Genetics in Medicine (2022) 24, 384–397

ELSEVIER

Genetics in Medicine

www.journals.elsevier.com/genetics-in-medicine

ARTICLE

Pathogenic variants in *RNPC3* are associated with hypopituitarism and primary ovarian insufficiency

Leyla Akin[1,2,*], Karine Rizzoti[3], Louise C. Gregory[4], Beatriz Corredor[5], Polona Le Quesne Stabej[6,7], Hywel Williams[8], Federica Buonocore[4], Stephane Mouilleron[9], Valeria Capra[10], Sinead M. McGlacken-Byrne[4], Gabriel Á. Martos-Moreno[5,11,12], Dimitar N. Azmanov[13,14], Mustafa Kendirci[2], Selim Kurtoglu[2], Jenifer P. Suntharalingham[4], Christophe Galichet[3], Stefano Gustincich[15], Velibor Tasic[16], John C. Achermann[4], Andrea Accogli[17,18], Aleksandra Filipovska[13,19], Anatoly Tuilpakov[20,21], Mohamad Maghnie[22,23], Zoran Gucev[16], Zeynep Burcin Gonen[24], Luis A. Pérez-Jurado[25,26], Iain Robinson[3], Robin Lovell-Badge[3], Jesús Argente[5,11,12,27], Mehul T. Dattani[4,26,28,**]

ARTICLE INFO

Article history:
Received 10 April 2021
Revised 15 July 2021
Accepted 27 September 2021
Available online 30 November 2021

Keywords:
Growth hormone deficiency
Hypopituitarism
Minor spliceosome
Primary ovarian insufficiency
U12-type spliceosome

ABSTRACT

Purpose: We aimed to investigate the molecular basis underlying a novel phenotype including hypopituitarism associated with primary ovarian insufficiency.

Methods: We used next-generation sequencing to identify variants in all pedigrees. Expression of *Rnpc3/RNPC3* was analyzed by in situ hybridization on murine/human embryonic sections. CRISPR/Cas9 was used to generate mice carrying the p.Leu483Phe pathogenic variant in the conserved murine *Rnpc3* RRM2 domain.

Results: We described 15 patients from 9 pedigrees with biallelic pathogenic variants in *RNPC3,* encoding a specific protein component of the minor spliceosome, which is associated with a hypopituitary phenotype, including severe growth hormone (GH) deficiency, hypoprolactinemia, variable thyrotropin (also known as thyroid-stimulating hormone) deficiency, and anterior pituitary hypoplasia. Primary ovarian insufficiency was diagnosed in 8 of 9 affected females, whereas males had normal gonadal function. In addition, 2 affected males displayed normal growth when off GH treatment despite severe biochemical GH deficiency. In both mouse and human embryos, *Rnpc3/RNPC3* was expressed in the developing forebrain, including the hypothalamus and Rathke's pouch. Female *Rnpc3* mutant mice displayed a reduction in pituitary GH content but with no reproductive impairment in young mice. Male mice exhibited no obvious phenotype.

Conclusion: Our findings suggest novel insights into the role of RNPC3 in female-specific gonadal function and emphasize a critical role for the minor spliceosome in pituitary and ovarian development and function.

© 2021 by American College of Medical Genetics and Genomics. Published by Elsevier Inc.

[*]Correspondence and requests for materials should be addressed to Leyla Akin, Department of Paediatric Endocrinology, Faculty of Medicine, Ondokuz Mayis University, 55200 Atakum, Samsun, Turkey. *E-mail address:* leylabakin@gmail.com

[**]Correspondence and requests for materials should be addressed to Mehul Dattani, Genetics and Genomic Medicine Research and Teaching Department, UCL Great Ormond Street Institute of Child Health, University College London, London WC1N 1EH, United Kingdom. *E-mail address:* m.dattani@ucl.ac.uk

Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gim.2021.09.019

1098-3600/© 2021 by American College of Medical Genetics and Genomics. Published by Elsevier Inc.

Introduction

Primary ovarian insufficiency (POI), characterized by amenorrhea with elevated gonadotropin concentrations, includes a spectrum ranging from 46,XX gonadal dysgenesis to premature menopause. Nonsyndromic POI occurs in 1% of women; however, early-onset forms manifesting as primary amenorrhea with the absence of pubertal development affect approximately 1 in 100,000 females.[1] To date, more than 50 genes have been associated with POI.[2] Overall, known genetic causes, including X chromosomal abnormalities, account for 20% to 25% of cases.[3-11]

Pre–messenger RNA splicing is an essential step in gene expression in all eukaryotes. Two distinct splicing machineries, the major (U2-dependent) and minor (U12-dependent) spliceosomes, recognize and excise either the major (U2-type) or minor (U12-type) class of introns. Minor introns constitute approximately 0.35% of all human introns and are present in approximately 700 to 800 genes.[12] *RNPC3* encodes the U11/U12-65K protein, a component of the U12-dependent spliceosome.[13]

In this study, we describe 15 patients from 9 pedigrees harboring variants in *RNPC3* with a hypopituitary phenotype, including severe growth hormone (GH) deficiency (GHD, previously described in Argente et al[14]), hypoprolactinemia, variable thyrotropin (also known as thyroid-stimulating hormone [TSH]) deficiency, and anterior pituitary hypoplasia (APH). POI was diagnosed in 8 of 9 affected females, whereas all males had normal gonadal function. We performed genetic and functional studies to elucidate the molecular basis underlying this novel phenotype.

Materials and Methods

Participants

Five first-degree consanguineous pedigrees, living in geographically close areas in Turkey, included 8 (5 females, 3 males) Turkish patients (T1-T8). Pedigree 6 included 3 previously described[14,15] Romanian-origin Spanish sisters (S1-S3). Pedigree 7 included 2 Turkish-origin Macedonian brothers (M1 and M2). Pedigree 8 contained a Russian patient R1, and pedigree 9 contained an Albanian-origin Italian patient I1. Pedigrees 6 to 9 were not consanguineous (Figure 1A).

Genetic studies

Next-generation sequencing (NGS) was performed in all pedigrees, followed by Sanger sequencing to confirm NGS-discovered variants and segregation of the identified variants in unaffected family members. Primer sequences are shown in Supplemental Table 1.

In situ hybridization studies

In situ hybridization (ISH) studies were performed in human and mouse embryos and adult mice to analyze *RNPC3*/*Rnpc3* expression in the hypothalamo-pituitary (HP) region and ovaries.

Mouse models

Using CRISPR/Cas9 technology, we generated mice carrying the p.Leu483Phe variant in the conserved RRM2 domain of murine *Rnpc3*. We also generated mice harboring the p.Pro350Arg variant in *Prmt6* (Figure 2, Supplemental Figure 1).

The methodology of the genetic, ISH, and mouse studies is detailed in the Supplemental Methods.

Results

Clinical assessment

Growth patterns

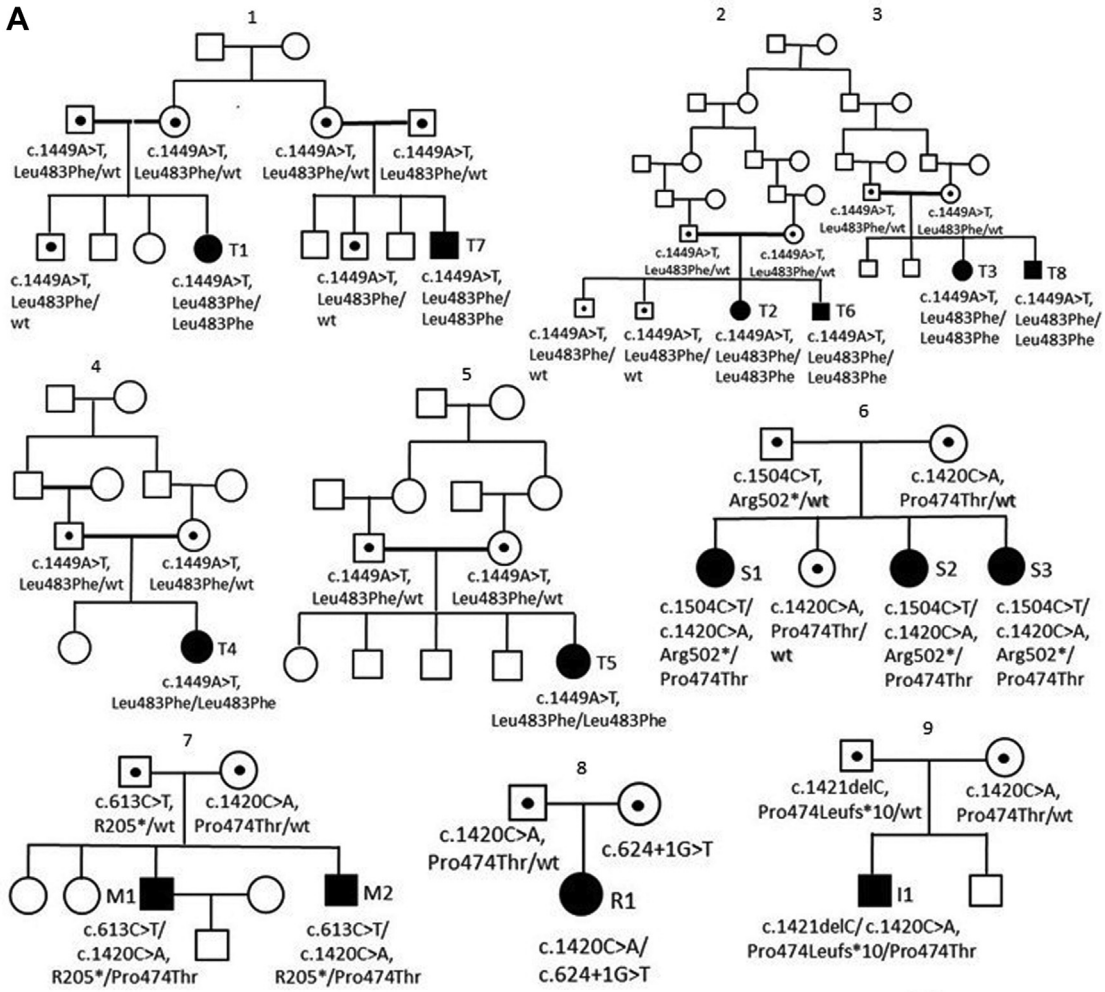
All patients were born with a normal birthweight but showed severe postnatal growth retardation (heights = –4.1 to –8.9 SD score [SDS] at presentation) with typical features of GHD (Table 1). Although IGF-1 and peak GH during provocation tests were undetectable at age 9.7 years, patient T6 showed growth without GH until age 15 years when the growth velocity decreased to 4 cm/y and recombinant human GH (rhGH) therapy was started. He did not achieve his target adult height (Supplemental Figure 2A).

