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 another transcription factors such as COUP-TF1, to regulate neuronal migration during cortical development. Recently, we found four 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. We investigated the role of FOXG1 variants by using *in utero* electroporation (IUE) to introduce mutant FOXG1 into embryonic cortices. We found FOXG1 variants cause different neuronal migration defects 3 days after IUE due to loss of function.

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

Fig. 1 FOXG1 Variants with Malformation of Cortical Development

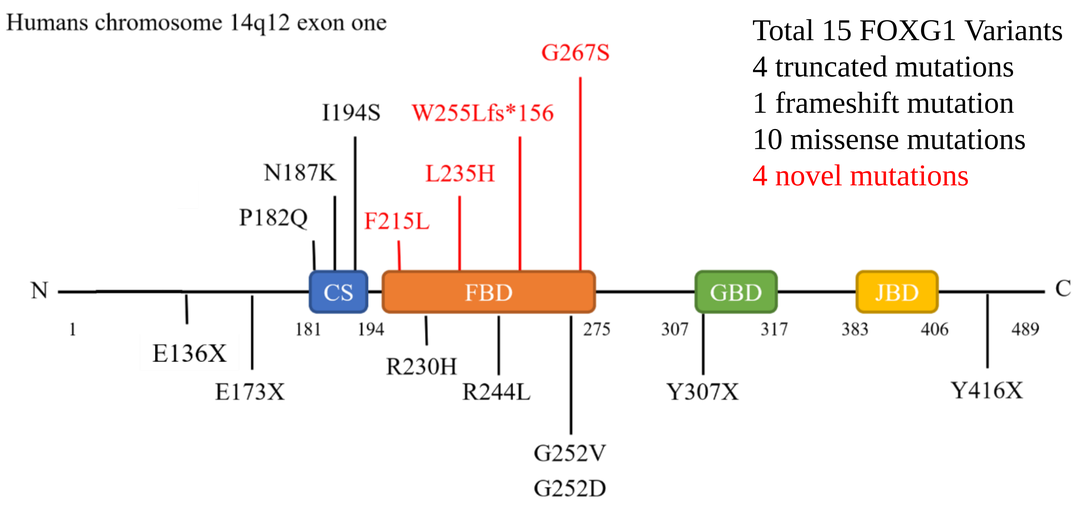
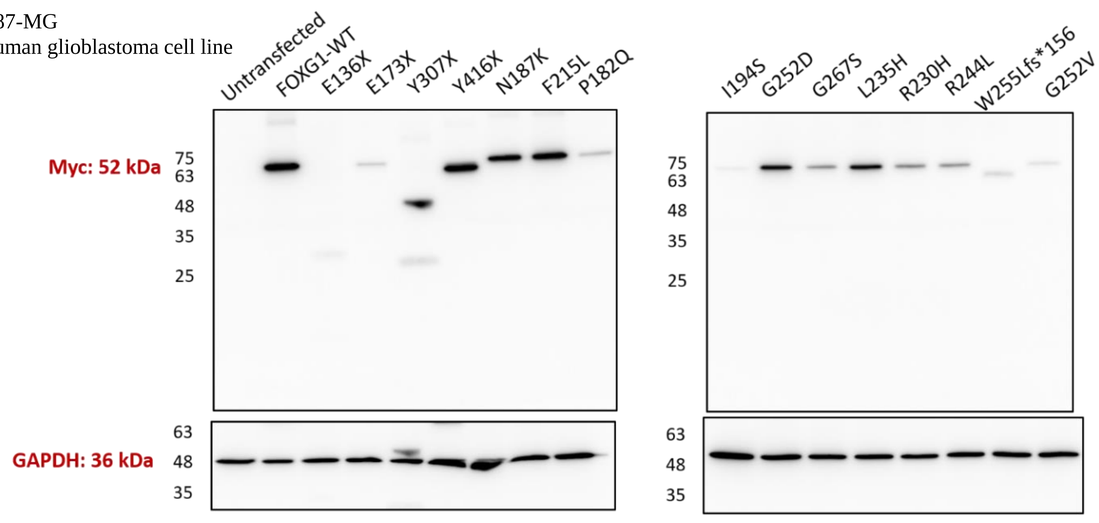


