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SHORT REPORT

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Re-analysis of whole genome sequencing ends a diagnostic odyssey: Case report of an *RNU4-2* related neurodevelopmental disorder

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Abstract

Despite increasing knowledge of disease-causing genes in human genetics, approximately half of the individuals affected by neurodevelopmental disorders remain genetically undiagnosed. Part of this missing heritability might be caused by genetic variants outside of protein-coding genes, which are not routinely diagnostically investigated. A recent preprint identified de novo variants in the non-coding spliceosomal snRNA gene *RNU4-2* as a cause of a frequent novel syndromic neurodevelopmental disorder. Here we mined 164 whole genome sequencing (WGS) trios from individuals with neurodevelopmental or multiple congenital anomaly disorders that received diagnostic genomic investigations at our clinic. We identify a recurrent de novo *RNU4-2* variant (NR_003137.2(*RNU4-2*):n.64_65insT) in a 5-year-old girl with severe global developmental delay, hypotonia, microcephaly, and seizures that likely explains her phenotype, given that extensive previous genetic investigations failed to identify an alternative cause. We present detailed phenotyping of the individual obtained during a 5-year follow-up. This includes photographs showing recognizable facial features for this novel disorder, which might allow prioritizing other currently unexplained affected individuals sharing similar facial features for targeted investigations of *RNU4-2*. This case illustrates the power of re-analysis to solve previously unexplained cases even when a diagnostic genome remains negative.

KEYWORDS
chromosome 12q, clinical genetics, deep-phenotyping, dysmorphology, neurodevelopmental disorder, non-coding genome, *RNU4-2*, WGS

1 | INTRODUCTION

While advances in sequencing technologies have greatly expanded the number of disease causing genes for neurodevelopmental disorders (NDDs), ~50% remain genetically undiagnosed. Reasons for this so called missing heritability include non-genetic factors, variants of unknown clinical significance in genes yet to be identified as disease-causing, somatic variants not present in the investigated tissue, or variants that might be missed upon whole exome sequencing (WES) based diagnostics, including deep intronic variants affecting

Rachel Schot and Federico Ferraro contributed equally to this study.

splicing of protein-coding genes or variants in the non-coding genome.[1–3] Although applying whole genome sequencing (WGS) can potentially overcome the shortcomings that WES brings along detecting variants outside of the exome, most diagnostics analysis pipelines still focus on the protein-coding parts of the genome, even when using WGS.[4,5] Therefore, at present, the full promise of WGS in diagnostics to identify non-coding causes of human disorders is yet to be fully leveraged. The importance of this is illustrated by a recent preprint, identifying de novo variants in the non-coding spliceosomal snRNA gene *RNU4-2* on chromosome 12q, as a cause of a novel syndromic NDD.[6] Leveraging large scale WGS data sets from unexplained NDD individuals, and looking for the most prevalent de novo variants, the authors of this study identified 119 individuals with NDDs harboring de novo variants in an 18 bp region of *RNU4-2*. This region maps to the T-loop and Stem III in the U4/U6 snRNA duplex that is severely depleted of variation in the general population. The phenotype of these individuals included moderate to severe global developmental delay, intellectual disability, short stature, microcephaly, seizures, hypotonia, brain abnormalities, involvement of other organs including the skeleton, and dysmorphic features for which at this stage no clinical photographs are available in literature. Given the prevalence of the variants in their cohorts, the authors estimate that de novo variants in *RNU4-2* might explain ~0.41% of all NDD individuals. This makes this new disorder likely one of the most prevalent rare disorders currently known, but likewise one that is currently widely undiagnosed given that *RNU4-2* is not captured in WES.

Here, we mined 164 diagnostic trio WGS from individuals that visited our clinical genetics clinic for diagnostics in Q1 of 2024 because of NDDs or multiple congenital anomalies. Among these 164 individuals, representing the average daily population seen at a Dutch tertiary university medical center, we identified one individual (1/164, 0.61%) harboring the most recurrent de novo *RNU4-2* variant.[6] We present an in-depth phenotype description of this case. We foresee that this detailed phenotype information will help clinicians recognizing this new disorder effectively, allowing prioritizing candidate individuals from their cohorts for targeted DNA investigations of *RNU4-2* potentially reducing their missing heritability.