Patients M1 and M2 (pedigree 7) presented with severe GHD at ages 28 and 20 months, respectively. Rapid growth ensued in the first 2 years on treatment, with heights in the 30th and 40th percentiles on the growth curve. Subsequently, growth continued without GH treatment during puberty. After cessation of rhGH therapy (age 9 years), the eldest boy (M1) had a 36 cm pubertal growth spurt, achieving a final height exceeding target height range. The younger brother (M2) achieved a pubertal growth spurt of around 30 cm after GH therapy discontinuation at 5.5 years, but his final height was at the lower end of the target height range (Supplemental Figure 2A). Retesting GH pituitary reserve showed low peak GH concentrations (<0.3 ng/mL).

Growth responses to rhGH therapy were excellent in all patients (Table 1).

Anterior pituitary function

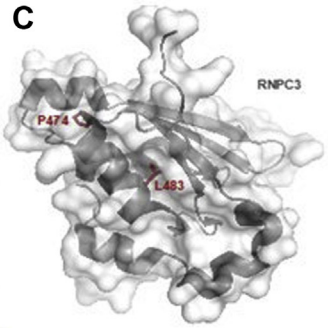
In all patients, basal IGF-I and IGFBP-3 concentrations were almost undetectable as were peak GH concentrations at provocation. Basal serum cortisol and adrenocorticotropic hormone concentrations were normal. In pedigrees 1 to 5, there was mild thyroid dysfunction (low serum free



B

	Variants Maternal allele (NM_017619.4)			Variants Paternal allele (NM_017619.4)		
Pedigree	Nucleotide change	Aminoacid change	gnomAD (v2.1.1) Allele frequency	Nucleotide change	Aminoacid change	gnomAD (v2.1.1) Allele frequency
1-5	c.1449A>T	p.Leu483Phe	not present	c.1449A>T	p.Leu483Phe	not present
6	c.1420C>A	p.Pro474Thr	0.00003185	c.1504C>T	p.Arg502*	0.00002024
7	c.1420C>A	p.Pro474Thr	0.00003185	c.613C>T	p.Arg205*	0.000005780
8	c.624+1G>T		not present	c.1420C>A	p.Pro474Thr	0.00003185
9	c.1420C>A	p.Pro474Thr	0.00003185	c.1421del	p.Pro474Leufs*10	0.000004017

C



D



Figure 1 A. Pedigrees 1 to 9 harboring the *RNPC3* variants. All affected patients are homozygous in pedigrees 1 to 5 and compound heterozygotes in pedigrees 6 to 9 for the *RNPC3* variants shown under each symbol, whereas the unaffected parents are heterozygotes for the relevant variants. Shaded squares and circles indicate affected family members: squares for males and circles for females. Heterozygote members are indicated with a dot in each symbol. Shapes joined by thick lines indicate consanguinity between those individuals. Pedigree numbers are given at the top of each pedigree, and each affected subject is marked with the relevant patient IDs relating to the text and tables. B. The pathogenic variants found in *RNPC3* in pedigrees 1 to 9. C. The structure of the C-terminal RNA recognition motif of the U11/U12

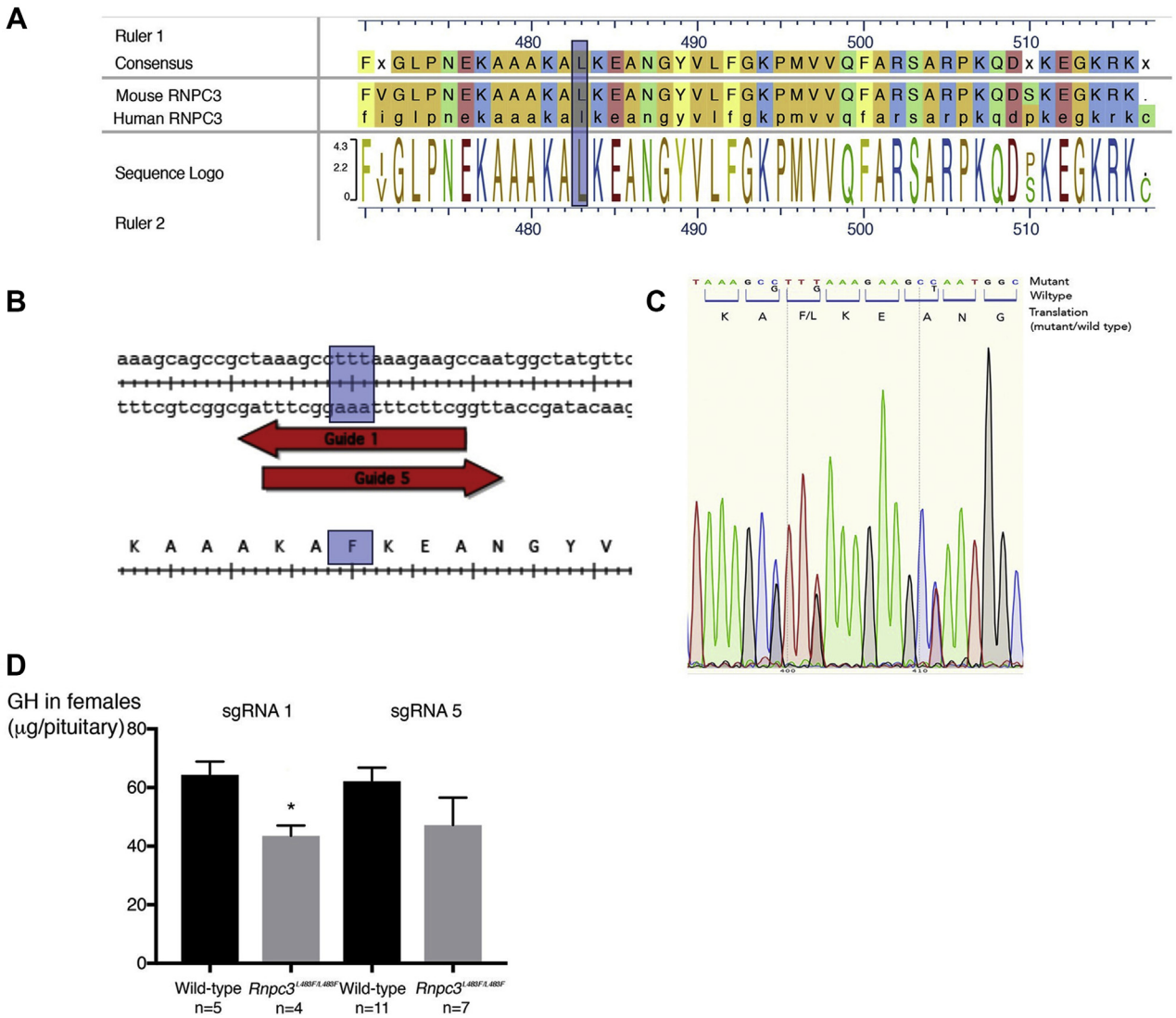


Figure 2 **Murine data including the methods and the results.** A. Alignment of the mouse and human RNPC3 protein sequences. The leucine residue at position 483 that is the position of the pathogenic variant in patients and the surrounding region are conserved between mouse and human. B. Two sgRNA were designed in which the leucine codon (ttg) was changed to a phenylalanine codon (ttt). C. Chromatogram of a heterozygous *Rnpc3* mutant mouse. Sanger sequencing showing changed nucleotides giving rise to the targeted variation (L to F) and introduction of 2 synonymous variants to avoid subsequent cutting by the sgRNA. D. Radioimmunoassays performed on female pituitaries comparing homozygous mutants and wild-type littermate controls. A decrease in GH levels was observed in both sgRNA1 and 5 strains, but it was only statistically significant in sgRNA1 (SEM = 64.28 ± 4.6 in controls vs SEM = 43.4 ± 3.5 in mutants; $P = .016$). GH, growth hormone; sgRNA, single-guide RNA.

thyroxine with a normal TSH or normal free thyroxine with a slightly elevated TSH) in all patients, and levothyroxine replacement was required in 2 of them. In patient I1, central hypothyroidism was detected at age 14.5 years and required levothyroxine replacement. Prolactin (PRL) concentrations were low in most patients (Table 1).

