A homozygous Y443C variant in the *RNPC3* is associated with severe syndromic congenital hypopituitarism and diffuse brain atrophy

Diğdem Bezen[1], Orkide Kutlu[2], Stephane Mouilleron[3], Karine Rizzoti[4], Mehul Dattani[5], Tulay Guran[6*], Gözde Yeşil[7*]

[1] Department of Pediatrics, Pediatric Endocrinology, University of Health Sciences, Prof. Dr. Cemil Taşçıoğlu City Hospital, 34384, Istanbul, Turkey. demboli4@yahoo.com

[2] Department of Internal Medicine, University of Health Sciences, Prof. Dr. Cemil Taşçıoğlu City Hospital, 34384, Istanbul, Turkey. orkidekutlu@windowslive.com

[3] Structural Biology Science Technology Platforms, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK. stephane.mouilleron@crick.ac.uk

[4] Stem Cell Biology and Developmental Genetics Lab, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK. karine.rizzoti@crick.ac.uk

[5] Genetics and Genomic Medicine Research and Teaching Department, UCL GOS Institute of Child Health, 30 Guildford Street, London WC1N 1EH. m.dattani@ucl.ac.uk

[6] Marmara University, School of Medicine, Department of Pediatric Endocrinology and Diabetes, 34722, Istanbul, Turkey. tulayguran@yahoo.com

[7] Department of Medical Genetics, Pediatric Genetics, Istanbul University, Istanbul Medical Faculty, 34452, Istanbul, Turkey. gozdeyesil@msn.com

*Denotes equal contribution

Acknowledgments: We are deeply grateful to the patient and family without whom this study could not have been performed.

Conflict of interest statement: The authors declare no conflict of interest.

Data availability statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethical statement: Written approval from Marmara University Ethics Committee was obtained prior to the short report (#01.04.2022.625).

Abstract: 200 words, **Table:** 1, **Figure:** 1, **Supporting information:** 1, **References:** 20

***Co-joint senior authors and co-corresponding authors to whom requests should be addressed:**

Professor Tulay Guran, MD
Marmara University School of Medicine,
Department of Pediatric Endocrinology and Diabetes
Fevzi Cakmak Mh. Mimar Sinan Cd. No 41. 34899
Ustkaynarca/Pendik
Istanbul, Turkey
Tel: +90 2166254545
Fax: +90 2164168855
E-mail: tulayguran@yahoo.com, tulay.guran@marmara.edu.tr

Assoc. Professor Gozde Yesil, MD
Department of Medical Genetics,
Pediatric Genetics, Istanbul University,
Istanbul Medical Faculty,
34452, Istanbul, Turkey
Tel: +90 212414 20 00
E-mail: gozdeyesil@msn.com

Abstract

Context: Biallelic *RNPC3* variants have been reported in a few patients with growth hormone deficiency, either in isolation or in association with central hypothyroidism, congenital cataract, neuropathy, developmental delay/intellectual disability, hypogonadism and pituitary hypoplasia.

Objective: To describe a new patient with syndromic congenital hypopituitarism and diffuse brain atrophy due to *RNPC3* mutations and to compare her clinical and molecular characteristics and pituitary functions with previously published patients.

Case Report: A twenty-year-old female presented with severe growth, neuromotor and developmental delay. Her weight, height and head circumference were 5135 gr (-25.81 SDS), 68 cm (-16.17 SDS), and 34 cm (-17.03 SDS), respectively. She was prepubertal, and had dysmorphic facies, contractures and spasticity in the extremities, and severe truncal hypotonia. There were no radiological signs of a skeletal dysplasia. The bone age was extremely delayed at 2 years. Investigation of pituitary function revealed growth hormone, prolactin, and thyroid-stimulating hormone deficiencies. Whole-exome sequencing revealed a novel homozygous missense (c.1328A>G; Y443C) variant in *RNPC3*. Cranial MRI revealed a hypoplastic anterior pituitary with diffuse cerebral and cerebellar atrophy.

Conclusion: The Y443C variant in *RNPC3* associated with syndromic congenital hypopituitarism and abnormal brain development. This report extends the *RNPC3*-related hypopituitarism phenotype with a severe neurodegenerative presentation.

Keywords: *RNPC3*, syndromic congenital hypopituitarism, brain atrophy, neuropathy, neurodegeneration

Introduction

The development of the pituitary gland is tightly regulated by various signaling molecules and transcription factors. Abnormal development of the pituitary gland may result in congenital hypopituitarism (CH) **(1, 2)**. Occasionally, CH may present as a component of a syndrome with extra-pituitary abnormalities. While a number of genetic mutations have been associated with phenotypes including congenital hypopituitarism, the underlying aetiology of CH remains elusive in the majority of patients **(3)**.