2 | CASE REPORT

The individual is a currently 5-year- and 4-month-old girl, born as the second child to non-consanguineous, healthy Dutch parents with an unremarkable family history. During pregnancy, an increased nuchal fold was observed (9.3 mm), leading to an invasive prenatal diagnostic that remained unremarkable. Ultrasound investigations at 30 3/7 gestational weeks showed a small head circumference (p5) with a small brain, mild ventriculomegaly, and mild cerebellar hypoplasia, confirmed on Magnetic Resonance Imaging (MRI) at gestational week 32 (Figure 1A). Gyration and pons were normal. Delivery was induced at 38 6/7 weeks and was uncomplicated, without cardiorespiratory issues. Birth length was 49 cm (−0.97 SDS), weight was 2.74 kg (−2.00 SDS), and head circumference was 34 cm (−0.83 SDS). An MRI at day 2 showed enlarged ventricles, most prominently at the occipital horns (Figure 1B). The transversal cerebellar diameter and gyration impressed as what would be expected upon 32–34 gestational weeks, with the vermis showing a normal cranio-caudal diameter, together indicating a mild cerebellar hypoplasia. Diffuse shortage of white matter was observed in both cerebral hemispheres. Given her clinical performance at that moment, she was discharged, but presented in the first week again at a peripheral hospital because of feeding issues, which led to several hospital admissions in the following weeks. At age 2 months, she again presented to our hospital with episodes of saturation drops, possible myoclonic seizures, and agitation. Electroencephalogram (EEG) showed no abnormalities and no clinical signs of seizures were noticed during the recording. Head circumference was then 36 cm (−2.0 SDS). Subsequent months were complicated by feeding issues requiring tube feeding, recurrent pneumonia, and poor sleep. At 3 months, hip dysplasia was treated with a Pavlik harness. At 5 months, she presented at the orthopedic surgeon, with the left leg no longer spontaneously moving. An x-ray showed a pathogenic old fracture at the left greater trochanter pathogenic old fracture. Continuous feeding problems required percutaneous endoscopic gastrostomy (PEG) tube placement at 9 months. Developmental delay was clearly evident at that time, with no eye contact, axial hypotonia, and limited spontaneous movement. Frequent airway infections continued. At age of 1 year, head circumference was 42.4 cm (−2.40 SDS), presenting again with axial hypotonia and increased peripheral muscle tonus. Routine blood investigations were unremarkable. Significant reflux occurred. Bilateral tympanostomy tubes were placed at 13 months given continuous ear infections. Polysomnography at 23 months showed central apnea. At 2 years and 10 months, she presented with an estimated developmental age of 8–10 months, being able to sit and stand with assistance. She made sounds but no words, with extensive drooling. Because of potential absence seizures (not captured on an EEG), she was treated with anti-epileptics at 3 years, but given adverse reactions of levetiracetam and valproic acid these were stopped, with no seizures re-appearing. At 3 years and 8 months, hip dysplasia was surgically corrected. A month later, she was again hospitalized because of saturation dips and suspicion of seizures. As electrocardiogram (ECG) and EEGs were unremarkable, this was interpreted as possibly autonomic dysfunction, triggering gabapentin treatment. At 4 years and 3 months, when again events suspicious for generalized tonic-clonic seizures occurred, an interictal EEG showed generalized seizure activity, with polyspikes and waves starting at the frontal lobe. Carbamazepine and valproic acid treatment were initiated, but no complete seizure control was obtained. MRI at 4 years and 4 months showed a progressive loss of cerebral white matter compared to earlier MRIs, small volume of the cerebellar hemispheres, thin corpus callosum, and normal gyration pattern (Figure 1C). At 4 years and 6 months, she could independently sit, but not stand (still revalidating from hip surgery), and play with her hands, not having achieved a tweezers grip. She did not use any words, made sounds, and pointed as means of