Brain magnetic resonance imaging

Brain magnetic resonance imaging revealed APH in 13 of 15 patients (Table 1). The posterior pituitary was eutopic in all patients. Patient I1 had an irregular sellar floor, mild hypoplasia of the splenium of corpus callosum, and mild cerebellar tonsil herniation on magnetic resonance imaging.

65K protein (PDB-ID: 3EGN) displayed in dark gray cartoon and transparent surface. Residues P474 and L483, discussed in the text, are displayed in red sticks. D. The conservation of substitutions *RNPC3* (p.Leu483Phe), (p.Pro474Thr), (p.Arg502*), (p.Arg205*), and (p.Pro474Leufs*10) across multiple species. AT, Arabidopsis thaliana; BTA, Bos taurus; CAN, Canis lupus; DR, Danio rerio; GG, Gallus gallus; gnomAD, Genome Aggregation Database; HSA, Homo sapiens; MM, Mus musculus; MMU, Macaca mulatta; PTR, Pan troglodytes; RN, Rattus norvegicus; wt, wild type.

Table 1 Auxological and hormonal parameters and anterior pituitary height on MRI of the patients at diagnosis and the growth response after rhGH treatment

Patient ID	Sex	Age (y)	Bone age (y)	Height (SDS)	Weight (SDS)	BMI (SDS)	Growth Velocity (cm/y)	Growth Velocity (SDS)	GH (ng/mL) Peak After Clonidine or Arginine	IGF-1 (ng/mL)	IGFBP-3 (mg/L)	Prolactin (ng/mL)	Cortisol (μg/dL)	TSH (U/L)	Free T4 (ng/dL) (Normal Range)	Anterior Pituitary Height on MRI (*n* for age)	First Year Growth After rhGH (cm/year)	Final Height (SDS)	Mid-Parental Height (SDS)	Height SDS Change[a]
T1	F	8.2	2.6	−8.9	−6.1	0.3	2.5	−2.6	<0.01	0.01	1.7	1.8	14.1	2	0.7 (0.7-1.7)	2.5 (4.4 ± 0.9)	6	−2.7	−1.6	6.8
T2	F	5.1	2	−8.7	−7.3	0.0	2.4	−3.1	0.01	0.01	<0.5	1.7	12.3	3	0.8 (0.8-2.0)	1.5 (4.9 ± 0.9)	17.5	−2.2	−1.5	6.7
T3	F	3	1	−6.5	−4.7	0.1	4.0	−2.3	<0.01	0.01	<0.5	1.2	10.8	7.5[b]	0.8 (0.8-2.0)	4 (4.2 ± 0.6)	17	GC	−2.1	5.1
T4	F	2.2	0.7	−4.4	−1.5	2.0	4.5	−2.5	0.40	<10	6.8	9.1	13.3	10.6[b]	1.0 (0.8-2.0)	NA	15.1	GC	0.9	3.7
T5	F	5.5	2	−6.5	−4.5	0.3	4.0	−2.1	0.12	<25	<0.5	3.2	16.1	2	0.9 (0.6-1.1)	3 (4.2 ± 1.0)	12.6	NA	−1.6	NA
T6	M	9.7	5	−7.2	−3.5	1.3	4.4	−0.9	<0.01	2.4	NA	1.1	14.0	3.1	0.7 (0.8-1.7)	2 (4.2 ± 1.2)	7.5	−4.9	−1.4	1.6
T7	M	6.6	2	−8.5	−8.8	−0.5	1	−4.2	0.01	<0.01	NA	NA	15.2	6.7[b]	1.0 (0.6-1.1)	3 (5.0 ± 0.7)	11.5	−1.8	−1.4	7.5
T8	M	1.1	0.6	−4.2	−2.0	1.1	6.7	−1.6	0.06	15.0	<0.5	2.8	17.4	1.7	0.8 (0.9-1.7)	2.5 (4.1 ± 0.7)	14.1	GC	−2.0	1.1
S1	F	14.0	10	−9.6	−2.6	2.8	1.7	−2.1	<0.5	36	0.8	0.7	20.4	4.2	0.9 (0.6-1.4)	3.5 (6.1 ± 1.0)	12.8	−1.8	−0.9	4.0
S2	F	7.6	4.5	−5.0	−1.8	0.1	1.5	−3.5	<0.5	18	<0.5	2.1	14.2	4.5	0.9 (0.6-1.4)	2.5 (4.6 ± 1.3)	14.2	0.9	−0.9	4.0
S3	F	5.5	2	−6.7	−3.5	−1.5	1.7	−4.0	<0.5	21	<0.5	1.8	20.0	3.2	0.9 (0.6-1.4)	2.5 (3.8 ± 1.0)	14.6	GC	−0.9	5.0
M1	M	2.3	0.5	−5.9	−3.1	1.1	2.5	−2.6	<0.3	NA	NA	NA	12.5	3.2	12.0[c] (4.5-12.5)	3.5 (4.2 ± 0.6)	22	0.0	−1.7	5.9
M2	M	1.8	0.8	−5.0	−1.9	1.3	3.0	−2.5	<0.3	NA	NA	NA	13.0	4.2	11.0[c] (4.5-12.5)	2.5 (4.1 ± 0.6)	13	−2.8	−1.7	2.2
R1	F	3	1.7	−4.1	−2.3	1.3	4.8	−1.8	0.6	10.9	—	18.3	22.9	1.8	1.3 (1.1-2.0)	3.0 (3.4 ± 1.0)	13.3	GC	−0.1	
I1	M	3.2	1.5	−6.5	−3.7	2.0	—	—	<0.05	14	—	<0.5	13.4	0.9	0.6 (0.8-1.9)	4 (5.0 ± 0.7)	15.7	−1.1	−0.8	4.5

Conversion factors from conventional units to SI units:

GH (ng/mL) to μg/L, multiply by 1.

IGF-1 (ng/mL) to nmol/L, multiply by 0.131.

Prolactin (ng/mL) to μg/L, multiply by 1.

Free T4 (ng/dL) to pmol/L, multiply by 12.871.

Total T4 (μg/dL) to nmol/L, multiply by 12.871.

Cortisol (μg/dL) to nmol/L, multiply by 27.588.

F, female; *GC*, growth continues; *GH,* growth hormone; *M*, male; *MRI*, magnetic resonance imaging; *NA*, not available; *rhGH*, recombinant human growth hormone; *T4*, thyroxine; *TSH*, thyroid-stimulating hormone; *SDS*, SD, score.

[a]Height SDS change from the onset of rhGH to the latest evaluation.

[b]Values above the upper limit of TSH.

[c]Total T4 (μg/dL). Prolactin normal range, 3.5 to 25 ng/mL. Cortisol normal range, 5 to 25 μg/dL.

