Characterisation of a recurrent missense mutation in the DNA-binding domain of the NFI transcription factors

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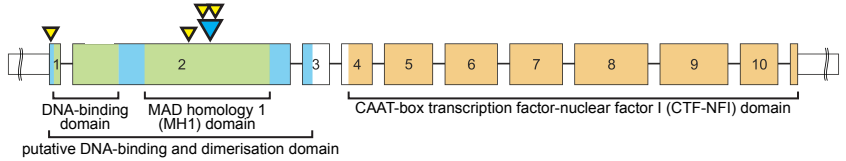
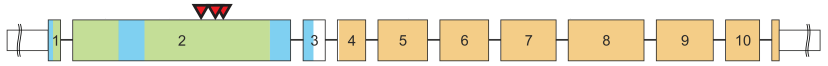
Background

Haploinsufficiency of the nuclear factor one (NFI) transcription factors is associated with neurodevelopmental phenotypes that include dysgenesis of the corpus callosum, macrocephaly and intellectual disability. Exome sequencing of individuals that present to the clinic with these phenotypes has led to the discovery of missense mutations affecting *NFIA*, *NFIB* and *NFIX* (Fig.1). The pathogenicity of mutations is often inferred based on the phenotypes of the proband, taking into consideration the absence of the identified variant in normal individuals as well as computational analyses of the likelihood of the variant being deleterious. While this approach has proven useful, it is largely dependent on assessing the phenotypes of an individual, which can be particularly challenging to implement for novel variants identified during prenatal development. Hence, we sought to develop standardised assays to test the pathogenicity of missense mutations affecting the NFI genes. We hypothesised that mutations that occur within the DNA-binding domain are likely to affect the ability of these transcription factors to bind to their cognate motif.

NFIA (ENST00000403491.7)



NFIB (ENST00000380953.5)



NFIX (ENST00000592199.5)

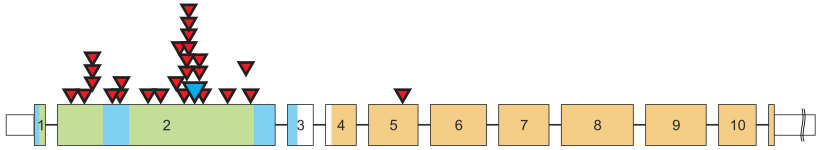


Fig. 1. Schematic depicting missense mutations discovered in the *NFI* genes. Each box represents an individual exon. Figure adapted from Zenker et al., 2019.

Results

Exome sequencing of an individual (Proband 1) with partial agenesis of the corpus callosum (Fig. 2), developmental delay and hypotonia identified a missense mutation in *NFIA*, c.361C>T (p.Arg121Cys) that was validated by Sanger sequencing (Fig. 3). We previously reported the occurrence of this mutation in another individual with partial agenesis of the corpus callosum and developmental delay (Zenker et al., 2019). A third individual with this mutation in *NFIA* is reported in the ClinVar database (ID: 265253), as well as four individuals with an identical mutation in *NFIX* (ID: 208724).

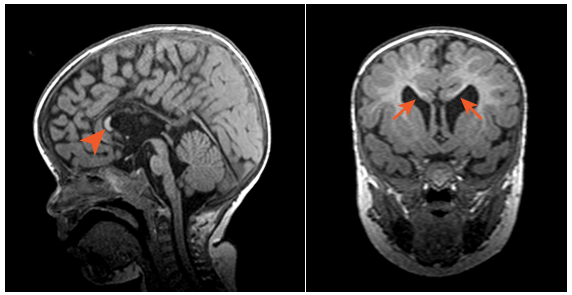


Fig. 2. Sagittal and coronal T1-weighted images of the proband at 13 months of age, depicting partial agenesis of the corpus callosum with only the genu present (arrowhead) and the presence of Probst bundles (arrows).

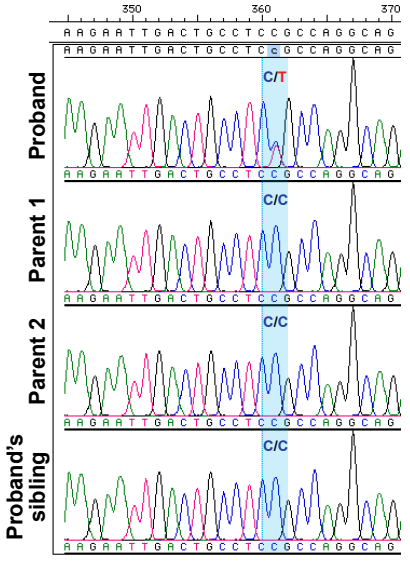


Fig. 3. The c.361C>T mutation in *NFIA* occurred *de novo* in Proband 1 and is absent in both parents and the proband's sibling.

We previously tested missense variants identified in *NFIB* for their ability to drive luciferase activity from the mouse *Gfap* promoter (Schanze et al., 2018). Utilising this assay, we observed reduced luciferase activity when the c.361C>T mutation was cloned into NFIX (Fig. 4). To further determine how this mutation affects the ability of these proteins to bind to the NFI cognate motif, quantitative gel shift assays are currently ongoing (see Roulet et al., 2000 for a detailed description of this assay to assess NFI binding).

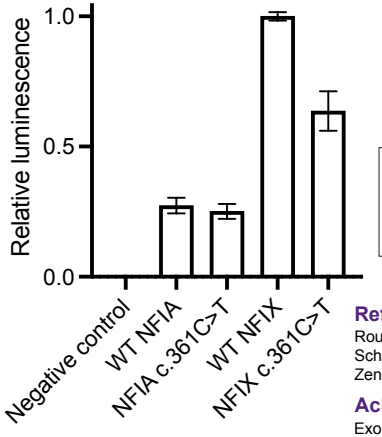


Fig. 4. Relative luciferase activity upon over-expression of wildtype (WT) or c.361C>T mutant NFIA and NFIX.

References

Roulet et al., J Mol Biol. 297(4), 833-848 (2000).
Schanze et al., Am J Hum Genet. 103(5), 752-768 (2018).
Zenker et al., Am J Med Genet. 181(4), 611-626 (2019).

Acknowledgements

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