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Gastric Biopsies During G-POEM in Patients with Refractory Gastroparesis: Histological, Transcriptomics and Meta-Transcriptomics Analysis, And Correlation with Clinical Outcomes

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ABSTRACT

Introduction: Gastroparesis (GP) is a chronic gastric motility disorder that is often underdiagnosed and underestimated, with major impact on quality of life. The underlying tissue mechanisms remain poorly defined. G-POEM is an emerging endoscopic therapy for refractory GP, but robust predictors of response are lacking. This study aimed to characterize gastric transcriptomic and metatranscriptomic profiles in patients with refractory idiopathic or diabetic GP.

Methods: In this single-center prospective study, we enrolled adults with refractory idiopathic or diabetic GP undergoing G-POEM (clinical cohort). All patients underwent standardized additional mucosal gastric biopsies from antrum, body, and fundus. Clinical success at 3 months after G-POEM was defined as a ≥1.0-point reduction in total GCSI and ≥25% improvement in at least one GCSI subscale.

A nested molecular cohort (9 GP patiens) was selected for RNA sequencing. A control group of patients without GP symptoms undergoing EGD for non–motility-related indications underwent the same biopsy protocol; 9 controls were age- (±3 years) and sex-matched to GP cases. Host transcriptomic and microbial meta-transcriptomic data were analyzed using differential expression, GSEA, UMAP, cell-type deconvolution, microbial alpha-diversity, and species-level differential analyses.

Results: Between February 2023 and June 2025, 21 refractory GP patients (57% idiopathic, 43% diabetic; median age 52 years, 71.4% female) undergoing G-POEM and 16 controls were enrolled. Clinical and functional success after G-POEM were 47.6% and 42.9%, respectively. G-POEM significantly improved GCSI, SF-36, and GES parameters.
In the molecular cohort, a marked and region-specific transcriptional shift was observed in the antrum of GP patients (1,793 genes upregulated and 1,006 downregulated vs controls). Pathway enrichment in the antrum revealed upregulation of programs related to tissue remodeling, cell migration and adhesion, vascular regulation and neuromuscular organization. Microbial alpha-diversity was preserved across groups and regions, although a distinct, region-specific shift in bacterial community composition at the species level was observed in the antrum of GP patients.

Conclusion: In refractory GP, G-POEM provides a moderate short-term clinical and functional benefit. GP patients exhibit a distinct, antral-specific transcriptomic reprogramming together with localized alterations in bacterial community composition. These findings support the concept of a regionally confined, functionally oriented molecular phenotype in GP, which may contribute to disease mechanisms and help inform future, more personalized therapeutic strategies.

ABSTRACT – Versione italiana

Introduzione: La gastroparesi (GP) è un disturbo cronico della motilità gastrica, spesso sottodiagnosticato, con un impatto rilevante sulla qualità di vita. I meccanismi tissutali alla base della malattia sono ancora poco definiti. La G-POEM è una nuova opzione terapeutica endoscopica per la GP refrattaria, ma mancano predittori affidabili di risposta. Questo studio ha avuto l'obiettivo di caratterizzare i profili trascrittomici e metatrascrittomici gastrici in pazienti con GP idiopatica o diabetica refrattari.

Metodi: In questo studio prospettico monocentrico sono stati arruolati pazienti con GP idiopatica o diabetica refrattaria sottoposti a G-POEM (coorte clinica). Sono state eseguite biopsie gastriche aggiuntive in antro, corpo e fondo. Sono stati raccolti sintomi (GCSI, SF-36), scintigrafia gastrica (GES) ed esiti della G-POEM. Il successo clinico a 3 mesi è stato definito come una riduzione ≥1 punto del GCSI totale e un miglioramento ≥25% in almeno un sottogruppo.
Una sottocoorte molecolare (9 pazienti con GP) è stata selezionata per il sequenziamento dell'RNA. È stato incluso un gruppo di controllo, sottoposto a EGD per altre indicazioni e sottoposto allo stesso protocollo di biopsie; 9 controlli sono stati appaiati per età (±3 anni) e sesso. I dati trascrittomici e metatrascrittomici sono stati analizzati mediante analisi di espressione differenziale, GSEA, UMAP, deconvoluzione dei tipi cellulari, alfa-diversità microbica e analisi differenziale a livello di specie.

Risultati: Tra febbraio 2023 e giugno 2025 sono stati arruolati 21 pazienti con GP refrattaria (57% idiopatica, 43% diabetica; età mediana 52 anni; 71,4% donne) sottoposti a G-POEM e 16 controlli. Il successo clinico e funzionale dopo G-POEM è stato rispettivamente del 47,6% e del 42,9%, con un miglioramento significativo di GCSI, SF-36 e dei parametri alla scintigrafia di svuotamento gastrico. Nella sottocoorte molecolare è emerso un marcato e specifico rimodellamento trascrittomico confinato all'antro, con sovra- e sotto-espressione di numerosi geni rispetto ai controlli. L'analisi funzionale ha evidenziato l'attivazione di vie coinvolte nel rimodellamento tissutale, nella migrazione e adesione cellulare, nella regolazione vascolare e nell'organizzazione neuromuscolare. La diversità microbica globale è risultata conservata, ma nell'antro dei pazienti con GP è stata osservata una chiara modifica della composizione batterica.

Conclusioni: Nella GP refrattaria, la G-POEM offre un beneficio clinico e funzionale moderato a breve termine. I pazienti con GP hanno mostrato una riprogrammazione molecolare antrale specifica, a supporto di un fenotipo molecolare confinato che potrebbe contribuire ai meccanismi di malattia e orientare future strategie terapeutiche personalizzate.

SOMMARIO

GASTROPARESIS

Gastroparesis (GP) is a chronic disorder characterized by delayed gastric emptying of solid food in the absence of mechanical gastric outlet obstruction (1). The hallmark symptoms include nausea and vomiting, early satiety, postprandial fullness, bloating, and epigastric or abdominal pain/discomfort (2).

GP substantially impairs quality of life, affecting up to 40% of patients and often leading to social withdrawal and psychological distress (3). Its prevalence has been estimated at 0.24% in the United States (4) and 0.13% in the United Kingdom (5), although the condition is likely underdiagnosed because of overlapping symptoms with other functional gastrointestinal disorders, particularly functional dyspepsia (FD)(6). In addition to its clinical burden, GP also carries a significant economic impact. A recent study conducted in the United States estimated an annual economic burden of US $4,000 to $9,000 per patient (7).

Although GP has traditionally been considered idiopathic, it may result from various underlying conditions, including diabetes mellitus, myopathic and neurological disorders, and connective tissue diseases (8–10). It can also occur after medical or surgical procedures or as a post-infectious complication (11–13). Recent evidence points toward an autoimmune pathogenesis in a subset of patients, supported by findings of immune-mediated fibrosis within the muscular layers, loss of enteric neurons, and depletion of interstitial cells of Cajal (ICCs) on full-thickness gastric biopsies (14-17).

Given its multifactorial and heterogeneous nature, GP remains a therapeutic challenge. Initial management relies on dietary modifications and pharmacologic treatment with prokinetic agents such as dopamine D2 receptor antagonists (e.g., metoclopramide, domperidone) or motilin receptor agonists (e.g., erythromycin) (18,19). However, the efficacy of medical therapy is limited, and up to 40% of patients fail to achieve adequate symptom relief (20). Consequently, more invasive interventions—both surgical and endoscopic—have been explored for refractory cases. Consequently, more invasive treatment modalities, including both surgical and endoscopic approaches, have been developed for patients with refractory gastroparesis. In this context, G-POEM has recently gained attention as an innovative, minimally invasive therapeutic strategy yielding promising outcomes (21,22).

1. Epidemiology and prognosis

The global epidemiology of GP remains uncertain, largely due to the substantial symptom overlap with FD and the limited availability and inconsistent use of standardized gastric emptying tests across clinical settings (6). Upper abdominal pain, discomfort, belching, bloating, and early satiety are symptoms commonly shared by both condition (23).

A population-based study from Minnesota evaluated the age-adjusted incidence of gastroparesis over a 10-year period, reporting 2.4 cases per 100,000 person-years in men and 9.8 per 100,000 person-years in women (4). The corresponding prevalence was 9.6 per 100,000 in men and 37.8 per 100,000 in women (4). Despite these estimates, many individuals with symptoms suggestive of GP may never undergo diagnostic testing. One analysis estimated that up to 1.8% of the general population could experience symptoms compatible with GP, whereas only 0.2% receive a formal diagnosis (24). These findings indicate that the true prevalence of the disease is likely underestimated.

The impact of GP on life expectancy remains unclear. Studies conducted in referral populations suggest that delayed gastric emptying does not influence mortality in patients with diabetes mellitus, even after long-term follow-up—12 years in one study (26) 25 years in another (27). Conversely, a community-based study from Minnesota reported reduced survival among individuals with GP of mixed etiology (4). Although mortality may not be directly increased, patients with GP experience substantial morbidity, characterized by frequent hospitalizations, recurrent emergency visits, and reduced functional capacity. Many patients suffer from malnutrition, dehydration, and complications related to poor oral intake or medication intolerance, often requiring enteral or parenteral nutritional support. This chronic disease burden translates into impaired quality of life and a significant socioeconomic impact, with higher healthcare utilization compared with the general population (3,4)

2. Risk factors

The higher incidence and prevalence of GP among women are well documented, although the underlying reasons remain uncertain. It has been proposed that gastric motility involves neuronal nitric oxide synthesis, a mechanism potentially modulated by estrogen regulation (28,29). Sex-related hormonal and autonomic differences have also been suggested, although direct evidence remains limited.

Few studies have investigated the relationship between body mass and GP, and available data are inconsistent. In patients with type 2 diabetes, obesity has been associated with a higher likelihood of reporting upper gastrointestinal symptoms resembling those of GP, although objective confirmation of delayed gastric emptying was not always obtained (30). Similarly, small studies have suggested a correlation between higher body mass index (BMI) and delayed gastric emptying, but these findings have not been consistently replicated (31). Interestingly, data from the NIH Gastroparesis Consortium showed that nearly half of patients with idiopathic GP were overweight or obese (32). This observation indicates that GP does not necessarily lead to weight loss and may occur across the entire BMI spectrum.

The influence of other modifiable risk factors, such as smoking and alcohol consumption, remains uncertain. Epidemiological data show comparable rates of tobacco and alcohol use among individuals with diabetes and healthy controls (32). However, a longitudinal follow-up study of 262 patients with GP managed according to standard care in the United States found that a history of smoking was significantly associated with poorer symptom improvement over 48 weeks(33).

3. Pathophysiology

Despite significant progress in understanding the mechanisms underlying GP, substantial knowledge gaps remain (34). GP is believed to result from impaired coordination within the gastric neuromuscular apparatus, involving smooth muscle cells, ICCs, and intrinsic and extrinsic neurons (34).

To contextualize the pathophysiological mechanisms of GP, an overview of normal gastric motor physiology is first necessary.

During the gastric phase of digestion, ingested food is initially retained in the proximal stomach, or fundus, during the so-called "lag phase" (34). This period allows the stomach to relax and accommodate the incoming meal. Fundic relaxation is mediated primarily by vagal efferent signaling, modulated through feedback from vagal afferents (1,34).

Once the proximal stomach has adapted to the meal, coordinated motor activity in the distal stomach begins. In this phase, known as gastric trituration, strong antral contractions mechanically reduce solid food to particles of 1–2 mm. These fragments are mixed with gastric secretions and propelled toward the pylorus for controlled emptying into the duodenum (35,36). This process depends on coordinated vagal input and cholinergic excitation of the antral musculature, counterbalanced by intrinsic inhibitory pathways mediated by nitrergic neurons (1,34).

The rhythmic pattern of antral contractions is governed by electrical slow waves—cyclic depolarizations that determine the timing and direction of peristaltic activity. Originating in the mid-corpus, slow waves propagate distally toward the pylorus, coordinating trituration and promoting efficient gastric emptying (37,38)

Slow-wave generation and propagation rely on ICCs, which function as pacemaker cells, and on platelet-derived growth factor receptor alpha (PDGFRα)–positive fibroblast-like cells. Together with smooth muscle cells, these elements form a multicellular electrical syncytium that synchronizes contractions from the proximal to the distal stomach (39).

The pyloric sphincter, a specialized muscular zone with high resting pressure, regulates gastric outflow (40). Functional studies have identified two distinct components within the pyloric musculature: a superficial circular layer influenced by the gastric slow wave and a deeper layer controlled by enteric motor neurons that operate independently of slow-wave propagation (34,41,42). Despite anatomical continuity, gastric and duodenal electrical activities remain largely independent across the pylorus, separated by a "slow-wave-free" gap characterized by a reduced number of ICCs (42,43).

Pyloric motility is regulated by both intrinsic and extrinsic neural inputs. Intrinsically, the myenteric plexus extends across the pyloric region and mediates excitatory (acetylcholine and

substance P) and inhibitory (nitric oxide and vasoactive intestinal peptide, VIP) signaling (42-45). Extrinsically, vagal fibers exert both excitatory and inhibitory influences throughout the stomach, including modulation of nitrergic pathways, whereas sympathetic input plays a minor role (46-48).

In GP, several of these mechanisms become disrupted. Histopathologic studies have documented a reduction of ICCs in full-thickness gastric biopsies, including those from the pylorus, in patients with GP (49). The loss of ICCs, along with neuronal and smooth-muscle alterations, is thought to play a major role in delayed gastric emptying and pylorospasm (49).

Beyond pyloric dysfunction, other abnormalities have been described in GP. Vagal neuropathy, loss of enteric neurons, and degeneration of smooth muscle fibers have been demonstrated in histopathologic studies (14–17). Inflammatory and immune-mediated changes, including macrophage infiltration and a reduction of anti-inflammatory macrophage phenotypes, have also been reported (15,16). These alterations contribute to impaired fundic accommodation, uncoordinated antral contractions, and overall gastric hypomotility.

4. Etiology

GP has a heterogeneous etiology. Diabetes mellitus is the most common identifiable cause, followed by myopathic and neurological disorders, connective tissue diseases, and postsurgical vagal injury after procedures such as vagotomy, esophagectomy, bariatric surgery, or Nissen fundoplication (1–4). Idiopathic GP accounts for approximately one-third of cases and refers to patients in whom no underlying cause can be identified despite thorough clinical evaluation (1–4)

4.1 Diabetic gastroparesis

Diabetic GP is a multifactorial disorder resulting from a combination of metabolic, neural, and myogenic injury. Chronic hyperglycemia, oxidative stress, autonomic neuropathy, and damage to intrinsic enteric neurons all contribute to impaired coordination within the gastric neuromuscular unit (1). Hyperglycemia plays a contributing—but not exclusive—role, as its effects often interact with long-standing neuronal and structural alterations. These mechanisms are shared by both type 1 and type 2 diabetes, which show a similar incidence of GP (1).

Epidemiologic data suggest an association between hyperglycemia and upper gastrointestinal symptoms (50). Poor glycemic control is reported in about 36% of patients with diabetic GP hospitalized in the United States (51). Kidney and pancreas transplantation have been shown to improve gastric emptying and gastrointestinal symptoms, supporting the hypothesis that glycemic control may positively influence disease outcomes (52).

Experimental studies demonstrate that acute hyperglycemia inhibits antral contractility and delays gastric emptying in a dose-dependent manner, even at postprandial glucose levels (53). Conversely, insulin-induced hypoglycemia (around 2.6 mmol/L) accelerates gastric emptying, likely through vagal activation as a compensatory mechanism (54).

However, the impact of chronic glycemic control on gastric emptying remains uncertain. In a cohort of 129 patients, HbA1c levels were not associated with delayed gastric emptying assessed by scintigraphy (55), and similar findings have been reported in type 2 diabetes (56). Most studies indicate that improving glycemic control alone does not significantly normalize gastric emptying (56,57), except for a single uncontrolled study (58).

Further research is needed to elucidate the long-term relationship between hyperglycemia, neuronal injury, and gastric motor dysfunction in diabetic GP.

4.2 Post-surgical gastroparesis

Post-surgical GP most often results from injury or entrapment of the vagus nerve, leading to impaired fundic relaxation, antral hypomotility, and pylorospasm (1). This complication is most

observed after fundoplication or bariatric surgery. Truncal vagotomy, once a frequent cause of postoperative GP, is now rarely performed following the decline of surgical treatment for peptic ulcer disease.

Laparoscopic sleeve gastrectomy (LSG) has been associated with the development of abnormal distal pacemaker activity in the stomach; however, available studies have not demonstrated this to cause delayed gastric emptying (59). On the contrary, LSG typically accelerates gastric emptying due to reduced gastric volume and altered gastric compliance (60). Less frequent causes of post-surgical GP include older procedures such as Billroth I and II gastrectomy, which occasionally involved vagotomy (1,13). Rarely, vagal denervation during partial esophagectomy for cancer or after heart transplantation may also result in delayed gastric emptying (13).

4.3 Iatrogenic gastroparesis

Iatrogenic GP most commonly results from pharmacologic interference with gastrointestinal motility. The main agents involved are μ-opioid receptor agonists and certain antidiabetic drugs, including amylin analogs (e.g., pramlintide) and glucagon-like peptide-1 (GLP-1) receptor agonists (e.g., liraglutide, exenatide) (61). In contrast, dipeptidyl peptidase IV (DPP-IV) inhibitors, such as vildagliptin and sitagliptin, improve glycemic control without impairing gastric emptying (61). Drugs used in Parkinson's disease, such as levodopa and anticholinergics, can also contribute to iatrogenic GP (1).

Opioids—such as codeine, oxycodone, and morphine—affect gastrointestinal motility at multiple levels. They delay gastric emptying by increasing pyloric sphincter tone and suppressing antral contractility, while also reducing coordinated propulsion along the GI tract (62). At the cellular level, opioids inhibit adenylate cyclase and presynaptic Ca^{2+} channels while activating K^{+} channels, leading to decreased acetylcholine release from excitatory neurons and reduced nitric oxide and purinergic signaling from inhibitory motor neurons (63). In the stomach, these effects increase pyloric tone and phasic pressure, resulting in delayed gastric emptying and symptoms such as early satiety, postprandial fullness, and nausea (62,64,65).

The use of opioids in GP represents a clinical paradox. Although opioids are known to delay gastric emptying, they are sometimes prescribed for the management of chronic abdominal or visceral pain, which can be severe and refractory to conventional treatments. In the Temple University cohort of 223 GP patients, 19.3% were on chronic opioid therapy, yet only 8.1% used opioids specifically for GP-related pain (66,67). Chronic users exhibited worse symptoms, more frequent hospitalizations, and poorer treatment response. This suggests a bidirectional relationship: patients with more severe disease are more likely to receive opioids, while opioids themselves may further impair gastric motility and exacerbate symptoms.

