Molecular mechanism of novel FOXG1 variants in causing cortical malformations

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Abstract

FOXG1 (Forkhead-box Protein G1) is an important transcriptional repressor which binds to transcription factors, such as COUP-TF1 and Tbr1, to regulate neuronal migration and cell fate during cortical development. Recently, we found three novel FOXG1 variants in patients with FOXG1-related syndrome which has been reported to cause malformation of cortical development and epilepsy. However, the relationship between FOXG1 variants and clinical symptoms still remains unclear. We aim to elucidate how FOXG1 variants affect transcriptional regulation functions and lead to cortical malformation.

To test transcriptional regulatory function of FOXG1 variants, luciferase assay was used to check FOXG1 regulatory activities of downstream targets. Foxg1 variants with severe clinical symptoms fail to repress COUPTF1 expression through transcriptional abnormality. In contrast, variants with mild clinical symptoms shown similar results with FOXG-WT. We investigated the role of FOXG1 variants by using *in utero* electroporation (IUE) to introduce mutant FOXG1 into embryonic cortices. Besides, FOXG1 variants cause different neuronal migration defects 3 days after IUE due to loss of function. In postnatal day 7, cells expressing severe FOXG1 variant shifted location from layer 4 to layer 2/3 and showed spiny stellate neuron morphology. Foxg1 variants also affected the connectivity of callosal projection through corpus callosum. These results were highly correlated with clinical symptoms of FOXG1 syndrome.

Disease causing FOXG1 variants not only affect transcriptional regulation of downstream transcription factors but also cause neuronal migration defects and cell fate change during corticogenesis. The correlation between *in vitro* and *in vivo* phenotypes and clinical symptoms may provide a reference in FOXG1 syndrome diagnosis.

Outline

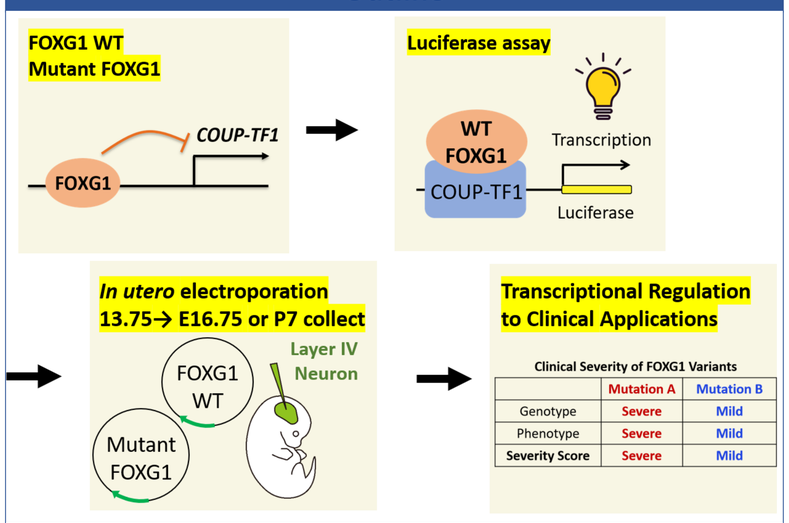
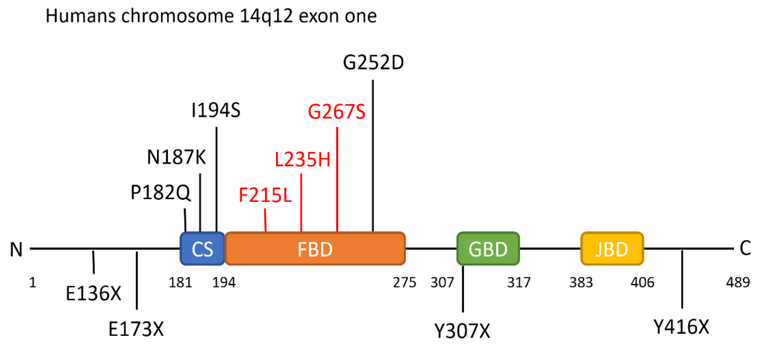