Pathogenic variants in components of the minor spliceosome have been associated with several diseases that include growth retardation. Pathogenic variants in *RNU4ATAC* are linked to microcephalic osteodysplastic primordial dwarfism type 1, Roifman syndrome and Lowry-Wood syndrome **(4-6)**. *RNPC3* codes for the U11/U12-65K protein, a component of the minor spliceosome. The minor spliceosome plays a role in the splicing of minor (U12-type) introns, which are present in ~700-800 genes in humans and represent about 0.35% of all introns **(7)**. Variants in *RNPC3* have previously been associated with isolated growth hormone deficiency **(8-10)**. Additionally, three siblings with novel biallelic *RNPC3* variants were recently reported to manifest panhypopituitarism **(11)**. More recently, biallelic mutations in *RNPC3* were reported in association with a phenotype including growth hormone deficiency, primary ovarian insufficiency and neuropathy **(12)**. The phenotype associated with biallelic *RNPC3* variants may therefore be variable.

Here we report a patient with a homozygous missense variant in *RNPC3*, presenting with panhypopituitarism with extreme growth failure and microcephaly due to diffuse cerebral and cerebellar atrophy. The clinical, biochemical, radiologic and molecular characteristics of the patient were investigated.

Case report

A twenty-year-old female presented with severe growth failure and developmental delay. She was born at term with a birth weight of 3300 g (0 SDS) to 2nd cousin parents. At initial examination her weight, height and head circumference were 5135 g (-25.81 SDS), 68 cm (-16.17 SDS), and 34 cm (-17. 03 SDS) respectively. She had the appearance of a prepubertal female. She had dysmorphic facies with extreme microcephaly, a small forehead, bitemporal narrowing, arched eyebrows, shallow orbits, long and straight eyelashes, big ears, maxillary hypoplasia, narrow palate, irregularly placed teeth, small and anteverted nose and microretrognathia. The elbow, wrist and knee joints were restricted and had a pes equinovarus deformity with a very small, rounded toe. The skin was soft, loose and transparent with visible veins, and was dry and fragile due to anhydrosis. Cutis marmaratus was also observed. Neurological examination revealed spasticity in all extremities, truncal hypotonia and lack of head control **(Figure 1A)**. There were no pathological reflexes. Despite extreme short stature there were no signs of a skeletal dysplasia on imaging **(Figure 1D-G)**. The bone age was extremely delayed at 2 years **(Figure 1H)**.

Endocrine evaluation of the patient revealed central hypothyroidism [sT4 0.25 ng/dL (0.7-1.66) and TSH 4.1 mU/L (0.51-4.8)]. Early morning ACTH [13.67 pg/mL (7.2-63.3)] and cortisol [11.62 mcg/dL (6-12)] concentrations were normal. The serum IGF1, IGFBP3 and PRL concentrations were found to be low [IGF1: 15.2 ng/mL (127-424), IGFBP3: 0.63 mg/L (2.9-7.2) and PRL: 0.43 ng/mL (4.7-23.2)]. L-dopa and clonidine growth hormone stimulation tests revealed a peak GH value of 0.03 ng/ml which reflects severe growth hormone deficiency. The basal LH value was <0.1 mIU/mL and the gonadotropin releasing hormone (GnRH) stimulation test showed hypogonadism **(Table 1)**. There was no history of polyuria and polydipsia and the urinary specific gravity was 1015. Pituitary MRI revealed a hypoplastic anterior pituitary gland with a height of 3.1 mm. The cranial MRI showed diffuse, symmetrical cerebral and cerebellar atrophy which is most significant in frontal and

temporal lobes. Decreased cortical sulci, decreased white matter volume, mega cisterna magna in posterior fossa and enlarged extra-axial cerebrospinal fluid (CSF) spaces secondary to diffuse atrophy were noted. The optic discs were atrophic and dysplastic bilaterally **(Figure 1B-C)**. She died unexpectedly at 21 years of age due to respiratory failure.

Materials and methods

Genetic studies

The Ethical Committee of Marmara University approved the study (#01.04.2022.625). Written informed consent for publication of the clinical details and clinical images of the patient was obtained from the mother.