4.4 Post-viral gastroparesis

Post-viral GP is an uncommon but recognized clinical entity. Although post-infectious dyspepsia has been described in the literature, evidence supporting a clear causal link between viral infection and GP remains limited. Reported viral triggers include Epstein–Barr virus, norovirus, herpesvirus, and cytomegalovirus (68). In some cases, post-viral GP occurs in the context of autonomic dysfunction—manifesting with postural hypotension or abnormal sweating—and is generally associated with a poorer prognosis (68). Conversely, when GP develops after a viral illness without features of dysautonomia, the course is usually self-limiting, with gradual resolution of symptoms over approximately one year (69,70).

However, available studies are few and heterogeneous, providing little information on the interval between infection and symptom onset, as well as on potential predisposing factors—genetic or acquired—that might increase susceptibility to post-viral GP (1).

4.5 Other etiologies

GP may also result from primary neuromuscular disorders affecting the stomach wall. These conditions can involve dysfunction of extrinsic nerves, intrinsic neurons, ICCs, or smooth muscle cells (8,9). Smooth muscle myopathies include infiltrative diseases such as scleroderma and amyloidosis, as well as degenerative or mitochondrial disorders like hollow visceral myopathy and mitochondrial cytopathy (9).

These diseases often lead to generalized gastrointestinal dysmotility involving the esophagus, lower esophageal sphincter (LES), and small intestine (8). In mitochondrial cytopathies, external ophthalmoplegia and skeletal muscle weakness may also be present. The impaired gastric emptying observed in these conditions likely reflects smooth muscle degeneration and fibrosis, which compromise contractility and coordination within the gastric wall (1).

5. Clinical signs and symptoms

GP is a chronic and often persistent condition, with only 28% of patients reporting sustained symptom improvement over time (33). The cardinal symptoms include nausea, vomiting, early satiety, postprandial fullness, bloating, abdominal pain, and visible gastric distension (71).

Symptom severity is commonly assessed using the Gastroparesis Cardinal Symptom Index (GCSI), which evaluates three subscales: nausea/vomiting, fullness/early satiety, and bloating/distension (72,73).

Approximately 15% of GP cases have a sudden onset of symptoms (74). Among all manifestations, nausea is the most frequently reported, affecting over 95% of patients with suspected GP (75). Its underlying mechanisms remain poorly understood. Functional MRI studies have demonstrated altered functional connectivity within the insula, suggesting a role for central nervous system pathways in the modulation of nausea and visceral perception (76).

Abdominal pain is another prominent feature, reported in up to 22% of patients. Those with significant pain often exhibit higher levels of somatization, depression, and anxiety (77). Pain management is challenging, as opioid use—reported in nearly 60% of patients with GP—is associated with worsened symptoms, delayed gastric emptying, and impaired quality of life (78,79).

GP symptoms frequently overlap with other disorders of gut–brain interaction (DGBIs), such as chronic unexplained nausea and vomiting (CUNV), cyclic vomiting syndrome, and, most notably, FD (80–82). Small bowel dysmotility is also common, with constipation reported in up to 60% of GP patients. Although constipation correlates with symptom severity and delayed intestinal transit, it is not directly related to gastric retention (83–86).

In idiopathic GP, approximately 86% of patients also meet the Rome IV criteria for FD, particularly for postprandial distress syndrome (87). Distinguishing GP from FD can be difficult, especially when epigastric pain predominates in patients with delayed gastric emptying. In a large study by the Gastroparesis Clinical Research Consortium (GpCRC) involving 944 patients, significant overlap was observed in symptom profiles, quality-of-life measures, and histopathological findings, suggesting that GP and FD may represent different points along a spectrum of gastric neuromuscular disorders (88). Furthermore, follow-up gastric emptying study demonstrated variability over time, with some GP patients normalizing and FD patients developing delayed emptying, reinforcing the concept of a dynamic disease continuum (88).

Despite this overlap, certain clinical and physiological distinctions exist. Huang et al. reported significantly longer gastric emptying times in GP compared with FD (89). Recent guidelines from the United European Gastroenterology (UEG) and the European Society of Neurogastroenterology and Motility (ESNM) propose that nausea and vomiting are more indicative of GP, whereas early satiety, postprandial fullness, and epigastric pain are more

characteristic of FD (2). These diagnostic distinctions aim to improve clinical stratification and guide tailored management strategies.

6. Diagnosis

In patients presenting with GP-like symptoms, upper gastrointestinal endoscopy represents the initial diagnostic step, primarily to exclude mechanical obstruction, malignancy, or peptic ulcer–related strictures (90). When further assessment is warranted, computed tomography (CT) can provide complementary information.

Once structural causes are excluded, gastric emptying scintigraphy (GES) remains the gold standard for evaluating gastric emptying (90).

6.1 Gastric emptying scintigraphy

GES is a functional nuclear medicine test that quantifies the rate of gastric emptying into the small intestine (91). The standard protocol involves ingestion of a low-fat, radiolabeled meal (commonly Technetium-99m–labeled egg whites), followed by serial imaging at 0, 1, 2, and 4 hours, according to the Tougas protocol (92,93). The inclusion of the 4-hour time point markedly improves diagnostic accuracy, increasing yield by approximately 50% compared with the 2-hour scan (94). The American College of Gastroenterology (ACG) recommends performing GES over at least three hours in symptomatic patients, with delayed gastric emptying defined as >10% retention at 4 hours (90).

To ensure reliable results, medications that affect gastric motility (e.g., prokinetics, antiemetics) should be discontinued at least 48 hours before testing (90).

GES presents several limitations, including inter-institutional variability in meal composition, imaging equipment, and analysis protocols, as well as concerns regarding radiation exposure and the limited physiological relevance of the standardized low-fat meal (255 kcal, 2% fat), which may contribute to underdiagnosis in some patients (95,96). Despite these challenges, GES remains the gold standard for diagnosing GP.

In 2008, the American Neurogastroenterology and Motility Society (ANMS) and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) introduced a staging system based on 4-hour retention values—mild (11–20%), moderate (21–35%), severe (36–50%), and very severe (>50%) (92).

A complementary clinical classification, developed by the ANMS and the American Gastroenterological Association (AGA), stratifies GP into:

- Mild, controlled with dietary modification,
- Moderate, requiring medical therapy,
- Gastric failure, refractory to pharmacologic and nutritional support (98).

However, several studies have shown that symptom severity does not consistently correlate with delayed gastric emptying, raising questions about the clinical–scintigraphic relationship

(92,94,97). This discrepancy suggests that GP symptoms may reflect a broader spectrum of gastric neuromuscular dysfunction rather than delayed emptying alone, highlighting the importance of integrating clinical, physiological, and imaging findings in patient assessment.

GES can also provide insight into intragastric meal distribution, differentiating proximal from distal retention. Proximal retention has been associated with early satiety, whereas distal retention correlates with nausea and vomiting (99).

To better quantify this, the Intragastric Meal Distribution (IMD) index has been proposed, calculated as the ratio of proximal to total gastric counts at a given time point, including baseline (IMD_0) (100). Lower IMD_0 values reflect impaired fundic accommodation and are linked to severe early satiety (99).

Emerging evidence suggests that these scintigraphic parameters may guide personalized therapy: Mandarino et al. found that patients with distal gastric dysfunction (lower pre-procedural IMD_0) achieved better functional outcomes after G-POEM for patients with refractory GP (101).

Further research is required to validate the clinical utility of these advanced parameters and to clarify the interplay between scintigraphic findings, neuromuscular dysfunction, and symptom expression in GP.

6.2 Wireless Motility Capsule

The Wireless Motility Capsule (WMC) system (SmartPill™, Medtronic, Dublin, Ireland) is a non-invasive ambulatory test that measures gastrointestinal transit using a single use capsule that records pH, pressure, and temperature (102). Recently approved by the FDA, WMC provides an indirect assessment of gastric emptying by detecting the rise in pH (>4) that marks capsule passage from the stomach to the duodenum (90,103).

GE time is determined by detecting a rise in pH from the acidic gastric baseline to levels above four, indicating the capsule's passage into the duodenum (103).

A study by Kuo et al. demonstrated a strong correlation ($r = 0.73$) between WMC gastric emptying times and 4-hour GES results in both healthy subjects and GP patients (104). In a larger cohort of 167 GP patients, WMC detected delayed gastric emptying in 34.6%, compared with 24.5% by GES ($p = 0.009$), with an overall agreement of 75.7% ($\kappa = 0.42$) (105). However, the clinical relevance of this higher sensitivity remains uncertain, as WMC findings do not strongly correlate with symptom severity (105).

An advantage of WMC is its ability to simultaneously measure motility and transit times throughout the GI tract, providing valuable information in GP patients with associated constipation (103). Nevertheless, the WMC cannot identify specific pathophysiological

mechanisms (e.g., impaired accommodation, antral hypomotility, pylorospasm), limiting its role in guiding targeted interventions (105).

Contraindications include dysphagia, Crohn's disease, prior GI strictures or fistulas, and recent abdominal surgery (<3 months) (83). Additionally, cost and limited availability currently restrict its widespread clinical use.

6.3 13C-gastric emptying breath test

The 13C-gastric emptying breath test (13C-GEBT) offers a non-radioactive and non-invasive alternative to GES for assessing gastric emptying of solids and liquids. It uses 13C-labeled substrates, such as 13C-octanoic acid (105) or 13C-spirulina platensis (106) for solids and 13C-acetate for liquids (107).

After ingestion, these compounds are metabolized in the small intestine and liver, producing $13CO_2$ that is exhaled and measured using isotope ratio mass spectrometry (108).

In the study by Szarka et al., breath samples collected at 45 and 180 minutes achieved 93% sensitivity for accelerated emptying, while measurements at 150 and 180 minutes showed 89% sensitivity for delayed emptying (109). The ACG guidelines endorse the 13C-spirulina breath test as a reliable tool for evaluating gastric emptying in suspected GP (90).

Advantages of 13C-GEBT include simplicity, absence of radiation, and the possibility of performing sample collection outside the clinical setting (108). However, results can be influenced by hepatic or pulmonary dysfunction, and the test provides only an indirect estimate of gastric emptying, without clarifying the underlying mechanism of delay (108,91).

6.4 Other diagnostic techniques

High-resolution electrogastrography (HR-EGG) is an emerging non-invasive method for assessing gastric myoelectrical activity. Studies have shown that GP patients often exhibit abnormal slow-wave patterns—including reduced 3-cycles-per-minute activity and gastric dysrhythmias—which can be detected and spatially mapped with HR-EGG (110-113). This technique holds promise for identifying gastric hypomotility phenotypes and guiding individualized treatments.

Transabdominal ultrasonography (US) can assess both gastric accommodation and emptying (114), whereas magnetic resonance imaging (MRI) allows detailed evaluation of antro-duodenal motility, correlating reduced motility with delayed gastric emptying (115). Other less commonly used modalities include the hepatobiliary iminodiacetic acid (HIDA) scan, which remains under validation (116), and single-photon emission computed tomography (SPECT), which can evaluate gastric tone but is limited by cost and radiation exposure (117).

Finally, the Endoluminal Functional Lumen Imaging Probe (EndoFLIP) has gained importance in assessing pyloric function in GP. By measuring the Pylorus Distensibility Index (P-DI), EndoFLIP can identify candidates for pylorus-targeted therapies and evaluate post-procedural outcomes (118,119).

A P-DI <9.2 mm^2/mmHg has been shown to predict clinical success following G-POEM (119), while increases in post-procedural cross-sectional area (CSA) correlate with symptomatic improvement and faster gastric emptying (120-122). Although highly promising, further research is required before EndoFLIP can be established as a standard tool for the functional phenotyping of GP patients eligible for targeted therapy.

7. Treatment

The management of GP aims to correct fluid, electrolyte, and nutritional imbalances, identify and treat the underlying cause of delayed gastric emptying (e.g., diabetes mellitus), and alleviate or eliminate symptoms (90).

Current therapeutic options include dietary modifications, pharmacological therapies that enhance gastric motility, antiemetic agents, and non-pharmacological approaches such as endoscopic or surgical procedures and gastric electrical stimulation.
Since no standardized treatment algorithm exists, a stepwise, individualized approach is typically recommended. According to the AGA, approximately 30% of patients develop refractory GP, defined as persistent symptoms and scintigraphic evidence of delayed gastric emptying despite dietary modifications and first-line therapy with metoclopramide (90).

7.1 Dietary modifications

Dietary intervention is considered the first-line treatment for all patients with GP, regardless of disease severity (90).

Because early satiety is a frequent and disabling symptom, patients are advised to consume small, frequent meals while avoiding foods high in fat or indigestible fibers, which can further delay gastric emptying (123).

A typical GP diet consists of three small meals per day plus two snacks, ensuring adequate caloric intake (123,124). Liquid or homogenized foods (e.g., soups, smoothies) are generally better tolerated, as liquid gastric emptying is often preserved (123). Conversely, high-fat solid meals tend to exacerbate symptoms, whereas small-particle-size diets have been shown to improve upper GI symptoms—such as nausea, vomiting, bloating, postprandial fullness, regurgitation, and heartburn—particularly in diabetic GP (125).

Approximately 40% of patients with GP symptoms exhibit disordered eating behaviors. Although a systematic review found no direct causal association between eating disorders and delayed gastric emptying measured by GES (126), recent guidelines emphasize the importance of screening for eating behavior disorders—especially in patients with unexplained weight loss or nutritional compromise (90).

7.2 Medical treatment

Pharmacological management primarily targets symptomatic relief and acceleration of gastric emptying for GP primarily aim to enhance GE to alleviate symptoms (90).

Among available agents, dopamine-2 (D2) receptor antagonists—particularly metoclopramide—have the strongest evidence base. Metoclopramide remains the only FDA-

approved drug for GP and has demonstrated significant improvement in both gastric emptying and symptom scores across different patient populations (127). Recent studies suggest that higher doses (10–20 mg) produce greater symptom reduction in diabetic GP compared with the standard 10 mg regimen (128). An adequate therapeutic trial is generally defined as ≥10 mg three times daily for at least four weeks (90).

The long-term use of metoclopramide is limited by the risk of extrapyramidal adverse effects, notably tardive dyskinesia, though recent data indicate a true incidence below 1% (≈0.1 per 1,000 patient-years) (129-131).

Domperidone, a peripheral D2 antagonist that does not cross the blood–brain barrier, has shown benefit particularly in diabetic GP, though evidence remains less robust (132). Its use requires caution due to potential cardiac arrhythmia risk (133). Domperidone is widely used in Europe, where it is available as a standard therapeutic option, whereas in the United States its use is restricted to compassionate or investigational settings.

Motilin receptor agonists (erythromycin, clarithromycin, azithromycin) also accelerate gastric emptying and provide short-term relief (≤4 weeks). However, prolonged use is limited by tachyphylaxis and a reported 15% increase in myocardial infarction risk (134). In clinical practice, these agents are most used as bridge therapy or rescue treatment in acute or refractory cases.

Growing interest has focused on 5-hydroxytryptamine 4 (5-HT_4) receptor agonists, which exert prokinetic effects through serotonergic stimulation of the enteric nervous system. Cisapride demonstrated efficacy in improving antroduodenal hypomotility by enhancing the migrating motor complex, fundic accommodation, and perception of gastric distension (135), However, it was subsequently withdrawn from the market due to serious cardiac AEs, including QT prolongation and arrhythmias, as documented in large FDA cohort analyses (136).

Among newer 5-HT_4 receptor agonists, Prucalopride—approved by the FDA for chronic constipation—has shown acceleration of gastric emptying in a small crossover RCT, but without corresponding improvement in symptoms (137). Similarly, Revexepride did not produce significant changes in either gastric emptying or symptom scores compared with placebo (138).

In contrast, Velusetrag has demonstrated moderate, dose-dependent benefits for both gastric emptying and symptom relief in diabetic and idiopathic GP, although the effect tends to diminish over time (139). Felcisetrag has yielded the most promising results to date, significantly improving gastric emptying half-time ($T_{½}$), small bowel transit, and colonic emptying compared with placebo in both diabetic and idiopathic GP (140). Nevertheless, these findings are still preliminary, and long-term data on clinical efficacy and safety are awaited.

Substantial interest also surrounds ghrelin receptor agonists, particularly Relamorelin, which mimics the physiological effects of ghrelin on gastric motility. A recent meta-analysis reported

significant overall improvements in GP symptoms—including early satiety, nausea, vomiting, and abdominal pain (141). In a phase IIb RCT involving patients with diabetic GP, Relamorelin significantly improved both gastric emptying and symptom control at 12-week follow-up (142). However, larger phase III trials have shown variable efficacy, and its clinical role remains under investigation.

For 5-HT_3 receptor antagonists such as Ondansetron and Granisetron, moderate efficacy has been observed in reducing gastroparesis-related nausea and vomiting in up to 76% of patients over a two-week treatment period, although these agents do not influence gastric compliance or postprandial accommodation (143,144). Neurokinin-1 (NK-1) receptor antagonists, including Aprepitant and Tradipitant, have also demonstrated improvement in nausea and vomiting over four weeks, though changes in gastric emptying have not been systematically evaluated (145,146).

7.3 Surgical and endoscopic treatment

Given the pathophysiological basis of GP— including pyloric dysfunction—pylorus-directed therapies have emerged as rational treatment options for patients with refractory GP unresponsive to dietary and pharmacological measures. These approaches aim to restore coordinated gastric emptying by reducing pyloric resistance and improving gastric outflow.

Laparoscopic pyloroplasty (LP) was the first structured pylorus-targeted intervention in GP. Large retrospective series reported improvement in gastric emptying in over 80% of patients (147–150). However, LP remains invasive, and approximately one-third of patients relapse during long-term follow-up (151). Moreover, evidence of its cost-effectiveness and comparative efficacy across GP phenotypes is limited.

The advent of minimally invasive endoscopic techniques has progressively replaced surgical approaches. Early endoscopic interventions, such as intrapyloric botulinum toxin injection, initially showed symptomatic benefit, especially in diabetic GP (152), but placebo-controlled RCTs failed to confirm long-term efficacy (153,154). Given its short duration of effect (≈3 months), the latest AGA guidelines no longer recommend its use (90).

Other options, including pyloric balloon dilation and transpyloric stenting, have demonstrated transient benefit in selected cases, but high recurrence and stent migration rates up to 60% limit their utility (155–157). Accordingly, both techniques are not recommended by ESGE guidelines (158).

7.4 Gastric Peroral Endoscopic Myotomy

Gastric Peroral Endoscopic Myotomy (G-POEM) has rapidly become the preferred pylorus-directed therapy for refractory GP. Technically, G-POEM replicates the principles of peroral endoscopic myotomy (POEM) for achalasia: a submucosal tunnel is created in the gastric antrum, followed by selective myotomy of the pyloric circular muscle fibers, and closure of the mucosal entry site with clips (159,160).

Early multicenter prospective studies reported clinical success rates up to 86% at six months, with normalization of gastric emptying in approximately half of the patients (161). A subsequent larger series (n = 75) demonstrated 56% success at 12 months, defined as ≥ 25% reduction in two GCSI subscales, with better outcomes in patients with baseline GCSI > 2.6 and gastric retention > 20% at 4 hours (162). A pooled meta-analysis of 10 studies involving 482 patients confirmed 61% overall success, with adverse events (AEs) around 8% and a significant increase in P-DI post-procedure (163). Similar findings were reported in a more recent meta-analysis by Mandarino et al., which included 13 studies and 952 patients, showing clinical success rates of 72% at 1 year, 71% at 2 years, and 58% at 3 years of follow-up (164).

