Characterising the transcriptional profile of the calcium channel gene CACNA1F in human brain

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Introduction

- Voltage-gated calcium channels (VGCCs) significantly affect many aspects of brain function including neuronal excitability and synaptic plasticity (1).
- Large-scale genetic studies implicate VGCCs in the pathophysiology of psychiatric disorders (2).
- Each VGCC gene encodes multiple functionally-distinct isoforms via transcriptional mechanisms (3).
- Brain-enriched VGCC isoforms might represent attractive therapeutic targets for psychiatric disorders (4), but the complement of VGCC isoforms present in human tissues, including brain, remains largely unknown.
- The CACNA1F gene encodes $Ca_V1.4$ which has been associated with educational attainment (5). It is not reported to be expressed in brain tissue but there is little direct investigation of this possibility in humans.
- We investigated whether there is evidence of expression at the CACNA1F locus in publicly-available brain RNASeq data produced by the Lieber Institute for Brain Development (LIBD)
- The LIBD data indicated the possible presence of a truncated isoform of CACNA1F in human postmortem dorsolateral prefrontal cortex

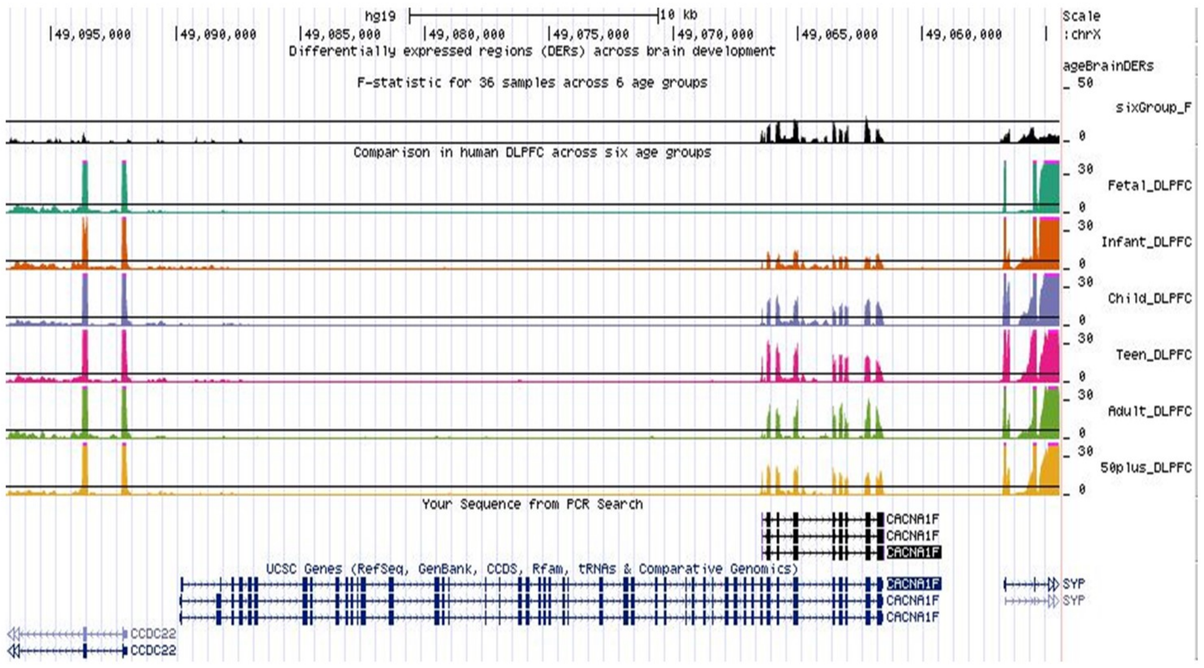

Figure 1 Genomic location of CACNA1F, showing the LIBD RNASeq data (coloured track). There is evidence of the presence of a truncated isoform, ~1298bp in length, that maps to the 3' end of CACNA1F in the dorsolateral cortex of infant (orange), child (purple), teen (pink), 18-49 year old (green) and 50+ year old (yellow), but not foetal (turquoise) samples. There is little evidence supporting expression of the full-length CACNA1F transcript in human brain at any age.

Methods

- RNA from 8 brain regions of 6 healthy controls was reverse transcribed to examine expression of truncated CACNA1F in human brain (see below)
- Primers were designed to target both the full-length CACNA1F and truncated CACNA1F mRNA, based on the LIBD RNASeq data
- We were able to amplify truncated, but not full length, CACNA1F
- Nanopore long-read sequencing was used to examine the diversity of truncated CACNA1F isoforms (see right), using a novel bioinformatics pipeline (3)

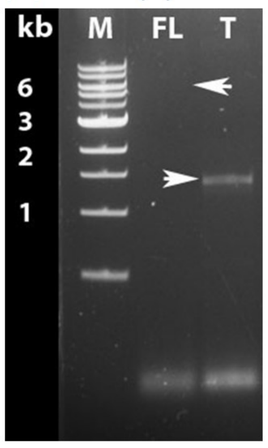

Figure 2. We were able to amplify truncated CACNA1F (in lane 'T', indicated with a black arrow) but not full-length CACNA1F (in lane 'FL', predicted size of 6002bp indicated with a white arrow) in human postmortem brain tissue. Lane 'M' contains a 1kb size marker, with selected size indicators (in kilobases; kb) indicated to the left.

