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A Rare Case of Osteosarcoma in an Individual with the Recurrent n.64_65insT Variant in the *RNU4-2* Gene

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Abstract

Background: Variants in the *RNU4-2* gene are one of the most common autosomal causes of Neurodevelopmental Disorders (NDD). Despite the increased recognition of their clinical impact, associated malignancies have not been previously reported. **Case Findings:** We present a male patient with the most common *RNU4-2* gene variant, n.64_65insT. At age 21, he was diagnosed with Osteosarcoma (OS), a condition not previously reported in affected individuals, and died 5 months later. **Clinical Implication:** This unique case expands the phenotype of *RNU4-2*-related neurodevelopmental disorder and highlights the importance of screening for cancer-related symptoms in patients with *RNU4-2* gene variants.

Keywords

RNU4-2 Gene, Neurodevelopmental Disorders, Osteosarcoma, Sarcomas, Cancer Screening, Spliceosome

1. Introduction

Two recent landmark papers describe variants in the U4 snRNA gene, *RNU4-2*, as a cause of one of the most prevalent single-gene NDD with an estimated prevalence of 0.40% of all these disorders [1] [2]. Both groups performed an analysis of genome data using participants from the 100,000 Genomes Project and discovered that variants in *RNU4-2*, which encodes U4 small nuclear RNA and is a critical component of the spliceosome, are one of the most common causes of devel-

opmental delay and intellectual disability. Some of the other major features included short stature, microcephaly, difficulties with speech, hypotonia, abnormal brain MRIs, seizures, vision issues, feeding difficulties, constipation, skeletal problems, and dysmorphic facial features [1] [2]. Since then, four other manuscripts have been published describing additional individuals and a distinctive dysmorphic facial pattern that has emerged from studying these individuals [3]-[6].

Osteosarcoma (OS) is the most common primary malignant bone tumor characterized by its aggressive nature. Its pathogenesis is multifactorial, involving both de novo mutations and established inherited predispositions [7]. The age-related incidence of OS is bimodal, with a primary peak during adolescence and young adults and a secondary, smaller peak in older adults. Globally, the incidence of OS is approximately 3.4 cases per million people per year. In the United States, the incidence of OS is approximately 1000 new cases diagnosed each year and accounts for approximately 0.049% of all new cancer diagnoses projected for 2025 [8].

2. Case Report

Two recent landmark papers describe variants in the U4 snRNA gene, *RNU4-2*, as a cause of one of the most prevalent single-gene NDD [1] [2]. Both groups performed an analysis of genome data using participants from the 100,000 Genomes Project and discovered that variants in *RNU4-2*, which encodes U4 small nuclear RNA and is a critical component of the spliceosome, are one of the most common causes of developmental delay and intellectual disability. Some of the other major features included short stature, microcephaly, difficulties with speech, hypotonia, abnormal brain MRIs, seizures, vision issues, feeding difficulties, constipation, skeletal problems, and dysmorphic facial features [1] [2]. Since then, four other manuscripts have been published describing additional individuals and a distinctive dysmorphic facial pattern that has emerged from studying these individuals [3]-[6].

Here we describe a patient from our center with the most common *RNU4-2* variant, n.64_65insT, who displayed many symptoms found in individuals previously described in the literature, but with a unique phenotype. At the age of 21, he was diagnosed with a sarcoma that had metastasized to his lungs, spine, and upper extremities, leading to his death 5 months later. We reached out to the corresponding authors of both landmark papers regarding cancer, and in the *Nature* paper they reported one individual with a possible low-grade cerebellar neoplasm who had the n.69C > T variant. To our knowledge, no other individuals with cancer have been reported in individuals with *RNU4-2* variants.

Our patient had an extensive, complicated medical history. He was born 5 weeks premature via spontaneous vaginal delivery, was jaundiced, hypotonic, and had breathing and feeding issues. His seizures began at three months and were described as "tonic stiffening, myoclonic jerks, upward eye deviation with decreased responsiveness". He was subsequently diagnosed with intractable focal epilepsy

with impaired awareness. His seizures were relatively controlled with carbamazepine and failed phenobarbital, diazepam, and gabapentin. An MRI at 9 years showed lateral and third ventricular enlargement with thinning of the corpus callosum.