The strongest evidence derives from a randomized, sham-controlled trial, in which 71% of G-POEM patients achieved ≥ 50% reduction in GCSI compared with 22% in the sham group ($p = 0.005$) (165). Gastric retention at 4 hours improved from 22% to 12% after the procedure, while no change was observed in controls. Subgroup analysis revealed the best responses in diabetic GP (89%), followed by idiopathic (67%) and post-surgical (50%) forms, reflecting the variable pathophysiological substrates of the disease (165).

Compared with other interventions, G-POEM offers greater durability and safety. In a propensity-matched study, it demonstrated higher clinical efficacy (76.6% vs 53.7%) and fewer AEs (4.3% vs 26.1%) than gastric electrical stimulation (166). Similarly, compared with surgical pyloromyotomy, G-POEM achieved superior reductions in GCSI scores and gastric retention, with shorter hospitalization and lower procedural costs (167).

Despite these encouraging results, patient selection remains a crucial issue. Baseline pyloric dysfunction measured by EndoFLIP (P-DI < 9.2 $mm^2/mmHg$) and higher gastric retention on scintigraphy have been associated with better outcomes (119-121). The integration of functional and morphological assessment through GES and EndoFLIP is expected to refine selection criteria and guide personalized treatment.

In terms of safety, G-POEM shows a favorable profile, with mostly minor AEs such as capnoperitoneum, bleeding, or limited mucosal perforation, all managed conservatively.

In conclusion, G-POEM represents a major advancement in the therapeutic management of refractory gastroparesis. Directly targeting pyloric dysfunction through a minimally invasive,

anatomy-preserving approach, it offers a durable, physiologically sound, and increasingly standardized alternative to surgical or pharmacological therapies.

Future studies should aim to define robust selection criteria, assess long-term durability, and explore combined strategies—such as G-POEM with prokinetic therapy or EndoFLIP-guided real-time calibration—to consolidate its role as the gold-standard pylorus-directed treatment for GP.

GASTRIC BIOPSIES DURING G-POEM IN PATIENTS WITH REFRACTORY GASTROPARESIS: HISTOLOGICAL, TRANSCRIPTOMICS AND META-TRANSCRIPTOMICS ANALYSIS, AND CORRELATION WITH CLINICAL OUTCOMES

1. Background

GP is a chronic gastric motility disorder characterized by delayed gastric emptying in the absence of mechanical obstruction (1). The condition manifests with nonspecific upper gastrointestinal symptoms, including nausea, vomiting, early satiety, bloating, and abdominal pain (1).

GP significantly impairs patients' quality of life and poses a substantial socioeconomic burden on both families and healthcare systems. In the United States, emergency department visits for GP increased from 15,459 in 2006 to nearly 36,820 in 2014 (7).

The etiology of GP is heterogeneous and multifactorial. In approximately one-third of patients, gastric dysmotility is related to diabetes mellitus (diabetic GP), although only 1–5% of diabetic individuals develop the disorder during their lifetime. Other secondary forms may arise from neurological or muscular diseases, connective tissue disorders such as scleroderma, or surgical vagal nerve injury following procedures like vagotomy, esophagectomy, or Nissen fundoplication. Nevertheless, more than 50% of cases remain idiopathic, where no clear underlying cause can be identified (96).

The estimated prevalence of GP in the general population ranges between 1.3% and 1.4%. However, only about 10% of affected individuals receive a correct diagnosis, while the majority are misclassified as FD, particularly within the postprandial distress syndrome subtype (96,168).

Clinical severity is commonly assessed using the Gastric Cardinal Symptom Index (GCSI), which quantifies the frequency and intensity of typical symptoms. The score can be applied at baseline and during follow-up to evaluate the response to treatment (72,73)

First-line therapy for GP relies on dietary modification and prokinetic or antiemetic drugs. Patients who remain symptomatic despite optimized medical therapy are defined as having refractory GP, for whom endoscopic or surgical interventions may be considered. The rationale of these procedures is to reduce pyloric tone and improve gastric outflow. According to the ESGE guidelines, botulinum toxin injection and pneumatic dilation are not recommended, G-POEM is suggested in carefully selected patients within tertiary referral centers (158).

Introduced in 2013, G-POEM involves endoscopic submucosal tunneling and full-thickness myotomy of the pyloric sphincter (159-162). The procedure has demonstrated a favorable safety profile and moderate mid- to long-term efficacy. A meta-analysis including 10 studies and 482

patients reported an 8% adverse event rate and a 61% one-year clinical success rate, defined by GCSI improvement (163). A more recent meta-analysis by Mandarino et al., comprising 13 studies and 952 patients, confirmed clinical success rates of 72% at 1 year, 71% at 2 years, and 58% at 3 years of follow-up (164)

Despite these encouraging results, long-term efficacy remains suboptimal, and a discrepancy between symptomatic and functional improvement persists. This underscores the need for reliable predictors of response to G-POEM.

Among the proposed tools, the EndoFLIP system, which measures pyloric compliance, has shown potential in predicting clinical outcomes. Recently, our group demonstrated that a low IMD_0 value at pre-procedural gastric scintigraphy—reflecting preferential food retention in the antrum—was associated with higher functional success after G-POEM (101). However, these findings are preliminary and require validation in larger prospective studies.

Advances in understanding GP pathophysiology have revealed multiple structural and molecular abnormalities, although the precise mechanisms remain unclear. Histopathological studies have identified loss or morphological distortion of interstitial cells of Cajal, degeneration of inhibitory (nNOS, VIP) and excitatory (acetylcholine, substance P) neurons, and the presence of inflammatory infiltrates within the myenteric plexus (15). Among these findings, macrophage dysregulation—characterized by reduced anti-inflammatory ($CD206^+$) and increased pro-inflammatory populations—has emerged as a potential key mechanism (169). An inflammatory signature of GP has also been supported by transcriptomic and proteomic analyses (170,171).

Nevertheless, to date no study has systematically integrated gastric mucosal histological and molecular profiling with detailed clinical and scintigraphic characterization in patients with refractory GP. A deeper understanding of transcriptomic and meta-transcriptomic alterations in may provide novel insights into GP mechanisms and support the development of more personalized management strategies.

Therefore, this study was designed to comprehensively characterize gastric histopathological, transcriptomic and meta-transcriptomic features in patients with refractory diabetic or idiopathic GP, and to explore their relationship with clinical and functional parameters.

2. Material and methods

2.1 Rationale of the study

As outlined in the *Background* section, the aim of this study was to define tissue-specific molecular signatures of refractory GP and to explore their relationship with clinical and functional features, including outcomes following G-POEM.

2.2 Study design and ethics

This was a single-center, prospective study conducted at the Gastroenterology and Digestive Endoscopy Unit, San Raffaele Hospital, Milan, Italy.

The study protocol, titled BXGPOEM 3.0 (*Version 04.11.2023*), was approved by the Ethics Committee of Lombardy 1 (*Comitato Etico Territoriale Lombardia 1*) on February 21, 2024 (CET Em. 61-2024).

The study was classified as a "low-risk". The only deviation from standard clinical practice consisted of collecting additional gastric biopsies for research purposes. No experimental or investigational techniques were applied, and all procedures followed institutional and international standards.

The principal investigator (PI) of the study was Dr. FVM.
Co-investigators included:

- Dr. AB, resident in Gastroenterology
- Dr. GA, resident in Gastroenterology
- Dr. EF, resident in Gastroenterology
- FA, Chief of Interventional Endoscopy Unit

2.3 Study population

The study population included patients with refractory diabetic or idiopathic GP and individuals undergoing upper endoscopy (esophagogastroduodenoscopy, EGD) for non–motility-related indications, serving as controls.

Both groups were prospectively enrolled according to predefined inclusion and exclusion criteria, and all participants provided written informed consent prior to enrollment.

2.3.1 Inclusion criteria

Cases (refractory GP):

- Age ≥ 18 years

- Confirmed diagnosis of diabetic or idiopathic refractory GP (lack of response to pharmacological and behavioral treatments for ≥ 6 months) (90)
- Signed informed consent

Controls:

- Age ≥ 18 years
- Absence of symptoms suggestive of GP or FD
- Normal gastric findings on both endoscopy and histology
- Signed informed consent

Patients undergoing EGD for non–motility-related indications were chosen as controls to allow gastric tissue sampling in individuals without symptoms suggestive of GP or FD, while avoiding ethically unjustified endoscopy in healthy volunteers.
Controls were recruited among patients undergoing EGD for routine clinical indications, including:

- Barrett's esophagus surveillance
- Follow-up of gastroesophageal reflux disease (GERD)
- Family history of gastric cancer
- Evaluation of esophageal stricture, including malignant stenosis.

2.3.2 Exclusion criteria

Cases (refractory GP):

- Pregnancy or breastfeeding
- Portal hypertension
- Autoimmune diseases
- Coagulopathy (INR > 1.5 or platelet count < 50,000/mm³)
- Inflammatory gastrointestinal diseases (eosinophilic esophagitis/gastritis, inflammatory bowel disease, gastric or duodenal ulcers, or celiac disease)
- Active Helicobacter pylori (HP) infection
- Previous esophagogastric surgery
- Cognitive impairment or inability to provide informed consent

Controls:

- Active HP pylori infection or peptic ulcer disease
- Autoimmune or inflammatory gastrointestinal diseases (as above)
- Current use of non-steroidal NSAIDs or prokinetic agents
- Previous esophagogastric surgery or endoscopic pyloric intervention
- Cognitive impairment or inability to provide informed consent

2.4 Study workflow

This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (172)

2.4.1 GP cases

Patients with suspected diabetic or idiopathic GP were referred to the attention of Dr. FA and FVM at the Endoscopy Unit, San Raffaele Hospital. It should be noted that this cohort represents only a subset of all patients presenting with GP symptoms and evaluated by physicians of the Department of Gastroenterology, where patient management and referral decisions are individualized by each physician. Nevertheless, all physicians within the Department are informed about the availability of the G-POEM procedure through previous dedicated informational meetings.

An overview of the study workflow is presented in *Table 1.*

Visit 1

During this visit, investigators assessed patients presenting with symptoms suggestive of GP. If symptoms were consistent, an outpatient EGD was proposed as part of standard care to exclude structural causes of gastric outflow obstruction.

Preliminary EGD

Patients underwent EGD according to routine clinical practice (90). In cases with negative endoscopic findings and no histological abnormalities explaining the GP symptoms, patients were subsequently scheduled for a GES according to the standard diagnostic algorithm.

GES

Details are provided in *Section 2.5.*

A diagnosis of GP was confirmed in the presence of delayed gastric emptying, defined as 4 hour-PGR >10% (90)

Visit 2- Medical and behavioral management of GP

Patients with a confirmed diagnosis of GP attended the visit, during which they completed the GCSI and SF-36 questionnaires as part of routine clinical practice.

Standard medical and behavioral therapy aimed at symptom relief and improvement of gastric motility was initiated and optimized over a 6-month period in accordance with international guidelines (90)

Visit 3 Confirmation of eligibility and enrolment

After 6 months, patients were re-evaluated.

During this visit, baseline data were collected, including reassessment with the GCSI and SF-36 questionnaires. In cases of persistent symptoms despite optimized therapy, G-POEM was proposed according to clinical judgment and current therapeutic guidelines for refractory GP (90,158)

The investigator reassessed the inclusion criteria, and in eligible cases, the study was proposed to the patient for enrollment. If the patient agreed to participate in the study, written informed consent was obtained.

Patients who were not eligible or who declined participation underwent G-POEM and follow-up according to routine clinical practice.

G-POEM

For enrolled patients, protocol gastric biopsies were systematically obtained during the same endoscopic session immediately before G-POEM and prior to mucosal incision, according to a standardized sampling protocol (see *Section 2.6*). This ensured a consistent temporal relationship between tissue collection and pyloromyotomy across all cases, minimizing variability related to peri-procedural factors. G-POEM was subsequently performed during the same procedure.

Technical details of G-POEM are described in *Section 2.7.*

Follow-up

After G-POEM, follow-up was conducted in accordance with standard institutional practice.

- *Visit 4 (3 months ± 30 days post-procedure)* – in-person or telephone: clinical assessment (GCSI and SF-36).
- *GES (3 months ± 30 days):* assessment of GES parameters.

The 3-month follow-up time point was selected to capture early clinical and functional response to G-POEM, in line with previous prospective studies and randomized trials evaluating short-term efficacy of pyloromyotomy. While longer-term outcomes are clinically relevant, this early assessment was considered appropriate for this exploratory study aimed at correlating baseline molecular features with initial treatment response.

2.4.2 Control subjects

Control subjects were recruited among patients undergoing outpatient EGD at our Unit for symptoms not suggestive of GP or FD, as specified in *Section 2.3.1*.

All participants discontinued proton pump inhibitor (PPI) therapy at least 15 days before the procedure, in accordance with institutional standards.

At the time of EGD, investigators verified eligibility based on the inclusion criteria and proposed the study. Participants who agreed to participate and provided written informed consent were enrolled.

For these patients, routine biopsy mapping for histology was performed according to standard clinical practice, and, in addition, the standardized biopsy protocol described for the study was applied (see *Section 2.6*).

Final inclusion of control subjects was confirmed after post-hoc histological review, excluding any abnormalities listed among the exclusion criteria (*Table 2*).

2.5 Gastric Emptying Study

For GP cases, GES was performed according to international consensus standards, as described below (92,93)

After an overnight fast (≥8 hours), patients ingested a standardized low-fat solid meal labeled with technetium-99m sulfur colloid (typically an egg-white sandwich, 120 kcal, 1.5 g fat) accompanied by 120 mL of water.
Scintigraphic images were acquired using a dual-head gamma camera in anterior and posterior projections immediately after ingestion, and at 1, 2, and 4 hours.
Radioactivity counts were decay-corrected, and geometric mean values were calculated.

The following parameters were recorded:

- Gastric half-emptying time (T½): time for half of the ingested radioactivity to leave the stomach.
- Percent Gastric Retention at 1, 2, and 4 hours (1 hour-, 2 hour-, and 4 hour-PGR): proportion of gastric activity at 1, 2, and 4 hours.
- IMD: ratio between proximal gastric and total activity. IMD_0 referred to baseline values.
- Retention Index (RI): ratio between the T½ of the proximal stomach and that of the entire stomach at baseline (92,93).

Delayed gastric emptying was defined as PGR > 10% at 4 hours, in accordance with standardized reference thresholds (90).

2.6 Endoscopic procedures

As previously mentioned, endoscopic procedures included both therapeutic G-POEM, performed in patients with refractory GP, and diagnostic EGD, performed in control subjects. All procedures were conducted at the Gastroenterology and Digestive Endoscopy Unit, San Raffaele Hospital, following institutional standards.

2.6.1 G-POEM (refractory GP)

Patients with refractory GP underwent preoperative evaluation including routine blood tests, electrocardiography (ECG), and anesthesiology assessment. All procedures were performed under general anesthesia, following administration of a single prophylactic dose of broad-spectrum antibiotics (158)

Procedures were carried out using high-definition gastroscopes equipped with a 3.2 mm working channel (Pentax Medical, Tokyo, Japan). Since June 2024, the Pentax Inspira series has been routinely adopted for all examinations. All procedures were performed by an experienced operator (F.A.), who has performed more than 200 POEM procedures.

Before initiating the procedure, gastric mucosal biopsies for molecular analyses were obtained according to a standardized sampling protocol (see *Section 2.6.3*).

Then G-POEM was performed, following the standard technique described in the literature (159-162) (*Figure 1*)

- Mucosal incision (2–3 cm) was made using a hybrid knife (T-Knife, ERBE Elektromedizin GmbH, Tübingen, Germany) connected to an electrosurgical generator (VIO 3, ERBE Elektromedizin GmbH, Tübingen, Germany) on the greater curvature of the gastric antrum, approximately 3-5 cm proximal to the pylorus.
- A submucosal tunnel was created toward the pyloric ring
- Pyloromyotomy was performed by dissecting the circular muscle fibers up to the duodenal bulb.
- The mucosal entry site was closed with endoscopic clips to ensure complete sealing.

After the procedure, patients were admitted to the Day Surgery or Week Surgery Unit (San Raffaele Hospital), and post-procedural care was provided according to institutional clinical practice. Antiemetic therapy and high-dose PPIs were routinely administered. Patients remained hospitalized for observation for the entire day in case of day-hospital management, or for approximately 24 hours if admitted to the Week Surgery Unit.

Discharge was generally scheduled within 12–24 hours for patients in good general condition and without complications. Hospitalization was prolonged only in the presence of fever, leukocytosis, or clinical suspicion of perforation.

After discharge, patients were instructed to follow a semiliquid diet for 3–4 days, then a soft diet for approximately 7 days, and to resume a normal diet within 10–14 days after the procedure.

2.6.2 EGD (controls)

All EGDs were performed in low-risk patients (ASA class I–II) on an outpatient basis under non-anesthesiologist-administered propofol (NAAP) sedation, according to institutional protocols. Continuous monitoring of vital parameters, including pulse oximetry, noninvasive blood pressure, and electrocardiography, was performed throughout the procedure. Supplemental oxygen was routinely administered.
All procedures were carried out using the same high-definition gastroscopes employed for G-POEM procedures, each equipped with a 2.8–3.2 mm working channel (Pentax Medical, Tokyo, Japan).

A systematic inspection of the upper gastrointestinal tract was performed, including the esophagus, stomach (antrum, body, and fundus), and duodenum up to the second portion, with standard photographic documentation of each anatomical site in accordance with routine clinical practice (173).

Gastric biopsies for histological evaluation were obtained according to routine clinical practice (174). When endoscopic abnormalities or other clinical indications were present, separate targeted biopsies for histological assessment were taken from the relevant sites, such as the esophagus or duodenum when appropriate. Subsequently, additional gastric biopsies were collected for molecular analyses as part of the study protocol (see *Section 2.6*).

Post-procedure, patients were observed in the recovery area until full recovery from sedation. Discharge was allowed on the same day after clinical reassessment, with instructions regarding diet and post-procedural care.

2.6.3 Biopsy protocol for molecular analysis

As previously mentioned, gastric biopsies for molecular evaluation were obtained prior to the G-POEM procedure in GP patients and during EGD in control subjects. These additional biopsies were performed exclusively for research purposes and were not part of routine clinical practice.

In both groups, biopsies were taken from each of the following gastric regions:

- Antrum: two biopsies taken approximately 3 cm proximal to the pylorus, one from the lesser curvature and one from the greater curvature.
- Body: two biopsies obtained from the mid-gastric corpus from the lesser and greater curvature.
- Fundus: two biopsies collected from the subcardial region and the gastric fundus.