Table 2 Pubertal status, serum gonadotropin concentrations and ultrasonographic findings of uterus and ovaries at the diagnosis and on follow-up in the female patients

Patient ID	T1			T2			T3			T4			T5	S1			S2		S3			R1
Age (y)	8	14	21	5.1	13	17.5	3.1	7.1	11.5	3.8	7.4	10.9	5.9	15.7	18.5	19.5	15.0	16	12.9	13.4	13.9	3
Tanner stage	1	2	5	1	2	5	1	1	2	1	1	1	1	2	5	5	4	5	1	3	4	1
FSH (U/L)	24.8	86.6	35.9	7.8	38.3	7.5	20.9	8.7	30.1	55.2	12.6	39.8	16.3	30.3	29.2	2.7	52.8	17.6	11.1	18.1	19.1	37.4
LH (U/L)	0.7	32.1	43.8	0.1	28.7	6.3	0.9	0.07	5.9	0.6	0.0	2.1	0.1	6.7	12.9	1.9	20.2	11.4	0.3	5.8	3.3	1.7
E2 (pg/mL)	<5	15	NA	<5	<5	34	NA	NA	<5	NA	<5	<5	<5	7.4	12	10.2	11	68	7.5	32	35	
(USG)																						
Uterus volume (mL)	0.2	0.6	5	0.5	4	4	0.3	0.3	0.4	0.3	0.3	0.3	0.2	1.4	5.9	19.3	17	39.2	NA	NA	29.2	0.4
Ovary-right																						
Volume (mL)	NV	0.06	2.2	NV	NV	NV	NV	NV	0.3	NV	NV	0.2	NV	1.7	0.5	0.5	3.3	1.6	NA	NA	2.5	NV
Follicle number	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	NA	NA	4	0
Follicle size (max mm)	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	4.5	2	NA	NA	3	-
Ovary-left																						
Volume (mL)	NV	0.07	1.9	NV	NV	NV	NV	NV	0.3	NV	NV	0.3	NV	NV	0.4	0.4	NV	1.9	NA	NA	2.3	NV
Follicle number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	NA	NA	3	0
Follicle size (max mm)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	NA	NA	2	-
E2 replacement	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
Age of spontaneous thelarche (y)		14			11.9			11.8			-		NA		15.7			11.6		13		-
Age at initiation of E2 therapy (y)		17			16.5			12.5			12.5		NA		19.2			15.0		-		-

For conversion of E2 (pg/mL) measurements to SI unit pmol/L, multiply by 3.671.

E, Estradiol; *FSH*, follicular stimulating hormone; *ID*, Identification; *LH*, luteinizing hormone; *NA*, not available; *NV*, not visible; *R*, Russion; *S*, Spanish; *T*, Turkish; *USG*, ultrasonography.

Ovarian/testicular phenotype

Pedigree 1 to 5

The proband in pedigree 1 (the eldest girl in the cohort) had elevated serum follicular stimulating hormone (FSH) levels and a normal luteinizing hormone (LH) concentration (24.8 and 0.7 IU/L, respectively) on presentation at age 8 years. Spontaneous puberty ensued at age 14 years, with FSH and LH concentrations of 86.6 IU/L and 32.1 IU/L, respectively. Breast development progressed slowly and arrested at Tanner stage 3 with undetectable serum estradiol concentrations. The other 4 female Turkish patients also had increased gonadotropin concentrations for their age; the levels were relatively decreased during the ages 3 to 6 years and then progressively increased thereafter. Pelvic ultrasonography showed a small uterus with undetectable or small ovaries and no follicles in all 5 girls (age range at investigations: 3 to 21 years) (Table 2). Pubarche started at a normal age, but the hair was sparse in all female patients. Menarche did not occur in any of the girls. All female patients had a 46,XX karyotype with no stigmata of Turner syndrome. Estrogen replacement therapy resulted in normal breast development; menarche with regular menstruation was achieved by cyclic estrogen/progestagen therapy.

In pedigree 6, patient S1 was prepubertal at age 15.4 years at the time of GHD diagnosis, with baseline FSH and LH concentrations of 30.3 IU/L and 6.7 IU/L, respectively, that peaked after gonadotropin-releasing hormone (GnRH) stimulation at 40.1 and 35.5 IU/L, respectively, with a concomitant low serum estradiol concentration (27.1 pmol/L [7.4 pg/mL; normal range, 10-400]). Three months after rhGH treatment commencement, puberty spontaneously developed, progressing to Tanner stage 4 with a spontaneous 4-day menarche after 16 months on therapy. No further menstrual cycles occurred and low serum estradiol concentrations and normal to high FSH and LH concentrations both at baseline and post-GnRH stimulation persisted, thus necessitating hormone replacement. Patient S2 spontaneously commenced puberty at 11.6 years, progressing to Tanner stage 4 at age 13 years, but remained amenorrhoeic up to her last evaluation (age 15.7 years). The patient had a baseline estradiol of 35.9 pmol/L (9.8 pg/mL). Baseline FSH and LH concentrations were 44.2 IU/L and 12 IU/L that peaked post-GnRH stimulation at 63.1 and 49.5 IU/L, respectively, and the patient also required estrogen replacement. Patient S3 commenced spontaneous puberty at age 13 years, progressing to Tanner stage 4 over a 12-month period, with spontaneous menarche at age 13.9 years and later regular menstrual cycles with mildly elevated FSH and LH concentrations up to her last evaluation at age 14.1 years. Normal sized/small ovaries but with sparse or absent follicles were found in all 3 siblings by ultrasonography. Russian patient R1 also had an elevated FSH concentration despite normal LH concentration at age 3 years. Pelvic ultrasonography revealed a small uterus with no visible ovaries (Table 2).

Five male patients in this cohort developed normal puberty that progressed to Tanner stage 5 with normal gonadotropin and testosterone concentrations. The youngest male (aged 2 years) also had a normal mini-puberty (Supplemental Table 2). Patient M1 fathered a healthy child at 30 years of age.

The details of clinical and laboratory data are given in Tables 1 and 2 and Supplemental Table 2 and in the Supplemental Results.

Additional findings/neuropathy

In pedigrees 1 to 5, sitting height and upper-lower segment ratios were slightly increased (+2.2 to +2.6 SDS) and physical examinations were otherwise normal except for a slightly high arched palate in all patients. On follow-up, patient T7 developed lower leg pain and bilateral pes cavus at age 22 years. He was diagnosed with polyneuropathy on the basis of nerve conduction studies with an elevated serum creatine phosphokinase (CPK) concentration (475 U/L; normal range, 35-195).

In pedigree 6, patient S2 presented with reduced deep tendon reflexes and elevated CPK concentrations and was diagnosed with mixed motor neuropathy at age 16 years. Electromyogram revealed moderately severe symmetric demyelination in both the upper and lower limbs.

In pedigree 7, at age 5 years, patient M1 became ataxic with frequent falls; increased CPK concentrations led to a diagnosis of myopathy. Two muscle biopsies were inconclusive, with degenerative nervus suralis changes on an electromyogram.

In pedigree 9, patient I1 manifested minor dysmorphisms, including prominent forehead, deep set eyes, prognathism, dental malocclusion, thin upper lip, and clinodactyly. He had mild global developmental delay that improved with time. During early childhood, he experienced a few generalized motor seizures that were responsive to valproate. He also had a steppage gait and reduced deep tendon reflexes with mildly increased CPK, suggesting an underlying neuropathy. Nerve conduction studies showed electrophysiological findings consistent with a length-dependent axonal neuropathy that was more significant in the lower limbs. An NGS panel that included more than 150 genes related to various neuropathies revealed no pathogenic variants.

Molecular assessment

Variants identified in *RNPC3* in the patients are shown in Figure 1B. The localization of the mutated residues within the hydrophobic core of the C-terminal RNA recognition domain of RNPC3 suggests that pathogenic variants could impair protein function and/or structure (Figure 1C).

In pedigree 1 to 5, in 6 patients (5 females, 1 male), NGS identified a novel homozygous missense variant c.1449A>T, p.Leu483Phe in *RNPC3*. This variant was not reported in any public database, including 1000 Genomes, dbSNP, Exome Variant Server, or the Genome Aggregation Database

