Abstract

FOXG1 (Forkhead Box G1) is a critical transcription factor for brain development, regulating progenitor cell proliferation, neuronal migration, and cortical circuit assembly. Pathogenic FOXG1 variants lead to FOXG1 syndrome, a neurodevelopmental disorder characterized by severe brain anomalies and cognitive impairments. Despite efforts to correlate genetic variants with clinical outcomes, the precise relationship remains elusive. Here, we analyzed clinical severity and brain anomalies in 14 individuals with FOXG1 variants, investigating how these variants impact FOXG1's properties and functions. We uncovered a strong correlation between the severity of brain anomalies in affected individuals and functional alterations of these variants. Variants with very low protein expression were associated with moderate-to-severe brain anomalies. These variants were then transfected to cultured cells to assess their protein expression and ability to repress COUP-TFI (NR2F1) expression-a function of FOXG1 validated through single-cell RNA-sequencing (scRNA-seq). Variants losing COUP-TFI repression ability by binding to COUP-TFI's enhancer region consistently caused moderate-to-severe brain anomalies. Furthermore, in utero electroporation (IUE) in embryonic mouse brains was employed to study their impact on neuronal migration and differentiation. Electroporation of wild-type Foxg1 delayed neuronal migration and altered their cell fate. Remarkably, variants associated with moderate-to-severe brain anomalies impaired these functions, while those with mild brain anomalies caused partial impairment. Using protein expression, COUP-TFI repression, and neuronal migration assays, we developed a patient stratification paradigm for predicting the severity of brain anomalies. The workflow successfully identified 92.3% of cases with brain anomalies, facilitating early diagnosis and guiding future therapeutic interventions.