All biopsies were obtained from macroscopically normal mucosa using sterile, single-use biopsy forceps (Radial Jaw 4, Boston Scientific, Marlborough, MA, USA). Minimal suction and gentle water irrigation were applied to optimize visualization and ensure adequate hemostasis.

2.6.4 Sample handling and storage

Immediately after collection, all biopsy specimens intended were placed in in sterile Eppendorf tubes and kept on ice. Samples were promptly transferred from the endoscopy suite to the research laboratory, where they were snap-frozen and stored at −80 °C until RNA extraction. Care was taken to minimize the time between biopsy collection and freezing to preserve RNA integrity and microbial viability for downstream molecular analyses.

2.7 Study variables and outcomes for patients with GP

For each GP patient, the following variables were collected:

- Demographic variables, including age, sex, and BMI
- Clinical variables, including GCSI and Short Form Healthy Survey (SF-36) (see *Clinical Variables*)
- Scintigraphic variables, assessed before and after the intervention (see *Functional Variables*)
- Procedural variables, including procedural time and AEs, classified according to the ASGE Lexicon (175).
- Outcome variables (see *Section 2.7.4*)

2.7.1 Clinical Variables

The following clinical variables were assessed:

- GCSI (72,73): a 9-item questionnaire evaluating the frequency of symptoms typically associated with GP. It consists of three subscales: postprandial fullness and early satiety (4 items), nausea/vomiting (3 items), and bloating (2 items). Each item is rated on a 0–5 Likert scale; the total GCSI score is calculated as the mean of the three subscale scores (*Table 3*)
- SF-36: a generic, multidimensional instrument assessing health-related quality of life, composed of 36 questions grouped into eight domains: physical functioning (10 items), role limitations due to physical health (4 items), role limitations due to emotional problems (3 items), energy/fatigue (4 items), emotional well-being (5 items), social functioning (2 items), pain (2 items), and general health perception (5 items). Higher scores indicate better perceived physical and mental health, whereas lower scores reflect greater functional limitation, pain, fatigue, or emotional distress.

  For the analysis, scores from individual domains were synthesized into two summary measures: the Physical Component Summary (PCS)—including physical functioning, role

physical, pain, and general health—and the Mental Component Summary (MCS)—including energy/fatigue, social functioning, role emotional, and emotional well-being.

2.7.2 Functional Variables

The following functional variables will be derived from GES (baseline and after 3 months):

- Gastric half-emptying time (t ½)
- PGR
- IMD
- RI.

For details and definitions, refer to *Section 2.5.*

2.7.3 Procedural Variables

- Procedure time: total duration of the G-POEM procedure (from endoscope insertion to removal).
- AEs: any peri- or post-procedural complication, classified according to the ASGE lexicon for endoscopic AEs (mild, moderate, severe, fatal) (175)

2.7.4 Outcome Variables

The study outcomes included:

- Clinical success: defined as a reduction of ≥ 1.0 point in the total GCSI score compared with baseline, and a ≥ 25% improvement in at least one of the GCSI subscales (postprandial fullness/early satiety, nausea/vomiting, or bloating) at 3 months after G-POEM (162)
- Functional success: defined as normalization of 4-hour PGR values ≥ 10% at 3 months post-procedure

2.8 Study variables for control patients

For each control subject, the following variables were collected:

- Demographic variables, including age and sex
- Indication for endoscopy
- Endoscopic findings.

As previously mentioned, final enrollment was confirmed only post hoc, after routine biopsies excluded histological abnormalities related to chronic inflammatory diseases of the gastrointestinal tract (excluding mild to moderate nonspecific gastritis).

2.9 RNA extraction, transcriptomic and meta-transcriptomic analyses

Gastric biopsies obtained during EGD, and G-POEM were subsequently processed for molecular analyses, including RNA extraction, transcriptomic, and meta-transcriptomic profiling, as detailed below.

2.9.1 RNA extraction

The RNA extraction phase was conducted at the laboratory of the Experimental Gastroenterology Unit, Vita-Salute San Raffaele University.

Total RNA was extracted from gastric biopsy samples using the *RNeasy® Mini Kit* (QIAGEN, cat. nos. 74104 and 74106), following the manufacturer's instructions (Quick-Start Protocol, Part 1 and Part 2).

Briefly, tissue samples (≤30 mg) were disrupted and homogenized in 350–600 µl of Buffer RLT supplemented with 10 µl β-mercaptoethanol (β-ME) per milliliter to inactivate RNases. Homogenization was performed using a TissueRuptor (QIAGEN) until complete tissue disruption was achieved. The lysate was then centrifuged for 3 minutes at maximum speed, and the supernatant was carefully transferred to a new tube.

An equal volume of 70% ethanol was added to the lysate, mixed thoroughly by pipetting, and up to 700 µl of the mixture was loaded onto an RNeasy Mini spin column in a 2 ml collection tube. The column was centrifuged for 15 seconds at ≥8000 × g, and the flow-through discarded.

Residual contaminants were removed by washing with 700 µl of Buffer RW1, followed by centrifugation for 15 seconds at ≥8000 × g. On-column DNase digestion was then performed using the RNase-Free DNase Set (QIAGEN) according to the manufacturer's protocol. Briefly, 80 µl of DNase incubation mix (10 µl DNase I stock solution + 70 µl Buffer RDD) was applied directly onto the membrane and incubated for 15 minutes at room temperature (20–30°C). The column was then washed with 350 µl Buffer RW1 and centrifuged for 15 seconds at ≥8000 × g.

Subsequent washes were performed with 500 µl of Buffer RPE, centrifuged for 15 seconds at ≥8000 × g, followed by a second wash with 500 µl Buffer RPE and centrifugation for 2 minutes at ≥8000 × g. Columns were then centrifuged for 1 minute at full speed to dry the membrane completely.

Finally, RNA was eluted with 30–50 µl of RNase-free water, incubated for 1 minute, and centrifuged for 1 minute at ≥8000 × g. If needed, the elution step was repeated using the same eluate to maximize RNA recovery.

Purified RNA was quantified using spectrophotometry (Nanodrop 2000, Thermo Fisher Scientific), and its integrity was verified by electrophoretic analysis. The Nanodrop system assesses both nucleic acid concentration and purity via absorbance measurements at 260 nm,

280 nm, and 230 nm. Specifically, the 260/280 ratio is used to detect protein contamination, while the 260/230 ratio assesses the presence of salts or organic compounds.

Biopsy samples were excluded prior to RNA extraction if deemed inadequate (e.g., insufficient tissue, visible damage, or improper preservation). Only high-quality specimens were processed, extracted, and subjected to spectrophotometric and electrophoretic quality control.

2.9.2 Transcriptomic and meta-transcriptomic analyses

Transcriptomic and meta-transcriptomic analyses were performed on biopsy samples, as previously reported (176). Samples were submitted to GENEWIZ (*Azenta Life Sciences, Leipzig, Germany*) for RNA sequencing and bioinformatic processing.

Briefly, total RNA was processed for library preparation using the TruSeq® Stranded Total RNA Library Prep kit (Illumina). FASTQ reads were adaptor-trimmed and quality-filtered with Trimmomatic (177) before alignment to the human reference genome (hg38) using STAR (178). Gene count normalization and differential expression analysis were conducted with DESeq2 (179). Functional enrichment and redundancy reduction analyses were performed using GeneSCF (180). Low-dimensional embedding of the data was carried out using t-distributed stochastic neighbor embedding (t-SNE) and Uniform Manifold Approximation and Projection (UMAP) algorithms.

For meta-transcriptomic analysis reads not aligning to the human genome were mapped to the complete collection of all available microbial genomes (https://*www.ncbi.nlm.nih.gov/genome/).* Relative abundances and differential analyses were performed using DESeq2. Prior to statistical analyses, classified reads were double-checked with FastQC (https://*www.bioinformatics.babraham.ac.uk/projects/fastqc/*) to confirm quality filtering and adaptor trimming, and subsequently submitted to BLAST to exclude possible in silico artifacts. Species alpha-diversity and dominance indices were calculated using the vegan package (*https://cran.r-project.org/web/packages/vegan*).

2.10 Study objectives

2.10.1 Primary objectives

- To compare gastric molecular profiles across different gastric sites of GP patients with those of age-and sex-matched non-gastroparetic controls.
- To explore their relationship with clinical symptoms, GES parameters and G-POEM outcomes.

2.10.2 Secondary objectives

- To evaluate the relationship between symptoms and GES parameters in the GP clinical cohort.

- To describe the clinical course, safety, and efficacy of G-POEM, including predictors of clinical and functional response.

2.11 Sample size

Given the lack of prior evidence on gastric transcriptomic or metatranscriptomic profiling in refractory GP, this study was designed as an exploratory pilot study without formal sample size calculation.

Two nested study populations were planned: a clinical cohort and a molecular substudy cohort.

2.11.1 Clinical cohorts

Based on expected referral patterns, resource availability, and procedural capacity at our center, approximately 20 consecutive patients with refractory GP undergoing G-POEM were anticipated for enrollment between January 2023 (Ethics Committee approval) and June 2025. This cohort was designed to assess clinical outcomes, safety, functional effects, and relationships between symptoms and gastric motility.

A control group of approximately 15 non-gastroparetic controls, undergoing elective EGD for unrelated indications, was also planned for inclusion.

2.11.2 Molecular substudy cohorts

A nested subset of GP patients from the clinical cohort was selected based on the availability of gastric biopsy samples with the highest RNA purity, as assessed via Nanodrop spectrophotometry (defined as a 260/280 absorbance ratio between 1.8 and 2.1 and a 260/230 ratio > 1.5). Each GP case was matched 1:1 with a non-gastroparetic control based on sex and age (±5 years), if control samples met the same RNA quality thresholds.
The aim of this substudy was to characterize gastric molecular signatures and compare expression profiles between GP and control groups.

An initial feasibility phase was conducted on biopsy samples collected up to June 2024 and included four GP patients and four controls. These samples were selected based solely on RNA quality, without applying matching criteria. This preliminary phase confirmed the reliability of the extraction, sequencing, and analytical protocols, and the results were presented at the seminar *"Gastroparesis and G-POEM: from clinical outcomes to the search for new disease markers"* on September 21, 2024.

Following this validation, the final analytic sample—consisting of 9 GP patients and 9 matched controls (54 gastric biopsies in total)—was assembled and deemed appropriate for the planned exploratory analyses. Further details are provided in Supplementary Appendix 1.

2.12 Statistical analysis

Due to the two-level study design, statistical analyses were performed separately for the clinical and molecular cohorts. All analyses were conducted using SPSS (version X; IBM Corp., Armonk, NY, USA) and R (version X; R Foundation for Statistical Computing, Vienna, Austria).

2.12.1 Clinical cohorts

Continuous variables were summarized as medians with interquartile ranges (IQR) due to the small sample size and the non-normal distribution of the data. Categorical variables were expressed as absolute and relative frequencies (%).

The normality of distributions was assessed using the Shapiro–Wilk test. A two-sided p-value < 0.05 was considered statistically significant.

In the GP clinical cohort, associations among demographic, clinical, quality-of-life, and GES parameters—and their relationships with post-procedural outcomes (clinical and functional success)—were evaluated using Pearson's correlation coefficient (ρ). Pre- versus post-procedural changes in clinical scores (GCSI, SF-36) and GES parameters (T½, 2-hour and 4-hour PGR) were assessed using the Wilcoxon signed-rank test.

Given the limited sample size, correlation analyses were considered exploratory, and the findings should be interpreted with caution.

2.12.2 Molecular substudy cohorts

Differential expression analyses were performed using DESeq2 after normalization and quality control, comparing GP versus control samples. All analyses were stratified by gastric region (antrum, body, fundus) to account for site-specific molecular variation. Significantly up- or downregulated human genes and microbial taxa were identified based on an adjusted p-value (FDR < 0.05), with multiple testing controlled using the Benjamini–Hochberg procedure.

To explore global similarity patterns among samples, unsupervised dimensionality reduction was performed using UMAP on both host transcriptomic and microbial meta-transcriptomic datasets. Functional enrichment of differentially expressed human genes was assessed using gene set enrichment analysis (GSEA), and the top enriched pathways were visualized according to normalized enrichment score (NES). For microbial taxa, differential analyses were conducted separately for bacteria, archaea, fungi, protozoa, and viral domains. Species meeting the

significance threshold (FDR < 0.05) were subsequently visualized using hierarchical clustering heatmaps. Microbial alpha diversity (Shannon and inverse Simpson indices) was calculated and compared across groups and gastric regions using the Kruskal–Wallis test.

For correlation analyses, rather than including all detected transcripts, a targeted subset of genes was selected by the authors on the basis of their statistical significance in the differential expression analysis and their biological relevance, as indicated by involvement in key pathways identified through GSEA. Molecular data were organized in long format, with each biopsy site (antrum, body, fundus) treated as a separate observation and patient-level variables (e.g., GCSI, GES parameters) replicated across sites. Associations between molecular features (transcriptomic and meta-transcriptomic) and clinical or GES parameters were assessed within the GP group using linear mixed-effects models to account for repeated measures per patient (model structure: feature ~ clinical_variable + site + (1|patient_id)). Similar models were used to evaluate associations between baseline molecular signatures and G-POEM outcomes.

3. Results

3.1 Study population and enrollment flow

Between February 2023 and June 2025, 55 patients with suspected idiopathic or diabetic GP referred to the Endoscopy Unit of San Raffaele Hospital and undergoing both EGD and GES were assessed for eligibility. Of these, 33 patients without a confirmed diagnosis of GP or with GP showing clinical improvement with behavioral and/or pharmacological therapy were excluded, leaving 25 patients with refractory GP. All these patients underwent G-POEM. Among them, 21 were finally enrolled in the clinical GP cohort after the exclusion of 3 patients not meeting inclusion criteria and 1 patient who did not provide informed consent.

During the same period, *5,443* patients with non–motility-related gastrointestinal symptoms underwent EGD at our Endoscopy Unit. Among these, 18 patients were enrolled, but 2 were subsequently excluded after post-hoc review of the histological findings of gastric biopsies, which revealed HP infection, resulting in 16 controls included in the study. Details of the enrolment from the clinical cohorts to the molecular substudy are provided in the *Supplementary Material* (*Appendix 1, Tables S1, S2*).

For the molecular substudy, 9 GP patients (27 biopsies; antrum, body and fundus) with the highest RNA integrity, as determined by Nanodrop spectrophotometry, were selected. Each case was 1:1 matched with a control based on sex and age (9 patients, 27 biopsies), as previously described (*Figure 2*).

3.2 Clinical GP cohort

3.2.1 Baseline characteristics

As described above, 21 patients were included in the clinical control cohort. The median age was 52 years (IQR 45–66), and the majority were female (n= 15, 71.4%). The BMI was 24.5 kg/m² (IQR 20.2–26.3), and the median body weight was 59 kg (IQR 50–67). Regarding etiology, slightly more than half of the cases were idiopathic (n = 12, 57.1%), while the remaining patients had diabetic GP (n= 9, 42.9%) (*Table 4*).

3.2.2 Symptom severity, quality of life, and gastric emptying

The median symptom duration was 36.0 months (IQR 24.0–60.0). The median GCSI total score was 3.11 (IQR 2.55–3.80), with the highest symptom burden in GCSI subscale C (bloating: 5.00, IQR 3.50–5.00), followed by subscale B (postprandial fullness: 3.50, IQR 2.00–4.25) and subscale A (nausea: 2.30, IQR 0.66–3.33).

The median SF-36 PCS score was 34.9 (IQR 30.6–51.9), and the median MCS score was 49.6 (IQR 28.9–61.7)

GES showed a median 2 hour-PGR of 76.00% (IQR 65.00–89.00) and 4 hour-PGR of 27.00% (IQR 22.00–54.00). The median gastric t ½ was 174.00 minutes (IQR 134.00–233.00), with an IMD_0 of 88.00% (IQR 72.00–94.00) and a RI of 92.00% (IQR 87.00–97.00) (*Table 5*)

3.2.3 Correlation among etiology, GCSI, GES, and SF-36

A significant correlation was found between GCSI subscale B (postprandial fullness/early satiety) and IMD_0 ($\rho = 0.562$, $p = 0.008$).
Diabetic patients showed higher GCSI-B scores compared with idiopathic cases ($\rho = 0.462$, $p = 0.035$) (*Table 6*)

3.2.4 G-POEM outcomes and adverse events

Procedural details and outcomes after G-POEM are shown in *Table 7.*

Clinical success at 3 months was achieved in 47.6% of patients (10/21), while functional success was observed in 42.9% (9/21). A total of 6 patients (28.6%) achieved both clinical and functional success, 4 patients (19.0%) achieved clinical but not functional success, 3 patients (14.3%) achieved functional but not clinical success, and 8 patients (38.1%) had neither outcome.

When comparing outcomes by etiology, rates of clinical and functional success were similar between diabetic and non-diabetic patients. Clinical success was observed in 5 of 9 diabetic patients (55.6%) and in 5 of 12 non-diabetic patients (41.7%). Functional success occurred in 4 of 9 diabetic patients (44.4%) and in 5 of 12 non-diabetic patients (41.7%).

The median procedural time for G-POEM was 50.0 minutes (IQR 42.0–62.0). Regarding AEs, one patient (4.7%) experienced post-procedural abdominal pain, classified as mild according to ASGE classification, which resolved with conservative management. Another patient (4.7%) developed an intra-procedural pneumoperitoneum, classified as moderate, and was successfully treated with peritoneal needle decompression. In both cases, the AEs did not result in prolonged hospitalization beyond standard practice, nor did they lead to long-term sequelae. No severe AEs were reported.

3.2.5 Correlation between clinical and GES features with outcomes

Higher baseline 4 hour-PGR was significantly associated with a lower probability of functional success ($\rho = -0.539$, $p = 0.012$).

Higher IMD_0 showed a borderline association with reduced functional success (ρ = –0.424, p = 0.056) (*Table 8*)

3.2.6 Pre- to post-G-POEM changes

GCSI total, GCSI-B, and GCSI-C scores significantly decreased after G-POEM (all p < 0.01). 2 and 4 hour-PGR also showed significant reductions post-treatment (p= 0.007 and p= 0.003, respectively). Both PCS and MCS-SF36 significantly improved after the procedure (p = 0.01 and p = 0.007, respectively) *(Table 9)*

3.3 Clinical control cohort

As previously mentioned, the clinical control cohort included 16 patients. The median age was 52.0 years (IQR 45.0–61.5), and the majority were female (13/16, 81.3%). Most procedures were performed for GERD evaluation (14/16, 87.5%), while the remaining cases were indicated for a family history of gastric cancer (2/16, 12.5%).

Regarding endoscopic findings, esophagitis was observed in 5/16 patients (31.3%), predominantly mild (Los Angeles grade A). In all cases, gastric endoscopic findings were normal.

3.4 Outcomes and safety of protocol biopsies

All biopsies performed as part of the protocol were successfully performed in both the clinical GP cohort and the clinical control study, and no AEs of any kind were reported, including bleeding, abdominal pain, or perforation.