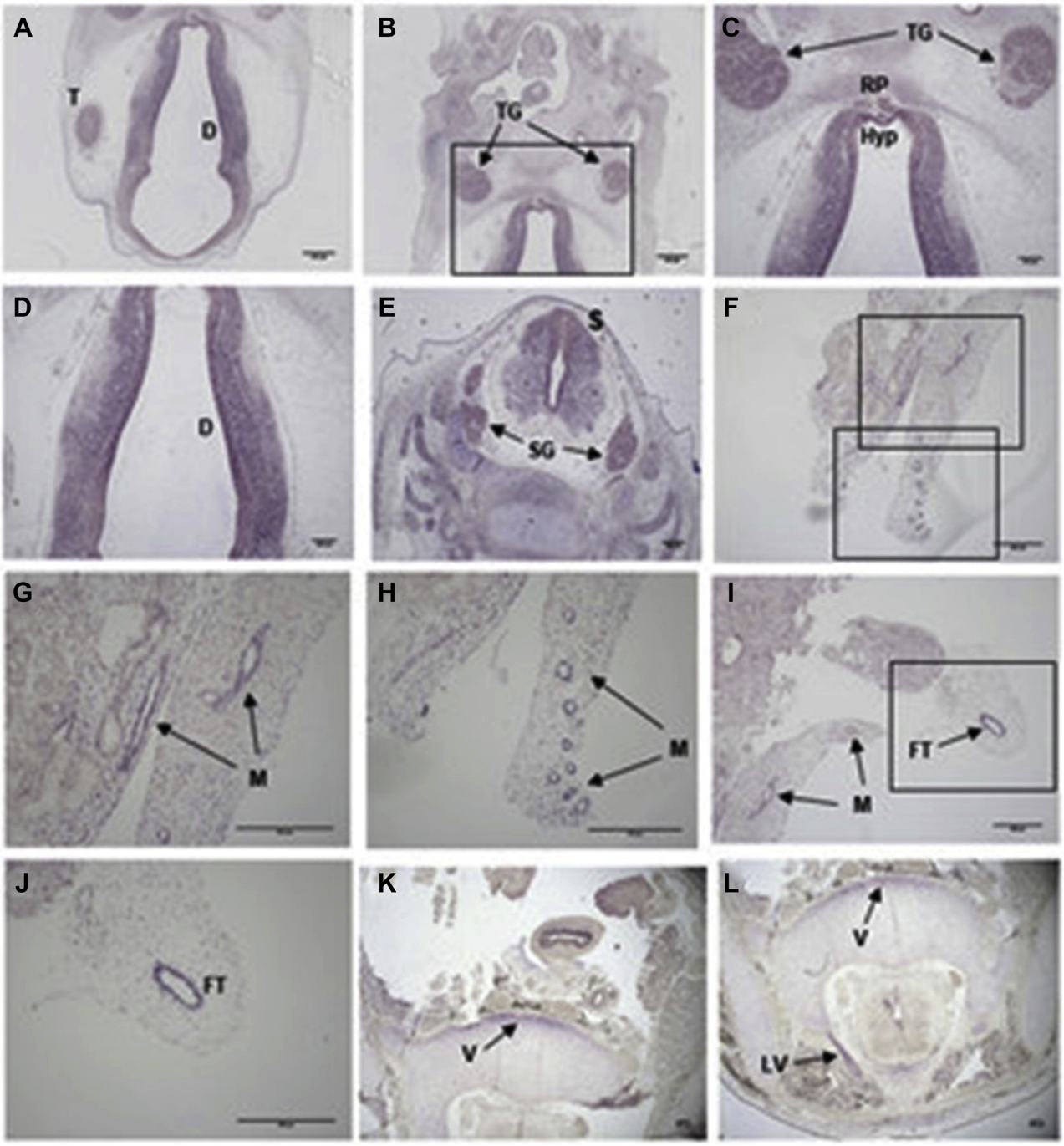


Figure 3 ***RNPC3*** **expression in the developing hypothalamic–pituitary axis during human embryogenesis.** In situ hybridization using the antisense probe against the human *RNPC3* mRNA transcript (*hRNPC3*) on human sections from different developmental stages during embryogenesis. A-D. CS19: high h*RNPC3* expression is seen in the T, D, TG, and RP. E. CS19: expression can be seen in the SC and SG. F-I. Week 9 after conception: h*RNPC3* mRNA transcripts are present in the M, the ducts that will develop into the kidney, indicated by the labeled arrows. The boxes in F correspond to G and H, respectively. I-J. Week 9 after conception: expression is noted in the presumptive developing FT. The box in I corresponds to J. K, L. Week 10 after conception: h*RNPC3* expression is seen in the C and LV, indicated by the labeled arrows. D, diencephalon; FT, fallopian tube; Hyp, hypothalamus; LV, lamina of the vertebrae; M, mesonephros; RP, Rathke pouch; S, spinal cord; SG, spinal ganglia; T, telencephalon; TG, trigeminal ganglia; V, vertebrae.

(v2.1.1) Browser (accessed on April 5, 2021). In addition, a homozygous missense variant in *PRMT6*, NM_018137.2:c.1049C>G, (p.Pro350Arg), was identified in the same individuals who possessed the homozygous *RNPC3* (p.Leu483Phe) variants. Both genes are located in close proximity to each other (approximately 4 Mb) (Supplemental Figure 3), and because of the large region of homozygosity spanning this locus, variants in both genes have co-segregated in these families. Both variants were confirmed by Sanger sequencing. Affected patients were homozygous for both

variants; the parents were heterozygous, and unaffected siblings were either heterozygous or homozygous wild type. In pedigrees 6 to 9, Sanger sequencing confirmed compound heterozygosity for 2 identified variants, 1 being the missense *RNPC3* c.1420C>A, p.Pro474Thr variant, in each pedigree. The novel variants in pedigrees 6 to 9 were predicted to be deleterious using different prediction programs detailed in Supplemental Appendix.

Conservation of the substituted amino acids in RNPC3 (p.Leu483Phe), (p.Pro474Thr), (p.Arg205*), (p.Arg502*) and (p.Pro474Leufs*10) across multiple species is shown in Figure 1D. The novel *PRMT6* (p.Pro350Arg) missense substitution is also conserved across 4 species and is not present in the homozygous state in control databases (1000 Genomes, Exome Variant Server, or the Genome Aggregation Database Browser).

Developmental expression pattern of *RNPC3*

The Human Protein Atlas states that *RNPC3* is variably expressed throughout the human body, including the pituitary gland, hypothalamus, and ovaries. ISH performed on human and mouse embryonic tissue in this study revealed *RNPC3/Rnpc3* expression in the telencephalon, diencephalon, trigeminal ganglia, spinal cord and spinal ganglia, the hypothalamus, and Rathke pouch (Figure 3A-E, Supplemental Figure 4). In addition, in embryonic sections of the pelvic area, *RNPC3* was expressed in the mesonephros (the preliminary kidney ducts), fallopian tube, and vertebrae (Figure 3F-L). Furthermore, *Rnpc3* was expressed in the ovaries and hypothalamus, predominantly in the ventromedial, dorsomedial, and arcuate nuclei, in adult mice. *Prmt6* was also shown to be expressed in the embryonic hypothalamus, pituitary, and ovaries as well as in the adult hypothalamus and ovaries in mice (Supplemental Figure 4).

Rnpc3 pathogenic variants are associated with GHD in female but not in male mice

Homozygous *Rnpc3* $^{p.Leu483Phe/\ p.Leu483Phe}$ mice generated by CRISPR/Cas9 technology were born in normal Mendelian ratios. Growth curves for the mutant male mice were normal. *Rnpc3* mutant female mice ($n = 5$) were slightly lighter than wild-type female mice ($n = 5$), although this difference was not statistically significant ($P = .09$) (Supplemental Figure 5). Radioimmunoassays performed on female pituitaries comparing homozygous mutants and wild-type littermate controls showed a decrease in GH levels in both single-guide RNA (sgRNA) 1 and 5 strains, reaching statistical significance only in sgRNA1 (SEM = 64.28 ± 4.6 in controls, $n = 5$, vs SEM = 43.4 ± 3.5 SEM in mutants, $n = 4$; $P = .016$) (Figure 2). There was no significant difference in the male GH levels in the sgRNA1 strain (SEM = 66.7 ± 9.0 in controls, $n = 7$, vs SEM = 61.5 ± 7.3 in mutants, $n = 7$). There was no significant difference in the LH, FSH, or PRL levels in homozygote mutant sgRNA1 females (XX, $n = 4$) vs wild-type littermates (XX, $n = 5$) (LH: SEM = 0.275 ± 0.04 μg vs SEM = 0.385 ± 0.02 μg/pituitary, $P = .6$; FSH: SEM = 55.44 ± 18.09 ng vs SEM = 41.21 ± 10.63 ng, $P = .9$; PRL: SEM = 7.34 ± 1.45 μg vs SEM = 9.52 ± 0.52 μg, $P = .4$). Histologic examination of sexually mature female ovaries revealed no abnormality.

Homozygous *Prmt6* $^{p.Pro350Arg/\ p.Pro350Arg}$ mice were also generated by CRISPR/Cas9. Growth curves for homozygous *Prmt6* $^{p.Pro350Arg/\ p.Pro350Arg}$ animals were normal (data not shown). Therefore, we only performed radioimmunoassays for GH in 1 of the 2 lines in females. There was no significant difference in pituitary GH content (GH: SEM = 72.9 ± 27.1 in wild type, $n = 2$, and SEM = 74.4 ± 6.1 in mutants, $n = 3$) (Supplemental Figure 1).

For fertility studies, 8-week-old females were mated with wild-type males for 10 weeks. There was no obvious difference in the average number of litters between *Rnpc3* $^{p.Leu483Phe/p.Leu483Phe}$ (3.8 ± 0.4 SD, $n = 5$) and wild-type (3.0 ± 0.8 SD, $n = 4$) females. The average number of pups/litter was also comparable (SD = 8 ± 1 pups/litter in *Rnpc3* $^{p.Leu483Phe/\ p.Leu483Phe}$ females, SD = 7.2 ± 2.6 in controls). Although these results suggest that fertility is initially normal in female *Rnpc3* mutants, it would be interesting to test fertility over a longer period.

