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A novel p.Ser282Pro *CPA1* variant is associated with autosomal dominant hereditary pancreatitis

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To the Editor

We read with great interest the recent publication by Németh et al. [1] in which the authors demonstrate that a misfolding human cationic trypsinogen (PRSS1) variant causes autosomal dominant hereditary chronic pancreatitis (CP) by inducing endoplasmic reticulum (ER) stress. Previously, the same mechanism was proposed for carboxypeptidase A1 (*CPA1*) gene variants strongly associated with early onset sporadic CP [2], however, association of misfolding CPA1 variants with familial or hereditary CP has not been demonstrated so far. Here, we report two Polish families with hereditary CP carrying a novel heterozygous c. 844T>C (p.Ser282Pro) *CPA1* variant. In Family 1, the index patient, her mother and uncle (half-brother of the mother) developed CP (Figure 1). The age of diagnosis was 17 years for the index patient and 51 and 31 years (with earlier abdominal pain) for the mother and the uncle, respectively. In Family 2 three members were affected by CP (Figure 1): the index patient, her mother and her father with ages of onset of 12, 32 and 34 years, respectively. In both families the diagnosis of CP was confirmed by imaging methods. CP risk factors such as alcohol abuse, smoking, injury, anatomical defects, metabolic and bile duct disorders were excluded.

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Each person gave informed consent for genetic testing. All exons of *CPA1*, *PRSS1*, *SPINK1*, *CTRC* and exons 4, 9, 10 and 11 of *CFTR* were analyzed by Sanger sequencing in the index patients from both families. Large unbalanced rearrangements in *PRSS1* and *SPINK1* were excluded by Multiplex Ligation-dependent Probe Amplification (MRC Holland). In the rest of the affected and unaffected family members sequencing of exon 8 of *CPA1* and exon 3 of *CTRC* was performed. In Family 1, all three affected individuals and the unaffected grandfather of the index patient (age 83 years) were heterozygous for the p.Ser282Pro *CPA1* variant (Figure 1). All other unaffected individuals available for testing did not carry the *CPA1* variant. In Family 2, the index patient inherited the p.Ser282Pro *CPA1* variant from the affected mother and the heterozygous c.180C>T (p.Gly60=) *CTRC* variant from the affected father who was negative for the *CPA1* variant (Figure 1). The heterozygous p.Gly60= *CTRC* variant is a weak CP risk factor increasing the disease risk about 2-fold. No other pathogenic variants were identified in the susceptibility genes tested in the index patients of the two families.

The functional effects of the p.Ser282Pro *CPA1* variant were compared to those of the pathogenic variant p.Asn256Lys previously shown to cause misfolding and ER stress [2]. HEK 293T cells transfected with wild-type and mutant CPA1 constructs only secreted the wild type proenzyme (Figure 2A), while both mutants were retained intracellularly and suffered degradation, indicative of misfolding (Figure 2B). Importantly, variants p.Ser282Pro and p.Asn256Lys induced ER stress to a comparable extent as judged by elevated mRNA levels of the chaperone BiP and increased splicing of the *XBPI* mRNA relative to wild type (Figure 2C).

In conclusion, we demonstrated that the novel p.Ser282Pro CPA1 variant causes hereditary CP by inducing ER stress in a similar manner to the previously reported misfolding p.Leu104Pro PRSS1 variant. The observations indicate that misfolding PRSS1 and CPA1 variants are similarly strong risk factors and ER stress is a highly relevant pathological mechanism in CP as suggested by Németh et al. and Witt et al. [1, 2].

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Figure 1.

Pedigrees of Polish families with hereditary chronic pancreatitis associated with the p.Ser282Pro *CPA1* variant. The arrow points to the index patient. Solid black symbols indicate family members with CP. Open symbol with a dot designates unaffected carrier. Crossed symbol indicates deceased family member. Nd, genotype not determined. Known carriers of the p.Gly60= *CTSC* variant are also indicated.

Figure 2.

Effect of the p.Ser282Pro (S282P) and p.Asn256Lys (N256K) variants on CPA1 secretion, intracellular CPA1 levels and ER stress markers in transfected HEK 293T cells. **(A)** Secretion of CPA1 to the growth medium was analyzed by SDS-PAGE and Coomassie Blue staining. Samples were loaded in duplicates. CPA1 activity in the conditioned medium is also indicated in milliOD/min units. **(B)** Intracellular and secreted levels of CPA1 were evaluated by western blotting. Twenty µg total cell lysate protein and 4 µL of 2mL conditioned media were loaded in duplicates. **(C)** Expression of BiP mRNA was measured by quantitative reverse-transcription (RT) PCR; levels of spliced XBP1 (sXBP1) was assessed by RT-PCR, agarose gel electrophoresis and densitometry of the spliced and unspliced bands. Average of three experiments with standard deviation is shown. For experimental details see Witt et al. [2].