nature portfolio

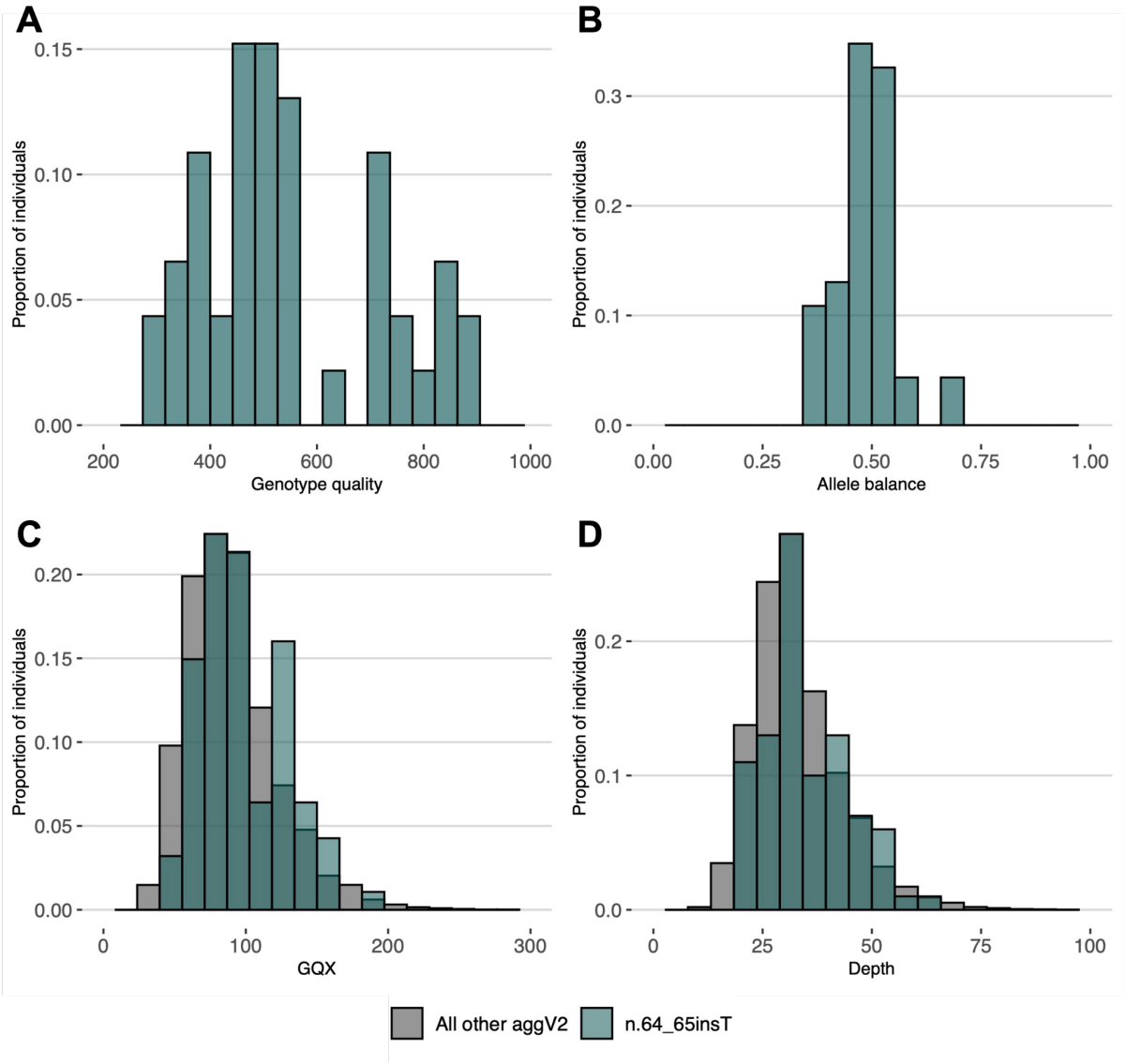
https://doi.org/10.1038/s41586-024-07773-7