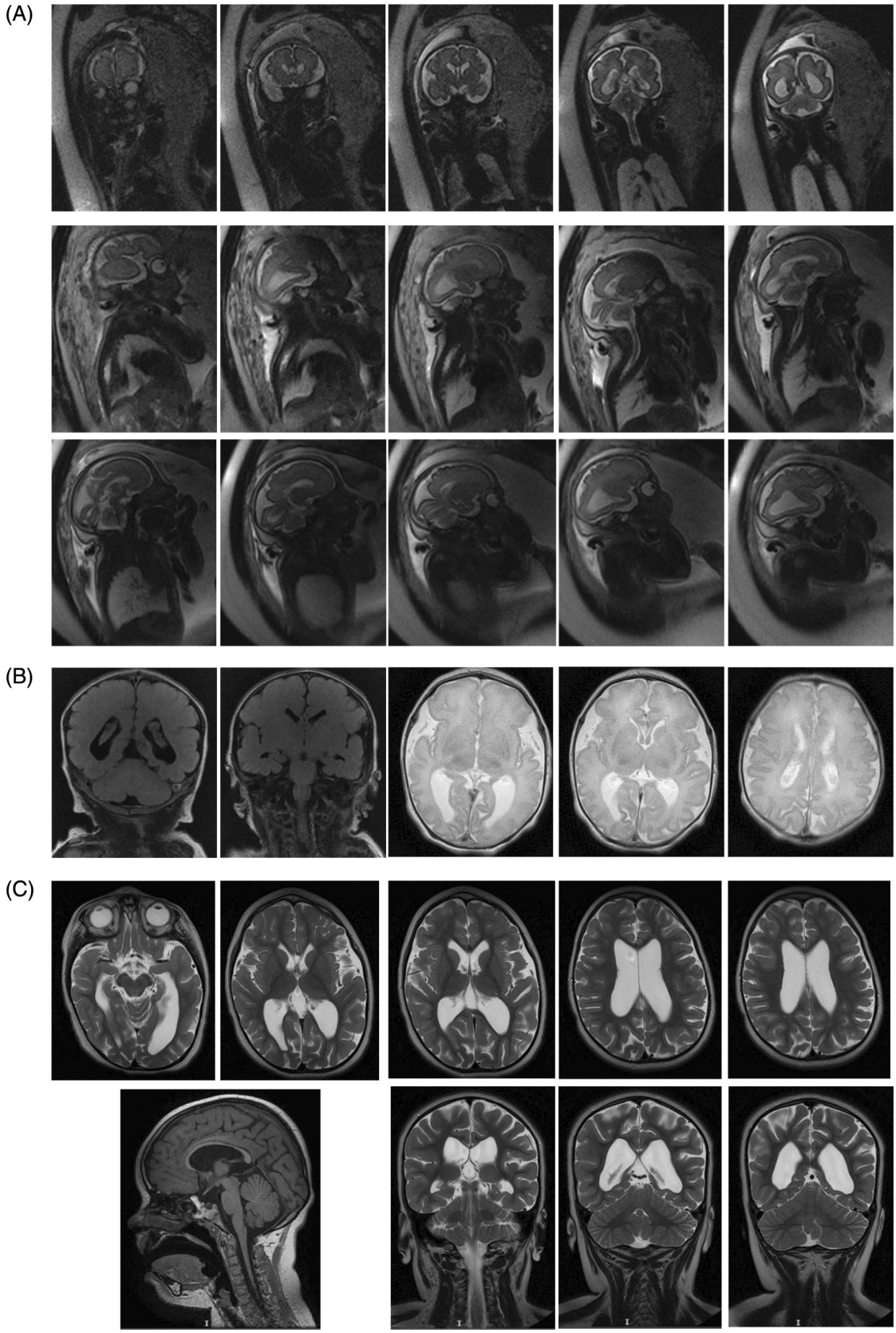

FIGURE 1 Brain imaging. (A) Fetal MRI at gestation week 32 showing a relatively small brain, mild ventriculomegaly, and mild cerebellar hypoplasia and normal pons. Gyration was normal. (B) MRI imaging (T2 Flair; T2 propellor) in various planes at day 2. Note the enlarged ventricles, most prominently at the occipital horns. (C) MRI imaging (T1-weighted; T2-propellor) in various planes at age 4 years and 4 months showed progressive loss of cerebral white matter, small cerebellar hemisphere volume, thin corpus callosum, and normal gyration pattern.

