A novel mutation in 5’ untranslation region of Makorin ring finger 3 gene associated with the familial precocious puberty

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Introduction and objectives

loss-of-function mutations in human MKRN3 were found to contribute to over 30% of cases of familial CPP. Here we reported a novel mutation of MKRN3 in 5’ UTR of a boy with familial CPP, and we identified that this mutation cause the reduction of serum MKRN3 which consistent with clinical manifestation. Our study not only further expand the mutational spectrum of MKRN3 but also confirm imprinted inheritance in male patients with familial CPP.

Methods

To further pinpoint the genetic basis of CPP in Chinese patients, genetic analyses were performed with a total amount of 107 individuals who manifested the defining clinical features of CPP. To further assess whether and how the disease associated mutation (-81C>T) might affect the expression of MKRN3, DNAs of the sequences derived from MKRN3 promoter of the health control or the proband were cloned into PGL3 basic luciferease reporter plasmids, resulting inPGL3-MKRN3-P1000-UTR (WT) and PGL3-MKRN3 -P1000-UTR (-81C>T), respectively.

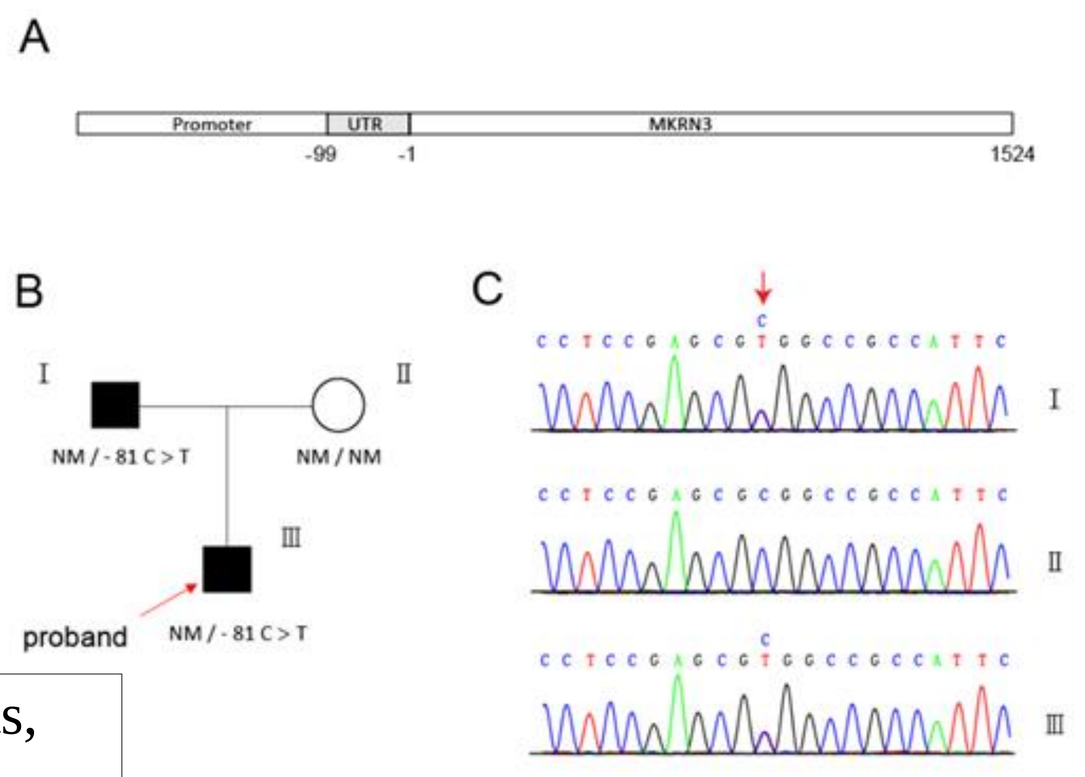
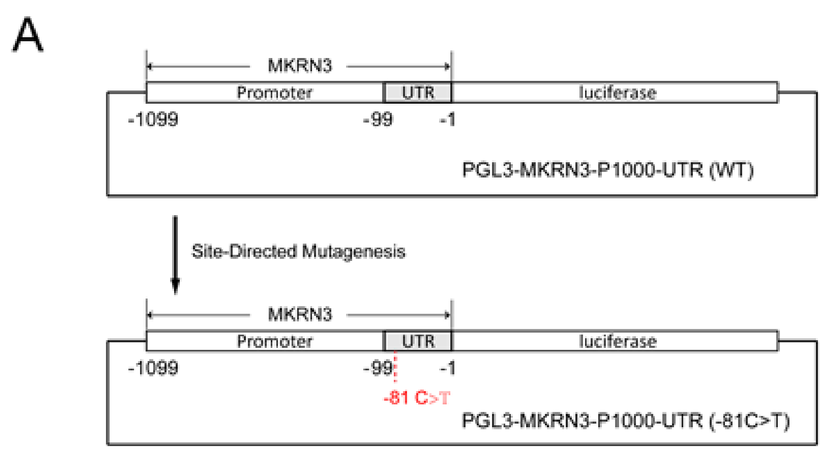