DNA was extracted from peripheral blood of the patient and from the only living parent, her mother. Whole exome sequencing was performed. Amplified PCR samples were used for gene libraries by using the NEXTERA XT (ILLUMINA, USA) kit protocol and ran on the NextSeq500 platform. Variants were analyzed using Illumina Variant Studio software, Alamut Visual and HGMD Professional databases and pathogenicity prediction of novel variants were evaluated using Polyphen2, SIFT and MutationTaster databases **(13, 14)**. The mean depth of coverage was 50-fold coverage and an average of 93% of target bases sequenced at $\geq 20\times$ coverage.

Given the rarity of the phenotype and due to parental consanguinity, we first queried for homozygous variations which were absent in public databases like dbSNP, 1000 Genomes Project and ExAC. We then filtered for candidates that were computationally predicted to be damaging according to SIFT, Polyphen2 and mutationtaster databases. The *RNPC3* gene was the only candidate gene associated with the phenotype. Besides the homozygous variant in *RNPC3*, there were 6 more homozygous variations identified in WES (*SLC10A2*; p.Ala190Thr, *TCHH*; p.Gln506_Leu507insGluArgArgGluGlnGln, *KCNN3*;

p.Gln76_Gln80dup, *KIF1A*; p.Glu917del, *CLDN16*; p.Ala56LeufsTer16, *AR*; p.Gln58_Gln60del), all of which were predicted benign according to ACMG criteria or reported benign in ClinVar. We have also checked pathogenic variants in the previously described genes for growth/ pituitary hormone deficiency and central hypothyroidism (*HESX1*, *LHX3*, *LHX4*, *POU1F1*, *PROP1*, *BTK*, *GH1*, *GHRHR*, *GHSR*, *OTX2*, *SOX2*, *SOX3*, *PAX6*, *BMP4*, *FGFR1*, *ARNT2*, *GLI2*, *FGF8*, *PROKR2*, *GPR161*, *IGSF1*, *NFKB2*, *PITX2*, *CHD7*, *TSHB*, *TRHR*, *THR*, *GNAS*) **(2, 15)**. No disease associated mutations or variations were found in these genes. Furthermore, there were no disease associated variants in 200 genes known to be associated with peripheral neuropathy and neurodegenerative diseases.

We have compared the clinical characteristics and pituitary function test results of our patient with all other previously reported patients with *RNPC3* mutations **(Supp. Table 1)**.

Results

We detected a novel homozygous missense change in exon 12 of *RNPC3* (NM_017619.3:c.1328A>G; NP_060089.1:Y443C) by whole exome sequencing. Sanger sequencing confirmed homozygosity in the patient and heterozygosity in the mother of the given variant **(Figure 1J)**. The variant was also absent in our in-house control exome data set of more than 100 patient with genetic and neurometabolic disorders. The variation is a variant of unknown significance according to ACMG criteria. The variant has not been reported in the gnomAD database and is predicted to be pathogenic according to 9 prediction databases including BayesDel_addAF, DANN, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MutationTaster, PrimateAI and SIFT and predicted benign by 2 databases DEOGEN2 and MVP. The variant also occurs in a highly conserved residue, and is predicted to be pathogenic due to the high degree of conservation of the residue **(Figure 1K)**.

Y443 is a large aromatic residue with its side chain buried inside the protein domain. On one side the Y443 sidechain is engaged in hydrophobic interactions with the aliphatic part of R442 and K481, two solvent exposed residues, and it also forms a hydrogen bond with the side chain of E485, another solvent exposed residue **(Figure 1L-M)**. On the other side, the Y443 contributes largely to form the hydrophobic core of the protein together with L394, I422, V424, L436, I439, F440, I471, L473, A478, A482, A486 and M496. Mutation of Y443 by a much smaller cysteine residue would have a dramatic effect on the protein hydrophobic core which has a crucial function to maintain the normal structure and function of U11/U12 65K protein, and it would also affect the conformation of R442, K481 and E485 at the surface of the protein.

Discussion

The patient described in this report further establishes the association between syndromic congenital hypopituitarism and *RNPC3* mutations, which are very rarely reported in the literature. Coexistence of severe microcephaly and anatomic brain abnormalities in our patient suggest the role of *RNPC3* in pituitary and brain development.

