Mutations of Gly277 in *CPA1*-related chronic pancreatitis: clinical clues for misfolding aetiology

We have been elated to see the recent flurry of publications by *Gut* on chronic pancreatitis (CP) associated with mutations of the pancreatic digestive enzyme carboxypeptidase A1 (CPA1).[1–4] These studies confirmed that *CPA1* gene variants are strong risk factors for CP and exert their pathogenic effect through promoting enzyme misfolding and endoplasmic reticulum (ER) stress, although the exact mechanism of pancreatic inflammation has remained unclear. Through the functional analysis of 63 *CPA1* variants in cell culture studies, we previously established that pathogenic variants caused diminished secretion (<10% wild type) and markedly upregulated heat shock protein family A member 5 (*HSPA5*, encoding the immunoglobulin heavy chain binding protein, BiP) mRNA expression, a marker of ER stress, as exemplified by the pathogenic reference variant p.Asn256Lys.[1 4] The *CPA1 N256K* mouse model carrying the p.Asn256Lys mutation in the native mouse *Cpa1* gene developed progressive CP with acinar atrophy, fibrosis, macrophage infiltration, acinar-to-ductal metaplasia and plasma amylase elevation.[5 6] Despite the increased mechanistic understanding of the pathogenic role of *CPA1* variants, specific clinical characteristics of misfolding-induced CP have not been reported to date.

In this letter, we describe three new paediatric cases carrying mutations that affect amino-acid Gly277 that lies in proximity to a misfolding-prone region of the protein.[7] One patient carried a novel heterozygous c.830G>C (p.Gly277Ala) variant, and two siblings carried the heterozygous c.829G>A (p.Gly277Ser) variant, which was reported previously in two cases.[4 8] We performed functional analysis using transfected HEK 293T cells according to our standard protocol.[1 4 7] We found that variants p.Gly277Ala and p.Gly277Ser both exhibited the same misfolding phenotype as the pathogenic reference variant p.Asn256Lys, characterised by loss of secretion (figure 1A), intracellular retention (figure 1B) and elevated BiP mRNA levels (figure 1C), indicating strong pathogenic potential.

Importantly, these three CP cases with uniquely homogeneous genetic background provided potential clues for the distinguishing clinical features of misfolding-associated CP (table 1). Thus, all cases were paediatric, with familial aggregation in case of the two siblings, consistent with prior reports that pathogenic *CPA1* variants cause early onset and hereditary CP.[7–10] The patients had imaging-confirmed CP with chronic pain, with only one or no episodes of acute pancreatitis requiring hospitalisation.

