RFC8.3

Central Precocious Puberty Caused by Novel Mutations in the Promoter and 5'-UTR region of the Imprinted *MKRN3* Gene

Pavlos Fanis[1,2], Nicos Skordis[3,1], Meropi Toumba[4,1], Nikoletta Papaioannou[1,2], Anestis Makris[1], Andreas Kyriakou[5], Vassos Neocleous[1,2] & Leonidas A. Phylactou[1,2]

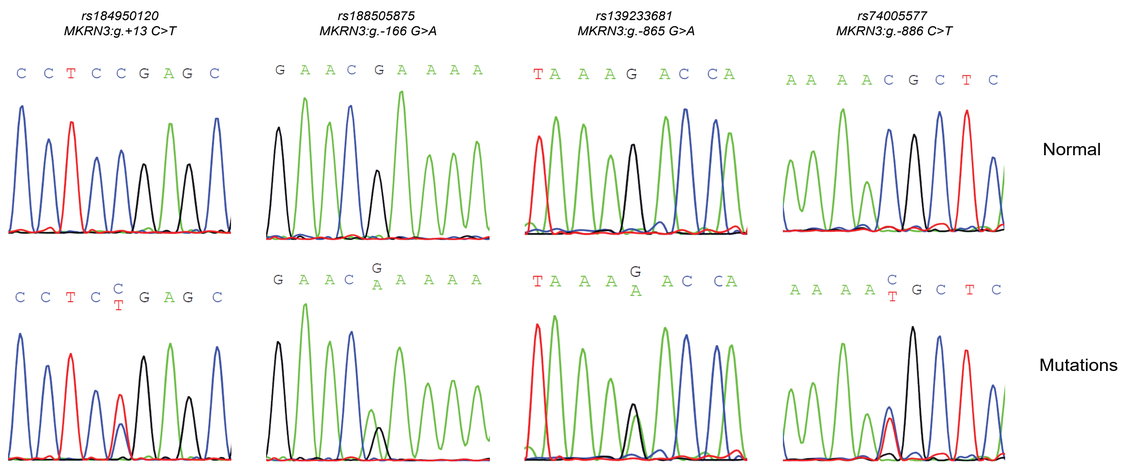
1. Department of Molecular Genetics, Function & Therapy, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
2. Cyprus School of Molecular Medicine, Nicosia, Cyprus
3. Department of Pediatric Endocrinology, Paedi Center for specialized Pediatrics, Nicosia, Cyprus
4. Department of Pediatrics, Iasis Hospital, Paphos, Cyprus
5. Developmental Endocrinology Research Group, School of Medicine, University of Glasgow, Glasgow, UK

Background

Central precocious puberty (CPP) is characterized by the premature activation of the hypothalamic- pituitary-gonadal axis due to the early activation of pulsatile Gonadotropin Releasing hormone (GnRH) secretion. CPP is clinically defined by the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in boys and is associated with a range of clinical and biological implications. The most common genetic causes of CPP are the reported loss-of-function mutations in the MKRN3 gene. Although most of the studies describe loss-of-function mutations in the coding region of MKRN3 gene, defects in the regulatory regions of the gene were described only in two recent studies. In this study, we report four novel heterozygous mutations located in the proximal promoter and 5'-UTR regions of the MKRN3 gene.

1. Genetic Screening of CPP patients

- The promoter/5'-UTR region of the *MKRN3* gene was screened in 73 index CPP girls
- Mutations in the coding sequence of the *MKRN3*, *KISS1*, *KISS1R* and *DLK1* genes previously excluded



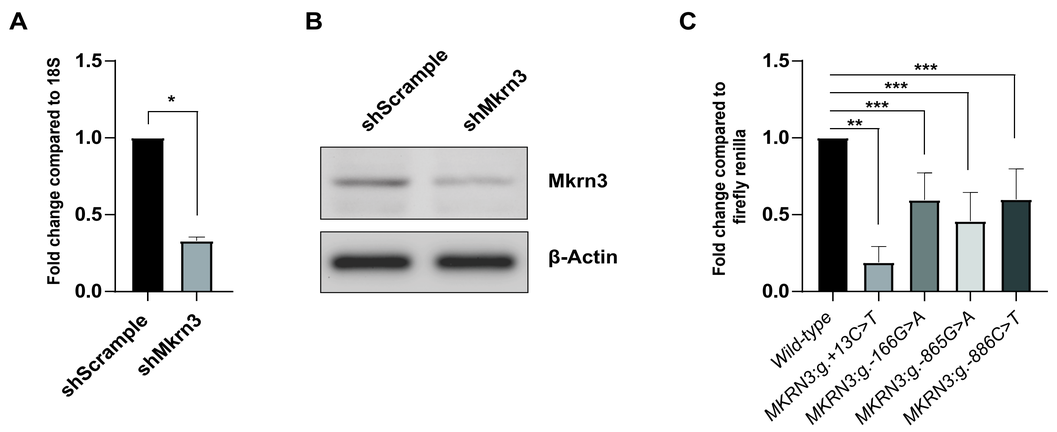
DNA sequencing analysis. Part of the sequencing electropherograms of the *MKRN3* proximal promoter showing the novel heterozygous mutations identified. For each mutation the corresponding normal sequencing electropherograms is showed.