## Supplementary information

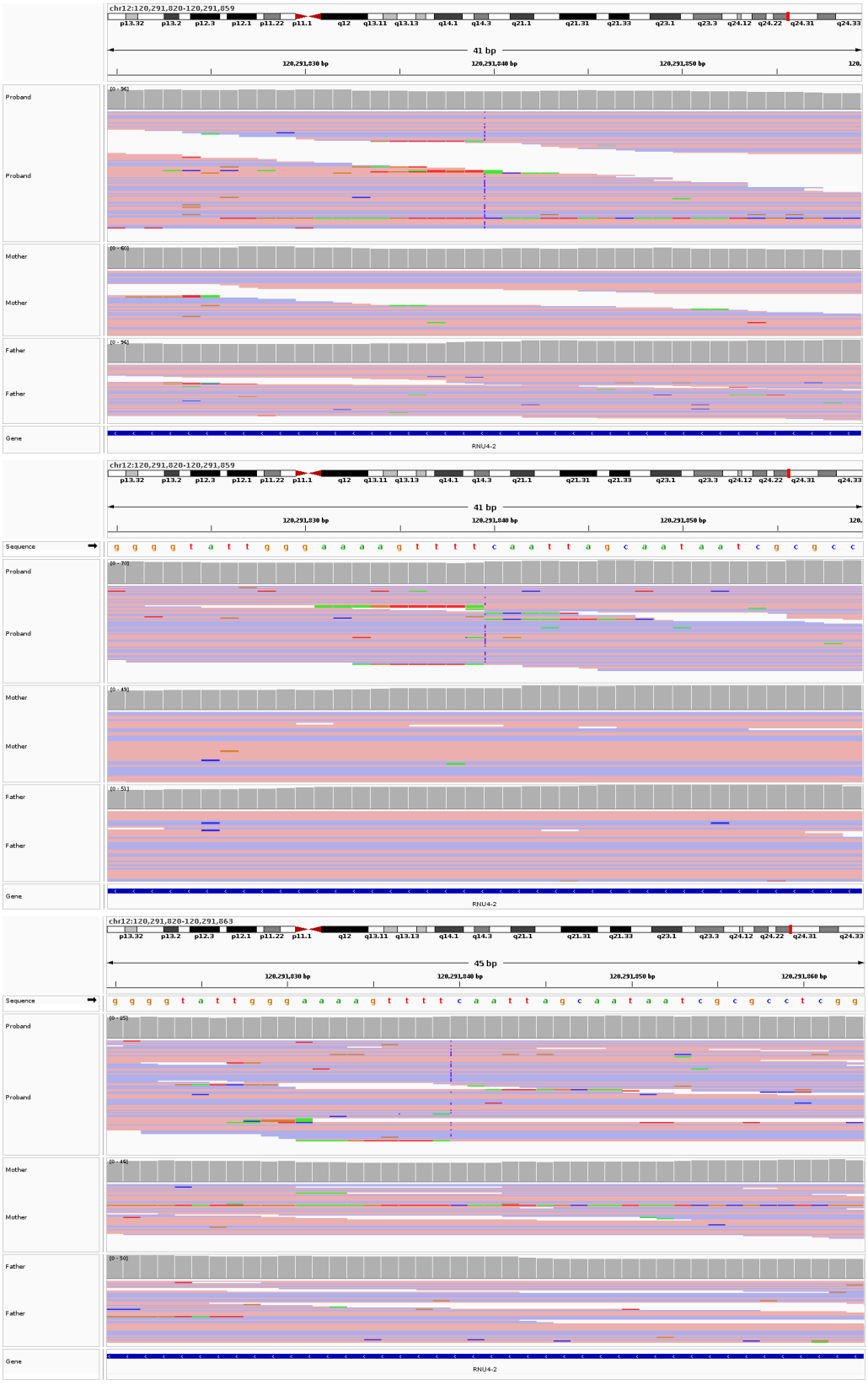
# De novo variants in the *RNU4-2* snRNA cause a frequent neurodevelopmental syndrome

