IDENTIFICATION AND FUNCTIONAL STUDY OF A HETEROZYGOUS MISSENSE MUTATION IN UMOD GENE IN AN ITALIAN FAMILY AFFECTED BY MEDULLARY CYSTIC KIDNEY DISEASE TYPE II.

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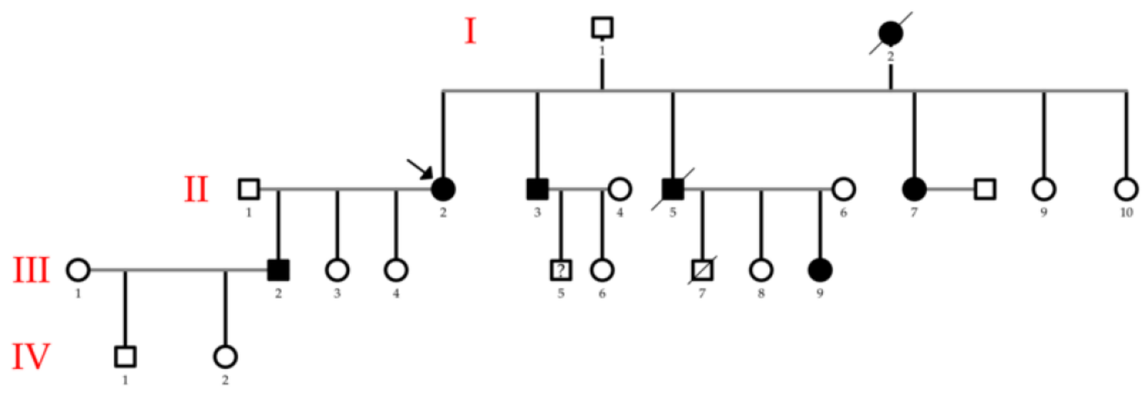
BACKGROUND AND AIM

The medullary cystic kidney disease type II (MCKD2) is an autosomal dominant disorder characterized by early onset of hyperuricemia, decreased fractional renal urate excretion and progressive interstitial nephropathy leading to end-stage renal disease. MCKD2 is caused by mutations in the UMOD gene, which encodes Uromodulin, a glycosylphosphatidylinositol-anchored protein that is expressed in the thick ascending limb of the loop of Henle and excreted in the urine. Uromodulin contains three epidermal growth factor (EGF)-like domains, a cysteine-rich region which includes a domain of eight cysteines and a zona pellucida (ZP) domain. Over 90% of UMOD mutations are missense, and 62% alter a cysteine residue, implicating a role for protein misfolding in the disease. In this study we investigated the presence of mutations in UMOD gene in four members of Italian family showing the phenotype of MCKD2.

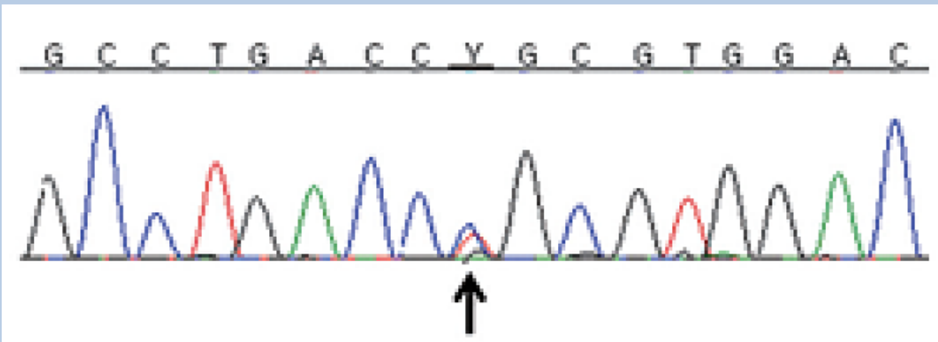
MATERIALS AND METHODS

Clinical data, blood samples of 4 affected members were collected. Genomic DNA was extracted from peripheral blood leukocytes by using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA), following the manufacturer's instructions. Mutation analysis of the UMOD gene was performed by polymerase chain reaction and direct sequencing. A mutant UMOD construct, containing the identify mutation was created by in vitro mutagenesis. Transient transfection studies were performed in human embryonic kidney cells. Expression was evaluated by reverse transcription polymerase chain reaction (RT-PCR), western blot and immunofluorescence.

