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Trends in Genetics

Spotlight

# ReNU syndrome – a newly discovered prevalent neurodevelopmental disorder

Vanessa F. Burns[1] and Elizabeth J. Radford[1,2,*]

**Two recent papers have identified genetic variants in the noncoding gene *RNU4-2* to cause a frequent neurodevelopmental disorder. This work will have a substantial impact on the rare disease community, leading to thousands of diagnoses worldwide. These studies also highlight the untapped diagnostic potential of noncoding regions.**

Genomic sequencing is now a routinely used diagnostic tool in the investigation of rare conditions. However, between 40% and 60% of individuals with rare neurodevelopmental disorders (NDDs) remain undiagnosed following genetic testing [1]. At present, clinical genetic tests almost exclusively focus on protein-coding genes. Accordingly, the vast majority of variants known to cause monogenic genetic conditions reside in protein-coding regions of the genome. However, with the introduction of large-scale genomic sequencing initiatives and the increasing use of whole-genome versus coding exome sequencing, the role of noncoding variation in disease is becoming increasingly apparent.

Genetic variation in noncoding regions has been shown to cause NDDs through diverse mechanisms, such as by altering the function of conserved *cis*-regulatory elements and by disrupting the expression or function of noncoding RNAs [2]. Noncoding RNAs are RNA transcripts that are not translated into protein. Noncoding RNAs contribute to a wide variety of cellular processes, including but not limited to the maintenance of germline genome integrity and the regulation of gene expression, RNA splicing, and translation. Dysregulation of noncoding RNAs is implicated in many human diseases, including cancer and neurological diseases [3]. Small nuclear RNAs (snRNAs) are a class of noncoding RNAs that play an important role in the removal of introns during pre-mRNA splicing as part of small nuclear ribonucleoprotein complexes [4]. *RNU4-2* encodes U4 snRNA, a critical component of the of the major spliceosome.

Two recent studies have identified *RNU4-2* as a novel NDD gene. One study, led by Yuyang Chen and Nicola Whiffin at the Big Data Institute and the Wellcome Centre for Human Genetics, Oxford, made the discovery using a cohort of roughly 9000 undiagnosed NDD probands from the 100 000 Genomes Project (100KGP) in Genomics England (GEL) [5]. Using a largely overlapping cohort, another group of scientists, led by Daniel Greene and Ernest Turro at Mount Sinai New York, made the same discovery [6]. Subsequent analyses performed by both groups identified a newly recognized NDD, termed 'ReNU syndrome,' linked to variation in the central region of *RNU4-2*. *RNU4-2* is not the first snRNA to be linked to human disease. *RNU4ATAC* and *RNU12*, components of the minor spliceosome, have, for example, been linked to autosomal recessive disorders [7]. However, what is particularly striking about these studies is the projected prevalence of this new disorder among patients with NDD. Although a rare condition, researchers from both groups estimate that *RNU4-2* variants may explain roughly 0.4% of individuals with NDD. This means that there are thousands of individuals globally who could receive this diagnosis.

Initial discoveries among 100KGP participants led Chen *et al.* and Greene *et al.* to identify a highly recurrent *de novo* single-base insertion (n.64_65insT) in 46 and 36 NDD probands, respectively. All individuals with this variant were significantly more likely to have intellectual disability (ID) and severe neurodevelopmental phenotypes, as compared with other NDD probands in GEL (Figure 1A). Both Chen *et al.* and Greene *et al.* found the central region of *RNU4-2* to be depleted of variation in population databases, including gnomAD, the All of US dataset, and the UK Biobank. This central region is defined by Chen *et al.* as an 18–base pair critical region (n.62-79) and by Greene *et al.* as two adjacent critical regions (n.62-70 and n.73-79). Inclusion of other insertions and single-nucleotide variants detected in the 18–base pair critical region led Chen *et al.* to identify 115 individuals among an expanded international cohort of undiagnosed NDD probands. Greene *et al.*, who conducted a genetic association analysis comparing rare variants in roughly 41 000 noncoding genes between 5529 unrelated probands and 46 401 control subjects, initially identified three *RNU4-2* variants (n.64_65insT included) with a statistically significant association with risk of ID. An expanded search identified a further seven variants and 27 NDD probands with a variant in the defined critical regions of *RNU4-2*.