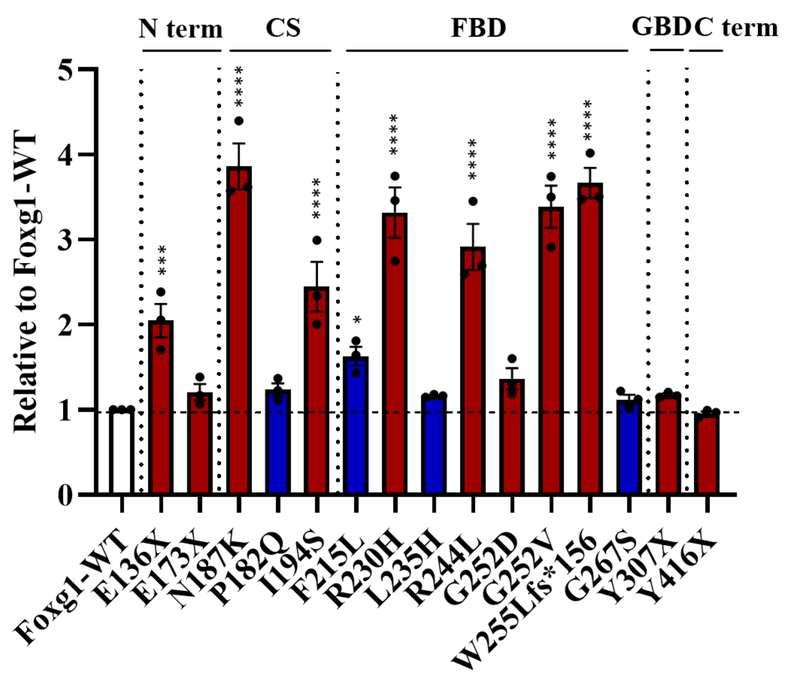
Fig. 2 Protein expression level of FOXG1 variants

U87-MG
Human glioblastoma cell line



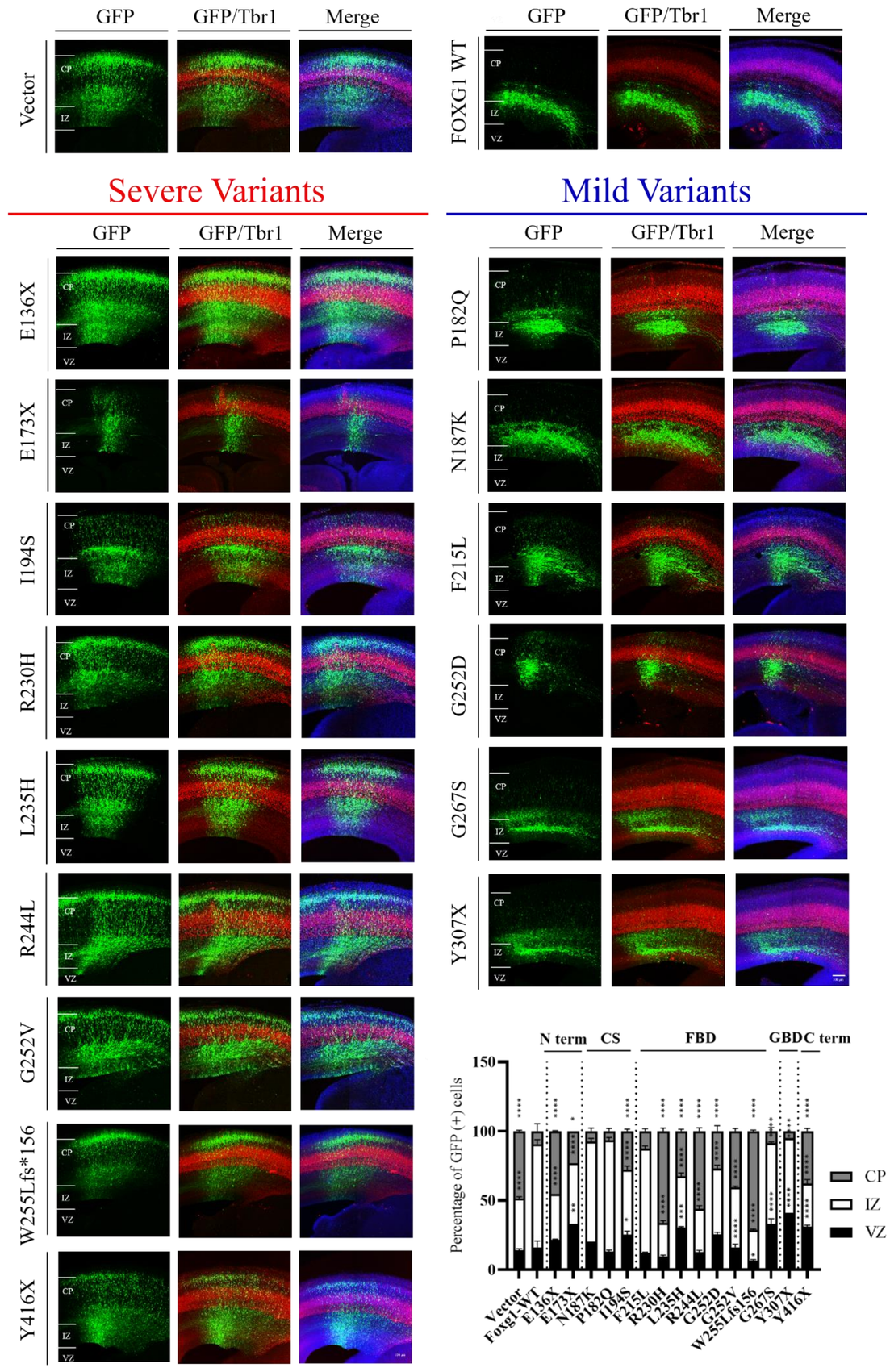
In comparison with WT FOXG1, 2 out of 4 truncated variants (E136X and E173X) showed lower expression. On the contrary, Y307X and Y416X showed similar expression compared to WT FOXG1. For missense variants, the protein expression was lower in 7 out of 10 variants (P182Q, I194S, G252D, G267S, L235H, R230H, R244L and G252V). The frameshift variant W255Lfs*156 also displayed a very low protein level.