Fig1:Genetic analysis of *MKRN3* gene in a CPP patient



Results

We found a novel mutation in the 5’-UTR of MKRN3 in CPP patient , A schematic view of the human MKRN3 gene locus including promoter, 5’ UTR and the coding region. Pedigree of a family with a novel -81C>T mutation in 5’-UTRof MKRN3 gene. Partial sequencing chromatographs of the 5’-UTR in MKRN3 gene.
The CPP-associated -81C>T mutation in 5’-UTR of MKRN3 compromises the expression of MKRN3 ,The strategy for constructing the luciferase reporters to determine the function of MKRN3 5’-UTR. Luciferase assays indicated that the -81C>T mutation in 5’-UTR compromised the expression of luciferase mRNA. Cells were co-transfected with the firefly luciferase reporters and the references at indicated amounts to eliminate the potential influence of dose-dependent effect of plasmid transfection. ELISA assays showed that serum level of MKRN3 protein was significantly lower in the proband that those in the nine age-matched control.

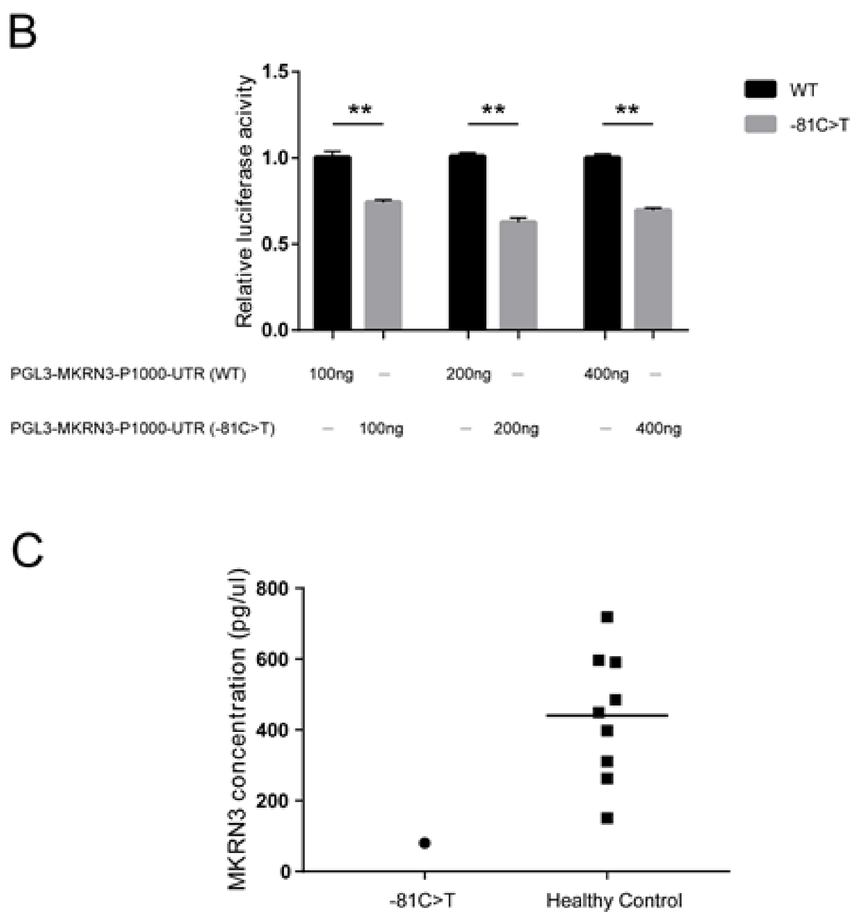


Fig2:Assessing the functional consequences of the disease-associated mutation in 5’ UTR of *MKRN3*

Conclusions

Our findings have revealed a novel mutation in *MKRN3*, which added a yet another region to check in the spectrum of mutations when studying the genetics of CPP. In the meantime, our work has led to the identification of a critical role of the 5’-UTR region in regulating the expression of *MKRN3* gene.