The phenotypic spectrum associated with *RNPC3* mutations has been better understood with the description of new affected patients. So far twenty patients from 8 families have been reported (**8-12)**. Isolated growth hormone deficiency was described in the initial patients **(8, 9)**. Akin *et al*. reported growth hormone, as well as variable TSH and PRL deficiencies, in their patients, as did Verberne *et al* **(11, 12)**. Similarly severe deficiencies of TSH, PRL and growth hormone in our patient supports the role of *RNPC3* mutations in the aetiology of some syndromic forms of congenital hypopituitarism, possibly with variable interindividual phenotypic expression. Compared to previously published patients, our patient had the most severe growth failure phenotype. Nevertheless ACTH and vasopressin secretion of the patient were normal at 20 years of age, and this is consistent with previously published patients,

suggesting spared function of corticotrophs and vasopressin-secreting cell lineages in these patients. Gonadotropin function of previous patients was variable; some had ostensibly low gonadotrophins **(11)** whereas others had elevated FSH values **(8, 12)**. Akın *et al*. reported five female patients from the same family with ovarian dysgenesis due to homozygous *RNPC3* mutations; however, histological examination of sexually mature ovaries of *Rnpc3* knockout mice revealed no abnormalities **(12)**. FSH secretion increased to 21.5 mIU/mL by GnRH stimulation in our patient at 20 years of age; nevertheless her bone age was 2 years which makes the interpretation of these data challenging.

A number of other clinical characteristics were reported in patients with *RNPC3* mutations including red hair, obesity and myopathy **(9)**, congenital cataract and intellectual disability (**11**). This study, to our knowledge, is the first to describe diffuse cerebral and cerebellar atrophy associated with a hypoplastic anterior pituitary. In both mouse and human, *Rnpc3/RNPC3* was expressed in the telencephalon, diencephalon, trigeminal ganglia, hypothalamus and Rathke's pouch (**12)**. Various datasets also demonstrate the high expression of RNPC3 in cerebral cortex, cerebellum, basal ganglia, amygdala and hippocampus (https://www.proteinatlas.org/ENSG00000185946-RNPC3/tissue). Pathogenic variants in components of the minor spliceosome have been associated with several human diseases associated with microcephaly including Lowry Wood syndrome, microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1) or Taybi-Linder syndrome (TALS) **(4, 5, 16)**. This suggests that aberrant/abnormal splicing of genes containing U12-type introns may impair global cell proliferation in brain. However, previous patients with *RNPC3* mutations were also reported to have less severe microcephaly **(8)**, although Yamada et al, described a patient with severe microcephaly and growth retardation due to compound heterozygous *RNPC3* mutations **(10)**. Cranial imaging findings of our patient supports the role of *RNPC3* in global brain development. Even more importantly, minor spliceosome

inactivation is reported to cause microcephaly, owing to cell cycle defects and death of self-amplifying radial glial cells **(17)** or reported to be linked with some neurodegenerative diseases like amyotrophic lateral sclerosis **(18, 19)**, and early onset cerebellar ataxia **(20)**. Polyneuropathy has already been reported in the majority of the patients with *RNPC3* mutations (**12)**. Although we could not confirm the polyneuropathy by electromyography or nerve conduction studies; skin findings, anhydrosis and deformities, and spasticity in extremities in the absence of skeletal abnormalities suggest severe polyneuropathy in our patient. Together with global cerebral and cerebellar atrophy in this patient, we think that *RNPC3* mutations which impair the function of minor spliceosome complex can be related to degeneration and atrophy of neural structures and that potential manipulation of the minor spliceosome pathway can be an important treatment target for neurodegenerative disorders. Phenotypic variation in microcephaly and severity of neurodegeneration in previously reported patients can be explained by the type or the effect of the RNPC3 variation, though this needs further functional evidence, which we cannot provide in this report. In summary, our findings suggest that *RNPC3* is a cause of syndromic congenital hypopituitarism. The brain phenotype of minor spliceosome-related disease due to *RNPC3* mutation might be broader than previously described.

Financial Support: None

Disclosure summary: The authors have nothing to disclose.

Author contributions: DB, MD, TG and GY designed the study. DB, OK, TG and GY clinically characterized the patient. DB, OK and TG conducted and analyzed biochemical measurements. GY performed and analyzed the sequencing data. SM and KR analysed the characteristics of mutant protein in silico. DB, MD, TG and GY prepared the draft manuscript. All authors contributed to the discussion of results, and edited and approved the final manuscript.

References:

1. Gergics P. Pituitary Transcription Factor Mutations Leading to Hypopituitarism. In: Genetics of Endocrine Diseases and Syndromes. Springer; 2019:263-298.

2. Ara LB, Katugampola H, Dattani MT. Congenital hypopituitarism during the neonatal period: epidemiology, pathogenesis, therapeutic options, and outcome. Front Pediatr. 2021: 8:600962. doi:10.3389/fped.2020.600962.