3.5 Molecular substudy

3.5.1 Cohorts

The molecular study cohort included 9 patients with refractory GP and 9 matched controls. *Table 10* shows the baseline characteristics, clinical symptoms, and GES parameters of the GP patients in the molecular cohort and their respective matched controls.

3.5.2 Preliminary analysis

As previously mentioned, a preliminary validation analysis was conducted using biopsy samples collected up to June 2024 (4 GP patients and 4 controls). Given the very small sample size, these data were intended solely to validate the technical workflow and were therefore not considered for biological interpretation. The results of this validation phase are reported in *Figures S1–S7*.

3.5.3 Human transcriptomic profiles

UMAP analysis of gastric transcriptomes showed no clear distinction between GP and control samples. In contrast, samples tended to cluster according to gastric region (antrum, body, fundus) in both groups *(Figure 3).*

3.5.4 Differential human gene expression

Differential expression analysis revealed a pronounced transcriptional shift in the antrum of GP patients, with 1,793 genes significantly upregulated and 1,006 downregulated compared with controls (FDR < 0.05). In the gastric body, a smaller set of 21 genes were significantly upregulated and none were downregulated, whereas no significantly differentially expressed genes were detected in the fundus *(Figure 4).*

3.5.5 Pathway enrichment in the antrum (GSEA)

In the antrum of GP patients, pathway enrichment analysis revealed a predominant upregulation of processes related to tissue remodeling, cell migration and adhesion, vascular regulation, and neuromuscular organization, including smooth muscle differentiation and contraction, endothelial development, and axonogenesis. Conversely, pathways associated with lipid and sterol metabolism, xenobiotic and retinoid metabolism, and epithelial homeostasis were significantly downregulated. In contrast, the gastric body showed a much weaker transcriptional signature, with enrichment restricted to a small number of pathways mainly related to VEGF receptor signaling, chemotaxis, and homophilic cell adhesion *(Figure 5).*

3.5.6 Cell-type deconvolution analysis

Cell-type deconvolution analysis did not reveal a global increase in immune cell infiltration in GP samples. The estimated proportions of basophils, erythrocytes, granulocytes, macrophages, mast cells, neutrophils, plasma cells and platelets were overall comparable between GP patients and controls across all gastric regions. A nominally significant increase in mesenchymal stromal cells (MSC) was observed in GP samples ($p = 0.02$) (*Figure 6*).

3.5.7 Microbial transcriptomic profiles (UMAP)

UMAP projections of microbial transcriptomes across kingdoms (archaea, bacteria, fungi, protozoa, and viruses) also showed no distinction between GP and controls. Samples again clustered primarily by gastric region *(Figure 7).*

3.5.8 Microbial alpha-diversity in GP and control samples across gastric regions

Alpha-diversity analysis based on the Shannon and inverse Simpson indices revealed no significant differences in microbial diversity between GP patients and controls, nor across gastric regions (Kruskal–Wallis $p = 0.91$ and $p = 0.83$, respectively) *(Figure 8).*

3.5.7 Microbial community composition

A clear and region-specific shift in bacterial community composition at the species level was observed in the antrum, where multiple bacterial taxa differed between GP patients and controls (FDR < 0.05). These changes involved several taxa, suggesting a broad, non-selective alteration of the antral microbial ecosystem; for this reason, a species-level heatmap is not shown. No significant differences in bacterial community composition were detected in the gastric body or fundus. Differential analysis across microbial kingdoms showed no significant differences in archaea, fungi, or protozoa between GP and control samples in any gastric region (FDR < 0.05), whereas viral species showed only minimal and inconsistent changes *(Figure 9).*

3.5.8 Exploratory correlation between transcriptomic markers, GP features and G-POEM outcomes

Based on the results of the pathway enrichment analysis, a subset of genes involved in neuromuscular function, vascular signaling, cell migration and tissue remodeling (including ZEB1, TNF, WNT4, TBX2, SMARCD3, SLC8A1, RYR2, PDGFR, NOTCH1, NOTCH4, NFATC4, MYOCD, KIT, EDNRA, ENG, and CXCL8) was selected for exploratory correlation analyses.

No statistically significant or consistent correlations were identified between the expression of the selected genes and baseline clinical characteristics, GES results, or post-procedural outcomes, including clinical or functional response to G-POEM.

4. Discussion

GP is a chronic disorder that profoundly affects patients' quality of life and represents a significant burden for healthcare systems. Individuals with GP frequently experience symptoms that often lead to repeated consultations, diagnostic assessments, and hospitalizations (4-6). Despite its substantial clinical and economic impact, GP remains insufficiently understood and characterized. The condition is often mistaken for FD, and key challenges persist in fully defining its phenotypic heterogeneity, particularly about differences in symptom patterns across etiologies and the correlation between clinical presentation and objective measures such as GES (92,94,97).

Conventional first-line pharmacological and behavioral treatments often fail to provide sustained symptom relief (20). This underscores the need for interventional alternatives. Historically, gastric electrical stimulation and surgery have been the main therapeutic options for treatment-refractory GP, though both are limited by variable efficacy and procedural risks. More recently, endoscopy has emerged as a promising minimally invasive strategy aimed at targeting the pylorus to improve gastric emptying. However, earlier endoscopic approaches, such as botulinum toxin injection and transpyloric stenting, have shown inconsistent and often short-lived benefits (21,22).

Adapted from the POEM technique for achalasia, G-POEM, which consists of an endoscopic pyloromyotomy, represents a significant advancement in interventional therapy for GP (159-162). However, clinical success rates remain suboptimal. This limited efficacy suggests that, in the pathophysiology of the disease, mechanisms beyond pyloric dysfunction may also be involved.

In this context, a deeper characterization of GP and the identification of reliable predictors of response to endoscopic therapy—whether clinical, physiological, or molecular—have become urgent unmet needs. Importantly, molecular and histological characterization of GP is still in its infancy, and no validated signature currently exists to stratify patients or guide personalized treatment.

At our institution, G-POEM was introduced in 2017, and to date approximately 60 procedures have been performed in patients with refractory GP. Building on this experience, we designed the current prospective study. We enrolled patients with refractory diabetic or idiopathic GP undergoing G-POEM (clinical cohort). In this cohort, we evaluated clinical and functional outcomes of G-POEM, as well as the correlation between symptom severity and GES parameters. From within this group, we identified a nested molecular cohort to explore associations between molecular signatures, clinical features, and GES parameters, and to identify potential predictors of treatment response. Additionally, by matching GP patients by age and sex to asymptomatic controls, we investigated differences in molecular profiles—including transcriptomic and metatranscriptomic patterns—between individuals with GP and those without motility disorders.

4.1 Molecular cohort

In our study, we observed a pronounced and highly region-specific transcriptional reprogramming of the antral mucosa in GP patients compared with controls, with 1,793 genes upregulated and 1,006 downregulated. Gene set enrichment analysis revealed a coordinated activation of pathways involved in tissue remodeling, cell migration and adhesion, vascular regulation, and neuromuscular organization, including smooth muscle differentiation and contraction, endothelial development, and axonogenesis. Conversely, pathways related to lipid and sterol metabolism, xenobiotic and retinoid metabolism, and epithelial homeostasis were significantly downregulated. This suggests that the antral mucosa in our GP patients has undergone a functional reprogramming towards a remodeling- and neuromuscular-oriented phenotype.

In contrast, the gastric body displayed a markedly weaker transcriptional response, with only a limited number of upregulated genes and pathway enrichment restricted to signaling processes mainly related to VEGF receptor activity, chemotaxis, and homophilic cell adhesion. Taken together, these findings highlight a striking regional heterogeneity of molecular alterations in GP, pointing to the antrum as the primary site of disease-associated transcriptional remodeling.

However, the absence of significant transcriptional changes in the gastric body and fundus should be interpreted cautiously, as limited statistical power in these regions cannot be excluded. While the magnitude of antral differential expression strongly supports region-specific involvement, smaller effects in other gastric compartments may have remained undetected.

The cellular and molecular mechanisms underlying GP remain only partially elucidated. Current evidence supports a multifactorial model in which abnormalities in neuromuscular transmission, vagal dysfunction, immune-mediated injury, and structural remodeling of the gastric wall converge to impair coordinated motility (14-17). Although the neuromuscular and interstitial compartments have been extensively investigated, the mucosal compartment has received comparatively little attention.

Nitric oxide (NO)–mediated neurotransmission represents one of the best-characterized pathways involved in GP. NO, synthesized by neuronal nitric oxide synthase (nNOS) within the myenteric plexus, plays a critical role in smooth muscle relaxation and in the regulation of gastric accommodation, pyloric relaxation, and peristalsis. Reduced nNOS expression and impaired NO production have been demonstrated particularly in diabetic and idiopathic GP. Light microscopy has revealed loss of nNOS-positive neurons in approximately 20% of diabetic GP and up to 40% of idiopathic GP cases (15), while electron microscopy has shown ultrastructural neuronal degeneration, including swollen mitochondria and disrupted nerve terminals (16).

Vagal neuropathy represents an additional well-established mechanism. In diabetic GP, autonomic dysfunction disrupts vagal signaling, impairing the coordination between antral

contraction and pyloric relaxation, a process further exacerbated by hyperglycemia (181). Supporting the relevance of this pathway, a pilot study demonstrated that noninvasive vagal nerve stimulation was associated with symptomatic improvement and accelerated gastric emptying in a subset of patients with idiopathic GP (182).

Another key component of GP pathophysiology is the depletion and dysfunction of ICCs, the gastric pacemaker cells responsible for slow-wave generation. Full-thickness gastric biopsies from patients with both diabetic and idiopathic GP have consistently demonstrated marked ICC depletion, often exceeding 50% in the antrum, together with muscle fibrosis (183,169). ICC loss has been associated with macrophage-mediated injury and oxidative stress, as well as with altered expression of the Ca^{2+}-activated chloride channel Ano1, including the slower-kinetic Ano1 Δ1,2,3 (5′) isoform, suggesting impaired electrical pacemaker function (184).

Structural and functional abnormalities of smooth muscle have also been described. Studies based on full-thickness biopsies reported increased collagen deposition and expansion of the connective tissue stroma in both diabetic and idiopathic GP (183,185). In a subset of patients, smooth muscle cells displayed ultrastructural alterations, such as lipofuscin accumulation, lamellar bodies, and mitochondrial swelling (15), along with thickening of the basal lamina in diabetic GP and altered expression of smooth muscle contractile proteins in idiopathic GP (186).

In contrast to the extensive body of literature focusing on neural and muscular alterations in GP, the gastric mucosa has received comparatively little attention. Positioned at the luminal interface, the mucosa plays an active role in epithelial barrier regulation, sensory signaling, local vascular control, and gut–brain communication. While mucosal alterations have been implicated in other disorders of gut–brain interaction, such as irritable bowel syndrome and FD (187,188), a systematic molecular characterization of the gastric mucosa in GP has, until now, remained largely unexplored.

Our data highlight the presence of highly site-specific molecular alterations selectively involving the antral mucosa in GP. The coordinated activation of pathways related to structural reorganization, cellular interaction, vascular dynamics, and elements of neuromuscular architecture — including programs associated with smooth muscle differentiation and neural patterning — suggests that the mucosa may mirror, or respond to, deeper full-thickness alterations occurring within the underlying neuromuscular layers. This finding raises the intriguing possibility that the mucosa, despite being anatomically superficial, may capture molecular signatures reflective of broader, wall-wide remodeling processes in GP.

This regionally restricted molecular signature is consistent with previous evidence from full-thickness gastric biopsies demonstrating profound alterations of the neuromuscular compartment in the antrum, including loss of ICCs (169) and structural remodeling of the gastric wall. Interestingly, our cell-type deconvolution analysis did not reveal a global increase in

immune cell infiltration, but showed a nominal increase in MSC, further supporting the concept of a remodeling-oriented antral microenvironment.

The mucosal changes observed in our study should therefore be interpreted within the broader pathogenic framework of GP as part of a complex, regionally confined reprogramming process that may both reflect and contribute to the disruption of normal antral–pyloric neuromuscular function.

To date, only one previous study has specifically evaluated mucosal immune signatures in idiopathic GP, focusing primarily on leukocyte infiltration (189). In contrast, our findings point toward a distinct, transcriptionally driven remodeling phenotype, complementing and extending existing histopathological evidence by highlighting the potential role of the mucosa as a molecular sensor of deeper gastric wall alterations.

Notably, no significant correlations were identified between the expression of the selected genes and baseline clinical features, GES parameters, or post–G-POEM outcomes. This lack of association may be explained by several factors. First, GP is a highly heterogeneous and multifactorial condition, and this complexity may have obscured clear relationships between molecular alterations and clinical manifestations. Second, the available outcome measures — including broad and partly subjective scores such as the GCSI — may not be sensitive enough to capture subtle or specific molecular–clinical links, particularly in the post-treatment setting. In addition, our analysis was limited to a subset of genes selected on the basis of pathway enrichment rather than the entire transcriptome, potentially resulting in the omission of other biologically relevant associations.

In parallel, our metatranscriptomic analysis revealed a heterogeneous bacterial landscape within the antral mucosa of GP patients, characterized by the differential representation of multiple taxa. We speculate that this pattern may, at least in part, reflect prolonged luminal stasis and altered intragastric ecology associated with delayed gastric emptying, rather than a primary, species-specific microbial driver of disease. However, additional mechanisms contributing to these alterations cannot be excluded.

Increasing attention has been directed toward the potential contribution of infections and the microbiota to the pathophysiology of GP. However, whether these alterations represent primary drivers of disease or secondary consequences of impaired gastric emptying remains unclear. Among infectious potentially involved in GP, HP is the most consistently implicated. In a retrospective cohort of patients with type 2 diabetes, successful eradication of HP was associated with improved gastric emptying (190). In experimental models, HP infection has been shown to reduce ICC density and disrupt ICC networks through downregulation of stem cell factor, a key regulator of ICC proliferation and c-kit signaling (191).

Limited and inconsistent data suggest possible links between GP and duodenal dysbiosis or small intestinal bacterial overgrowth (SIBO). This association may be explained by the fact that delayed gastric emptying can predispose to bacterial overgrowth in the small intestine. In one cohort, SIBO was detected in up to 60% of GP patients (192), while another study reported a prevalence of 39%, with hydrogen production correlating with symptoms such as bloating and early satiety (193). Nevertheless, these studies are heterogeneous and largely observational, and causality remains unproven.

Following these observations, microbiota-targeted therapies, including probiotics, have been explored in GP. However, the available trials are small, heterogeneous, and methodologically limited (194,195).

Ultimately, our transcriptomic analysis revealed a distinct and highly localized molecular reprogramming at the antral level. While it cannot be excluded that part of this signature reflects secondary effects of delayed gastric emptying and prolonged luminal stasis, its consistent regional confinement supports the presence of a spatially restricted biological process in GP.

Future longitudinal and mechanistic studies will be essential to clarify the causal significance of these alterations and to determine whether they may be leveraged as biomarkers or therapeutic targets in refractory GP.

4.2 Clinical cohort

Our clinical cohort included 21 patients with refractory GP who underwent G-POEM. The demographic characteristics of the cohort reflected the known epidemiology of GP: the median age was 52 years, and the majority were female (71.4%). From a clinical view, patients showed a substantial symptom burden, with a median GCSI total score of 3.11. Quality of life was similarly compromised, as indicated by median SF-36 scores of 34.9 for the PCS and 49.6 for the MCS. These values confirm that patients in our study were significantly affected both physically and psychologically, consistent with the multidimensional impact of refractory GP reported in the literature.

Noteworthy findings emerged from the analysis of correlations between GP symptoms and GES data. First, diabetic patients showed higher scores on the GCSI-B subscale (postprandial fullness and early satiety) compared with those with idiopathic GP. This observation aligns with the hypothesis that diabetic GP may be more strongly associated with impaired gastric accommodation and early-phase emptying delay, likely due to autonomic neuropathy affecting fundic relaxation and antral contractility.

Second, a significant association was found between GCSI-B and IMD_0. This finding is physiologically plausible, as a higher IMD_0 reflects impaired fundic accommodation or reduced antral propulsion, leading to proximal gastric retention and the clinical perception of postprandial

fullness and early satiety. By contrast, a higher IMD_0 is less likely to be associated with nausea and vomiting (GCSI-A), which are often linked to delayed global gastric transit or neurohormonal dysregulation, and bloating (GCSI-C), which may arise from mechanisms such as visceral hypersensitivity or altered gas handling (99).

In our study, clinical success at 3 months—defined as a ≥1-point reduction in total GCSI score and a ≥25% reduction in at least one subscale—was achieved in 47.6% of patients. This rate falls slightly below the 50–90% short-term success range reported in earlier studies. In the first large multicenter prospective study on G-POEM reported by Khashab et al., which included 30 patients with refractory GP of different etiologies (idiopathic, diabetic, and post-surgical), the clinical success rate at 6 months reached 86% (161). Similarly, a subsequent US-based multicenter study involving 75 patients reported a more modest clinical response rate of 56% at 12 months (162). These variations likely reflect methodological differences, including patient selection, distribution of GP subtypes, and definitions of clinical success.

Our findings also revealed discordance between clinical and functional outcomes: 4 patients (19.0%) achieved clinical improvement without corresponding improvement in GES parameters, while 3 patients (14.3%) experienced functional normalization without symptom relief. This mismatch reflects the well-recognized dissociation between symptom severity and gastric emptying time in GP. Importantly, it is also well established that not all patients who report clinical improvement after G-POEM show normalization of gastric emptying (161).

The mismatch between clinical and GES outcomes has raised reasonable concerns regarding the true therapeutic value of G-POEM over last years. To address this gap, Martinek et al. conducted the first RCT comparing G-POEM to a sham procedure in 41 patients with refractory GP. The study provided robust support for the efficacy of G-POEM, with a clinical success rate of 71% at 6 months in the G-POEM group versus 22% in the sham group ($p = 0.005$) (165). This trial offered high-quality evidence that G-POEM is not merely a placebo effect, but a valid and effective therapeutic option in appropriately selected patients (165).

In our cohort, clinical and functional success rates were similar between diabetic and non-diabetic patients. Clinical success occurred in 55.6% of diabetic and 41.7% of idiopathic cases, while functional success was observed in 44.4% and 41.7%, respectively. These findings differ from the study by Martinek, which reported higher efficacy in diabetic GP compared to idiopathic and post-surgical phenotypes (89% vs. 67% and 50%, respectively). Nonetheless, both studies were limited by small sample sizes ($n = 21$ in ours, $n = 22$ in Martinek's), warranting cautious interpretation (165).

The identification of patients most likely to benefit from G-POEM remains one of the major clinical challenges. Although several factors have been proposed as potential predictors, including GCSI >2.6 (162), early symptomatic response (162), high BMI (196), long disease

duration (196), and concomitant psychiatric or narcotic medication use, the current evidence is inconsistent. For example, high baseline 4-hour PGR has been associated with both favorable (162,197) and unfavorable (198,199) outcomes across different studies. Such conflicting findings highlight the need for more robust and reproducible predictors of treatment response.