Figure 1. The Symptoms and Distinct Protein Level of FOXG1 Variants

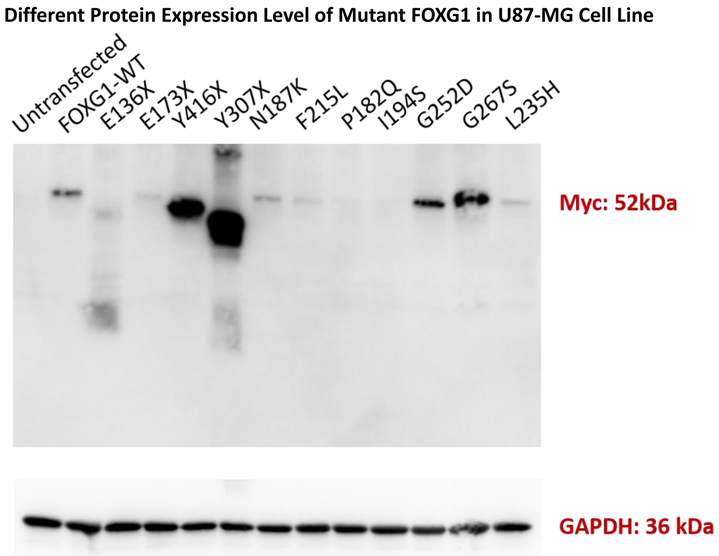
A. 11 FOXG1 Variants with Clinical Symptoms in Cortex and Corpus Callosum



B. Phenotypic Characterization of New and Published Patients with FOXG1 Syndrome

DNA change	a.a change	Domain	Clinical Symptoms	Brain MRI
c.406G>T	**E136X**	N-terminal		c.c anomaly
c.517G>T	**E173X**	N-terminal		Cortical & c.c anomaly
c.561C>A	**N187K**	FBD, CS		Cortical & c.c anomaly
c.581T>G	**I194S**	FBD	**Severe**	Cortical & cc anomaly
c.755G>A	**G252D**	FBD		Cortical anomaly
c.920dupA	**Y307X**	C-terminal		Cortical & c.c anomaly
c.1248C>G	**Y416X**	C-terminal		Cortical & c.c anomaly
c.545C>A	**P182Q**	FBD, CS	**Mild**	no cortical/c,c anomaly
c.645C>A	**F215L**	FBD	**Mild**	Normal (our case)
c.704T>A	**L235H**	FBD	**Mild-Moderate**	N/A
c.799G>A	**G267S**	FBD	**N/A**	N/A

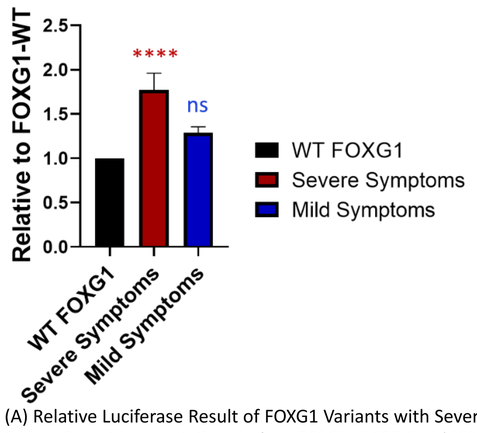
C. Different Protein Expression Level of Mutant FOXG1 in U87-MG Cell Line