In the format provided by the authors and unedited

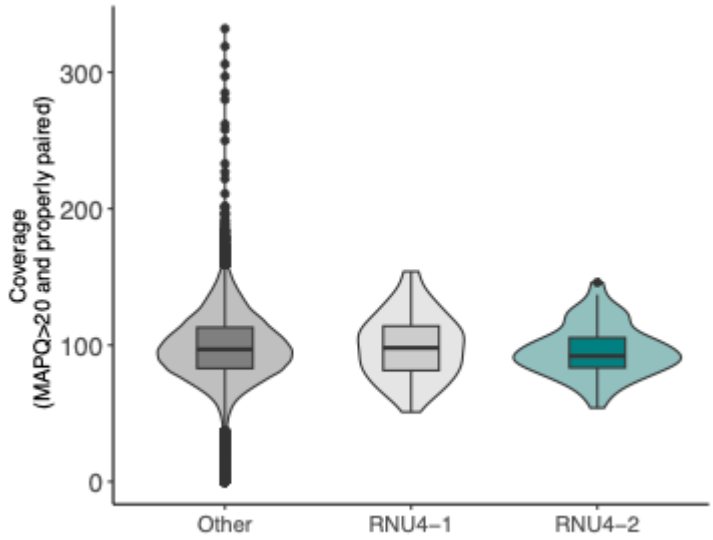
**Supplementary Figures**



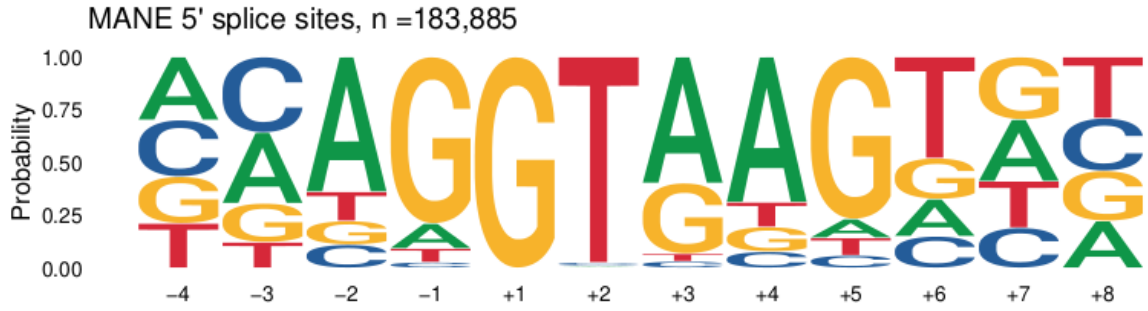

Supplementary Figure 1: Quality control metrics for the variant calls in all 46 individuals with the n.64_65insT variant. (A) genotype quality scores, (B) allele balance, (C) GQX score (defined as “Empirically calibrated variant quality score for variant sites, otherwise the minimum of genotype quality assuming variant position and genotype quality assuming non-variant position”), and (D) sequencing depth (the number of reads covering the variant position. (C) and (D) includes a background distribution of these metrics in all other sequenced genomes in the GEL aggregated variant dataset (grey).