Another key issue concerns the definition of clinical success following G-POEM. Clinical response is typically evaluated using the GCSI score; however, this scale includes nonspecific gastrointestinal symptoms that overlap with other functional disorders. Moreover, the most recent definition of success (162), despite being widely adopted, is somewhat arbitrary and lacks comprehensive validation, raising concerns about its clinical applicability.

In recent years, novel diagnostic tools have gained attention as potential strategies to identify GP patients who may benefit from pyloromyotomy. Among these, EndoFLIP (endoluminal functional lumen imaging probe) has shown promise.
In the study by Jacques et al., a preoperative pyloric distensibility index (DI) <9.2 mm²/mmHg predicted G-POEM failure with 72% sensitivity and 100% specificity (119). Similarly, in a study by Yang et al. including 90 patients with refractory GP, a DI threshold of 7.35 mm²/mmHg demonstrated a specificity of 80.8% and sensitivity of 60.6% in predicting treatment response (200). However, in both studies, clinical response was defined as an improvement of ≥1 point in the GCSI.

GES also holds potential as a predictive tool, especially via regional parameters such as IMD_0. In a previous publication lead by our group, involving 17 patients, we have shown that a lower preoperative IMD_0 was associated with higher rates of post-G-POEM functional response (>30% reduction in 2-hour retention) (91). The present study confirms this trend, with a borderline association between higher IMD_0 and reduced functional success ($\rho = -0.424$, $p = 0.056$). A higher IMD_0 reflects predominant proximal retention, often indicative of impaired fundic accommodation, which may be less amenable to pyloromyotomy. Thus, it is reasonable to assume that patients with more distally predominant gastric dysfunction may benefit the most from G-POEM.

Despite these ongoing uncertainties surrounding G-POEM, our results support its overall effectiveness in improving clinical symptoms, gastric emptying, and quality of life. Total GCSI scores and the subscales B and C decreased significantly after treatment (all $p<0.01$), while reductions in 2 and 4 hour-PGR were similarly significant ($p=0.007$ and $p=0.003$). These clinical and physiological gains were mirrored by improvements in both physical and mental quality-of-life domains (SF-36 PCS and MCS: $p=0.01$ and $p=0.007$).

Our study also reinforces the safety and technical feasibility of G-POEM. The median procedural time was 50.0 minutes (IQR 42.0–62.0). Only two minor AEs (4%) occurred, one case of mild post-procedural abdominal pain and one intra-procedural pneumoperitoneum, both

managed promptly and without sequelae. These data support G-POEM as a precise and minimally invasive technique that can be safely performed in an advanced endoscopic setting, reinforcing its role as a promising therapeutic option for patients with refractory GP.

4.3 Strenghts and limitations

This study has several important strengths, although some limitations should be acknowledged. A key strength is the prospective enrolment of patients with refractory GP, which allowed for standardized clinical characterization and biological sample collection. Moreover, this is among the first studies to explore gastric transcriptomic signatures in patients with refractory GP, providing novel insight into region-specific molecular alterations. An additional strength is the accurate matching between the GP cohort and healthy controls in terms of age and sex, thereby minimizing potential confounding.

The main limitation is the relatively small sample size, which was primarily related to funding constraints and the highly specialized, time-intensive nature of advanced endoscopic procedures. In addition, the correlation analysis between molecular data, GP features, and G-POEM outcomes was restricted to a subset of genes selected based on pathway enrichment, rather than the entire transcriptome. This analysis is planned to be expanded in subsequent phases of the project. Furthermore, a direct molecular comparison between diabetic and idiopathic GP was not performed.

Finally, this was a single-center study conducted in a tertiary referral setting, which may have introduced referral bias toward more severe or treatment-refractory cases. Consequently, the findings may not be fully generalizable to the broader GP population, particularly patients with milder disease managed in primary or secondary care. Multicenter studies with larger cohorts will be required to validate these results.

5. Conclusion

Our study provides novel evidence of disease-specific molecular signatures in patients with GP. We identified a distinct antral mucosal profile characterized by a a marked transcriptomic reprogramming involving tissue remodeling, cellular interaction, vascular regulation, and neuromuscular organization, together with a localized, non–species-specific shift in bacterial community composition. Our findings highlight the antrum as a site of selective molecular and ecological alteration in GP. This regional signature suggests a functional reprogramming of the antral mucosa that may mirror, or respond to, deeper neuromuscular and structural changes occurring within the gastric wall. While causality cannot be firmly established, these data expand current concepts of GP pathogenesis and support the potential relevance of antral, region-specific mechanisms that may inform future mechanistic studies and therapeutic strategies.

In parallel, our clinical cohort provided valuable insights. The overall clinical success rate of G-POEM was below 50%, and symptomatic improvement was often discordant from objective changes in gastric emptying. Our results further emphasize the need for improved patient selection. Scintigraphic measures, including IMD0, again emerged as promising non-invasive parameters for risk stratification. Nevertheless, additional tools will likely be required. In the future, technologies such as EndoFLIP, which directly assess pyloric function, may help identify patients most likely to benefit from endoscopic myotomy.

In the field of GP, our findings support the need for a more comprehensive molecular and functional characterization before further refining endoscopic treatment algorithms. Linking mucosal and molecular alterations to downstream neuromuscular and pyloric dysfunction will be important for identifying biologically and functionally distinct disease subgroups. Such an integrated approach may facilitate the development of stratified treatment pathways, in which pylorus-directed therapies are reserved for patients with objective evidence of antral or pyloric impairment, while alternative medical, dietary, or microbiota-targeted strategies are tailored according to the predominant pathogenic mechanism. Ultimately, this refined phenotyping may contribute to a more personalized, mechanism-based approach to care, replacing empirical strategies with biologically and physiologically informed interventions.

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Tables

Table 1. Workflow of GP cases

	Screening	Pharmacological and behavioral treatment	Enrollment	Treatment	3 month post-treatment
Visit 1	X				
EGD*	X*				
Gastric emptying study	X*				X*
Visit 2 (GCSI and SF-36 assessment)		X*			
Visit 3 (6 months after Visit 2) (GCSI and SF-36 assessment)			X**		
G-POEM* (+ biopsies)**				X*/**	
Visit 4 (GCSI and SF-36 assessment)					X*

EGD Esophagogastroduodenoscopy

*According to routine clinical practice

** In addition to standard clinical practice, as per study protocol

Table 2. Workflow of controls

	Screening	**Enrollment**
EGD (+ biopsies)**	X*	X**

EGD Esophagogastroduodenoscopy

*According to routine clinical practice

** In addition to standard clinical practice, as per study protocol

Table 3. Structure of the Gastric Cardinal Symptom Index (GCSI)

Domain	Items (n)	Symptoms assessed	Score range
Postprandial fullness **Early satiety**	4	Postprandial fullness, inability to finish a normal-sized meal, excessive fullness after eating, loss of appetite	0–5 per item
Nausea **Vomiting**	3	Nausea, retching, vomiting	0–5 per item
Bloating	2	Stomach bloating, visible abdominal distension	0–5 per item
Total GCSI score	9	Mean of the three subscale scores	0–5

GCSI Gastric Cardinal Symptom Index

Table 4. Baseline characteristics of the GP clinical cohort

GP patients (n=21)	**Median (IQR) or n (%)**
Age (years)	52 (45-66)
BMI, kg/m²	24.5 (20.2–26.3)
Sex Female Male	 15 (71.4%) 6 (28.6%)
Body weight, kg	59 (50–67)
Etiology Diabetes Idiopathic	 9 (42.8%) 12 (57.1%)

GP gastroparesis, IQR Interquartile range, BMI Body Mass Index

Table 5. Pre-procedural clinical and GES features of the GP clinical cohort

GP patients (n=21)	**Median (IQR)**
Duration of symptoms, months	36.00 (24.00–60.00)
GCSI total	3.11 (2.55–3.80)
GCSI subscale A	2.30 (0.66–3.33)
GCSI subscale B	3.50 (2.00–4.25)
GCSI subscale C	5.00 (3.50–5.00)
SF-36 PCS	34.9 (30.60–51.9)
SF-36 MCS	49.6 (28.90–61.7)
GES 2h-PGR (%)	76.00 (65.00–89.00)
GES 4h-PGR (%)	27.00 (22.00–54.00)
GES t ½ (minutes)	174.00 (134.00–233.00)
GES RI (%)	92.00 (87.00–97.00)
GES IMD (%)	88.00 (72.00–94.00)

GP gastroparesis, IQR Interquartile range, GCSI Gastric Cardinal Symptom Index, SF-36 PCS Short Form-36 Physical Component Summary, SF-36 MCS Short Form-36 Mental Component Summary, GES Gastric Emptying Study, PGR Percent Gastric Retention, RI Retention Index, IMD Intragastric Meal Distribution

Table 6. Correlation among etiology, clinical features, and GES parameters in GP clinical cohort

Variable 1	**Variable 2**	**Correlation coefficient (ρ)**	**p-value**
GCSI-B	IMD	0.562	**0.008**
Etiology (diabetic vs idiopathic)	GCSI-B	0.462	**0.035**

GCSI Gastric Cardinal Symptom Index

Table 7. Procedural details and outcomes of G-POEM

GP patients (n=21)	**Median (IQR) or n (%)**
Clinical success	47.6% (10/21)
Functional success	42.9% (9/21)
Procedural time (minutes)	50.0 (42.0–62.0)
Adverse events (ASGE lexicon classification) Mild Moderate Severe	 4.7% (1/21) 4.7% (1/21) 0 (0%)

GP gastroparesis, IQR Interquartile range

Table 8. Correlation between clinical and GES features with clinical and functional outcomes in GP clinical cohort

Variable 1	Variable 2	Correlation coefficient (ρ)	p-value
4h-PGR	Functional success	-0.539	**0.012**
IMD	Functional success	-0.424	0.056

PGR Percent Gastric Retention, IMD Intragastric Meal Distribution

Table 9. Pre- vs post-G-POEM changes

Variable	Trend	Statistic (W)	p-value
GCSI total	↓	17.0	**<0.001**
GCSI-A	↓	44.0	**0.07**
GCSI-B	↓	23.0	**0.002**
GCSI-C	↓	7.5	**<0.001**
2 hour-PGR	↓	39.5	**0.007**
4 hour-PGR	↓	35.0	**0.003**
SF-36 PCS	↑	32.0	**0.01**
SF-36 MCS	↑	29.0	**0.007**

GCSI Gastric Cardinal Symptom Index, PGR Percent Gastric Retention, SF-36 PCS Short Form-36 Physical Component Summary, SF-36 MCS Short Form-36 Mental Component Summary

Table 10. Baseline characteristics, clinical and functional features, and post-G-POEM outcomes of the 9 GP patients and their matched controls

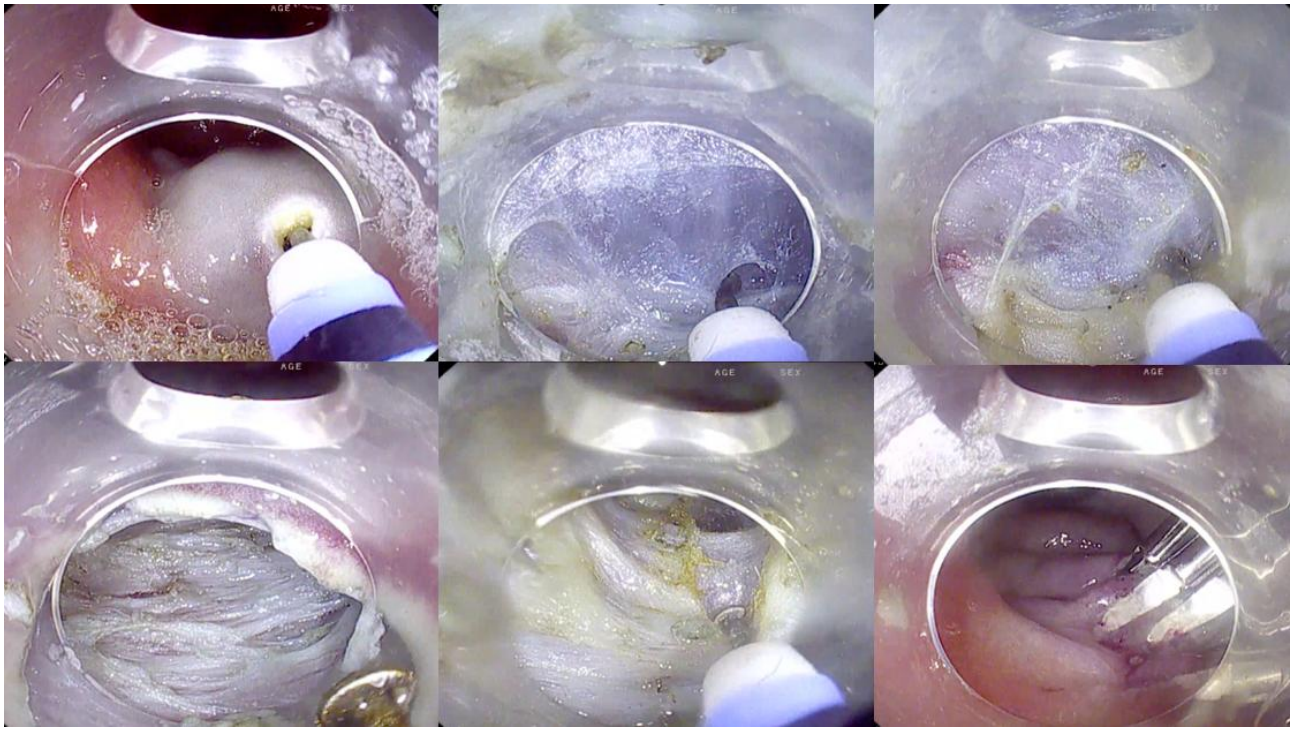
Code	Date enrolled	Age	Sex	BMI	Eziology	Duration symp (months)	GCSI tot	GCSI A	GCSI B	GCSI C	GES 2h-PGR	GES 4h-PGR	GES t ½ (min)	GES RI	GES IMD_0	SF-36 PCS	SF-36 MCS	Clinical success	Functional success	Code	Date enrolled	Age	Sex
GP 1	11/05/2023	19	F	20.2	I	24	3	1	4	4	79%	30%	221	94%	90%	63.8	49.6	Yes	Yes	Cnt 7	04/04/2024	18	F
GP 5	29/02/2024	56	F	26.3	I	36	2.33	0	2	5	65%	21%	134	98%	72%	41.4	45.6	Yes	No	Cnt 6	04/04/2024	54	F
GP 7	29/03/2024	47	F	20.5	I	6	3.86	3.33	4.75	3.5	58%	12%	136	97%	94%	34.9	57.2	No	Yes	Cnt 2	18/01/2024	45	F
GP 8	29/03/2024	62	M	31.4	D	30	1.16	1	0.5	2	79%	12%	174	89%	65%	45.4	67.8	Yes	Yes	Cnt 8	18/04/2024	58	M
GP 17	20/01/2025	32	F	19.0	D	48	4.8	4.7	4.8	5	97%	67	31	90%	99%	8.8	23.4	Yes	No	Cnt 9	19/04/2024	36	F
GP 18	27/01/2025	62	F	30.0	D	12	4.11	3.33	4	5	98%	92%	500	92%	90%	30.6	53.4	No	No	Cnt 16	21/02/2025	66	F
GP 19	17/02/2025	76	M	21.8	I	30	2,36	1,33	2,25	3,5	95%	64%	45	100%	88%	24.4	14.4	Yes	No	Cnt 13	21/01/2025	71	M
GP 20	10/03/2025	30	F	22.2	I	120	3.0	0.66	3.3	5	64%	23%	112	87%	62%	55.4	61.7	No	Yes	Cnt 15	21/02/2025	28	F
GP 21	31/03/2025	50	F	26.4	I	24	3,33	0	5	5	68%	24%	166	42%	97%	33.8	63.1	Yes	Yes	Cnt 10	02/10/2024	46	F

BMI Body Mass Index, F female, M male, D diabetic, I idiopathic; GCSI Gastroparesis Cardinal Symptom Index (total and subscales A–C); GES gastric emptying scintigraphy; PGR percent gastric retention, t½, half-emptying time, RI Retention Index; IMD_0 Intragastric Meal Distribution at baseline; SF-36 PCS/MCS, physical and mental component summary scores

Each GP case (shown in black) was matched 1:1 with a non-gastroparetic control (shown in red) based on sex and age (±5 years), selected among samples with adequate RNA purity (260/280 ratio 1.8–2.1; 260/230 ratio >1.5). This table reports demographic data, clinical features (including BMI, symptom duration, and GCSI scores), and functional parameters from GES. Clinical and functional success after G-POEM is also shown. Matching was performed once on the final pooled sample to optimize comparability.