*RNU4-2* variants observed in individuals with NDD map to a region of U4 responsible for unwinding the duplex formed between U4 and U6 snRNAs (Figure 1B). Both studies propose that the highly recurrent single-base insertion, situated in a complex quasi-pseudoknot secondary structure, may destabilize the U4/U6 base-pairing interaction and/or disrupt receipt of the 5′ splice site during spliceosome activation. Consistent with this hypothesis, Chen *et al.* observed an increase in the use of unannotated 5′ splice sites in individuals with the highly recurrent single-base insertion variant. Five of the genes implicated in these abnormal splicing events were known NDD genes or

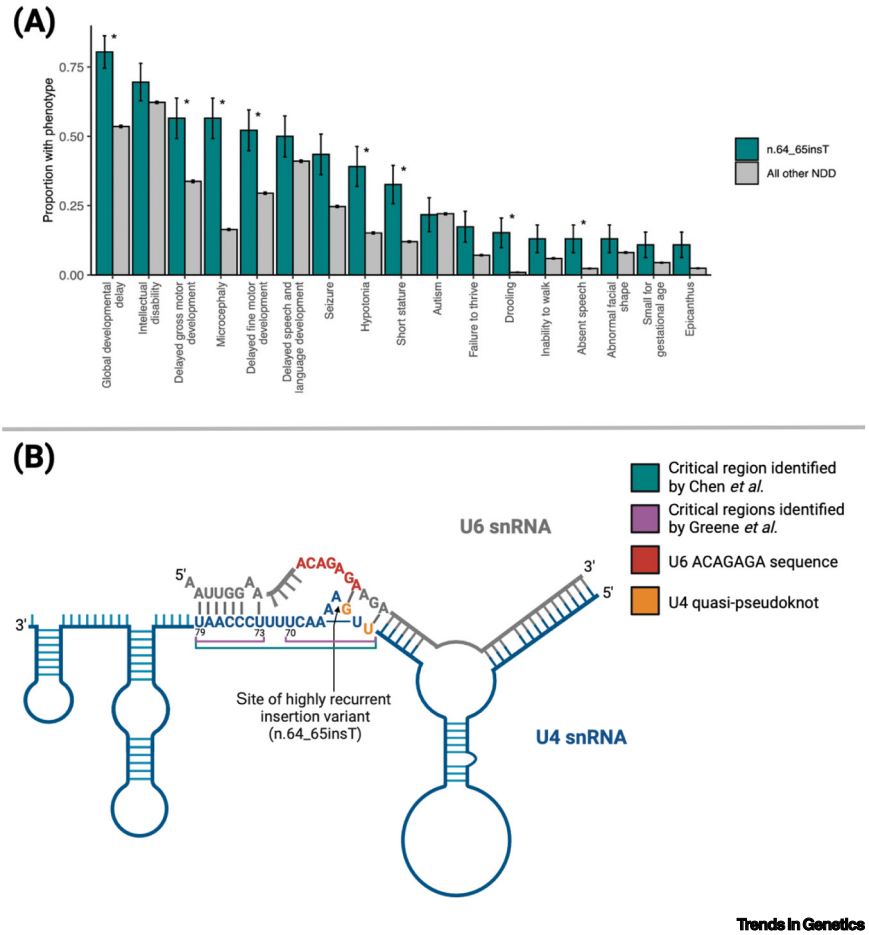


Figure 1. Clinical features and structural context corresponding to highly recurrent *RNU4-2* insertion variant. (A) Proportion of individuals with Human Phenotype Ontology (HPO) terms corresponding to phenotypes observed in five or more individuals with the highly recurrent n.64_65insT variant compared with all other individuals with neurodevelopmental disorders (NDDs) in the Genomics England (GEL) database. Multiple HPO terms are significantly enriched in individuals with the n.64_65insT variant after Bonferroni adjustment (marked with an asterisk), indicating that individuals with the n.64_65insT variant have more phenotypic similarity than the GEL NDD cohort. Multiple terms relating to global developmental delay, intellectual disability, hypotonia, seizure, microcephaly, autism, and short stature have been collapsed into single phenotypes. Error bars indicate ±1 standard error. Enriched HPO terms highlight the clinical features to look for in patients who may benefit from specific genetic testing for *RNU4-2* variants. This figure is taken, with permission, from Chen *et al.* [5] (http://creativecommons.org/licenses/by/4.0/). (B) Schematic of U4 small nuclear RNA (snRNA) (blue) binding to U6 snRNA (gray), highlighting the central critical region(s) enriched for *RNU4-2* variants in NDD probands. The arrow points to the location of the highly recurrent n.64_65insT variant, situated within a quasi-pseudoknot structure, in the context of nearby secondary structures. The U6 ACAGAGA sequence is also highlighted, which receives the 5′ splice site during spliceosome activation. Created using BioRender (https://biorender.com). Abbreviation: bp, base pair.

had previously been associated with NDD. RNA-sequencing analyses by both groups show *RNU4-2* to be highly expressed in the human brain. Chen *et al.* assayed transposase-accessible chromatin with sequencing (ATAC-seq) demonstrating high chromatin accessibility at *RNU4-2*. High levels of transcription alongside a propensity for secondary structure formation may explain the high recurrence rate of this insertion variant. Further work will be necessary to pinpoint the precise cause.

