Novel p.K374E variant of *CPA1* causes misfolding-induced hereditary pancreatitis with autosomal dominant inheritance

We read the publication written by Lin *et al*[1] with great interest. The authors suggest that the majority of functionally defective carboxypeptidase A1 (*CPA1)* mutations can elicit reduced expression due to nonsense-mediated decay (NMD) and therefore, only a small subset of the earlier reported *CPA1* variants[2] will predispose to chronic pancreatitis (CP) via the misfolding-dependent pathway.[3] This paper seemingly offered an explanation for the earlier finding of Wu *et al*[4] where the authors reported no association with CP of rare functionally defective *CPA1* variants in a large Chinese cohort.

However, it was earlier reported that functionally impaired variants of the *CPA1* gene were strongly associated with sporadic cases of non-alcoholic, early-onset CP in European, Indian and Japanese populations.[5] In addition, members of two Polish families carrying the p.S282P variant of the *CPA1* gene developed hereditary CP.[6] Protein misfolding and consequent endoplasmic reticulum (ER) stress were described as a potential disease mechanism of hereditary pancreatitis caused by loss-of-function variants of *CPA1* and *PRSS1* genes.[3 6 7] Recently, the finding that a knock-in mouse model carrying the misfolding-causing p.N256K *CPA1* variant developed CP confirmed the significance of the misfolding-dependent pathway in the disease mechanism[8] and revealed a novel opportunity for *in vivo* investigation of pancreatitis.[9]

As independent confirmation of the significance of the misfolding-dependent pathway, we report here the discovery of a novel missense variant c.1120A>G (p.K374E) in the *CPA1* gene associated with hereditary pancreatitis in a family of US origin.

The index patient is a 16-year-old female patient with idiopathic acute recurrent pancreatitis (ARP). She developed the first attack of acute pancreatitis (AP) at the age of 15 with a total of three documented attacks. Her brother developed the first episode of AP at the age of 12 with a total of three documented attacks. The index patient's mother was diagnosed with diabetes in her late 20s and had a single episode of AP aged 40. Next-generation sequencing of the known disease-associated genes (*CASR, CEL, CFTR, CLDN2, CPA1, CTRC, PRSS1, SBDS, SPINK1* and *UBR1*) was carried out in the index patient, and the novel variant c.1120A>G (p.K374E) in exon 10 of the *CPA1* gene was found in heterozygous form. No other pathogenic variant was found in the index patient. Targeted analyses for the presence of *CPA1* p.K374E variant were performed in all family members. Each family member who developed pancreatitis carried the p.K374E variant of the *CPA1* gene in heterozygous form (figure 1). The index patient's older sister also carried the novel variant but without any episode of AP. No smoking and/or alcohol consumption was reported as a