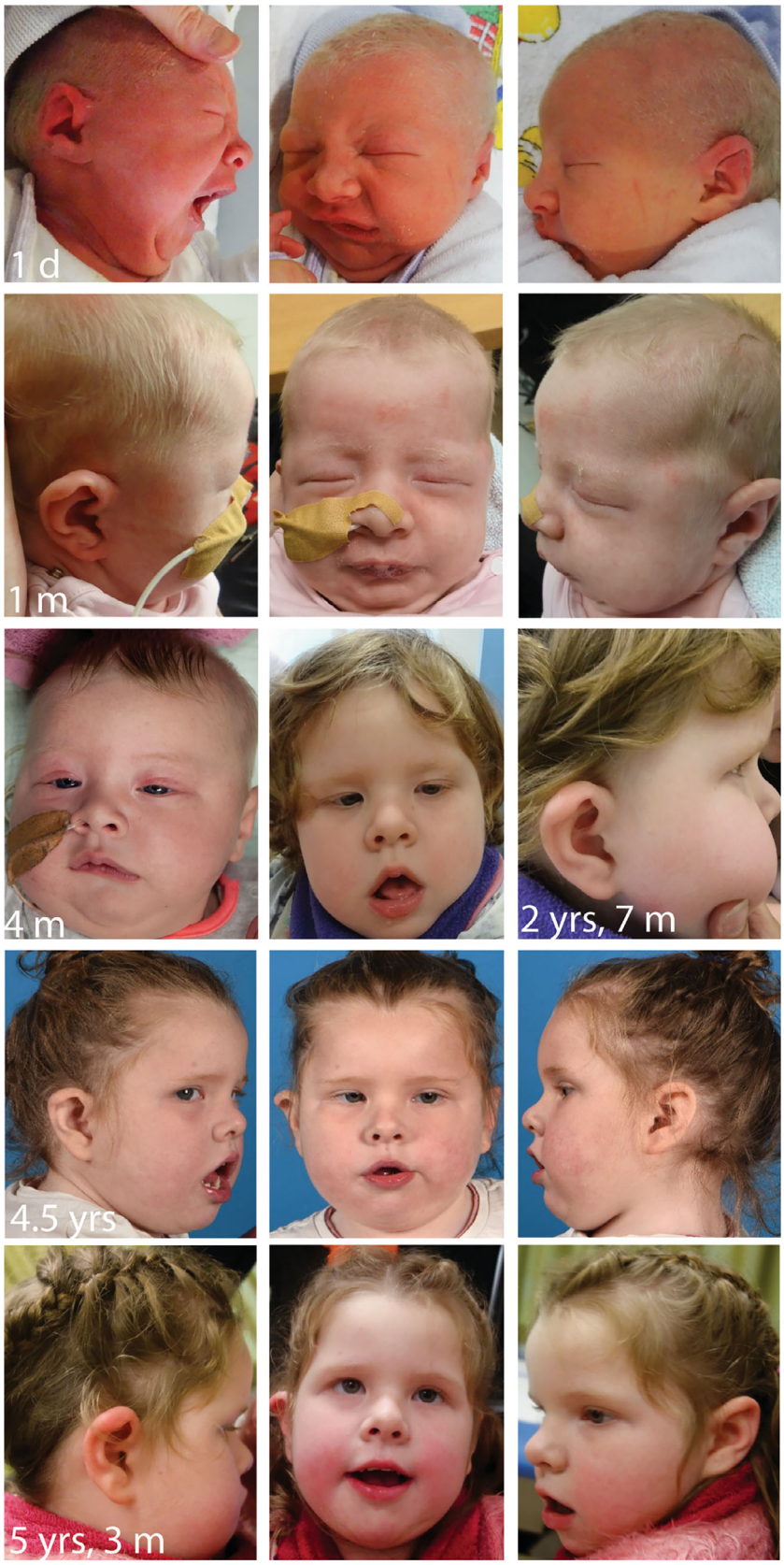

FIGURE 2 Photographs of the affected individual collected over a period of >5 years. Note the dysmorphologies becoming more pronounced over time, including prominent forehead, deep-set eyes, broad nasal bridge with hypoplastic alae nasae and anteverted nares, bilateral epicanthal folds, pronounced philtrum with a tented open mouth with thick upper and lower lips, downturned mouth corners, prominent protruding tongue, full cheeks, and cupped ears. [Colour figure can be viewed at wileyonlinelibrary.com]

communication. She was clinically diagnosed with severe cognitive impairment. At that time, her length was 99 cm (−2.12 SDS) and weight 16.2 kg (−0.71 SDS). Ophthalmological investigations were unremarkable. At 5 years and 1 months, a cluster of generalized tonic-clonic seizures occurred. Finally, at 5 years and 4 months, she was able to shortly stand and do a few steps.