Reanalysis of the RNA sequencing data for POI and peripheral neuropathy phenotypes

To test the splicing efficiency of U12-type introns in candidate genes that might be involved in 2 novel phenotypes, namely POI and/or peripheral neuropathy, we reanalyzed the RNA sequencing (RNA-seq) data obtained previously from the total RNA extracted from mononuclear blood cells of 2 patients (S1 and S2) and 4 controls.[14] We listed the U12-type intron–containing genes that were documented or candidates involved in POI and/or peripheral neuropathy. The splicing efficiency and intron retention of U12-type introns were quantified with respect to U2-type introns per gene, normalized by gene expression. Although several candidate genes did not have sufficient expression levels in blood cells to detect abnormalities, abnormal processing of U12 introns in patient cells were striking for *HARS* and *GARS* (Supplemental Table 3), reported to be involved in autosomal dominant neuropathies.[16,17]

Discussion

Using NGS, we identified a series of biallelic pathogenic variants in *RNPC3* in 15 patients from 9 families with severe GHD. Most affected females had evidence of POI ($n = 8$), whereas males ($n = 6$) had normal pubertal development and gonadotropin concentrations, with fertility documented in the eldest male patient.

Long thought to be a default pathway occurring in the absence of SRY, ovarian development is now known to be an active process involving several regulatory genes.[18] POI, with gonadal dysgenesis at the most severe end of the phenotypic spectrum, can be caused by genetic factors. To date, variants in >50 genes have been associated with POI, overall accounting for a small proportion of patients; the etiology remains to be elucidated in most cases.[2] These gene variants can affect various processes such as gonadal development, DNA replication/meiosis and repair, hormonal signaling, immune function, and metabolism.[19] In this study, we reported that pathogenic variants in *RNPC3* encoding U11/U12-65K, a specific protein component of the minor spliceosome, are associated with POI through a pathway that is distinct from the previously reported mechanisms.

The U12-dependent spliceosome includes 4 unique small nuclear RNAs designated as U11, U12, U4atac, and U6atac and 7 unique proteins, all found on the U11/U12 di-small nuclear ribonucleoprotein, which recognizes the first intron.[20,21] Compound heterozygous pathogenic variants in *RNPC3* were initially reported to cause isolated GHD.[14] Both variants, namely missense (p.Pro474Thr) and nonsense/truncating (p.Arg502*), were shown to reduce formation of the U12-type intron recognition complex, leading to splicing defects in a subset of minor introns.[14,22] A more recent study described compound heterozygosity for 2 variants in *RNPC3* in 3 siblings from a Caribbean pedigree that was associated with GHD and TSH and prolactin deficiencies as well as learning difficulties and congenital cataracts. Pubertal delay because of apparent hypogonadotrophic hypogonadism was described in 2 siblings, but biochemical data showing abnormal gonadotropin secretion and details of pubertal development and any treatment required to progress puberty were not provided.[23] During the submission process of this paper, another patient with severe primordial microcephalic dwarfism and intellectual disability was reported to be carrying compound heterozygous variants in *RNPC3*. She was reported to be prepubertal at age 11 years; GHD and prolactin deficiency were documented, but biochemical data of gonadotropin concentrations were not provided.[24]

Since the first 3 publications of *RNPC3* variants in cases with GHD,[14,23,24] we have identified 12 more patients, 4 compound heterozygotes for p.Pro474Thr together with a second disruptive change and 8 homozygotes with p.Leu483Phe variant. The first 3 Spanish siblings reported[14] presented with severe isolated GHD at ages 14 years, 7.6 years, and 5.5 years, respectively. The eldest patient developed spontaneous puberty at age 15.7 years, shortly after commencement of rhGH treatment, and had menarche at age 16.7 years. However, no further menstruation occurred, and the diagnosis of POI was later established. The other 2 siblings entered puberty spontaneously at ages 11.6 years and 13 years, respectively. Nevertheless, their gonadotropin concentrations also increased progressively, and ultrasonographic investigations revealed normal/small ovaries with sparse or absent follicles. In contrast, proband 1 in the Turkish pedigree presented with POI at age 14 years. The other 4 female patients harboring the same variant also showed evidence of ovarian insufficiency. Although spontaneous puberty started at ages 11.9 years and 11.8 years in patients T2 and T3, respectively, the ovaries were undetectable or very small with no follicles in all Turkish patients throughout the follow-up period, and hypergonadotropic hypogonadism developed eventually. The Russian patient also had elevated FSH and pubertal LH concentrations at age 3 years, consistent with the findings in the Turkish girls during the prepubertal period, although a definitive diagnosis of POI cannot be made as yet.

U12-type introns are particularly present in genes related to information processing functions, such as DNA replication and repair, transcription, RNA processing, and translation.[25] The impairment of minor class splicing usually leads to intron retention and less frequently to exon skipping and alternative splicing.[26] These disruptions often result in frameshifts, formation of premature stop codons, and decreased expression and function of the affected genes.[27,28]

We hypothesized that splicing defects in genes involved in ovarian development or function may be implicated in POI in our female patients with *RNPC3* variants. Therefore, we searched for all the U12-type intron–containing genes possibly or proven to be associated with POI and/or ovarian development, by examining the most updated human and animal studies in the literature. Several candidate genes were shortlisted, including *DIAPH2, EXO1, SPO11, NUP107*, and *PTEN*. Given the tissue-specific importance of Nup107 for ovarian development and female-specific sterility in knockdown animals,[29,30] *NUP107* seems to be a strong candidate to possibly explain the POI in our patients and requires further study. In patients S1 and S2, reanalysis of RNA-seq revealed increased retention of U12-type introns in *MCMBP,* encoding a component of the mini-chromosome maintenance complex, which acts as a regulator of DNA replication (Supplemental Table 3). Therefore, this gene might also be a good candidate for this phenotype, although it has not yet been reported to be associated with POI.

Of the approximately 50 genes in which variants clearly cause POI in humans, only half have relevant mouse models.[19] In this study, in the mouse model harboring the homozygous p.Leu483Phe variant, although there was female-specific GHD, the ovaries were grossly normal and fertility was not impaired in young mice. Eight-week-old $Rnpc3^{p.Leu483Phe\ p.Leu483Phe}$ female mice that were mated with males for 10 weeks appeared to have normal ovaries. It should, however, be noted that subfertility was reported in 12-month-old $Nup107^{p.Arg356Cys}$ mutant mice with normal ovarian structure and size at age 6 months.[30] Hence, these mice may be too young to manifest an ovarian phenotype. Another possible reason for the lack of an ovarian

phenotype in mice may lie in the difference between human and murine reproductive phenotypes, as observed in Turner syndrome, in which humans are mostly infertile and mice are fertile but have a reduced oocyte pool and reproductive lifespan.[31]

In this cohort, almost all patients had APH with a normal stalk and a eutopic posterior pituitary of normal hyperintensity. The expression studies performed on human embryonic sections in this study further suggest an important role for *RNPC3* during HP development, with strong expression throughout the diencephalon, hypothalamus, and Rathke pouch (Figure 3A-D).

Assessment of pituitary function revealed that the corticotropic axis and gonadotropin production and secretion were intact in all patients. Overt central hypothyroidism was diagnosed in 2 patients, whereas mild thyroid dysfunction was observed in a further 3 patients. Interestingly, however, PRL concentrations were strikingly low in most affected patients. The phenotype of combined pituitary hormone deficiency involving somatotrophs, thyrotrophs, and lactotrophs is consistent with the findings in the Caribbean pedigree,[23] although the TSH deficiency appeared to be more severe in the latter pedigree.

The growth response to rhGH therapy was excellent in all patients with *RNPC3* variants. In 10 patients who were treated with rhGH regularly, the height SDSs changed between 1.1 and 7.5 after 1 to 9 years. Growth almost ceased in 1 female patient (T4) during interruption of therapy for 21 months at around age 10 years, with growth acceleration on recommencing rhGH. In contrast, 2 of the males displayed normal growth when off GH treatment despite manifesting severe biochemical GHD. This relatively milder GHD phenotype in male patients may be consistent with the lack of a phenotype observed in the *Rnpc3* mutant male mice we generated. This may reflect a difference in the extent of GHD between males and females as was also observed with gonadal susceptibility in humans.