3. Blum WF, Klammt J, Amselem S, et al. Screening a large pediatric cohort with GH deficiency for mutations in genes regulating pituitary development and GH secretion: Frequencies, phenotypes and growth outcomes. EBioMedicine. 2018;36:390-400. doi:10.1016/j.ebiom.2018.09.026.

4. Edery P, Marcaillou C, Sahbatou M, et al. (2011). Association of TALS developmental disorder with defect in minor splicing component U4atac snRNA. Science 2011;332: 240–243. doi.org/10.1126/science.1202205.

5. Farach LS, Little ME, Duker AL, et al. The expanding phenotype of *RNU4ATAC* pathogenic variants to Lowry Wood syndrome. American Journal of Medical Genetics.2018;176;465– 469. doi: 10.1002/ajmg.a.38581.

6. He H, Liyanarachchi S, Akagi K, et al. Mutations in U4atac snRNA, a component of the minor spliceosome, in the developmental disorder MOPD I. Science. 2011;332:238–240. doi.org/10.1126/science.1200587.

7. Turunen JJ, Niemela EH, Verma B, et al. The significant other: Splicing by the minor splicesome. Wiley Interdiscip Rev RNA. Jan-Feb 2013;4(1):61-76. doi:10.1002/wrna.1141.

8. Argente J, Flores R, Gutierrez-Arumi A,et al. . Defective minor spliceosome mRNA processing results in isol

ated familial growth hormone deficiency. EMBO Molecular Medicine. 2014;6:299–306. doi: 10.1002/emmm.201303573.

9. Gucev Z, Polenakovic M, Tasic V, et al. Severe isolated growth hormone deficiency and myopathy in two brothers with *RNPC3* mutation. European Society of Paediatric Endocrinology Abstracts. 2015;924-P3. doi: 10.3252/pso.eu.54espe.201.

10. Yamada M, Ono M, Ishii T, et al. Establishing intellectuel disability as the key feature of patients with biallelic *RNPC3* variants. Am J Med Genet.2021;185A:1836-40. doi: 10.1002/ajmg.a.62152.

11. Verberne EA, Faries S, Mannens MMA, et al. Expanding the phenotype of biallelic *RNPC3* variants associated with growth hormone deficiency. Am J Med Genet A. 2020;182(8):1952-56. doi: 10.1002/ajmg.a.61632.

12. Akin L, Rizzoti K, Gregory LC, et al. Pathogenic variants in RNPC3 are associated with hypopituitarism and primary ovarian insufficiency. Genet Med. 2022;24(2):384-397. doi:10.1016/j.gim.2021.09.019.

13. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7.20. doi:10.1002/0471142905.hg0720s76.

14. Vaser R, Adusumalli S, Leng SN, Sikic M, Ng PC. SIFT missense predictions for genomes. Nat Protoc. 2016;11(1):1-9. doi:10.1038/nprot.2015.123.

15. Bosch I Ara L, Katugampola H, Dattani MT. Congenital Hypopituitarism During the Neonatal Period: Epidemiology, Pathogenesis, Therapeutic Options, and Outcome. Front Pediatr. 2021;8:600962. Published 2021 Feb 2. doi:10.3389/fped.2020.600962.

16. Kilic E, Yigit G, Utine GE, et al. A novel mutation in RNU4ATAC in a patient with microcephalic osteodysplastic primordial dwarfism type I. Am J Med Genet A. 2015;167A(4):919-921. doi:10.1002/ajmg.a.36955.

17. Baumgartner M, Olthof AM, Aquino GS, et al. Minor spliceosome inactivation causes microcephaly, owing to cell cycle defects and death of self-amplifying radial glial cells. Development. 2018;145(17):dev166322. Published 2018 Aug 28. doi:10.1242/dev.166322.

18. Buratti E. The minor spliceosome could be the major key for FUS/TLS mutants in ALS. EMBO J. 2016;35(14):1486-1487. doi:10.15252/embj.201694763.

19. Onodera O, Ishihara T, Shiga A, Ariizumi Y, Yokoseki A, Nishizawa M. Minor splicing pathway is not minor any more: implications for the pathogenesis of motor neuron diseases. Neuropathology. 2014;34(1):99-107. doi:10.1111/neup.12070.

20. Elsaid MF, Chalhoub N, Ben-Omran T, et al. Mutation in noncoding RNA RNU12 causes early onset cerebellar ataxia. Ann Neurol. 2017;81(1):68-78. doi:10.1002/ana.24826.