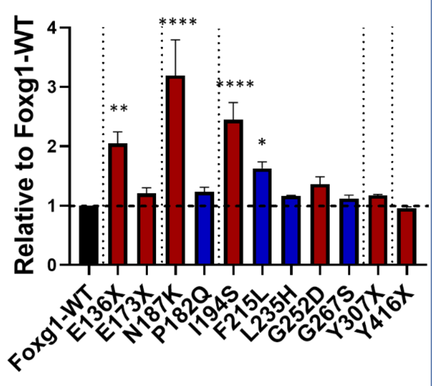
(A) 11 FOXG1 Clinical data were offered from Dr. Wang-Tso Lee, National Taiwan University Children's Hospital, including 4 missense mutation, 4 nonsense mutation and 3 unpublished mutations. FOXG1 located in human chromosome 14q12 exon one. Four major domains in FOXG1 include Forkhead Binding Domain (FBD, amino acids 181-275), Conserved Site 1 (CS, amino acids 181-194), Groucho-Binding Domain (GBD, amino acids 307–406), JARID1B-Binding Domain (JBD, amino acids 383–406). (B) General pathology of these patients shown dysgenesis of corpus callosum and epilepsy and malformation in cortical development. (C) Distinct protein level expression in FOXG1 variants. pCMV-Myc-tagged FOXG1 WT and mutant FOXG1 are transfected into U87-MG cell line to verify the expression by western blotting.

Figure 2. FOXG1 Variants with Severe Clinical Symptoms Fail to Repress COUP-TF1 Expression

A. FOXG1 Variants with Severe Symptoms shown Significantly High Luciferase Level compared to FOXG1 WT



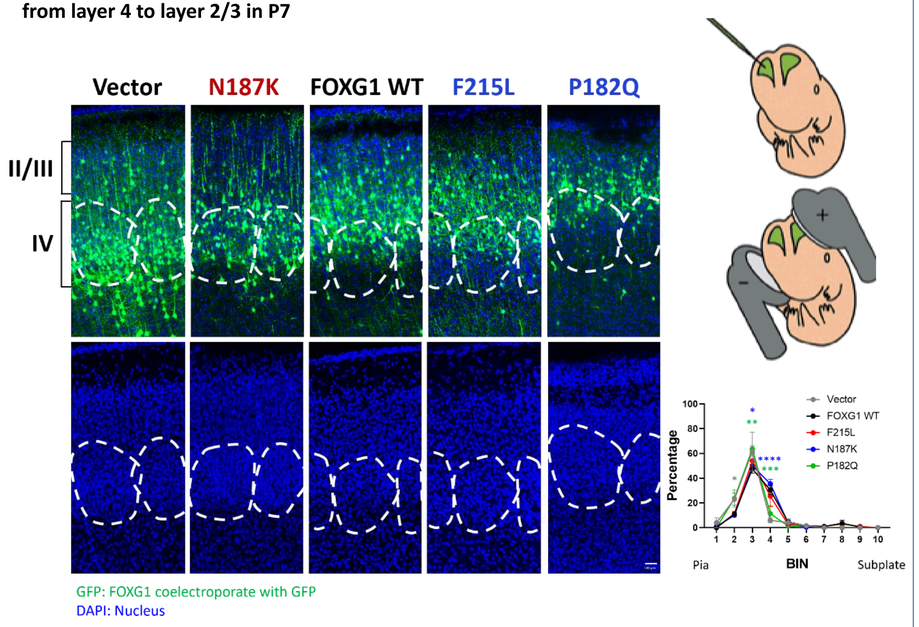
B. FOXG1 Variants with Severe and Mild Symptoms shown distinct luciferase level.



(A) Relative Luciferase Result of FOXG1 Variants with Severe and Mild Symptoms Compared to FOXG1 WT. (B) Severe Symptoms mutation such as E136X, N187K and I194S shown high luciferase result compare to control group, which mutant FOXG1 can not suppress COUP-TF1 expression properly. In addition, F215L mutation with mild symptoms also show relatively higher luciferase level than FOXG1 WT. However, some of the variants did not affect FOXG1 interact with COUP-TF1. Error bars represent SEM. *p<0.05, **p<0.01, ***P < 0.001, ****P < 0.0001. ANOVA test.