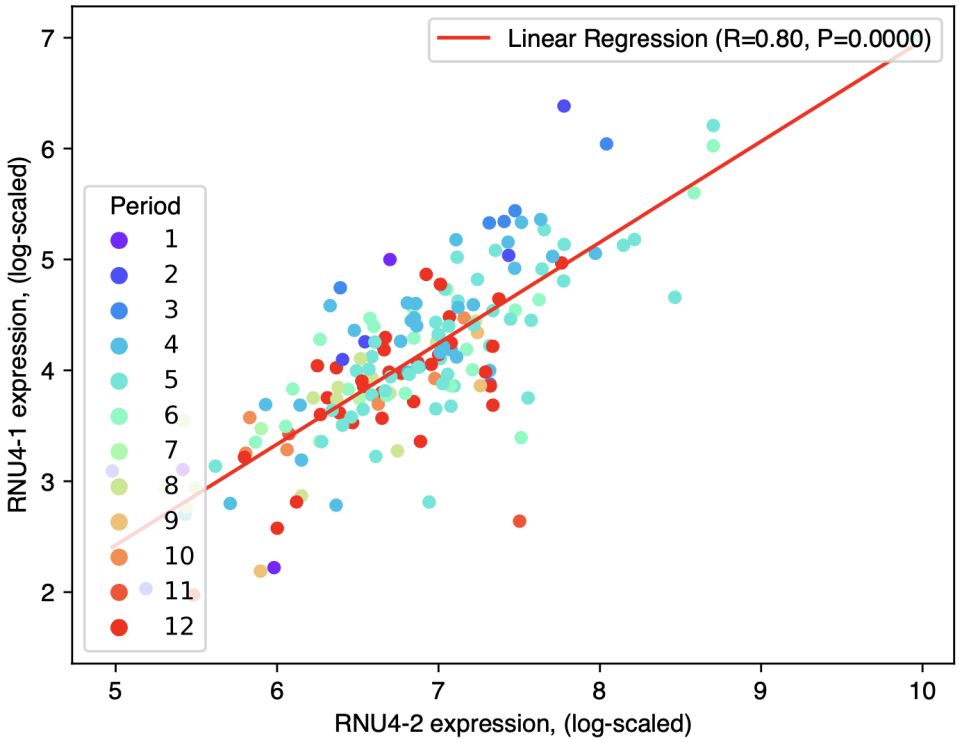

Supplementary Figure 2: Example IGV plots of the region surrounding the n.64_65insT variant in three trios demonstrate that the variant is detected with high confidence in the probands and is absent from the parents. Soft clipped reads are shown in colour.




Supplementary Figure 3: Number of sequencing reads with mapping quality scores (MAPQ) >20 across *RNU4-2* (teal), *RNU4-1* (light grey) and 999 random intergenic regions of the same length (dark grey) in probands with *RNU4-2* variants (n=57). Centre line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers.




Supplementary Figure 4: DNA sequence motifs around all canonical 5’ splice sites in MANE transcripts[41].




Supplementary Figure 5: Correlation between *RNU4-1* and *RNU4-2* expression in RNA-seq data from human cortex across prenatal and postnatal development from BrainVar[25]. 'Period' refers to developmental stages, spanning from embryonic development to late adulthood, that were defined previously[42].

**Supplementary Tables**

| Variant | Gene | Symbol | Allele count | ICD10 | ICD9 | Current age (5 yr range) |
|---|---|---|---|---|---|---|
| chr12:120291839-T-TA | ENSG00000202538 | *RNU4-2* | AC=1 | D64.9 Anaemia, unspecified; E83.3 Disorders of phosphorus metabolism; I10 Essential (primary) hypertension; I20.9 Angina pectoris, unspecified; I25.1 Atherosclerotic heart disease; I25.9 Chronic ischaemic heart disease, unspecified; I83.9 Varicose veins of lower extremities without ulcer or inflammation; L72.0 Epidermal cyst; R03.0 Elevated blood-pressure reading, without diagnosis of hypertension; R04.0 Epistaxis; Z30.2 Sterilisation; Z72.1 Alcohol use; Z82.3 Family history of stroke; Z86.4 Personal history of psychoactive substance abuse; Z86.6 Personal history of diseases of the nervous system and sense organs; Z86.7 Personal history of diseases of the circulatory system; Z92.1 Personal history of long-term (current) use of anticoagulants; Z95.5 Presence of coronary angioplasty implant and graft | | 70-75 |
| chr12:120291839-T-TG | ENSG00000202538 | *RNU4-2* | AC=1 | K30 Dyspepsia; K44.9 Diaphragmatic hernia without obstruction or gangrene; L82 Seborrhoeic keratosis | | 75-80 |
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | | | 65-70 |
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | B98.0 Helicobacter pylori [H.pylori] as the cause of diseases classified to other chapters; E66.9 Obesity, unspecified; E78.0 Pure hypercholesterolaemia; F32.9 Depressive episode, unspecified; F41.9 Anxiety disorder, unspecified; I10 Essential (primary) hypertension; K21.9 Gastro-oesophageal reflux disease without oesophagitis; K29.6 Other gastritis; K30 Dyspepsia; K44.9 Diaphragmatic hernia without obstruction or gangrene; K76.0 Fatty (change of) liver, not elsewhere classified; K76.8 Other specified diseases of liver; M19.9 Arthrosis, unspecified; M47.92 Spondylosis, unspecified (Cervical region); M54.9 Dorsalgia, unspecified; R10.4 Other and unspecified abdominal pain | | 65-70 |
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | C44.5 Skin of trunk; C61 Malignant neoplasm of prostate; D04.3 Skin of other and unspecified parts of face; N40 Hyperplasia of prostate; Z46.6 Fitting and adjustment of urinary device | | 70-75 |
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | | | 70-75 |

