Trends in Genetics

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Spotlight

A new era for the dark genome

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A landmark study by Quinodoz *et al.* revealed that variants in non-coding small nuclear RNA genes, *RNU4-2* and four *RNU6* paralogs, represent a previously unrecognized cause of autosomal dominant retinitis pigmentosa. This uncovers pleiotropy in *RNU4-2* variants and expands the genetic architecture of Mendelian disease into the 'dark genome'.

Vision is widely regarded as the most valued of the five senses, with population-based surveys showing that vision loss profoundly affects quality of life [1]. Among the leading causes of vision impairment are inherited retinal diseases (IRDs), which affect approximately 1 in 2000 individuals worldwide and are associated with over 460 genes and loci [2]. However, despite major advances in sequencing technologies, approximately 30–50% of patients remain without a molecular diagnosis, complicating genetic counseling, therapeutic decision-making, and prolonging the diagnostic odyssey for patients and families. This missing heritability likely reflects both limitations of current diagnostic pipelines and undiscovered disease-causing genes and variant classes.

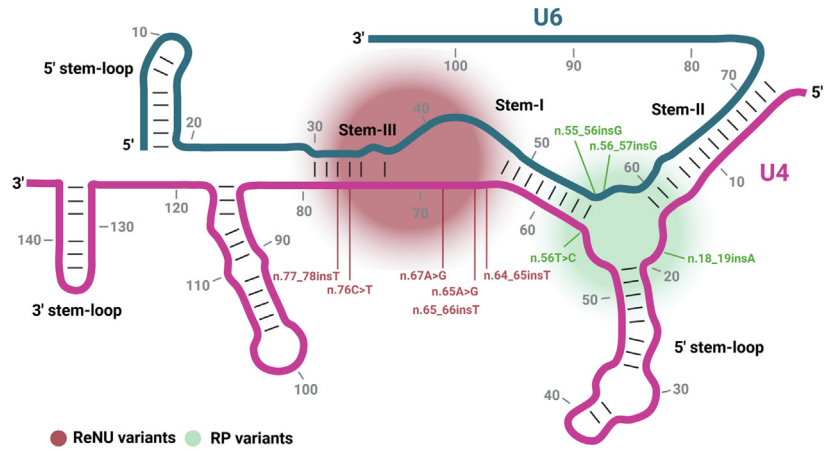
Among potential contributors to missing heritability, noncoding RNAs (ncRNAs) are increasingly recognized for their involvement in human disease, as demonstrated by the identification of pathogenic variants in small nuclear RNAs (snRNAs) as a prevalent cause of neurodevelopmental diseases (NDDs) [3]. Yet, diagnostic approaches largely focus on protein-coding regions, and even when whole-genome sequencing is performed, variants in ncRNAs remain difficult to interpret because of limited functional annotation and the lack of established variant interpretation frameworks.

In this context, Quinodoz *et al.* provide compelling evidence that pathogenic variants in snRNA genes represent a previously unrecognized cause of retinitis pigmentosa (RP), a genetic disorder causing progressive blindness [4]. The study began with a nonconsanguineous family affected by autosomal dominant RP (adRP) that lacked a molecular diagnosis despite genome sequencing. When no variants were found in known RP genes, extended variant filtering revealed a rare insertion in *RNU4-2*, which encodes U4 snRNA. Subsequently, screening of 4721 unsolved IRD cases across international cohorts identified additional families carrying the same and a second recurrent *RNU4-2* variant, providing a diagnosis for 41 individuals across 15 families.

U4 snRNA is one of several components of the spliceosome, the ribonucleoprotein complex responsible for pre-mRNA splicing. U4 forms a duplex with U6 snRNA and, together with U5 and associated ribonucleoproteins, constitutes the tri-small nuclear ribonucleoprotein (snRNP) complex [5]. As previous studies have already associated pathogenic variants in genes encoding spliceosomal proteins (PRPF3, PRPF4, PRPF8, and PRPF31) with adRP [6], the identification of pathogenic variants in U4 pointed toward perturbation of spliceosome function. Building on this, the authors hypothesized that variants in U6 snRNAs might also contribute to RP. Indeed, recurrent pathogenic variants were identified in four *RNU6* paralogs, explaining disease in 52 additional families and 112 individuals. Collectively, deleterious variants in *RNU4-2* and *RNU6* genes are estimated to explain approximately 1.4% of previously undiagnosed RP cases.