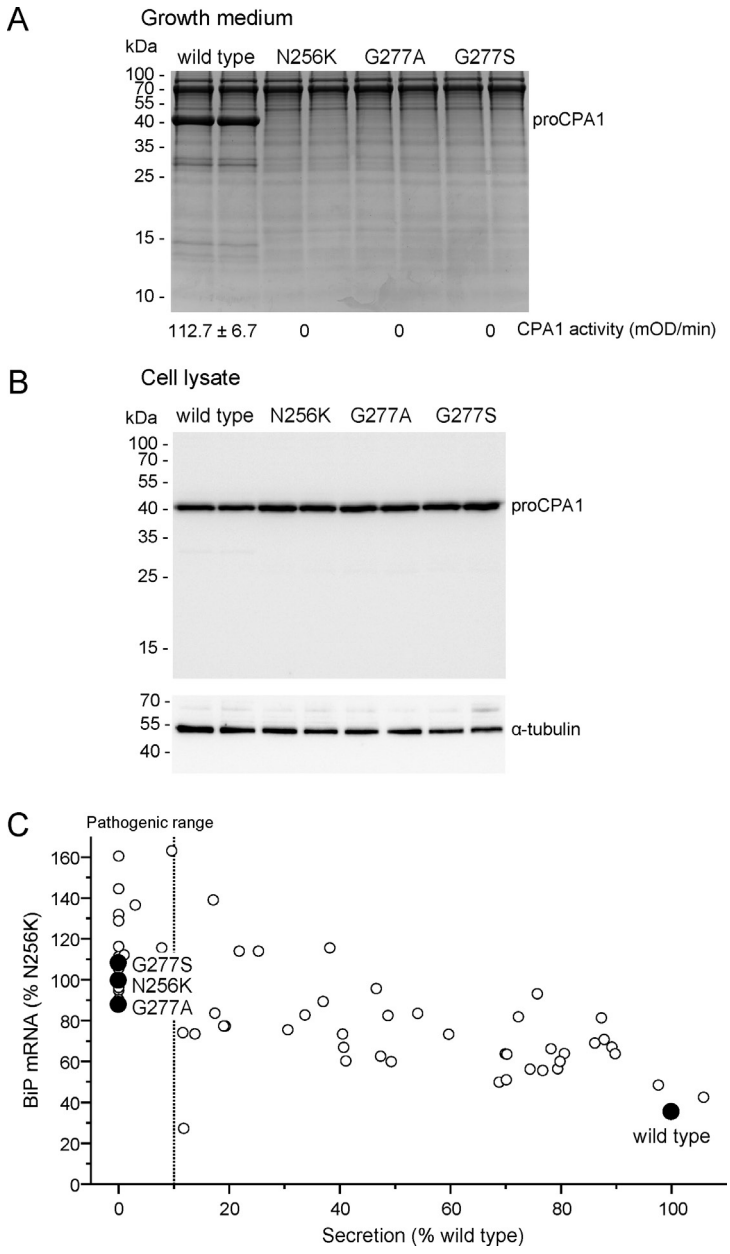

Figure 1 Effect of *CPA1* variants p.Gly277Ala (G277A), p.Gly277Ser (G277S) and p.Asn256Lys (N256K) on proCPA1 secretion and BiP mRNA expression in transiently transfected HEK 293T cells. Transfections (n=6) and measurements were carried out as described previously.[7] Conditioned media and cells were harvested after 48 hours. (A) Conditioned media were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and Coomassie Blue staining. A representative gel is shown. CPA1 enzyme activity in the conditioned medium was measured after activation with trypsin and chymotrypsin C. (B) Western blot analysis of cell lysates. As loading control, α-tubulin was measured. A representative blot is shown. (C) Total RNA was isolated from cells, BiP mRNA levels were measured by reverse-transcription quantitative PCR, expressed as % of the pathogenic reference variant p.Asn256Lys (N256K) and graphed as a function of secretion.[1 4] The mean±SD (n=6) values were wild type 35.2±8%, p.Asn256Lys 100±11.3%, p.Gly277Ala 88.8±20.4%, p.Gly277Ser 110.5±37.2%. The smaller, empty symbols represent the missense *CPA1* variants analysed previously, which are shown for comparison.[1 4] BiP, immunoglobulin heavy chain binding protein; *CPA1*, carboxypeptidase A1; proCPA1, procarboxypeptidase A1; mOD, milliOD; OD, optical density.

Table 1 Clinical characteristics of patients with *CPA1* variants p.Gly277Ala and p.Gly277Ser

Patients	1	2	3
CPA1 variant	p.Gly277Ala	p.Gly277Ser	p.Gly277Ser
Gender	Male	Male	Female
Current diagnosis	CP	CP	CP
Age of first AP	<10 years	early 10s	early 10s
AP episodes requiring hospitalisation	None	1	None
Pain	Chronic	Chronic	Chronic
Persistent lipase/amylase elevation	Yes	Yes	Yes
Family history (pancreatitis)	No	Sibling	Sibling
EPI	Yes	Yes	Yes
Endocrine insufficiency	No	No	No
Other risk factors	No	Pancreas divisum	No
Type 3C diabetes	No	No	No

Patients 2 and 3 are siblings. CP was diagnosed based on MRI (patient 1) and endoscopic ultrasound (patients 2 and 3) findings. EPI was diagnosed based on faecal elastase (patient 1) and endoscopic pancreatic function (patients 2 and 3) tests.
AP, acute pancreatitis; CP, chronic pancreatitis; *CPA1*, carboxypeptidase A1; EPI, exocrine pancreatic insufficiency.

Remarkably, all cases had demonstrable exocrine pancreatic insufficiency, which, besides parenchymal atrophy, might also indicate a functional acinar cell defect elicited by the misfolding *CPA1* variants. Finally, persistently elevated serum lipase and/or amylase levels were observed, even at baseline, some of which did not exceed the three times upper normal limit. The scarcity of acute pancreatitis episodes and the persistent serum enzyme elevations in these clinical cases are reminiscent of the *CPA1 N256K* mouse model phenotype, where progressive CP with elevated plasma amylase was evident without detectable acute episodes.[5 6] In contrast to trypsin-dependent CP associated with *PRSS1* or *SPINK1* mutations, the clinical characteristics of misfolding-induced CP have not been described to date. Our observations with this small cohort of *CPA1*-related paediatric CP point to potentially unique differences in disease presentation which may be leveraged in clinical management.

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REFERENCES

1 Sándor M, Sahin-Tóth M. Functional predictors of pathogenicity of missense *CPA1* variants in chronic pancreatitis. *Gut* 2024;73:1589–90.
2 Wang Y-H, Masson E, Liao Z, *et al*. Strengths and limitations of AlphaMissense in *CPA1* missense variant classification. *Gut* 2024;73:e42.
3 Shen F, Zhao H, Deng M, *et al*. Familial pancreatitis associated with a splice-site variant in *CPA1* *Gut* 2025;74:325–7.
4 Sándor M, Scheers I, Masamune A, *et al*. AlphaMissense versus laboratory-based pathogenicity prediction of 13 novel missense *CPA1* variants from pancreatitis cases. *Gut* 2025;74:678–9.
5 Hegyi E, Sahin-Tóth M. Human *CPA1* mutation causes digestive enzyme misfolding and chronic pancreatitis in mice. *Gut* 2019;68:301–12.
6 Wilhelm SJ, Curry GE, Matiwala N, *et al*. Diverse Misfolding Mutant Digestive Enzymes Cause Chronic Pancreatitis Through Common Pathways. *Cell Mol Gastroenterol Hepatol* 2025;20:101638.
7 Sándor M, Thiel FG, Schmid M, *et al*. Novel p.G250A Mutation Associated with Chronic Pancreatitis Highlights Misfolding-Prone Region in Carboxypeptidase A1 (CPA1). *Int J Mol Sci* 2022;23:15463.
8 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.
9 Németh BC, Orekhova A, Zhang W, *et al*. Novel p.K374E variant of *CPA1* causes misfolding-induced hereditary pancreatitis with autosomal dominant inheritance. *Gut* 2020;69:790–2.
10 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.