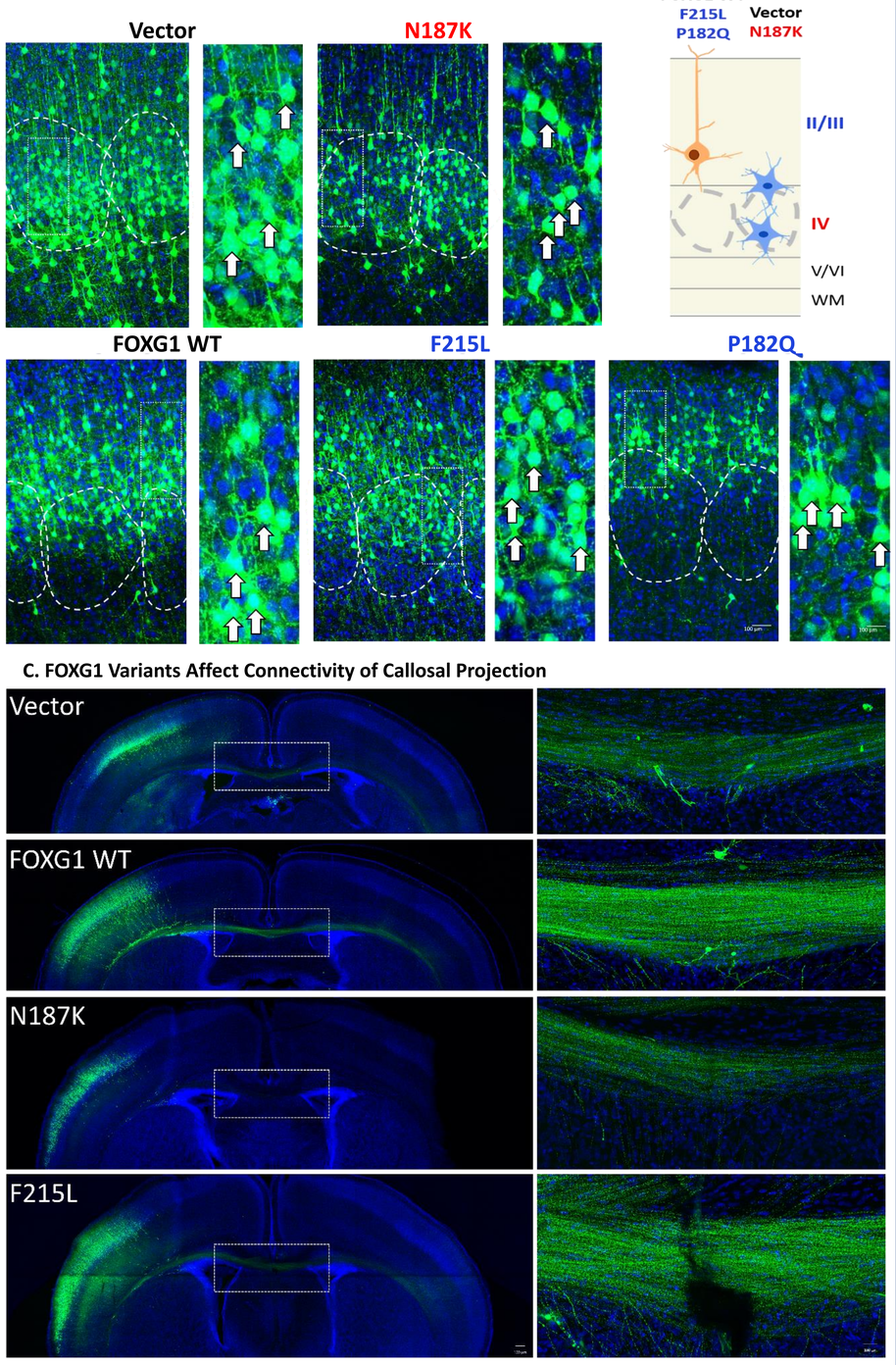
Figure 3. Severe Symptom of FOXG1 variant cause Cell Fate Change and Neuronal Migration Defect due to Loss of Function