Fig. 3 FOXG1 Variants with Severe Clinical Symptoms Fail to Repress COUP-TF1 Expression



Cells transfected with WT FOXG1, the luciferase activity was low, consistent with a repressor role of FOXG1 on COUP-TF1 expression. We found 8 out of 15 FOXG1 variants (E136X, N187K, I194S, F215L, R230H, R244L, G252V and W255Lfs*156) exhibited a relatively high luciferase activity, indicating that these 8 FOXG1 variants lost their function to suppress COUP-TF1. In contrast, the other 6 variants E173X, P182Q, F215L, L235H, G252D and G267S showed low luciferase activities similar to FOXG1-WT. Interestingly, mutations located in CS and FBD shown a significantly higher luciferase activities compared to FOXG1-WT. GBD and C-terminal truncated variants Y307X and Y416X also shown a similar luciferase activities similar to FOXG1-WT.

Fig. 4 FOXG1 Variants Overexpression cause Different Neuronal Migration Defects due to Loss of Function



To investigate the potential effect of FOXG1 variants in brain development, we first tested the effects of FOXG1 variants on neural progenitors by conducting *in utero* electroporation (IUE) during embryonic E13.75 and collected after 3 days. We found 9 FOXG1 severe variants (E136X, E173X, I194S, R230H, L235H, R244L, G252V, W255Lfs*156, Y416X) showed GFP+ cells migrated into cortical plate (CP) due to loss of function, same as Vector. 6 mild variants (P182Q, N187K, F215L, G252D, G267S, Y307X) showed GFP+ cells were arrested in intermediate zone (IZ) or ventricle zone (VZ), same as FOXG1-WT. These observations suggested that some of these disease-associated variants may cause neuronal migration defect.

Conclusion

1. Disease-causing FOXG1 variants may change protein expression level.
2. FOXG1 variants located in CS and FBD shown a significantly higher luciferase activities. Variants located in GBD and C-terminal shown a similar luciferase activities to FOXG1-WT, suggested GBD and C-terminal may have a less effects on COUP-TF1 promoter binding ability compared to CS and FBD.
3. In neuronal migration, severe FOXG1 variants' cells migrated into CP due to loss of function. Mild variants' cells stayed in IZ/VZ similar to FOXG1-WT.

	Protein Level	Transcriptional Level	In utero electroporation
Severe Variants	Low	High	In CP
Mild Variants	High	Low	In IZ/VZ

Discussion

Most of the severe variants shown low protein level, high luciferase level and located in CP based on IUE results. However, severe missense variants E173X and L235H exhibited low luciferase level. This observation suggested that FOXG1 mutation not only affect the transcriptional activity of COUP-TF1, but also other factor such as TBR1 (T-Box Brain Transcription Factor 1).

Significance

By verifying the effects of FOXG1 dysfunction on transcriptional activities and neural migration, this study will elucidate the effects of FOXG1 variants in neural development and provide information of potential treatment and clinical diagnosis for FOXG1- syndrome.