Already after birth, dysmorphic features were noticed that became more pronounced upon aging (Figure 2). This included prominent forehead, deep-set eyes, broad nasal bridge with hypoplastic alae nasae and anteverted nares, epicanthal folds, prominent philtrum with a tented open mouth with thick upper and lower lips with downturned mouth corners, prominent protruding tongue, full cheeks and cupped ears.

Extensive diagnostic investigations during >5 years included an unremarkable noninvasive prenatal testing, quantitative fluorescent PCR for chromosomal aneuploidy, SNP-array, and Noonan gene panel during pregnancy. Postnatal trio WES analysis in 2019, focusing initially on genes related to multiple congenital anomalies followed by exome-wide investigations, did not identify any (likely) pathogenic variant. A newly generated trio exome in 2023 also remained negative. Additional investigations included a clinical RNA-seq on patient-derived fibroblasts[7] not pointing to a disease-relevant variant or gene expression change, analysis of mtDNA which was normal, and unremarkable metabolic investigations. FGF21 was increased once (7900 pg/mL, normally 0–200 pg/mL), during an infection. Finally, clinical trio WGS in 2024, of which the diagnostic analysis focuses on single nucleotide variants, indels, copy number variants, and structural variants that affect >5000 known disease genes was reported as normal. Upon further re-analysis in the department's Discovery Unit, a de novo NR_003137.2(*RNU4-2*):n.64_65insT variant affecting *RNU4-2* was identified (Figure 3). This variant has previously been reported as the most frequent likely pathogenic variant in *RNU4-2* in the recent preprint,[6] and is absent in 76 215 genome-sequenced individuals in gnomADv4.0. Interestingly, in line with the recent preprint, re-analysis of the generated fibroblasts RNA-seq did not show signs of large-scale intron retention (not shown). This is somewhat in contrast with the observed U12-type intron retention detected in *RNU4ATAC*-opathy.[8] However, possible existence of compensatory roles, or tissue-specific or more subtle effects, reasonable considering the much higher prevalence of U2-type introns compared to U12-type ones, might explain the so far unidentified downstream effect on splicing of the *RNU4-2* variants.[6]

3 | DISCUSSION

We present an in-depth clinical characterization obtained during >5 year follow-up of an individual affected by a recently described disorder caused by de novo variants in *RNU4-2*. The features of the affected individual largely overlap with what has been recently reported,[6] consisting of severe developmental delay, hypotonia, microcephaly, seizures, brain abnormalities including ventriculomegaly, mild cerebellar hypoplasia, and progressive white matter loss, feeding problems, recurrent infections, short stature, difficult to treat hip dysplasia with pathological fracture and dysmorphic features. We expand the currently still sparse literature on this new syndrome, providing further evidence for the facial gestalt, consisting of a prominent forehead, deep-set eyes, broad nasal bridge, hypoplastic alae nasae with anteverted nares, epicanthal folds, pronounced philtrum with tented open