A. Cells expressing Severe FOXG1 variant stay in layer 4, mild FOXG1 variants shifted location from layer 4 to layer 2/3 in P7



GFP: FOXG1 coelectroporate with GFP
DAPI: Nucleus

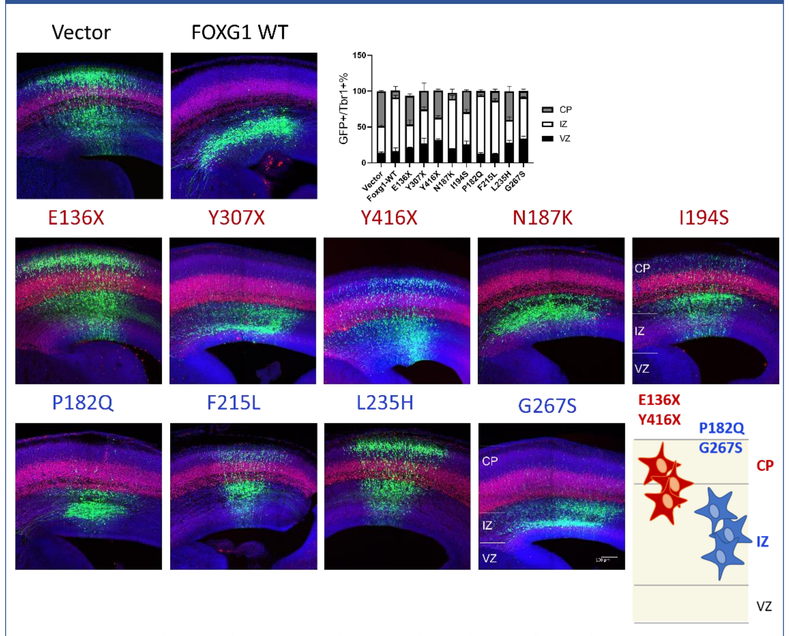
B. Overexpressed Severe FOXG1 Variant Change Cell Fate from Pyramidal Neuron to Spiny Stellate Neuron Morphology



C. FOXG1 Variants Affect Connectivity of Callosal Projection

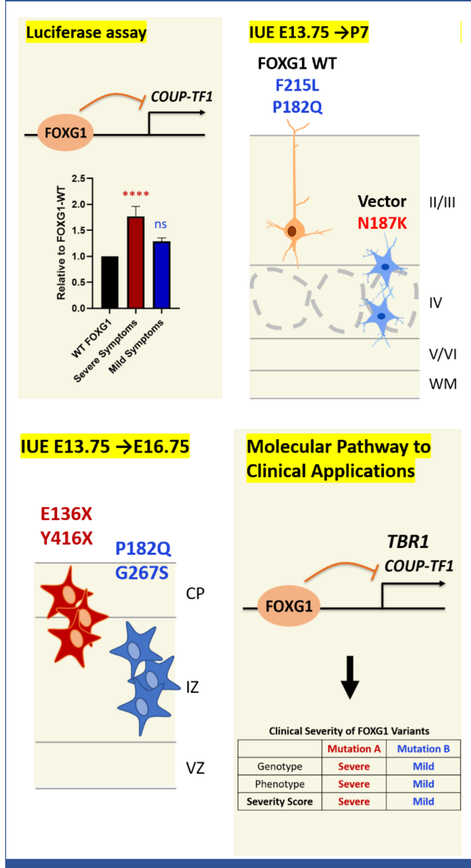
(A) In postnatal days 7 (P7), severe Symptom mutation N187K GFP cells are located in layer IV, same as in vector group. FOXG1 variants with mild clinical symptoms show relatively same results as FOXG1 WT. Dash-lined circle represented layer IV barrel cortex. Quantitative analysis of the distribution of GFP cells in P7 cortices. Cortical plates are divided into 10 BINs from the pia to the subplate. (B) We further observe the morphology of GFP cells in P7 cortices. N187K with severe symptoms locate in layer IV as spiny stellate neuron, same with vector group. However, F215L and P182Q with mild clinical symptoms change their cell fate to pyramidal neuron. (C) In P7 mice cortices, overexpress FOXG1 WT can form proper and oriented axons in corpus callosum. On the other hand, FOXG1 variants such as N187K and F215L fail to produce a well-formed callosal projection in corpus callosal. All slices were stained with DAPI (blue) to show the cell nuclei. Bars = 100 µm. Error bars represent SEM. *p<0.05, **p<0.01, ***P < 0.001, ****P < 0.0001. ANOVA test.