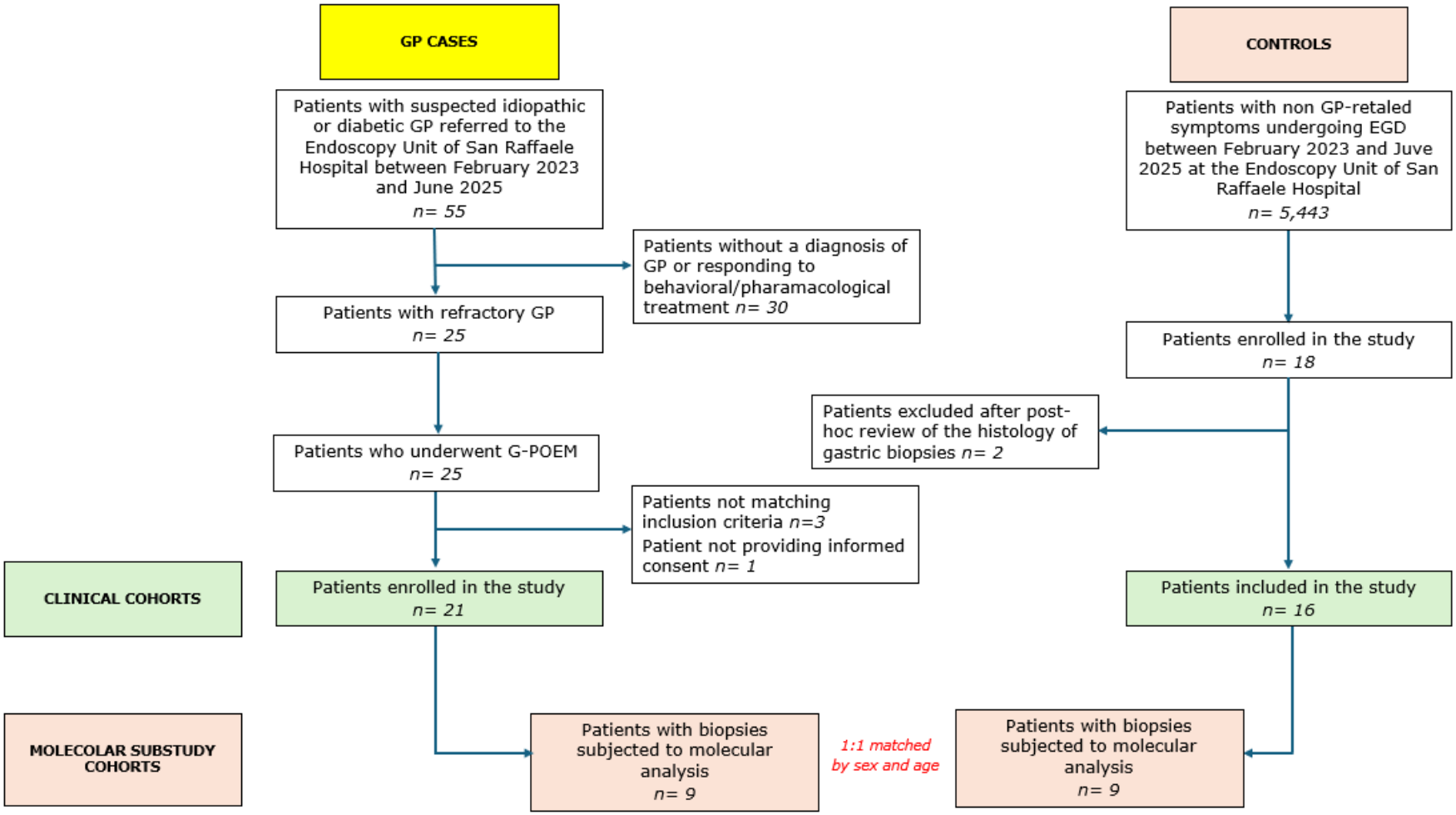
Figures

Figure 1. Key steps of the G-POEM procedure



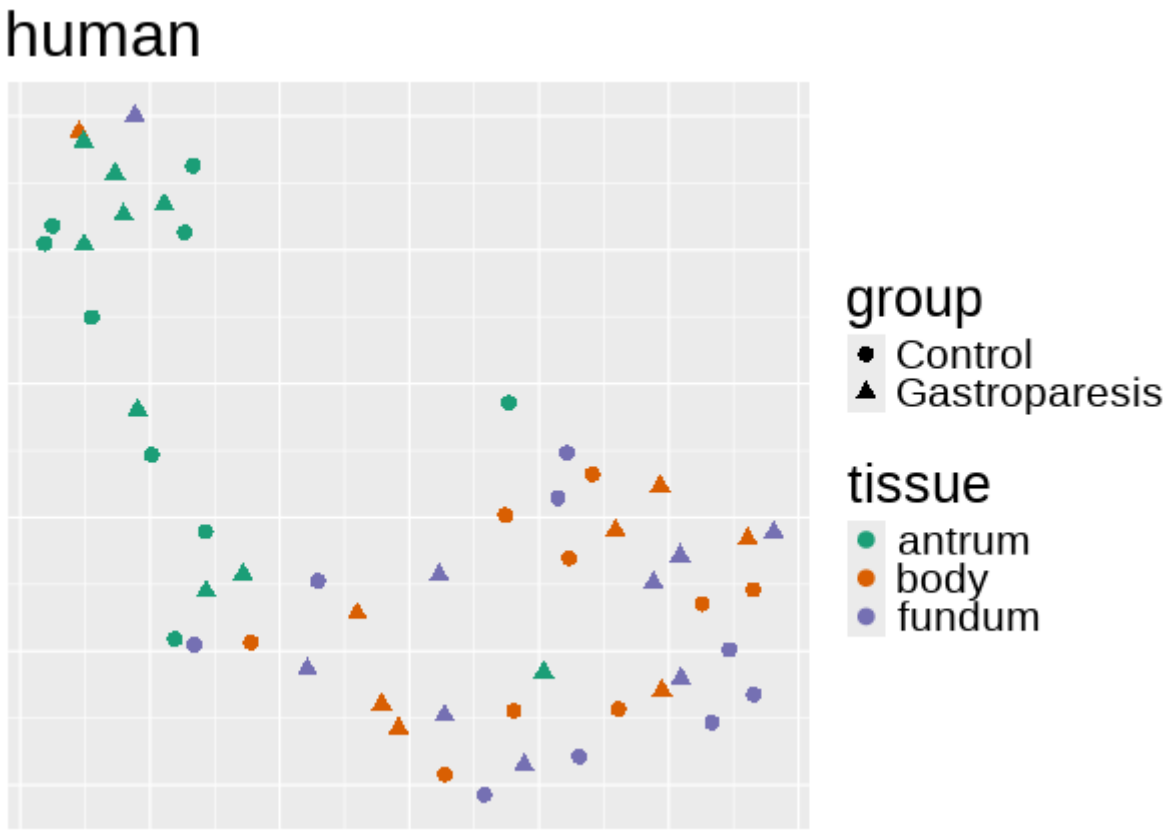
Submucosal injection and cushion, mucosal incision and entry into the submucosal space, tunneling toward the pylorus, identification and myotomy of the pyloric muscle, closure of the mucosal entry.

Fig.2. Study workflow (STROBE diagram)



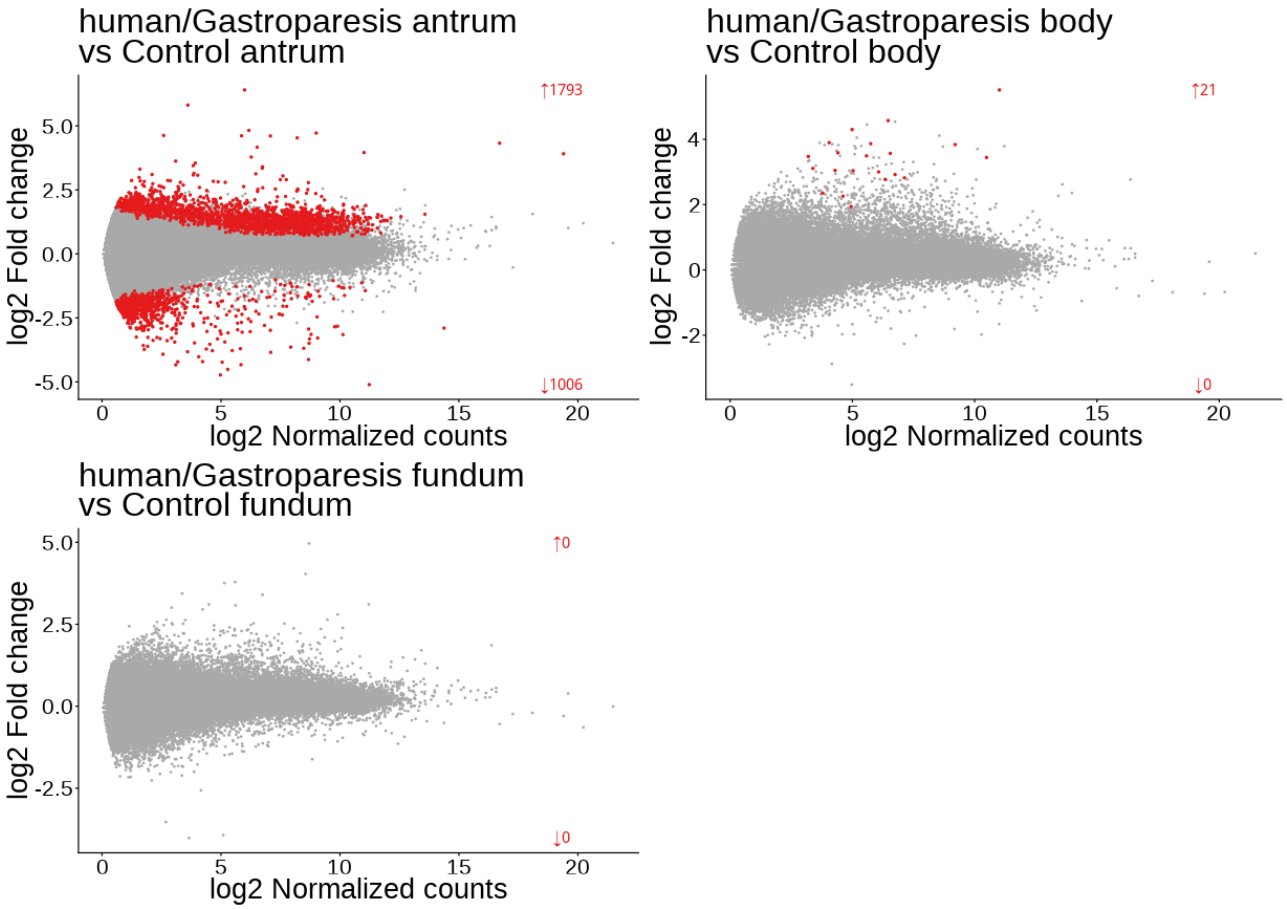
Flow diagram illustrating study design and sample processing.

Figure 3 UMAP projection of gastric biopsy transcriptomes from GP patients and controls



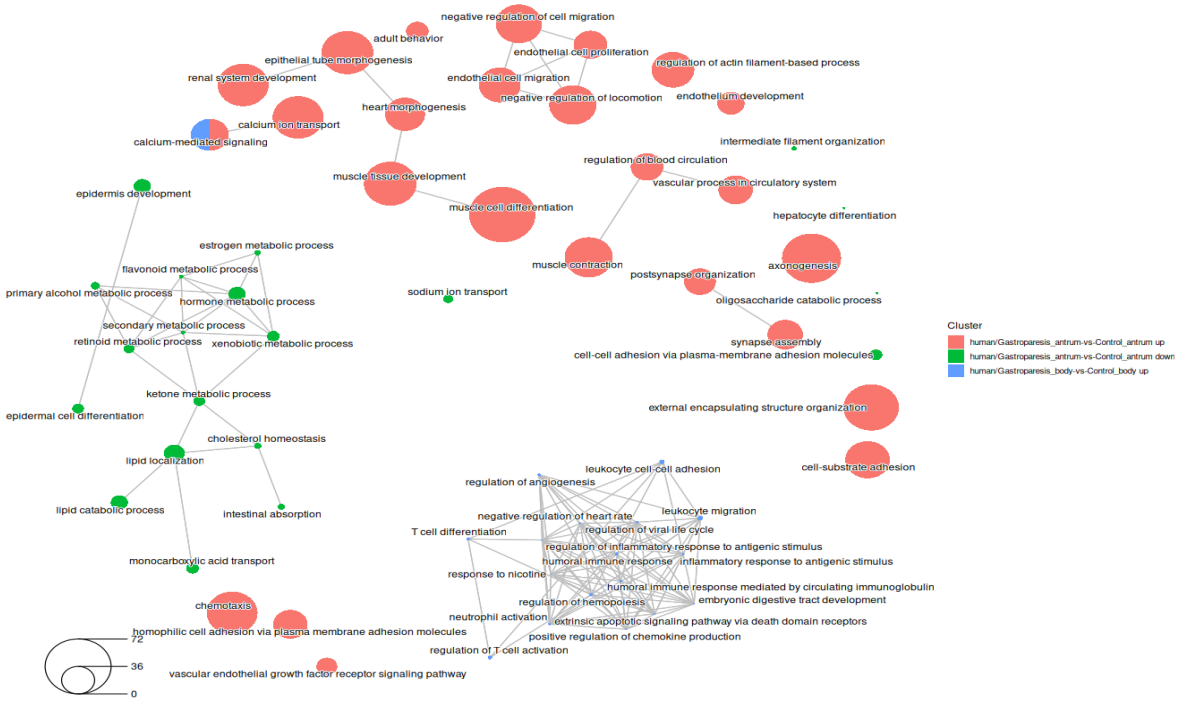
This UMAP plot displays the transcriptional profiles of gastric mucosal samples, with points coloured by anatomical site (antrum, body, fundus) and shaped by group (controls = circles, GP= triangles). No clear global separation is observed between GP and control samples. In contrast, samples tend to group partially by gastric region.

Figure 4. Differential human gene expression in GP versus controls across gastric regions



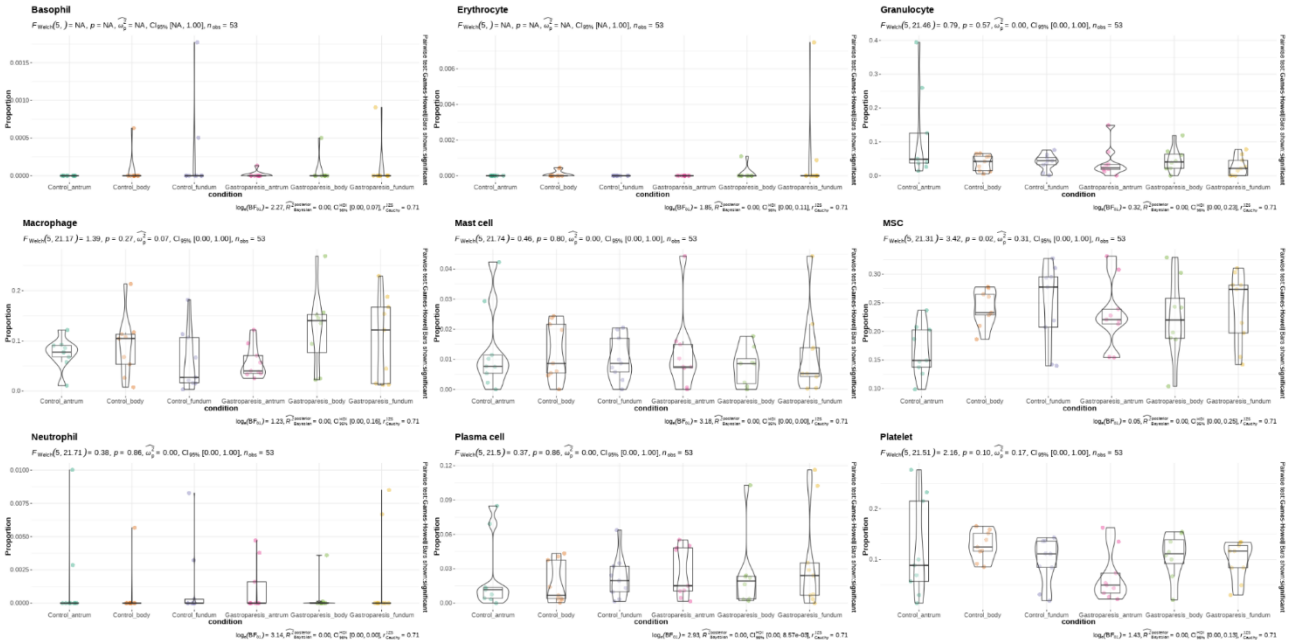
These plots show the relationship between gene expression levels (log_2 normalized counts) and differential expression (log_2 fold change) in GP compared with controls for each gastric region. In the antrum, a marked transcriptional shift is evident, with 1,793 genes upregulated and 1,006 genes downregulated (FDR < 0.05). In contrast, the gastric body of GP patients shows only 121 significantly upregulated genes and no significantly downregulated genes compared with controls. No statistically significant transcriptomic changes are detected in the fundus. Overall, these data indicate that transcriptomic alterations in GP are predominantly confined to the antral region.

Figure 5. Enriched biological pathways in the antrum of GP patients compared with controls (GSEA analysis)



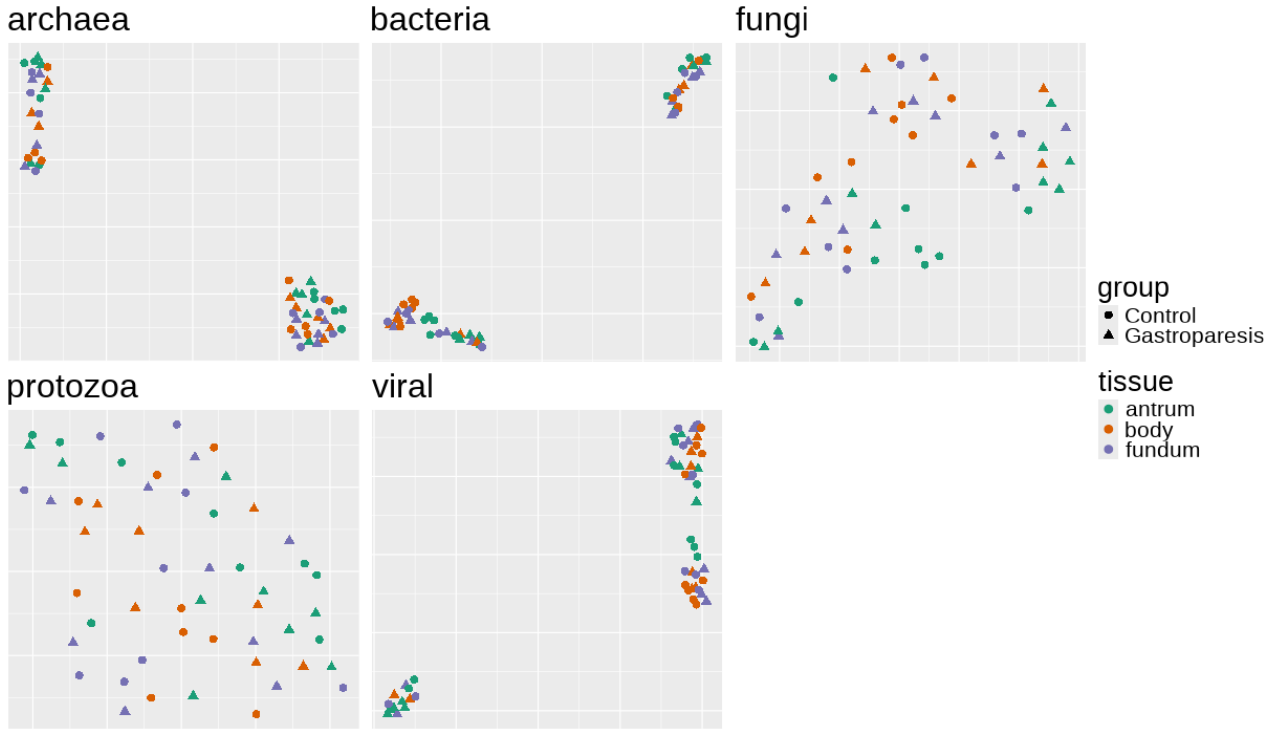
This network visualisation illustrates the main biological processes enriched in GP compared with controls, based on differentially expressed genes across gastric regions. Each node represents a pathway, with node size reflecting the number of associated genes and colours indicating the direction and location of dysregulation (red: upregulated in antrum; green: downregulated in antrum; blue: upregulated in body). In the antrum, enriched pathways are predominantly related to tissue remodelling, regulation of cell migration and adhesion, vascular processes, and neuromuscular organisation, including muscle differentiation, contraction, and axonogenesis. In contrast, the gastric body shows a much weaker and more limited enrichment pattern, mainly involving VEGF receptor signalling, chemotaxis, and homophilic cell adhesion.