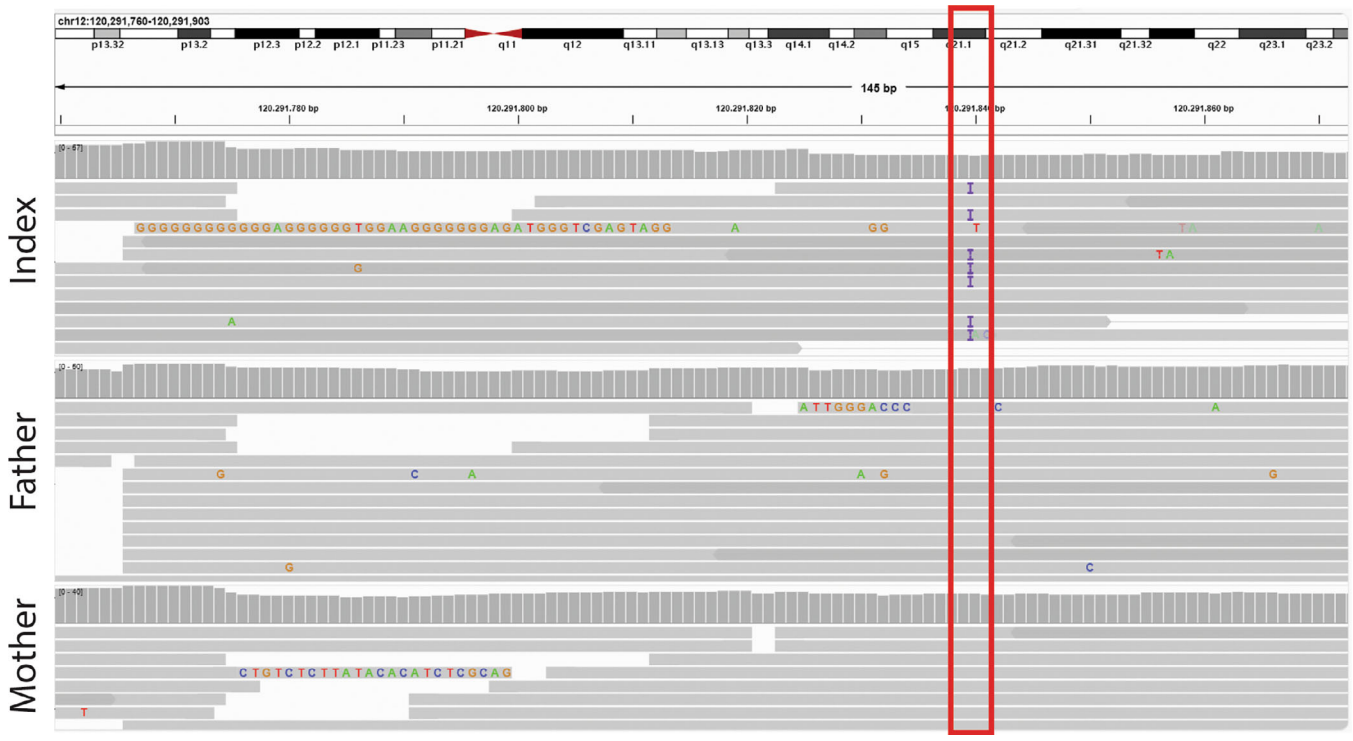

FIGURE 3 IGV browser view showing WGS tracks for the affected individual (index) and the unaffected parents, showing a de novo nucleotide insertion GRCh38/hg38:chr12:120291839T>TA, NR_003137.2(*RNU4-2*):n.64_65insT found in the index but absent in her parents (red box). [Colour figure can be viewed at wileyonlinelibrary.com]

mouth with downturned corners, thick upper and lower lips, protruding tongue, full cheeks, and cupped ears. Based on these dysmorphologies, we expect this to be a recognizable disorder that shares facial similarities with Pitt-Hopkins (OMIM #610954), ATRX (OMIM #301040), and Angelman syndrome (OMIM #105830). Access to additional photographs from individuals affected with this new disorder will allow to delineate the facial gestalt and to prioritize undiagnosed individuals based on shared dysmorphic features for targeted Sanger analysis of *RNU4-2* even if WGS is not available, which should be feasible given that this intron-less non-coding gene only encompasses 141 nucleotides (GRCh38/hg38, chr12:120,291,763-120,291,903). Given the predicted high-prevalence of this disorder that we confirm here mining a rather small set of 164 trio WGS identifying one case, we expect that many unexplained individuals with NDDs will be diagnosed shortly with this novel disorder ending their diagnostic odysseys.

4 | METHODS

All investigations occurred clinically. Using genome-wide technologies for diagnostic purposes was previously approved by the Erasmus MC institutional review board (MEC-2012-387). Informed consent was obtained for all diagnostics, and written informed consent was obtained from parents for publication of medical data including photographs, in line with the Declaration of Helsinki. Exome based diagnostic investigations occurred at Erasmus MC Clinical Genetics diagnostics, as described.[9] Clinical RNA-seq was performed as previously described.[7] Trio WGS occurred as previously described.[10] To find variants in *RNU4-2*, an aggregated VCF file was generated from 164 diagnostic trio WGS, and screened for variants in *RNU4-2*.

AUTHOR CONTRIBUTIONS

RS, FF, and GG performed genomic investigations. KD performed phenotyping. TSB conceived the study and wrote the manuscript with input of all authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14574.

DATA AVAILABILITY STATEMENT

All available clinical data are presented herein. All data generated or analyzed are included in this article, with the exception of raw genomic and RNA-sequencing data that due to privacy regulations and consent, cannot be publicly made available. Some images have been uploaded to the GestaltMatcher Database[11] (case 11 443 https://db.gestaltmatcher.org/id/11443, retrieved: 02.05.2024).

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