Effect of *De Novo* Variants on DEAF1 Activity

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Introduction

.•**Deformed Epidermal Autoregulatory Factor 1** (DEAF1) is a transcription factor that is highly expressed in the **central nervous system.** Mutations in the DEAF1 gene result in phenotypic spectrum of disorders including intellectual disability, speech impairment, behavioral problems, and autism.

•DEAF1 has multiple structures including **SAND** (SP-100, AIRE, Nucp 41/75, and DEAF1), **MYND** (Myeloid Translocation Protein, Nervy, and DEAF1), a **nuclear localization signal**, and a **nuclear expert signal**.

Objectives

- Determine the impact of three different patient-identified *de novo DEAF1* variants on the transcriptional activity of the DEAF1 protein.

Methods

Cell Culture

Human Embryonic Kidney (HEK) 293T cells were plated in a 24 well plate (~75k cells/well).

Cell Transfection

Cells were transfected with control (pcDNA3), WT-DEAF1, or indicated DEAF1 mutant expression plasmids with DEAF1 prom (repression) or BRSK2 prom (activation) and RSV-renilla (normalizing) using the calcium phosphate technique.

Media was replaced 24 hour later.

Dual Luciferase Assays

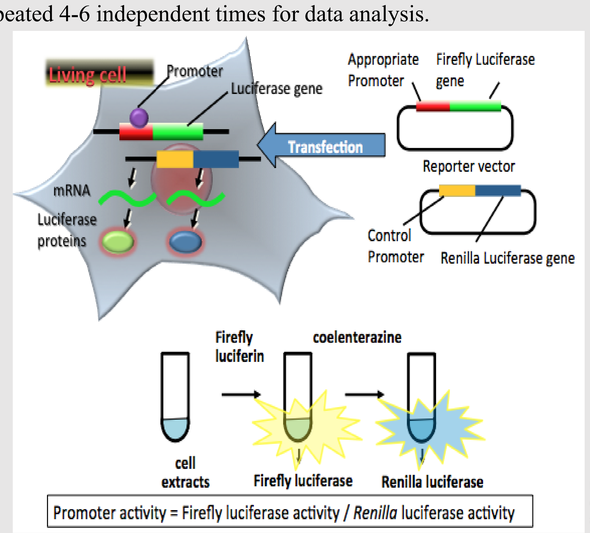
Cell lysates were prepared using 1x lysis buffer and luciferase assays were performed with the dual luciferase reporter assay system (Promega).

Data Analysis

DEAF1 or *BRSK2* prom light units were divided (normalized) by RSV-renilla light units.

Performed in duplicates to take the average.

Repeated 4-6 independent times for data analysis.



http://photobiology.info/Ohmiya.html

Results

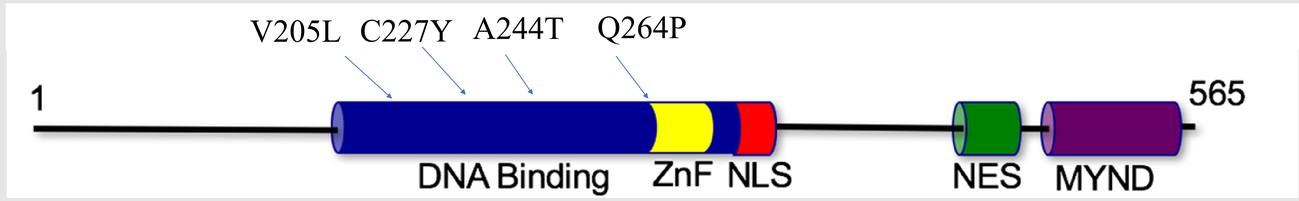


Figure 1. An overview of different structures of the DEAF1 gene including SAND domain, zinc finger homology domain, nuclear localization signal, nuclear export signal, and MYND domain. The positions of p.V205L, p.C227Y, and p.A244T are in the SAND domain. Identified mutations are located within the DNA binding domain.

