Functional Evaluation of Distal Cis-Acting Regulatory Elements Within the CYP2C Locus Using CRISPR interference

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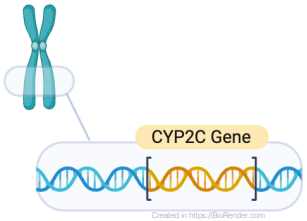
BACKGROUND

- CYP2C genes metabolize many clinically important medications
- Coding variants do not fully explain variability in drug response.
- Distal cis-acting regulatory elements (dCREs) may regulate CYP2C expression and contribute to interpatient variability.
- The regulatory roles of CYP2C dCREs remain poorly understood.



METHODS

1. Candidate dCREs
Six candidate dCREs selected from prior functional genomic analyses

2. gRNA Designing & Cloning
Guide RNAs designed to target each dCRE and cloned into CRISPRi expression vectors

3. Lentiviral Production
CRISPRi lentiviruses produced in HEK293T cells

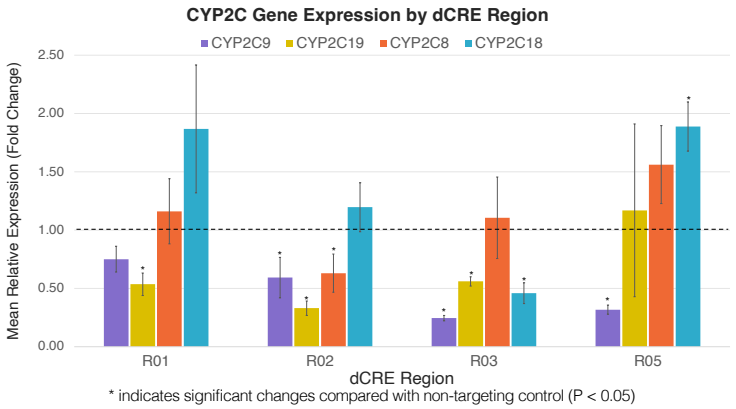
4. Huh-7 Cell Transduction
Lentiviruses transduced into Huh-7 liver cells

5. Harvest Huh7 cells for RNA preparation
Total RNA extracted from transduced cells

6. qPCR Analysis
Expression of CYP2C genes measured by qPCR with β-actin as housekeeping control

7. Data Analysis
Relative expression calculated using ΔΔCt method. Student t-test vs. non-targeting control: P<0.05

RESULTS



* indicates significant changes compared with non-targeting control (P < 0.05)

Figure 1. Changes in CYP2C gene expression after targeting each candidate dCRE. Huh7 cells were transduced with a CRISPRi lentivirus targeting CYP2C9, CYP2C19, CYP2C8, or CYP2C18, and their gene expression was compared with a negative control lacking a human genome target. Values represent mean fold change ± SD relative to non-targeting control (P < 0.05).

Table 1. Significant Regulatory Effects of candidate dCREs

dCRE	CYP2C9	CYP2C19	CYP2C8	CYP2C18
R01		↓		
R02	↓	↓	↓	
R03	↓	↓		↓
R05	↓			↑

↓ = decreased expression; ↑ = increased expression; blank cells indicate no significant change (P ≥ 0.05).

Key Findings:

- R02 and R03 demonstrated the broadest regulatory activity.
- R05 produced opposing effects on CYP2C9 and CYP2C18.
- Multiple dCREs regulate individual CYP2C family members.
- The CYP2C locus exhibits complex distal regulatory control.

CONCLUSIONS

- Multiple dCREs regulate each CYP2C gene expression.
- Each dCRE can regulate multiple CYP2C gene expression, supporting complex distal regulatory control within the CYP2C locus.
- Regulatory variation within these regions may contribute to pharmacogenomic variability beyond coding variants.

FUTURE DIRECTIONS

- Evaluate regulatory variants within validated dCREs.
- Incorporate regulatory variation into pharmacogenomic prediction models.

CLINICAL REVELANCE

Regulatory variation within validated dCREs may contribute to variability in CYP2C-mediated drug metabolism not explained by coding variants alone. These regions represent potential targets for future pharmacogenomic investigation and clinical prediction models.

REFERENCES