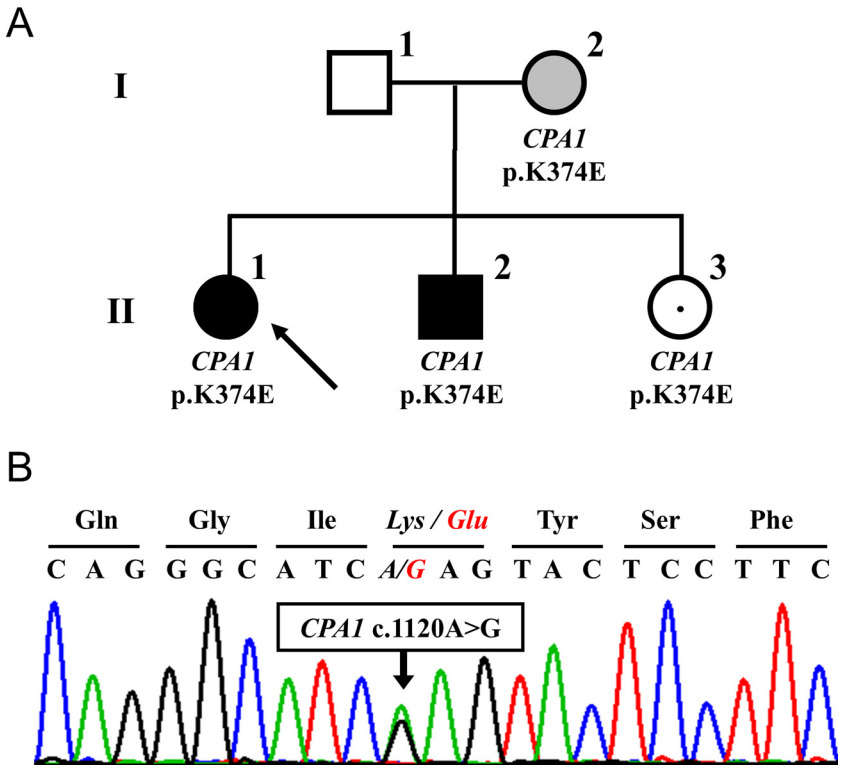

Figure 1 (A) Pedigree of a family with hereditary pancreatitis associated with the *CPA1* c.1120A>G (p.K374E) variant. The arrow points to the index patient with acute recurrent pancreatitis. Solid black symbols indicate affected family members with acute recurrent pancreatitis; the solid grey symbol indicates the subject with one acute pancreatitis attack. The open symbol with a dot designates unaffected carrier. (B) Electropherogram of a heterozygous carrier of the c.1120A>G *CPA1* variant.

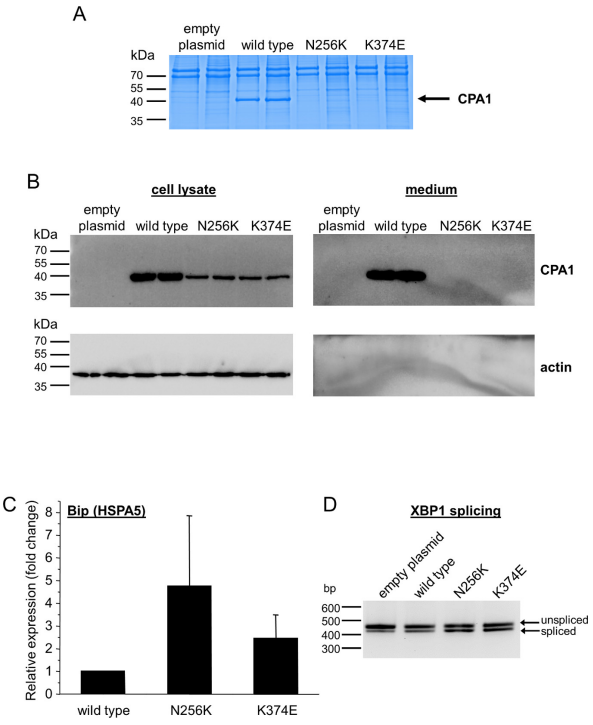

Figure 2 Effect of the p.Asn256Lys (N256K) and p.Lys374Glu (K374E) variants on CPA1 secretion, intracellular CPA1 levels and endoplasmic reticulum (ER) stress markers in transiently transfected HEK 293T cells. (A) Secretion of CPA1. Duplicate samples of 400 µL growth medium were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Blue staining. (B) Intracellular and secreted levels of CPA1 were evaluated by western blotting. Twenty micrograms total cell lysate protein and 2 µL of 2 mL conditioned media were loaded in duplicates. Two gels of cell lysate and two gels of growth medium were run with the same set of samples. The membranes were probed separately for CPA1 and actin. (C) Expression of BiP (HSPA5) mRNA was measured by quantitative reverse transcription (RT) PCR. Average of three independent experiments with SD is shown. (D) RT-PCR of XBP1 splicing was visualised by 2% agarose gel electrophoresis with ethidium bromide staining. A representative gel of three independent experiments is shown. For further experimental details see Witt *et al*.[5]

potential aetiology for ARP in any family member.

Functional analysis of the *CPA1* p.K374E variant was carried out in transiently transfected HEK293T cells as described elsewhere.[5] Our in vitro experimental findings were summarised in figure 2. Briefly, by using sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blot analysis of the cell lysate and growth medium, we detected significant secretion defect of the novel *CPA1* variant. The p.K374E and p.N256K variants caused elevation of ER stress markers compared with the wild-type CPA1 in transiently transfected HEK293T cells measured by reverse transcription PCR.