He was always underweight, had short stature, and microcephaly (at twenty years old 25 kg, 133.2 cm, head circumference 48.6 cm (all < 1 percentile)). His history includes asthma, obstructive sleep apnea treated with CPAP, scoliosis, osteoporosis with a history of pathological fractures, congenital hip dysplasia, hypotonia, hypothyroidism, delayed puberty, gastroesophageal reflux status post Nissen fundoplication, dysphagia and gastrostomy-tube dependence, hypoglycemia requiring continuous feeding, constipation, bilateral cataracts and retinal detachment, blindness in the right eye, hiatal hernia, quadriplegic cerebral palsy, autism spectrum disorder, and sensory integration disorder. Dysmorphic features included short and upslanting palpebral fissures, large posteriorly rotated low-set ears, microphthalmia, and short fingers and toes with camptodactyly.

At 17 years old, a buccal swab showed respiratory chain complex IV abnormalities and increased mitochondrial content. However, the findings were not definitive for mitochondrial disease. He was started on the mitochondrial cocktail, though no improvement was seen. At age 18, he had a right femoral fracture in bed thought to be caused by a seizure. He underwent rod placement with complications of wound healing. He was later diagnosed with metastatic osteosarcoma at age 21 that started in his right femur and spread to his spine and lungs. Palliative radiation for one week was ineffective and stopped. At the time of his cancer diagnosis, he was 60 pounds and 4.5 feet tall. Surgery and additional radiation were thought to be detrimental to his overall health. Exome sequencing at 21 years of age was negative. Trio genome sequencing and reanalysis eight years later identified the heterozygous *de novo RNU4-2* variant, n.64_65insT. The variant is classified as pathogenic according to ACMG/AMP guidelines, fulfilling criteria PVS1, PS2, PM2, and PP4 [9].

3. Methodology

Trio base whole-genome sequencing was performed on extracted DNA using sequencing-by-synthesis (2 × 150 bp reads) next-generation sequencing at a minimum mean sequencing depth of 40×. These data were processed using TruSight Software Suite (TSS) v2.5 on the Illumina DRAGEN Bio-IT Platform v3.8.4 including read alignment to the GRCh37/hg19 genome assembly, variant calling, variant annotation by Nirvana v3.17, and comprehensive variant filtering.

4. Discussion

The maternal cancer lineage included breast, thyroid, stomach, oral, skin, and kidney cancers, along with brain and nasal tumors. No cancers were reported on the paternal side. The extensive maternal cancer history in this family raises the possibility of an unrecognized hereditary cancer syndrome independent of the pro-

band's *RNU4-2* variant. Genome sequencing in the proband showed no variants of interest in over 70 genes associated with sarcomas, including the following genes associated with hereditary osteosarcomas: *p53*, *RB1*, *RECQL4*, *RECQL2*, and *RECQL3*. Although variants in *RNU4-2* have not been directly associated with osteosarcoma, Kärkkäinen *et al.* found increased expression levels of the gene in invasive local breast cancer compared to benign breast tissue, and Li *et al.* found increased levels in colon cancer compared to normal tissue [10] [11]. Furthermore, alterations in genes encoding small nuclear RNAs (snRNAs) of the spliceosome have increasingly been implicated in human disease, including malignancy [12]. Suzuki *et al.* identified recurrent hotspot mutations in the U1 snRNA gene (*RNU1-1*) in patients with medulloblastoma [13]. Additionally, recent papers have associated osteoporosis in patients with *RNU4-2* and suggest a broad impact from the disruption of normal splicing, which potentially impacts cellular pathways involved in growth factor signaling and epigenetic regulation [14] [15]. Of significance, our patient had numerous pathological fractures secondary to his osteoporosis. Moreover, there are several NDD with well-defined genetic pathways known to be associated with an increased risk for cancer, such as the RASopathies, tuberous sclerosis complex, and *PTEN* hamartoma tumor syndrome.

No studies have revealed a similar connection to *RNU4-2* and its formation of the spliceosome. Our patient's osteosarcoma could be unrelated to his underlying genetic disorder. However, given the novelty of this *RUN4-2* variant, our patient's case—a rare aggressive cancer with a high mortality rate if undetected—and that many of the individuals with this variant were diagnosed in the first and second decades of life, longitudinal cancer or sarcoma surveillance may be relevant. Further research is warranted.

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Conflicts of Interest

Illumina provided funding for the study, and its employees contributed to the project.

Informed Consent

Study participants consented to a research study approved by the Western Institutional Review Board, now called WCG IRB (WIRB protocol #20120789).

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