| | | | | | | |
|---|---|---|---|---|---|---|
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | A09.9 Gastroenteritis and colitis of unspecified origin; H26.4 After-cataract; I45.1 Other and unspecified right bundle-branch block; M13.94 Arthritis, unspecified (Hand); M67.4 Ganglion; R00.1 Bradycardia, unspecified; R55 Syncope and collapse | | 80-85 |
| chr12:120293173-T-TA | ENSG00000200795 | *RNU4-1* | AC=6 | I20.9 Angina pectoris, unspecified; I21.9 Acute myocardial infarction, unspecified; I25.1 Atherosclerotic heart disease; I48.0 Paroxysmal atrial fibrillation; J45.9 Asthma, unspecified; K20 Oesophagitis; R07.4 Chest pain, unspecified; R19.4 Change in bowel habit; R63.4 Abnormal weight loss; Z50.0 Cardiac rehabilitation; Z87.1 Personal history of diseases of the digestive system; Z87.4 Personal history of diseases of the genito-urinary system | 78909 Abdominal pain (other and unspecified) | 70-75 |
| chr12:120293173-T-TG | ENSG00000200795 | *RNU4-1* | AC=2 | R56.8 Other and unspecified convulsions | | 55-60 |
| chr12:120293173-T-TG | ENSG00000200795 | *RNU4-1* | AC=2 | M54.12 Radiculopathy (Cervical region) | | 55-60 |

Supplementary Table 1: ICD10 and ICD9 codes for individuals with single base pair insertions between codons 64 and 65 of *RNU4-2* and *RNU4-1* in the UK Biobank.

| **symbol** | **gene** | **chr** | **start** | **end** | **mean CPM** |
|---|---|---|---|---|---|
| *RNU4-2* | ENSG00000202538 | 12 | 120291763 | 120291903 | 3574.85 |
| *RNU4-1* | ENSG00000200795 | 12 | 120293097 | 120293237 | 629.12 |
| *RNU4ATAC* | ENSG00000264229 | 2 | 121530881 | 121531007 | 5.43150 |
| *U4* | ENSG00000274197 | 11 | 130114251 | 130114335 | 2.07471 |
| *U4* | ENSG00000277986 | 2 | 231711525 | 231711647 | 1.47215 |
| *U4* | ENSG00000276103 | 1 | 150608507 | 150608623 | 1.02479 |
| *U4* | ENSG00000278374 | 8 | 123454981 | 123455107 | 0.94882 |
| *U4* | ENSG00000273744 | 6 | 106271634 | 106271720 | 0.90575 |
| *U4* | ENSG00000283442 | 1 | 155894281 | 155894386 | 0.62500 |
| *U4* | ENSG00000272215 | 5 | 179492076 | 179492157 | 0 |

Supplementary Table 3: Mean expression of U4 genes in prefrontal cortex across all samples in BrainVar[25].

**Other Supplementary files and legends**

Supplementary Table 2: Detailed clinical information for 49 individuals with *RNU4-2* variants. SNVs are highlighted in pink, and alternate insertions are in blue. Blank spaces indicate that data were not provided.

**References**


25. Werling, D. M. *et al.* Whole-Genome and RNA Sequencing Reveal Variation and Transcriptomic Coordination in the Developing Human Prefrontal Cortex. *Cell Rep.* **31**, 107489 (2020).

41. Morales, J. *et al.* A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* **604**, 310–315 (2022).

42. Kang, H. J. *et al.* Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–489 (2011).