2. Clinical and laboratory findings

Clinical and laboratory characteristics for six girls with MKRN3 mutations

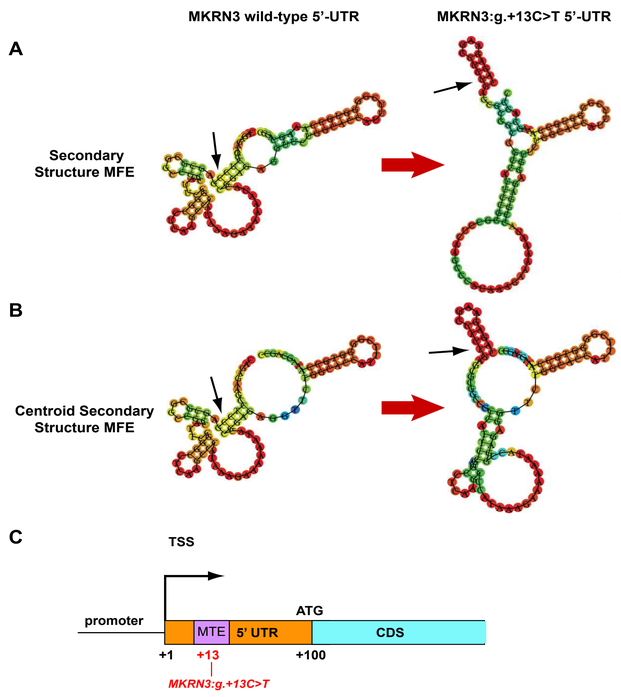
Proband	*MKRN3* mutation	Age at onset (y)	Age at referral (first visit) (y)	Stage of breast development	Stage of pubic hair	Stage of axillary hair	Bone age (years)	LHRH test FSH/LH(U/L)			MRI	Pelvic Ultrasound	Comments /Other symptoms
								0	30	60			
1	MKRN3:g.-865G>A	5.4	7.38	B3	P2	A1	10	1.95/5.3	8.9/9	7.7/7	No	Pubertal	Hearing impairment/cochlear implants/first cousin from father's side same clinical picture
2	MKRN3:g.-865G>A	7	7.8	B4	P3	A1	12	3.2/2.4	14/19	12/21	Normal	Pubertal	
3	MKRN3:g.-865G>A	N/A	9.5	B5	P4	A2	11.5	5.1/4.8	-	-	No	Pubertal	Patient came at age 9.5 y with menarche
4	MKRN3:g.-865G>A	6.1	8.8	B3	P2	A2	9.6	3/4	15/19	16/15	No	Normal	
5	MKRN3:g.-886C>T	N/A	8.3	B4	P4	A4	N/A	3/3	5/12	4/9	Normal	Normal	Ovarian volume: post pubertal
6	MKRN3:g.+13C>T	7.6	7.6	B2	P2	A1	8.5	0.25/3.5	-	-	No	No	Patient came back at age 9.1 y with menarche /Obesity-Insulin resistance

3. *MKRN3* promoter/5'-UTR mutations reduce the promoter activity



A and B. shRNA knockdown efficiency on (A) RNA and (B) protein Mkrn3 levels. GnRH expressing GN11 cells were treated with the indicated shRNA.
C. The *MKRN3* promoter/5'-UTR mutations reduce the promoter activity in GN11 cells. The MKRN3 promoter reporter gene constructs containing the indicated *MKRN3* mutations were transiently transfected in GN11 cells. Luciferase activities were calculated relative to the wild - type *MKRN3* promoter reporter construct. Results are the average of three independent experiments with each sample assayed in triplicate.
*P<0.0001; **P<0.001; ***P<0.05.

4. *In silico* analyses of the novel *MKRN3:g.+13C>T* 5'-UTR mutation



MKRN3 5'-UTR in silico predictions

(A) and (B) predicted MKRN3 5'-UTR mRNA secondary structures showing the Minimum Free Energy (MFE) and centroid MFE secondary structure, respectively, for the wild-type and *MKRN3:g.+13C>T* mutant.

(C) Position of the Motif Ten Element (MTE) in relation with the *MKRN3:g.+13C>T* mutation. Black arrows indicate the position of the *MKRN3:g.+13* nucleotide. The novel 5'-UTR *MKRN3:g.+13C>T* mutation would lead to the loss of a putative MTE binding site that can promote transcription by RNA polymerase II.

References/Acknowledgements

Fanis P, Skordis N, Toumba M, Papaioannou N, Makris A, Kyriakou A, Neocleous V, Phylactou LA. (2019).
Central Precocious Puberty Caused by Novel Mutations in the Promoter and 5'-UTR region of the Imprinted MKRN3 Gene.
(Submitted to Frontiers in Endocrinology).

This work was supported by the A.G. Leventis Foundation