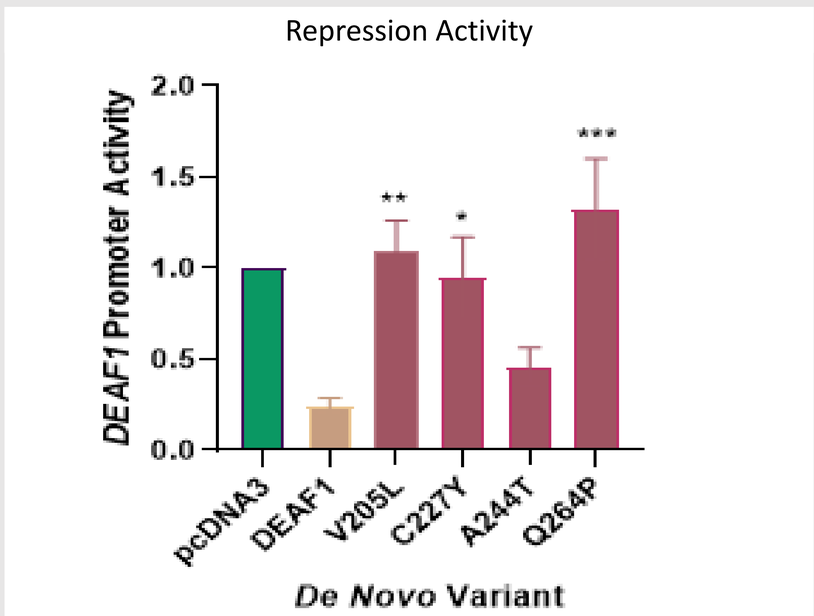


Figure 2. **De novo variants p.V205L and p.C227Y altered DEAF1 repression activity on the DEAF1 promoter.** Compared with wild type, the repression activity of p.V205L and p.C227Y was lost and the repression activity of p.A244T was not significantly altered.
N=6 *p<0.05 compared to DEAF1.
One way analysis of variance with Dunnett's multiple comparison of Wild Type DEAF1 compared to each mutant.

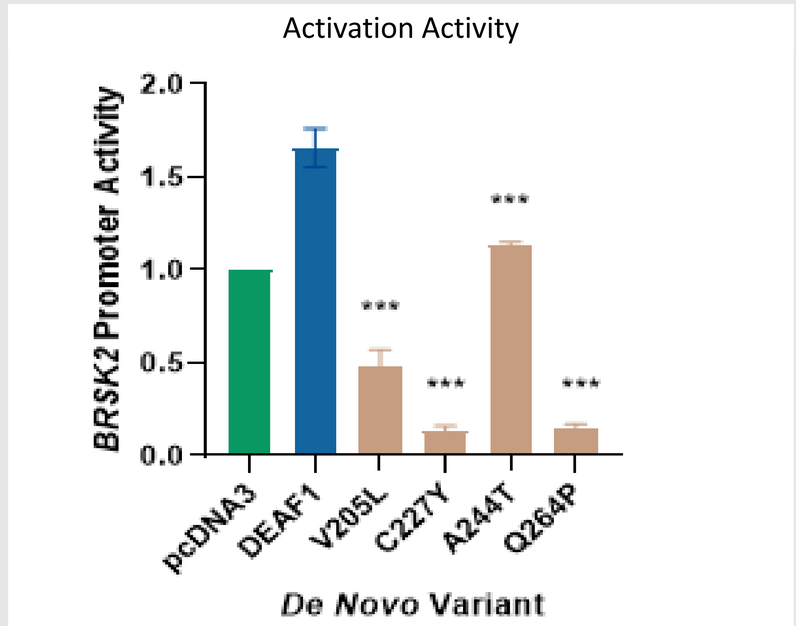


Figure 3. **De novo variants p.V205L, p.C227Y, and p.A244T significantly altered DEAF1 activation activity on the BRSK2 promoter.** Compared with wild type, the activation activity of de novo variants were lost. The differences in the variant effects on activation activity is apparent.
N=4 *p<0.05 compared to DEAF1.
One way analysis of variance with Dunnett's multiple comparison of Wild Type DEAF1 compared to each mutant.

Conclusion

-DEAF1 represses its own promoter activity while activating the BRSK2 promoter.

-DEAF1 variants p.V205L, p.C227Y, and p.A244T significantly changed the activation activity of DEAF1 while p.V205L and p.C227Y changed the repression activity of DEAF1.

- The altered function of DEAF1, due to these identified mutations, could potentially result in the intellectual disability, speech impairment, behavioral problems, and autism spectrum disorder described in these individuals.

Acknowledgements

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