Finally, where it was possible to phase the *de novo RNU4-2* variants, Chen *et al.* observed that they occurred exclusively on the maternal allele. There are a variety of possible explanations for this. *RNU4-2* variants may be selected against in the male germline, or the *RNU4-2* locus may be subject to genomic imprinting (a phenomenon that results in parent-of-origin–specific gene expression). It is possible that paternally inherited *RNU4-2* variants are embryonic lethal.

The clinical syndrome associated with *RNU4-2* has already been known by several different names. However, affected patients and their families have chosen the name ReNU syndrome, pronounced 'renew,' to symbolize the renewal of hope for a better future. From a clinical perspective, ReNU syndrome is characterized by moderate to severe global developmental delay, ID, dysmorphic facial features, short stature, microcephaly, hypotonia, and a variety of seizure types (Figure 1A). Given the projected prevalence of this condition, there are a large number of individuals worldwide who could receive this diagnosis. In a recent GEL podcast, Lindsay Pearse, whose son Lars recently received this diagnosis, shared that 'being undiagnosed had become quite a big part of our identity, and so now that's kind of shifting a little bit that we have this new diagnosis and are part of a new community' [8]. However, because the central region of *RNU4-2* is not currently included within the regions analyzed by standard exome-based tests, realizing the impact of these findings for the NDD community will require retesting many patients. Going forward, in the UK, *RNU4-2* is now included within the severe microcephaly, ID, and early onset or syndromic epilepsy genetic testing panels.

This research underlies the importance of large-scale patient cohorts and population genomic databases in making clinically

impactful discoveries in rare disease. It also highlights how little we understand the noncoding portion of our genome and the extent to which noncoding variants contribute to rare monogenic disorders. Previous studies had suggested that variation in fetal brain-active regulatory regions may contribute to roughly 2% of undiagnosed NDD [9]. However, the findings that *RNU4-2* variants alone may contribute up to 0.4% of NDD suggest that many more individuals may await a diagnosis caused by noncoding variation. Interpreting variation in noncoding regions is particularly challenging because functional models for noncoding regions do not presently exist in the same way that they do for protein-coding regions. Advancing our understanding will require expanding population databases and ensuring equitable representation of diverse genetic ancestries. Scalable experimental approaches that directly interrogate the functional consequence of genetic variants also have an important role to play [10]. Overall, this research marks a significant discovery that will have a lasting impact on families affected by genetic disease across the world.

## Declaration of interests

The authors have no interests to declare.

[1]Wellcome Sanger Institute, Hinxton, CB10 1SA, UK
[2]Department of Paediatrics, University of Cambridge, Level 8, Cambridge Biomedical Campus, Cambridge, CB2 0QQ, UK

*Correspondence:
er10@sanger.ac.uk (E.J. Radford).

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## References

1. Wright, C.F. *et al.* (2023) Genomic diagnosis of rare pediatric disease in the United Kingdom and Ireland. *N. Engl. J. Med.* 388, 1559–1571
2. Zhang, F. and Lupski, J.R. (2015) Non-coding genetic variants in human disease. *Hum. Mol. Genet.* 24, R102–R110
3. Nemeth, K. *et al.* (2024) Non-coding RNAs in disease: from mechanisms to therapeutics. *Nat. Rev. Genet.* 25, 211–232
4. Guthrie, C. and Patterson, B. (1988) SPLICEOSOMAL snRNAs. *Annu. Rev. Genet.* 22, 387–419
5. Chen, Y. *et al.* (2024) De novo variants in the RNU4-2 snRNA cause a frequent neurodevelopmental syndrome. *Nature* 632, 832–840
6. Greene, D. *et al.* (2024) Mutations in the U4 snRNA gene *RNU4-2* cause one of the most prevalent monogenic neurodevelopmental disorders. *Nat. Med.* 30, 2165–2169
7. Benoit-Pilven, C. *et al.* (2020) Clinical interpretation of variants identified in RNU4ATAC, a non-coding spliceosomal gene. *PLoS One* 15, e0235655
8. Callachand, N. (2024) *'How has a groundbreaking genomic discovery impacted thousands worldwide?' Behind the Genes*, Genomics England https://www.genomicsengland.co.uk/podcasts/how-has-a-groundbreaking-genomic-discovery-impacted-thousands-worldwide
9. Short, P. *et al.* (2018) *De novo* mutations in regulatory elements in neurodevelopmental disorders. *Nature* 555, 611–616
10. Weile, J. and Roth, F.P. (2018) Multiplexed assays of variant effects contribute to a growing genotype-phenotype atlas. *Hum. Genet.* 137, 665–678