<u>Postmortem samples:</u>

8 brain regions

6 control individuals (see table)

- Cerebellum
- Striatum
- Dorsolateral prefrontal cortex
- Thalamus
- Cingulate cortex
- Occipital cortex
- Parietal cortex
- Superior temporal cortex

ID	Sex	Ethnicity	Age at death
5238	M	AA	37
5244	M	AA	21
5298	M	AA	50
5346	F	AS	25
5579	F	CAUC	41
5717	M	CAUC	51

Acknowledgements

This study was funded by a MRC Project Grant awarded to EMT and WH, and PJH, and supported by the Oxford Health NIHR Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the National Health Service, NIHR or the Department of Health.

Results

- We identified 152 truncated CACNA1F isoforms expressed in human brain
- 21 of these isoforms contain novel (i.e. unannotated) exons

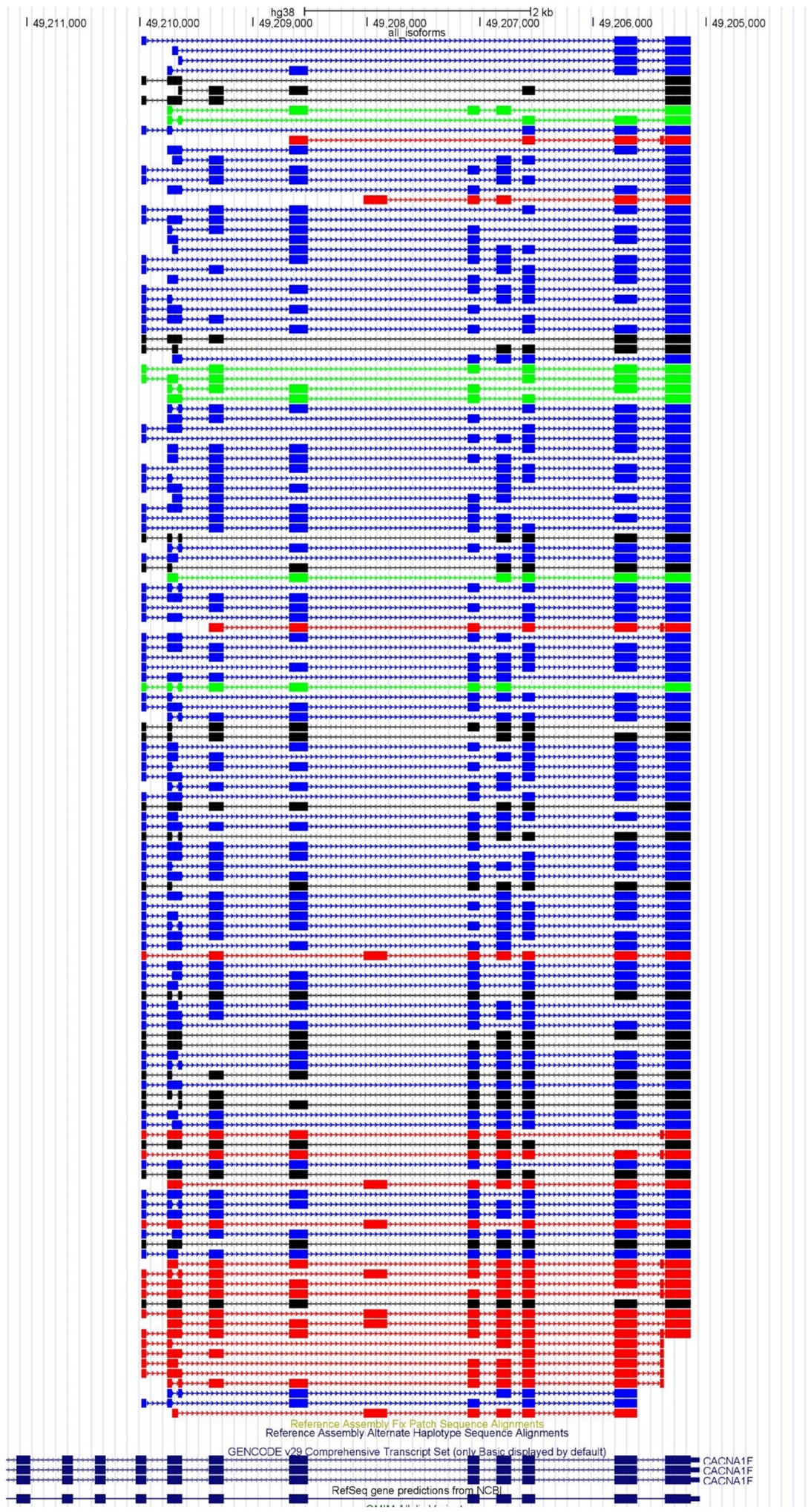

Figure 3. Truncated CACNA1F isoforms are expressed in human brain. These include isoforms that are predicted to be coding (black), those including a novel exon (red), those with a frame disruption (blue), and those with an early in-frame stop codon (green).

Conclusions

- We demonstrate the expression of truncated mRNAs expressed from the CACNA1F locus in human brain
- In addition, similar to our recent findings for CACNA1C (3), we have identified a wealth of unannotated isoforms and exons.
- The function of the truncated isoform is unknown. However, other VGCC genes are reported to encode transcription factors from their 3' ends (6).
- These findings provide further evidence of the incomplete nature of current genomic annotations, especially in human brain.

References

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