# A Novel *MKRN3* Frameshift Mutation in a Bulgarian Girl with Central Precocious Puberty

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**Introduction**: Precocious puberty is defined as the onset of pubertal signs in girls younger than 8 years of age and in boys younger than 9 years of age. Central precocious puberty (CPP) is due to an early activation of the hypothalamic-pituitary-gonadal axis. It is 20 times more common in girls and 90% of the female cases are considered to be idiopathic (ICPP) (1). Different genes have been proposed as candidate genes in ICPP, but there is not enough evidence for their role in the etiology.

In 2013 Abreu et al. first described the role of the *MKRN3* gene in pubertal initiation. They detected three frameshift and a missense mutation in five families with familial CPP (2). A year later, Macedo et al. reported five novel mutations - four frameshift variants and one missense variant (3). Following studies in patients with ICPP detected different defects in the *MKRN3* gene (4, 5, 6, 7, 8)

The *MKRN3* gene is an intronless gene located on chromosome 15q11.2. Only the paternal allele of the gene is expressed, which leads to paternal inheritance pattern in families with mutations in *MKRN3*. This gene encodes Makorin RING - finger protein 3 which is thought to influence pubertal timing by inhibition of the pulsatile GnRH secretion (2).

**Objectives:** The aim of our study was to search for mutations in *MKRN3* in cases of sporadic CPP. We screened 10 Bulgarian girls diagnosed with ICPP for mutations in *MKRN3*. All patients had pubertal signs before the age of 8 years, advanced linear growth and bone age, elevated basal levels of luteinizing hormone or/and pubertal stimulated levels of luteinizing hormone, normal brain image on magnetic resonance. Seven relatives from *MKRN3* affected families were also subjected to genetic testing.

**Methods:** DNA was isolated from peripheral blood. The coding exons and boundaries of genes *MKRN3* were amplified by PCR reaction The products were purified by ExoSAP-IT (Affymetrix USB products), and sequenced by ABI BigDyeTerminator Cycle Sequencing Kit (v3.1).

**Results:** Two heterozygous *MKRN3* mutations were found.

The first one is a novel frameshift mutation (c.1053_1056delACAG, p.Arg351Serfs*44) (Figure 1). On protein level, it generates stop codon, resulting in premature termination of the protein synthesis. The patient had early onset and fast progression of pubertal signs - breast enlargement at the age of 3.8 years; accelerated linear growth (HSDS +2.9); advanced bone age; elevated sex hormone levels. She started treatment with GnRH analogue and has satisfactory clinical and hormonal control. Familial genetic analysis revealed that the father and the grandfather were also mutation carriers (Figure 2A). Both of them had normal pubertal development.

The second mutation (c.482delC, p.Pro161Argfs*10) was previously reported in two unrelated Brazilian girls (4). It was subsequently identified in patient's father and older brother who reported normal pubertal timing (Figure 2B).

Other studies in families with CPP show that paternally inherited *MKRN3* mutations affect both girls and boys (3). However girls seem to be more severely affected than boys. Two authors have reported cases of girls with precocious puberty and their brothers with early puberty (5, 9). We are the first to report two asymptomatic males with paternally inherited *MKRN3* mutations.