A striking aspect of this work is the pleiotropy of *RNU4-2*. *De novo* variants in this gene were previously identified as a cause of ReNU syndrome, a severe NDD [3,7]. Remarkably, RP-associated variants cluster in the three-way junction of the U4/U6 duplex, which is important for assembly with other snRNP proteins, whereas ReNU-associated variants localize to stem III and the T-loop, regions involved in splice-site recognition [3] (Figure 1). Based on this spatial separation, Quinodoz *et al.* propose that RP-associated variants may exert a comparatively milder pathogenic effect than those causing ReNU syndrome. Functional analyses further support this mechanistic divergence. Unlike ReNU-associated variants, which lead to systematic misrecognition of donor splice sites by the spliceosome, resulting in widespread splicing failure [8], RP-associated variants did not produce major global splicing abnormalities. Instead, immunoprecipitation experiments revealed an increased association of RP-associated snRNAs with the di-snRNP markers (SART3 and PRPF31), accompanied by unchanged or reduced binding to the tri-snRNP component SNRNP200. These findings suggest that RP-associated snRNA variants induce a delay or partial block at the di-snRNP stage, impairing the transition to the fully assembled tri-snRNP complex. Consistent with this, a recent saturation genome editing study of *RNU4-2* demonstrated strong functional effects for ReNU variants on cell fitness, whereas RP variant n.56T > C exhibited scores within the neutral range, suggesting a milder pathogenic impact [9]. Because these effects were relatively modest, the authors propose a dominant-negative or gain-of-function mechanism in which subtle alterations in snRNP assembly dynamics are sufficient

(A)



(B)

Feature	ReNU syndrome	Retinitis pigmentosa
Affected region	T-loop + stem III (n.62–79)	Three-way junction (n.18–19, n.55–57)
Proposed mechanism	Splice-site recognition errors	Delayed tri-snRNP assembly
Inheritance mode	*De novo*, maternal allele	Both *de novo* and inherited
Onset of disease	Congenital/childhood	Adolescent

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Figure 1. Spatial segregation of *RNU* variants is associated with disease phenotype. (A) Schematic of the U4/U6 di-snRNA duplex with nucleotide positions indicated. RP-associated variants (green) cluster in the three-way junction (n.18–19, n.55–57), a region critical for tri-snRNP assembly, whereas ReNU-associated variants (red) localize to stem III and the T-loop (n.62–79), regions involved in splice-site recognition. (B) Summary of key genotype-phenotype distinctions between ReNU syndrome and retinitis pigmentosa arising from variants in the same gene. This figure was created using BioRender (2026) (https://BioRender.com/uhd4nte). RP: retinitis pigmentosa; snRNP: small nuclear ribonucleoprotein.

to trigger retinal degeneration without catastrophic splicing failure.

How can such a modest biochemical defect cause photoreceptor degeneration while sparing other tissues? The answer likely lies in the extraordinary splicing burden of the retina [10]. Quinodoz *et al.* show that *RNU4-2* is more highly expressed than *RNU4-1* across retinal layers, with U4 and U6 snRNAs reaching substantially higher levels in the neurosensory retina and Retinal pigment epithelium (RPE) than in the choroid, consistent with previous reports of elevated snRNA expression in the retina compared with other tissues [6]. Crucially, RNA-seq from the peripheral blood of patients with RP revealed no major splicing abnormalities, unlike the systematic splice-site misrecognition in ReNU. These variants likely exert tissue-specific effects that become rate-limiting only in photoreceptors, where continuous outer segment renewal and high rates of alternative splicing place an exceptional demand on spliceosome throughput. The relevance of the spliceosome machinery in retinal diseases is further demonstrated by the clinical similarities between patients harboring variants in snRNA or PRPF genes, showing earlier onset than most adRP forms alongside high rates of cystoid macular edema and cataracts.

Nevertheless, whether RP and ReNU are entirely separable remains an open question, as Chen *et al*. noted that some ReNU patients already display visual abnormalities, though most were too young to manifest classical RP [7].

Altogether, the findings by Quinodoz *et al.* have immediate clinical implications. For the 67 solved pedigrees in this study, identifying a genetic cause provides long-awaited diagnostic closure and enables informed reproductive choices, including preimplantation genetic testing. However, important questions remain, particularly regarding the structural consequences of RP-associated variants on spliceosome architecture and assembly, and whether disease arises through gain-of-function or dominant-negative mechanisms—insights critical for developing targeted therapies. Beyond IRDs, this work represents a broader conceptual shift in human genetics by demonstrating that highly penetrant Mendelian disorders can arise from pathogenic variations hiding in plain sight in the noncoding genome. These findings emphasize the need to expand genetic analyses beyond protein-coding regions and highlight the increasing need for new annotation tools to close the diagnostic gap and correctly interpret those variants contributing to hereditary disease, with significant implications for future diagnostics and therapeutic development.

Acknowledgments

The work of M.S. is supported by Stichting Ushersyndroom, the Usher Syndrome Society, and the Lifelong VISION project (funded by the Ministry of Education, Culture, and Science of The Netherlands, Gravitation Grant No. 024.006.034). D.C. is supported by the Italian Telethon Foundation and by the Italian Ministry of University and Research under the project PRIN2022 PNRR (project P2022KYM38). M.B.C. is supported by a postdoctoral grant from the Research Foundation Flanders (FWO 12AJK26N).

Declaration of interests

The authors declare no competing interests.

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https://doi.org/10.1016/j.tig.2026.03.003

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