Furthermore, a history of mild developmental delay and/or ataxia or neuropathic/myopathic symptoms led to the diagnosis of a motor neuropathy in patients M1, I1, T7, and S2 on the basis of abnormal nerve conduction studies and elevated CPK concentrations. Recently, a variant in the U12 small nuclear RNA gene (*RNU12*), probably affecting the binding site for the 65K protein, has been associated with early-onset cerebellar ataxia. RNA-seq analyses indicated increased U12-type intron retention along with U12 small nuclear ribonucleoprotein upregulation.[32] There is mounting evidence suggesting that the minor spliceosome contributes to the pathophysiology underlying neurodegenerative disorders such as spinal muscular atrophy.[33,34] Taking into account that patients with pathogenic variants in *RNPC3* from 4 different pedigrees had similar neurological findings, we speculate that splicing defects in some U12-type introns in neurons may contribute to this phenotype in our patients. Reanalysis of the RNA-seq data of our patients S1 and S2 revealed significantly defective splicing of *HARS1* and *GARS1* in patients when compared with controls (Supplemental Table 3). Further functional studies are needed to investigate any possible mechanistic relationship of this phenotype to *RNPC3* variants. Furthermore, the occurrence of rapidly progressive glomerulonephritis in patient M2 from pedigree 7 remains to be understood in terms of causality or coexistence. Conceivably, the *RNPC3* expression seen in the mesonephros of the human embryonic kidney may explain this phenotype. Likewise, the strong expression seen in the spinal cord and spinal ganglia in human embryonic sections (Figure 3E) and additionally in the vertebrae in pelvic sections (Figure 3K and L) of human embryos supports a contributory role of RNPC3 in neurologic phenotypes.

In summary, 2 of 3 previously reported Spanish patients with *RNPC3* variants developed ovarian insufficiency on follow-up with elevated gonadotropins and small ovaries observed in the third patient. Thereafter, 6 other girls from different families (5 Turkish and 1 of Russian origin) have been identified with the same phenotype of severe GHD associated with proven/possible POI. Causality is supported by the identification of different *RNPC3* variants in patients with a similar phenotype from different ethnic origins.

To conclude, we have further expanded the phenotypic spectrum associated with *RNPC3* variants. A novel homozygous missense pathogenic variant (p.Leu483Phe) in *RNPC3* was associated with GHD, hypoprolactinemia, variable TSH deficiency, and APH in humans. Most female patients also present with POI, whereas males have normal pubertal development and gonadal function, including fertility. Data in humans are partially supported by murine studies that show female-restricted GHD but normal gonadal function and by *RNPC3* expression in the HP region of the human brain, further supporting a role for this gene in HP development. Currently, we cannot explain the differences between the males and females, but this could be the foundation for future studies. Our findings suggest novel insights into the role of RNPC3 in ovary-specific gonadal function and emphasize a critical role of the minor spliceosome in the processing of genes required for pituitary and ovarian development and function.

Data Availability

The variants described in this publication have been submitted to ClinVar.

http://www.ncbi.nlm.nih.gov/clinvar/?term=SCV001571473[clv_acc]
http://www.ncbi.nlm.nih.gov/clinvar/?term=SCV001571474[clv_acc]
http://www.ncbi.nlm.nih.gov/clinvar/?term=SCV001571475[clv_acc]
http://www.ncbi.nlm.nih.gov/clinvar/?term=SCV001571476[clv_acc]
http://www.ncbi.nlm.nih.gov/clinvar/?term=SCV001571477[clv_acc].

Acknowledgments

We thank all the patients and their parents for contributing to this study. We thank Lynne Overman and Steven Lisgo for their helpful discussion and contribution toward the human expression results. We also thank Deborah Schneider-Luftman for performing the statistical analysis for the growth chart in mouse studies, Muge Gulcihan Onal for performing DNA extractions in the Turkish patients, and Ms. Natasha Henden (LabPLUS, Auckland City Hospital, New Zealand) for assistance with Alamut Software.

This work was supported by the Francis Crick Institute that receives its core funding from Cancer Research UK (FC001107), the United Kingdom Medical Research Council (FC001107), and the Wellcome Trust (FC001107). The human embryonic sections were provided by the Joint MRC/Wellcome Trust (Grant MR/R006237/1) Human Developmental Biology Resource (HDBR) (http://hdbr.org). M.T.D receives funding from the Great Ormond Street Hospital Children's Charity and the Medical Research Foundation, United Kingdom (grant# 535963). J.C.A is a Wellcome Trust Senior Research Fellow in Clinical Science (209328/Z/17/Z). S.M.M.-B is supported by a Wellcome Trust Clinical Training Fellowship at the University College London (216362/Z/19/Z). Research at the Great Ormond Street Hospital benefits from funding received from the National Institute for Health Research, Great Ormond Street Hospital Biomedical Research Centre (Grant IS-BRC-1215-20012). The views expressed are those of the authors and not necessarily those of the National Health Service, National Institute for Health Research, or Department of Health. D.N.A was supported by Raine Medical Research Foundation clinical research fellowship (CRF018). A.F was supported by the National Health and Medical Research Council and Cancer Council of Western Australia. L.A received grants from the European Society for Paediatric Endocrinology (ESPE), The Scientific and Technological Research Council of Turkey (TUBITAK), and Turkish Paediatric Endocrinology Society for her sabbatical leave in the United Kingdom.

"This research was funded in part, by the Wellcome Trust [FC001107, MR/R006237/1, 209328/Z/17/Z, 216362/Z/19/Z]. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission."

Author Information

Conceptualization: L.A., M.T.D.; Formal Analysis: P.L.Q.S., H.W., A.A., V.C., A.F., V.T., D.N.A., S.G., L.A.P.-J., S.M.; Investigation: L.A., J.A., M.M., Z.G., G.A.M.-M., A.T., S.K., M.K., B.C., L.C.G., K.R., R.L.B., C.G., I.R., Z.B.G., F.B., J.P.S., J.C.A., S.M.M.-B.; Supervision: M.T.D.; Writing-original draft: L.A., M.T.D.; Writing-review and editing: L.A.L., K.R., L.C.G., B.C., P.L.Q.S., H.W., F.B., S.M., V.C., S.M.M.-B., G.A.M.-M., D.N.A., M.K., S.K., J.P.S., C.G., S.G., V.T., J.C.A., A.A., A.F., A.T., M.M., Z.G., Z.B.G., L.A.P.-J., I.R., R.L.B., J.A., M.T.D.

Ethics Declaration

This study has been approved by the Institutional Review Board of Erciyes University. All the institutions involved in human participant research received local Institutional Review Board approvals. Written informed consent was obtained from all the participants and their parents. This study complied with the World Medical Association Declaration of Helsinki and National Institutes of Health Belmont report. All experiments carried out on mice were approved under the United Kingdom Animal (Scientific Procedures) Act (Project licence 80/2405 and 70/8560).

Conflict of Interest

All authors declare that they have no conflicts of interest.

Additional Information

Additional Information The online version of this article (https://doi.org/10.1016/j.gim.2021.09.019) contains supplementary material, which is available to authorized users.

Affiliations

[1]Department of Paediatric Endocrinology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey; [2]Department of Paediatric Endocrinology, Faculty of Medicine, Erciyes University, Kayseri, Turkey; [3]Stem Cell Biology and Developmental Genetics Lab, The Francis Crick Institute, London, United Kingdom; [4]Genetics and Genomic Medicine Research and Teaching Department, UCL Great Ormond Street Institute of Child Health, University College London, London, United Kingdom; [5]Departments of Paediatrics and Paediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, Madrid, Spain; [6]GOSgene, Genetics and Genomic Medicine Research and Teaching Department, UCL Great Ormond Street Institute of Child Health, University College London, London, United Kingdom; [7]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand; [8]Division of Cancer and Genetics, Genetics and Genomic Medicine, School of Medicine, Cardiff University, Cardiff, United Kingdom; [9]Structural Biology Science Technology Platforms, The Francis Crick Institute, London, United Kingdom; [10]Unit of Medical Genetics, IRCCS

Giannina Gaslini Institute, Genova, Italy; [11]Department of Paediatrics, Universidad Autónoma de Madrid, Madrid, Spain; [12]CIBER Fisiopatología Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain; [13]Centre of Medical Research, The University of Western Australia and Harry Perkins Institute of Medical Research, Perth, Western Australia, Australia; [14]Department of Diagnostic Genomics, PathWest, QEII MedicalCentre, Perth, Western Australia, Australia; [15]Istituto Italiano di Tecnologia – IIT, Genova, Italy; [16]University Children's Hospital, Medical School, Skopje, North Macedonia; [17]Division of Medical Genetics, Department of Specialized Medicine, Montreal Children's Hospital, McGill University Health Centre (MUHC), Montreal, QC, Canada; [18]Department of Human Genetics, McGill University, Montreal, QC, Canada; [19]Telethon Kids Institute, Perth Children's Hospital, Nedlands, Western Australia, Australia; [20]Department of Endocrine Genetics, Research Centre for Medical Genetics, Moscow, Russia; [21]Department of Inherited Endocrine Disorders, Endocrinology Research Centre, Moscow, Russia; [22]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, Italy; [23]Department of Paediatrics, IRCCS Giannina Gaslini Institute, Genoa, Italy; [24]Oral and Maxillofacial Surgery, Genome and Stem Cell Center, Erciyes University, Kayseri, Turkey; [25]Genetics Unit, Universitat Pompeu Fabra, Hospital del Mar Research Institute (IMIM) and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain; [26]South Australian Health and Medical Research Institute (SAHMRI), The University of Adelaide, Adelaide, South Australia, Australia; [27]IMDEA Food Institute, Campus of International Excellence UAM+CSIC, Madrid, Spain; [28]Department of Paediatric Endocrinology, Great Ormond Street Hospital for Children, London, United Kingdom