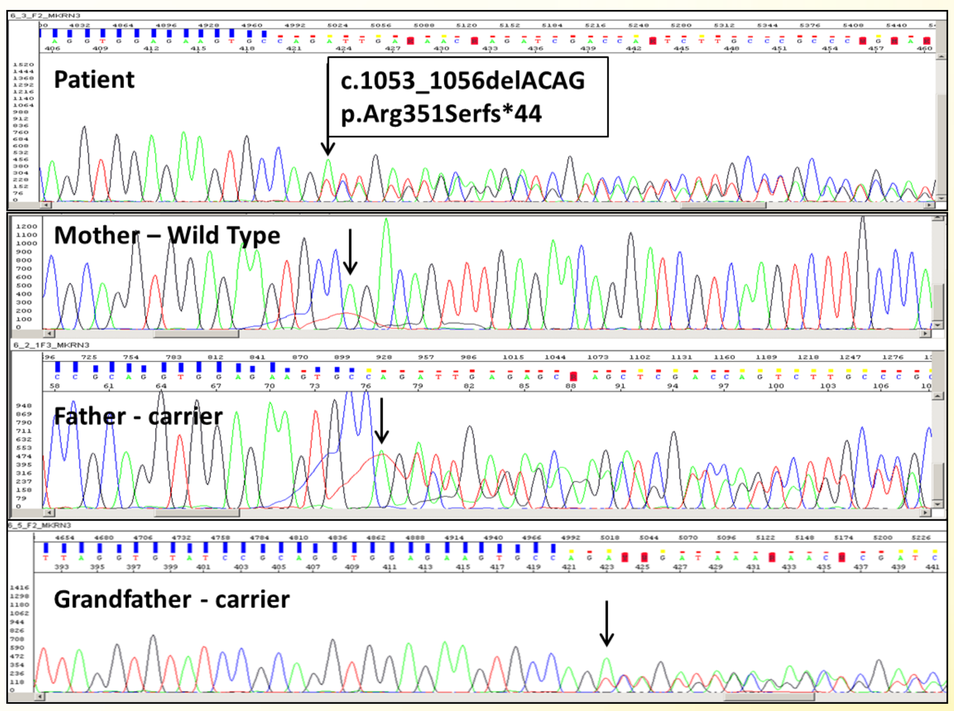


Figure 1. Arrows are showing the MKRN3 frameshift mutation p.Arg351Serfs*44 in the patient, her father and grandfather.

**Conclusion:** We report a novel mutation in gene MKRN3 (p.Arg351Serfs*44) with a deleterious effect in a girls with CPP. Although paternally inherited MKRN3 mutations are responsible for CPP in females, it seems that they do not necessarily lead to precocious pubertal development in males. We assume that this is due to any of the epigenetic mechanisms involved in gene expression control, particularly in this 15q11-q13.3 critical region.

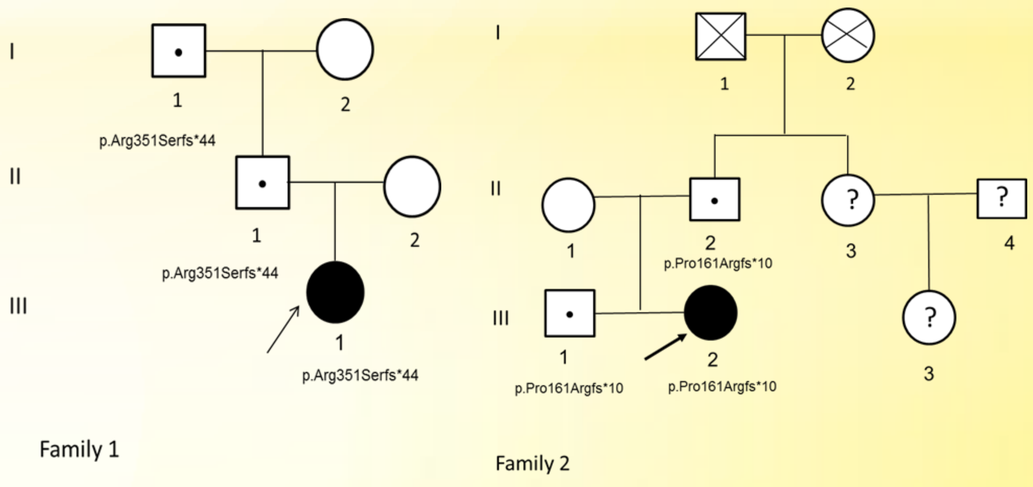


**Figure 2.** Pedigrees of the families with identified mutations in the *MKRN3* gene. Squares indicate male family members. Circles - females. Symbols with black circles - asymptomatic carriers, symbols with an X indicate deceased family members, symbols with a question mark - family members with unknown phenotype. The arrow indicates the proband.

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