In summary, we identified and functionally characterised the novel c.1120A>G (p.K374E) variant in the *CPA1* gene. We confirmed that ER stress-related missense *CPA1* variants can be responsible for hereditary pancreatitis. Therefore, without disputing the role of NMD in cases of non-sense *CPA1* variants, our results underline the significance of the misfolding-dependent pathway in the pathogenesis of pancreatitis.

Balázs Csaba Németh,[1,2] Anna Orekhova,[3] Wenying Zhang,[4,5] Shannon A Nortman,[5] Tyler Thompson,[6] Péter Hegyi,[2,7] Maisam Abu-El-Haija[4,6]

[1]First Department of Medicine, University of Szeged, Szeged, Hungary
[2]Momentum Gastroenterology Multidisciplinary Research Group, Hungarian Academy of Sciences and University of Szeged, Szeged, Hungary
[3]Department of Molecular and Cell Biology, Henry M Goldman School of Dental Medicine, Boston University, Boston, Massachusetts, USA
[4]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
[5]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[6]Division of Pediatric Gastroenterology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[7]Institute for Translational Medicine and First Department of Medicine, Medical School, University of Pécs, Pécs, Hungary

Correspondence to Dr Balázs Csaba Németh, University of Szeged First Department of Medicine, Szeged, Korányi fasor 8-10., H-6720, Hungary; nemeth.balazs@med.u-szeged.hu

Acknowledgements The authors acknowledge the help of Professor Miklós Sahin-Tóth for providing laboratory space for functional analysis, helpful discussions and advice.

Contributors Study concept and design: BCN and MAEH. Acquisition, analysis or interpretation of data: all authors. Drafting of the manuscript: all authors. Critical revision of the manuscript for important intellectual content: all authors. Obtained funding: BCN, PH, MAEH, and funds by the Molecular Genetics Laboratory at Cincinnati Children's Hospital Medical Center. Administrative, technical or material support: all authors. Study supervision: BCN and MAEH. Final approval of manuscript as submitted: all authors. Guarantors of the article: BCN and MAEH.

Funding Hungarian National Research, Development and Innovation Fund (FK124632 to BCN and K116634 to PH); Momentum Grant of the Hungarian Academy of Sciences (LP2014-10/2014 to PH); Economic Development and Innovation Operative Programme Grant of the National Research, Development and Innovation Office (GINOP 2.3.2-15-2016-00048 to PH); National Institutes of Health (R43 DK 105640-01 to MAEH).

Disclaimer The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval This study was reviewed and approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board (IRB number 2018-7699).

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Németh BC, Orekhova A, Zhang W, *et al*. *Gut* 2020;**69**:790–792.

Received 21 March 2019
Revised 2 April 2019
Accepted 4 April 2019
Published Online First 20 April 2019

Gut 2020;**69**:790–792. doi:10.1136/gutjnl-2019-318751

REFERENCES

1 Lin JH, Boulling A, Masson E, *et al*. Most unambiguous loss-of-function *CPA1* mutations are unlikely to predispose to chronic pancreatitis. *Gut* 2020;69:784–5.
2 Németh BC, Sahin-Tóth M. Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G466–73.
3 Sahin-Tóth M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. *Curr Opin Gastroenterol* 2017;33:390–5.
4 Wu H, Zhou DZ, Berki D, *et al*. No significant enrichment of rare functionally defective CPA1 variants in a large Chinese idiopathic chronic pancreatitis cohort. *Hum Mutat* 2017;38:959–63.
5 Witt H, Beer S, Rosendahl J, *et al*. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat Genet* 2013;45:1216–20.
6 Kujko AA, Berki DM, Oracz G, *et al*. A novel p.Ser282Pro *CPA1* variant is associated with autosomal dominant hereditary pancreatitis. *Gut* 2017;66:1728–30.

7 Németh BC, Patai ÁV, Sahin-Tóth M, *et al*. Misfolding cationic trypsinogen variant p.L104P causes hereditary pancreatitis. *Gut* 2017;66:1727–8.
8 Hegyi E, Sahin-Tóth M. Human *CPA1* mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. *Gut* 2019;68:301–12.
9 Rosendahl J. Unfolding the mystery of digestive enzyme misfolding in chronic pancreatitis. *Gut* 2019;68:181–2.