A GENEALOGICAL TREE

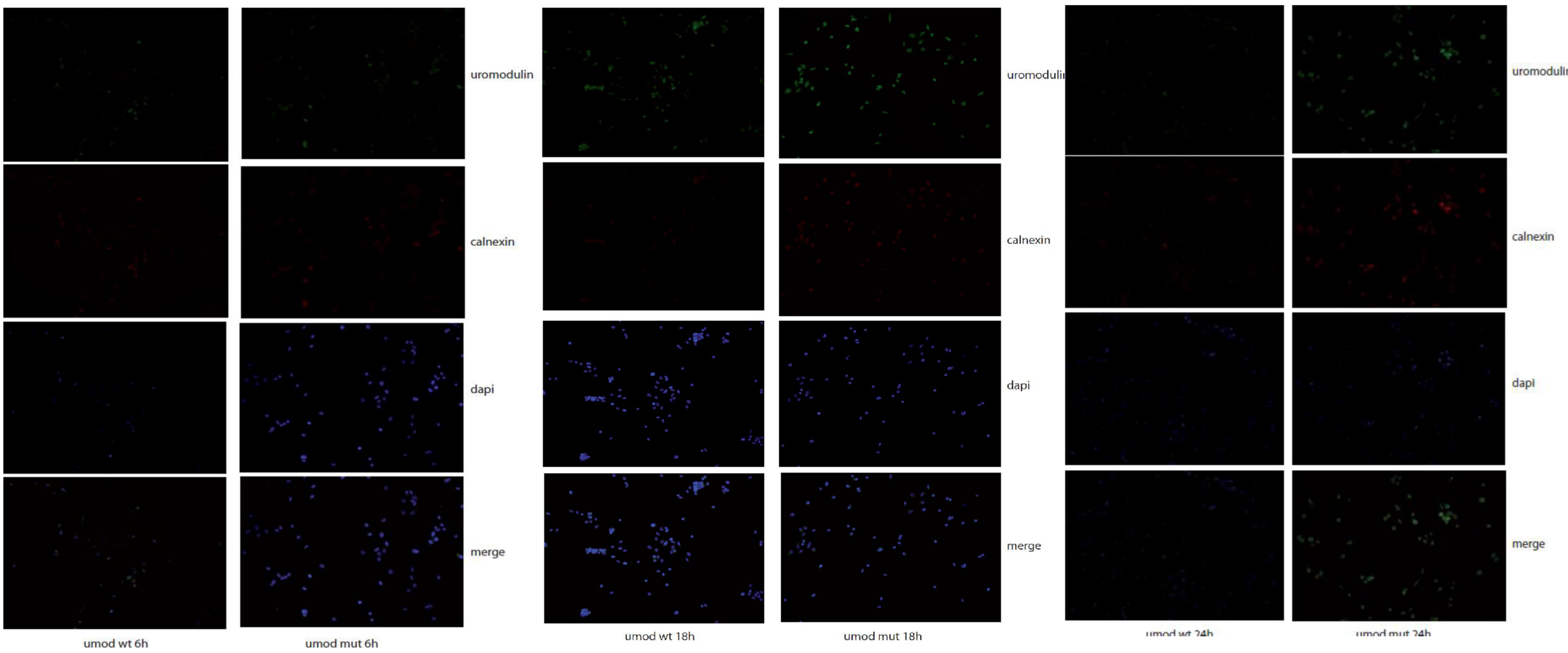


B



c.187T>C; p.Cys63Arg

C



RESULTS

(**B**) Sequence analysis revealed a heterozygous missense variation (c.187T>C; p.Cys63Arg) in exon 3 that altered an evolutionary conserved residue in the UMOD. (**C**) Functional studies showed that the mutant protein was retained in the endoplasmic reticulum and was not excreted to the cell medium, as opposed to the wild-type protein.

CONCLUSIONS

Collectively, our results suggested that the variant may be the causative mutation in this family. In addition, approximately two-thirds of known mutations lead to an alteration of a cysteine residue of uromodulin, and all such variants have been found to cause UMOD-associated kidney disease.

REFERENCES

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