References

1. Le Quesne Stabej P, Williams HJ, James C, et al. STAG3 truncating variant as the cause of primary ovarian insufficiency. *Eur J Hum Genet*. 2016;24(1):135–138. http://doi.org/10.1038/ejhg.2015.107.
2. Jiao X, Ke H, Qin Y, Chen ZJ. Molecular genetics of premature ovarian insufficiency. *Trends Endocrinol Metab*. 2018;29(11):795–807. http://doi.org/10.1016/j.tem.2018.07.002.
3. Beysen D, De Paepe A, De Baere E. FOXL2 mutations and genomic rearrangements in BPES. *Hum Mutat*. 2009;30(2):158–169. http://doi.org/10.1002/humu.20807.
4. Jenkinson EM, Rehman AU, Walsh T, et al. Perrault syndrome is caused by recessive mutations in CLPP, encoding a mitochondrial ATP-dependent chambered protease. *Am J Hum Genet*. 2013;92(4):605–613. http://doi.org/10.1016/j.ajhg.2013.02.013.
5. Caburet S, Arboleda VA, Llano E, et al. Mutant cohesin in premature ovarian failure. *N Engl J Med*. 2014;370(10):943–949. http://doi.org/10.1056/NEJMoa1309635.
6. de Vries L, Behar DM, Smirin-Yosef P, Lagovsky I, Tzur S, Basel-Vanagaite L. Exome sequencing reveals SYCE1 mutation associated with autosomal recessive primary ovarian insufficiency. *J Clin Endocrinol Metab*. 2014;99(10):E2129–E2132. http://doi.org/10.1210/jc.2014-1268.
7. Kasippillai T, MacArthur DG, Kirby A, et al. Mutations in eIF4ENIF1 are associated with primary ovarian insufficiency. *J Clin Endocrinol Metab*. 2013;98(9):E1534–E1539. http://doi.org/10.1210/jc.2013-1102.
8. Wang J, Zhang W, Jiang H, Wu BL. Primary Ovarian Insufficiency Collaboration. Mutations in HFM1 in recessive primary ovarian insufficiency. *N Engl J Med*. 2014;370(10):972–974. http://doi.org/10.1056/NEJMc1310150.
9. AlAsiri S, Basit S, Wood-Trageser MA, et al. Exome sequencing reveals MCM8 mutation underlies ovarian failure and chromosomal instability. *J Clin Invest*. 2015;125(1):258–262. http://doi.org/10.1172/JCI78473.
10. Wood-Trageser MA, Gurbuz F, Yatsenko SA, et al. MCM9 mutations are associated with ovarian failure, short stature, and chromosomal instability. *Am J Hum Genet*. 2014;95(6):754–762. http://doi.org/10.1016/j.ajhg.2014.11.002.
11. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. *Hum Reprod Update*. 2015;21(6):787–808. http://doi.org/10.1093/humupd/dmv036.
12. Turunen JJ, Niemelä EH, Verma B, Frilander MJ. The significant other: splicing by the minor spliceosome. *Wiley Interdiscip Rev RNA*. 2013;4(1):61–76. http://doi.org/10.1002/wrna.1141.
13. Zhao E, Li J, Xie Y, et al. Cloning and identification of a novel human RNPC3 gene that encodes a protein with two RRM domains and is expressed in the cell nucleus. *Biochem Genet*. 2003;41(9-10):315–323. http://doi.org/10.1023/b:bigi.0000006032.04031.d0.
14. Argente J, Flores R, Gutiérrez-Arumí A, et al. Defective minor spliceosome mRNA processing results in isolated familial growth hormone deficiency. *EMBO Mol Med*. 2014;6(3):299–306. http://doi.org/10.1002/emmm.201303573.
15. Martos-Moreno GÁ, Travieso-Suárez L, Pozo-Román J, et al. Response to growth hormone in patients with RNPC3 mutations. *EMBO Mol Med*. 2018;10(7):e9143. http://doi.org/10.15252/emmm.201809143.
16. Safka Brozkova D, Deconinck T, Griffin LB, et al. Loss of function mutations in HARS cause a spectrum of inherited peripheral neuropathies. *Brain*. 2015;138(Pt 8):2161–2172. http://doi.org/10.1093/brain/awv158.
17. Antonellis A, Ellsworth RE, Sambuughin N, et al. Glycyl tRNA synthetase mutations in Charcot–Marie–Tooth disease type 2D and distal spinal muscular atrophy type V. *Am J Hum Genet*. 2003;72(5):1293–1299. http://doi.org/10.1086/375039.
18. Knarston I, Ayers K, Sinclair A. Molecular mechanisms associated with 46,XX disorders of sex development. *Clin Sci (Lond)*. 2016;130(6):421–432. http://doi.org/10.1042/CS20150579.
19. Tucker EJ, Grover SR, Bachelot A, Touraine P, Sinclair AH. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. *Endocr Rev*. 2016;37(6):609–635. http://doi.org/10.1210/er.2016-1047.
20. Sikand K, Shukla GC. Functionally important structural elements of U12 snRNA. *Nucleic Acids Res*. 2011;39(19):8531–8543. http://doi.org/10.1093/nar/gkr530.
21. Padgett RA. New connections between splicing and human disease. *Trends Genet*. 2012;28(4):147–154. http://doi.org/10.1016/j.tig.2012.01.001.
22. Norppa AJ, Kauppala TM, Heikkinen HA, Verma B, Iwaï H, Frilander MJ. Mutations in the U11/U12-65K protein associated with isolated growth hormone deficiency lead to structural destabilization and impaired binding of U12 snRNA. *RNA*. 2018;24(3):396–409. http://doi.org/10.1261/rna.062844.117.
23. Verberne EA, Faries S, Mannens MMAM, Postma AV, van Haelst MM. Expanding the phenotype of biallelic RNPC3 variants associated with growth hormone deficiency. *Am J Med Genet A*. 2020;182(8):1952–1956. Published correction appears in *EMBO Mol Med*. 2020;12(9):e13132. Published correction appears in *EMBO Mol Med*. 2020;12(9):e13133. https://doi.org/10.1002/ajmg.a.61632.

24. Yamada M, Ono M, Ishii T, et al. Establishing intellectual disability as the key feature of patients with biallelic RNPC3 variants. *Am J Med Genet A*. 2021;185(6):1836–1840. http://doi.org/10.1002/ajmg.a.62152.
25. Verma B, Akinyi MV, Norppa AJ, Frilander MJ. Minor spliceosome and disease. *Semin Cell Dev Biol*. 2018;79:103–112. http://doi.org/10.1016/j.semcdb.2017.09.036.
26. Chang WC, Chen YC, Lee KM, Tarn WY. Alternative splicing and bioinformatic analysis of human U12-type introns. *Nucleic Acids Res*. 2007;35(6):1833–1841. http://doi.org/10.1093/nar/gkm026.
27. Patel AA, McCarthy M, Steitz JA. The splicing of U12-type introns can be a rate-limiting step in gene expression. *EMBO J*. 2002;21(14):3804–3815. http://doi.org/10.1093/emboj/cdf297.
28. Niemelä EH, Frilander MJ. Regulation of gene expression through inefficient splicing of U12-type introns. *RNA Biol*. 2014;11(11):1325–1329. http://doi.org/10.1080/15476286.2014.996454.
29. Weinberg-Shukron A, Renbaum P, Kalifa R, et al. A mutation in the nucleoporin-107 gene causes XX gonadal dysgenesis. *J Clin Invest*. 2015;125(11):4295–4304. http://doi.org/10.1172/JCI83553.
30. Ren Y, Diao F, Katari S, et al. Functional study of a novel missense single-nucleotide variant of NUP107 in two daughters of Mexican origin with premature ovarian insufficiency. *Mol Genet Genomic Med*. 2018;6(2):276–281. http://doi.org/10.1002/mgg3.345.
31. Heard E, Turner J. Function of the sex chromosomes in mammalian fertility. *Cold Spring Harb Perspect Biol*. 2011;3(10):a002675. http://doi.org/10.1101/cshperspect.a002675.
32. 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. http://doi.org/10.1002/ana.24826.
33. Boulisfane N, Choleza M, Rage F, Neel H, Soret J, Bordonné R. Impaired minor tri-snRNP assembly generates differential splicing defects of U12-type introns in lymphoblasts derived from a type I SMA patient. *Hum Mol Genet*. 2011;20(4):641–648. http://doi.org/10.1093/hmg/ddq508.
34. Jutzi D, Akinyi MV, Mechtersheimer J, Frilander MJ, Ruepp MD. The emerging role of minor intron splicing in neurological disorders. *Cell Stress*. 2018;2(3):40–54. http://doi.org/10.15698/cst2018.03.126.