Figure 4. FOXG1 variants cause different neuronal migration defects 3 days after IUE due to Loss of Function



We overexpressed vector in E13.75 and collected the tissues after 3 days, GFP cells migrate to cortical plate successfully. In FOXG1 WT group, GFP cells tend to stay in intermediate zone due to neuronal migration delay. In FOXG1 severe variants group, E136X and Y416X show no neuronal migration delay because of FOXG1 loss of function. In FOXG1 mild variants group, F215L and L235H have a miner level of neuronal migration delay. However, P182Q and G267S show a similar result compared to FOXG1 WT group. All slices were stained with DAPI (blue) to show the cell nuclei. Bars = 100 µm. Bar graph with individual data points showing the percentage of GFP+ cells in the CP, IZ, and VZ days after electroporation (n = 3 pregnant females in each condition). Error bars represent SEM.

Summary



1. FOXG1 variants cause FOXG1 fail to suppress downstream COUP-TF1 properly.
2. In P7 results, FOXG1 variants with severe symptoms eventually cause cell fate changed because the defect function of FOXG1.
3. After IUE three days, severe and mild FOXG1 variants cause distinct level of neuronal migration defects.
4. Develop an algorithm to calculate FOXG1 variants' severity score in order to provide a reference in FOXG1 syndrome diagnosis.

Discussion

- FOXG1 syndrome is a complicated disease with a wide spectrum. In this study, we elucidate transcriptional regulation level of FOXG1 variants.
- According to FOXG1 variants' protein expression level, Y416X, Y307X, G252D and G267S show high protein expression level. Moreover, missense mutation located at Forkhead-DNA binding domain such as N187K, F215L, P182Q and I194S, have lower expression level compared to FOXG1 WT. These FOXG1 variants also shown a certain level of neuronal migration defects. Perhaps these FOXG1 variants may cause some defects in structure and eventually affect protein stability. We still need to develop a severity score of FOXG1 variants to further investigate.
- Furthermore, we found out some FOXG1 variants with severe or mild symptoms fail to repress COUP-TF1 expression. However, there are still some mutations shown similar results in luciferase and IUE experiments. We speculate that COUP-TF1 might not be the only target in FOXG1 regulatory pathway. Due to previous ChIP Sequencing data, we found out FOXG1 can directly repress Tbr1. We plan to verified FOXG1 variants with Tbr1 in luciferase assay to acquire a bigger picture of FOXG1 syndrome to provide more information to clinical applications.

Acknowledgement

Special thanks to my professor Jin Wu Tsai who gave the golden opportunity to do join the lab and work in this project to fulfill my scientific research career. Your fruitful feedback and enthusiasm in research really inspire me. Moreover, I want to thanks to all the members in FOX genes group, Fang-Shin Nian, Haw-Yuan Cheng, Chiao-Wen Nian, Hui-Chin Huang and Hong-Jun Zhao who help me a lot not only in this project, but also in my life. Thanks again to all the people who ever supported me.

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