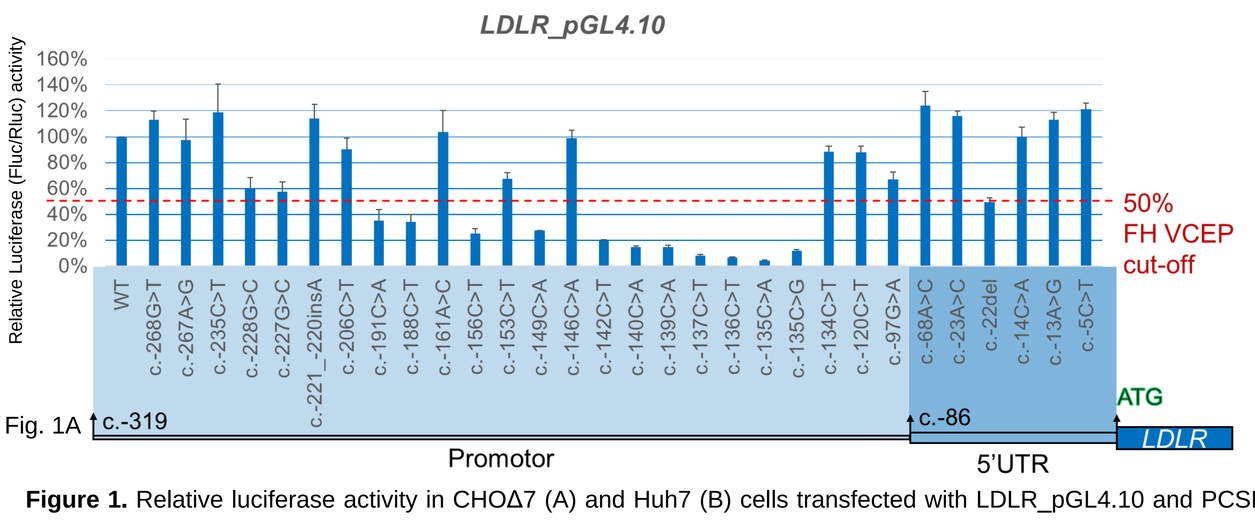
Background and Aims

FH is a genetic disorder of lipid metabolism caused by pathogenic variants in *LDLR*, *APOB*, and *PCSK9*. While diagnostic efforts traditionally focus on coding variants, non-coding regions, such as promoters and 5'UTRs, remain understudied despite their importance. This work aims to characterise 100 variants in the promotor/5'UTR of *LDLR* and *PCSK9*.

Methods



Results



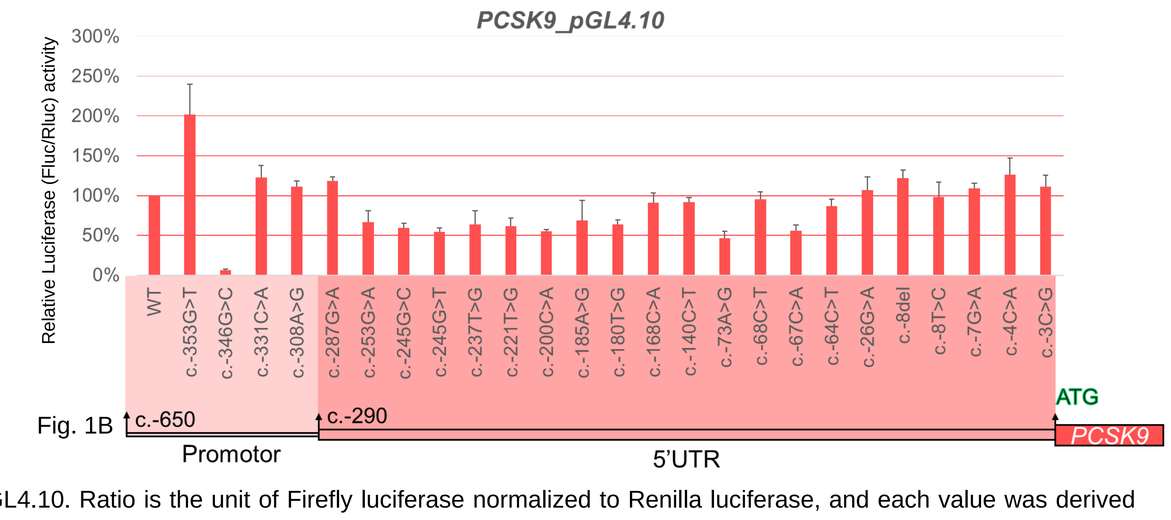


Figure 1. Relative luciferase activity in CHOΔ7 (A) and Huh7 (B) cells transfected with LDLR_pGL4.10 and PCSK9_pGL4.10. Ratio is the unit of Firefly luciferase normalized to Renilla luciferase, and each value was derived from, at least, three independent experiments. The ratio of all variants was compared to the correspondent wild-type (LDRL-wt or PCSK9-wt), arbitrarily set to 1 (100%). Results are expressed as mean ± standard deviation.

For ***LDLR***, 24 variants in the promoter and 6 in the 5'UTR were analysed. Twelve of these showed a reduction in relative luciferase activity to below 50% of wt levels, the established threshold set by the FH VCEP for applying the PS3_supporting criterion. No changes were observed at the mRNA level (data not shown). The functional impact of these variants appears to be associated with their position in the regulatory sequence: all *LDLR* variants showing reduced activity are located either within the SREBP1/2 binding site (variants from c.-191 to c.-149), the SP1 binding site (variants from c.-142 to c.-135), or - in the case of variant c.-22del - predicted to introduce a novel uAUG that is out of frame with the canonical translation start site.

For ***PCSK9***, 5' Rapid Amplification of cDNA Ends (5' RACE) was performed to experimentally define the transcription start site and delineate the 5'UTR - representing, to our knowledge, the first experimental validation of the *PCSK9* 5'UTR (data not shown). A total of 4 promoter variants and 21 5'UTR variants were investigated. Although no established cut-offs currently exist for *PCSK9* variant interpretation, two promoter variants exhibited notable effects: c.-353G>T, which increased promoter activity approximately two-fold compared to WT, and c.-346G>C, which resulted in a near-complete loss of activity

Conclusions

So far, this study provides novel insights into the functional impact of 30 *LDLR* and 25 *PCSK9* promoter/5'UTR variants on gene expression and their potential contributions to the FH phenotype. Among the ***LDLR*** variants, **12 showed reduced expression and meet the criteria for PS3_supporting, reinforcing their potential pathogenicity**. For ***PCSK9***, the variant **c.-353G>T may contribute to a FH phenotype** through increased expression, whereas **c.-346G>C** is associated with markedly reduced expression and **may be associated with a hypocholesterolaemia phenotype**.

These regulatory variants can influence gene expression by disrupting transcription factor binding sites in promoters or by introducing upstream open reading frames in the 5'UTR, thereby interfering with normal protein synthesis. By elucidating how these non-coding variants affect LDL-C levels through altered expression of *LDLR* and *PCSK9*, **this work underscores the importance of functional assays in variant classification—particularly for regulatory variants, which remain underrepresented in clinical genetic testing**. Importantly, we have established a robust experimental pipeline to systematically test and characterise any newly identified variant in these non-coding regions, enabling the expansion of the collection of functionally validated variants relevant to FH.

In total, this study aims to functionally assess 100 promoter/5'UTR variants. All mutant constructs have been generated, and luciferase assays are currently in progress.

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