Figure 6. Cell-type deconvolution analysis



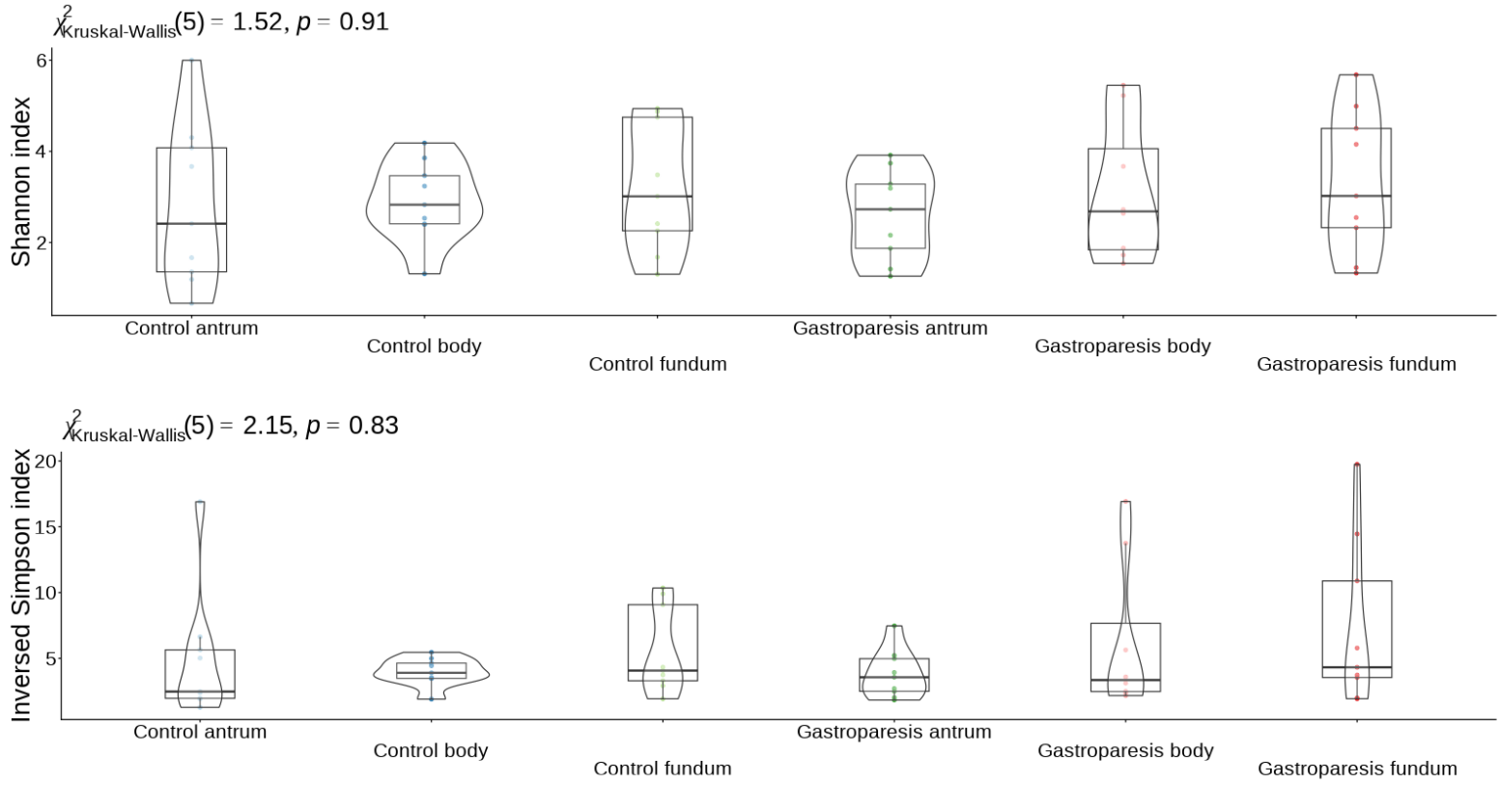
This figure shows the estimated proportions of major immune and non-immune cell populations derived from deconvolution of bulk transcriptomic data in control and GP samples from the antrum, body, and fundus. Across all regions, the relative abundance of basophils, erythrocytes, granulocytes, macrophages, mast cells, neutrophils, plasma cells, and platelets was comparable between GP patients and controls. A nominally significant increase in mesenchymal stromal cells (MSCs) was observed in GP samples (Welch's test, p = 0.02).

Figure 7 UMAP projections of archaeal, bacterial, fungal, protozoal, and viral transcriptomic signatures in GP patients and controls



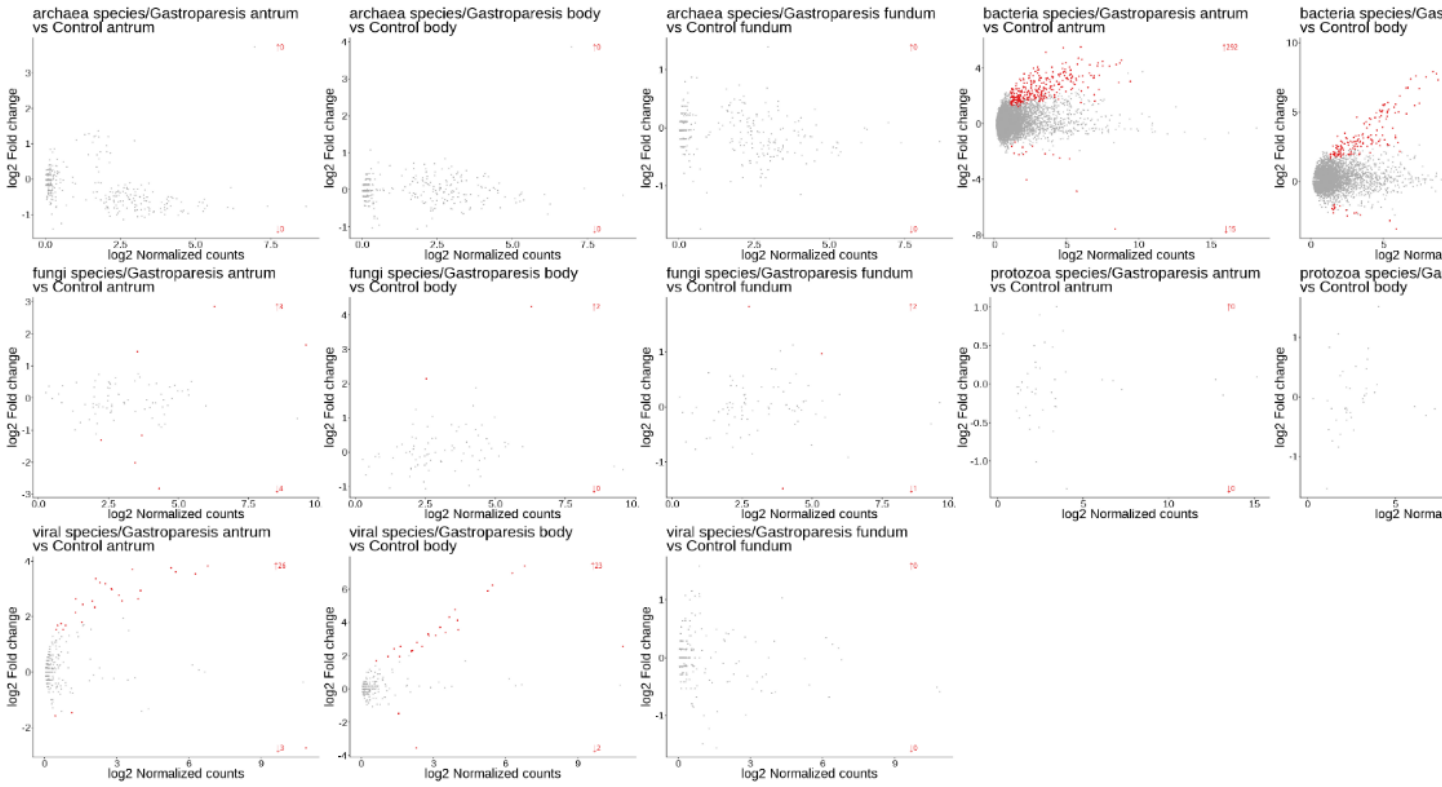
These UMAP plots display the microbial meta-transcriptomic profiles across different kingdoms (archaea, bacteria, fungi, protozoa, and viruses), with samples coloured by gastric region and shaped by group. Similar to the human transcriptomic data, no clear clustering is observed according to disease status. Instead, samples tend to show partial grouping by anatomical site, indicating that microbial community structure is influenced more by gastric region than by the presence of GP.

Figure 8. Microbial alpha-diversity in GP and control samples



These violin plots illustrate microbial alpha-diversity, measured by the Shannon index and inverse Simpson index, in control and GP samples from the antrum, body, and fundus. No significant differences are observed between groups or across anatomical regions.

Figure 9. Microbial community composition in GP versus controls across gastric regions



These plots show the results of differential species-level analyses for archaea, bacteria, fungi, protozoa and viruses in GP compared with control samples across gastric regions. Each point represents a detected species, plotted by $\log_2$ normalized abundance and $\log_2$ fold change. A clear, region-specific shift is observed only in bacterial species within the antrum, where multiple taxa are significantly dysregulated in GP (FDR < 0.05). In contrast, no significant differences are detected in the body or fundus, and no consistent disease-associated alterations are observed for archaea, fungi or protozoa. Viral changes appear minimal and inconsistent.

Supplementary matherial

Appendix 1

Overall, among the 21 GP cases and 16 controls included in the clinical cohort, 17 GP cases and 12 controls were eligible for Nanodrop spectrophotometric assessment. The remaining 4 cases and 4 controls were excluded due to contamination or insufficient tissue quality.

The selection and processing were conducted in two phases, as previously described. During the initial feasibility phase (June 2024), 9 GP cases and 12 controls had been enrolled; of these, 8 GP cases and 8 controls met the RNA integrity thresholds. From this subset, the 4 highest-quality case–control pairs were selected for sequencing (Table 1s).

A second extraction phase took place in June 2025, by which time an additional 12 GP cases and 4 controls had been enrolled. Among these, 8 GP cases and all 4 controls were suitable for RNA purity assessment; the remaining 4 GP samples were excluded due to contamination or suboptimal quality. From the eligible samples in both phases, the 5 GP cases with the highest RNA purity and 5 controls were selected. These were then used to complete the 1:1 sex- and age-matched cohort (±3 years), resulting in the final analytic subset of 9 GP patients and 9 matched controls (Table 2s). This final cohort was used for all exploratory molecular analyses.

Table S1. Results of RNA extraction and spectrophotometric assessment (June 2024)

Samples	**Conc.**	**Ratio**	
BXGPOEM GP cs	ng/mL	260/280	230/260
GP 1 m a	669,94	2,07	2,23
GP 1 m c	235,00	2,04	1,56
GP 1 m f	375,67	2,07	1,73
GP 3 m a	139,48	2,10	2,13
GP 3 m c	181,69	2,09	1,98
GP 3 m f	19,44	2,06	0,06
GP 4 m a	295,71	2,08	1,88
GP 4 m c	230,36	2,09	1,94
GP 4 m f	133,27	2,11	0,47
GP 5 m a	104,66	2,08	1,94
GP 5 m c	352,52	2,07	1,98
GP 5 m f	375,25	2,05	2,10
GP 6 m a	386,74	2,06	2,00
GP 6 m c	75,33	2,09	1,76
GP 6 m f	30,81	2,25	0,25
GP 7 m a	244,54	2,09	2,15
GP 7 m c	411,39	2,06	2,22
GP 7 m f	548,84	2,06	1,81
GP 8 m a	33,29	2,15	2,00
GP 8 m c	378,74	2,09	1,83
GP 8 m f	95,6	2,02	1,99
GP 9 m a	147,57	2,07	2,19
GP 9 m c	294,12	2,07	0,78
GP 9 m f	217,09	2,06	1,99
Samples	**Conc.**	**Ratio**	
BXGPOEM controls	ng/mL	260/280	230/260
Ctrl 1 m a	306,6	2,06	2,11
Ctrl 1 m c	191,2	2,08	1,20
Ctrl 1 m f	369,0	2,07	1,15
Ctrl 2 m a	444,1	2,07	2,28
Ctrl 2 m c	1034,0	2,07	2,24
Ctrl 2 m f	705,8	2,06	1,87
Ctrl 3 m a	325,0	2,07	1,94
Ctrl 3 m c	436,1	2,06	1,21
Ctrl 3 m f	261,9	2,06	1,76
Ctrl 4 m a	720,4	2,09	2,26
Ctrl 4 m c	488,6	2,09	2,14
Ctrl 4 m f	455,8	2,06	2,24
Ctrl 5 m a	711,0	2,06	2,07
Ctrl 5 m c	685,5	2,08	2,18
Ctrl 5 m f	957,6	2,07	2,24
Ctrl 6 m a	362,0	2,06	1,75
Ctrl 6 m c	878,8	2,08	2,27
Ctrl 6 m f	515,1	2,08	2,15
Ctrl 7 m a	311,2	2,06	1,75
Ctrl 7 m c	439,7	2,04	2,20
Ctrl 7 m f	704,4	2,07	1,90
Ctrl 8 m a	245,4	2,07	1,82
Ctrl 8 m c	619,6	2,09	1,95
Ctrl 8 m f	488,1	2,08	1,89

This table reports the RNA extraction results from gastric mucosal biopsies of GP patients and non-gastroparetic controls, including RNA concentration (ng/mL) and purity ratios (260/280 and 230/260).

Samples highlighted in red indicate those selected for downstream molecular analyses based on RNA purity thresholds (260/280 ratio 1.8–2.1 and 260/230 ratio >1.5).

The abbreviations used in the table describe the anatomical origin of each sample. The letter "m" denotes mucosa, while subsequent letters indicate the gastric region: "a" for antrum, "c" for body, and "f" for fundus. The labels "GP" and "Ctrl" refer to samples from GP patients and controls, respectively. For example, the sample labeled *GP 4 m* c refers to a mucosal biopsy taken from the body of the stomach in a GP patient, specifically the fourth patient in the series.

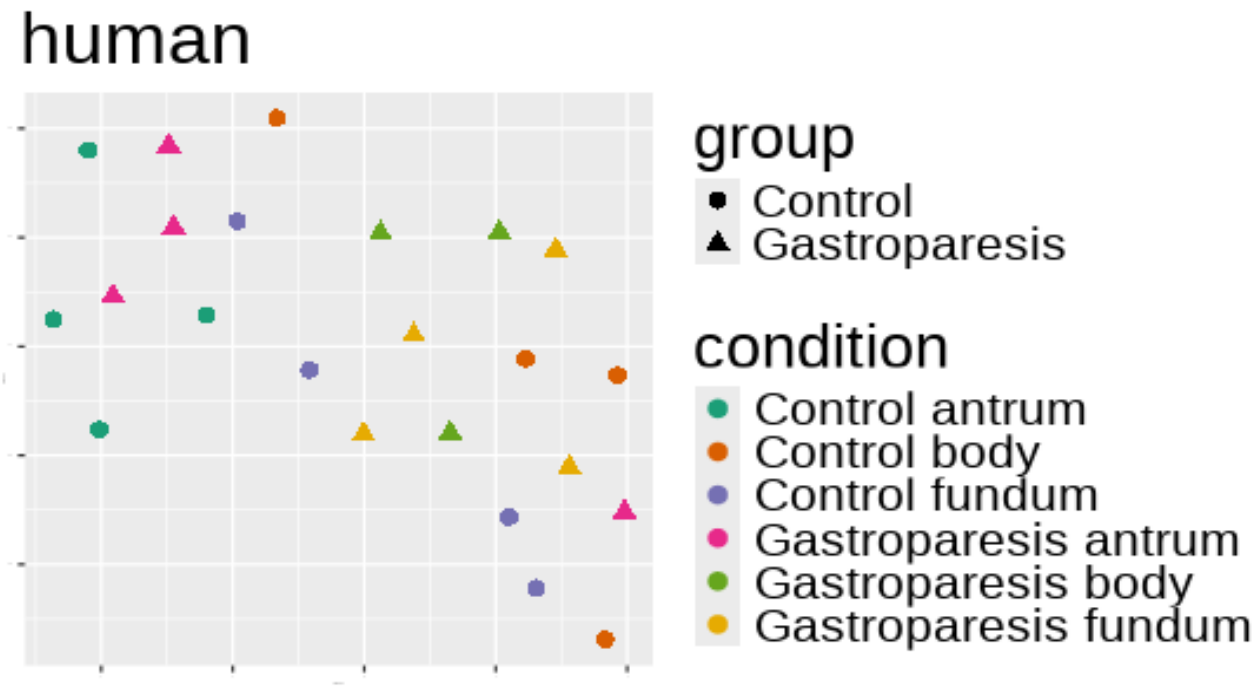
Table S2. Results of RNA extraction and spectrophotometric assessment (June 2025)

Samples	**Conc.**	**Ratio**	
BXGPOEM GP cs	ng/mL	260/280	230/260
GP 10 m a	401,2	2,12	1,30
GP 10 m c	372,2	2,07	1,40
GP 10 m f	677,8	2,05	2,24
GP 12 m a	477,9	2,05	1,98
GP 12 m c	502,7	2,05	1,86
GP 12 m f	654,1	2,06	2,08
GP 16 m a	74,3	2,11	0,37
GP 16 m c	187,9	2,07	0,47
GP 16 m f	412,6	2,05	2,20
GP 17 m a	322,5	1,64	1,89
GP 17 m c	467,9	2,05	1,88
GP 17 m f	392,5	2,08	1,09
GP 18 m a	299,6	2,06	1,91
GP 18 m c	81,5	2,12	1,99
GP 18 m f	491,1	2,07	1,97
GP 19 m a	337,2	2,07	2,04
GP 19 m c	963,2	2,07	2,13
GP 19 m f	406,2	2,06	2,24
GP 20 m a	324,1	1,87	1,99
GP 20 m c	901,3	2,07	1,93
GP 20 m f	856,2	2,07	2,28
GP 21 m a	417,3	2,07	1,77
GP 21 m c	780,5	2,07	1,83
GP 21 m f	869,3	2,07	1,89
Samples	**Conc.**	**Ratio**	
BXGPOEM controls	ng/mL	260/280	230/260
Ctrl 9 m a	295,2	2,07	1,93
Ctrl 9 m c	798,2	2,08	1,96
Ctrl 9 m f	932,0	2,08	1,92
Ctrl 10 m a	146,8	2,06	1,61
Ctrl 10 m c	433,3	2,05	2,06
Ctrl 10 m f	678,3	2,09	1,93
Ctrl 13 m a	493,9	2,12	1,97
Ctrl 13 m c	543,3	2,05	2,08
Ctrl 13 m f	272,4	2,04	1,79
Ctrl 15 m a	172,2	2,07	1,94
Ctrl 15 m c	106,7	2,04	1,91
Ctrl 15 m f	867,6	2,06	1,92
Ctrl 16 m a	162,0	2,06	1,68
Ctrl 16 m c	480,0	2,05	1,82
Ctrl 16 m f	391,6	2,07	2,12

This table reports the RNA extraction results from gastric mucosal biopsies of GP patients and non-gastroparetic controls, including RNA concentration (ng/mL) and purity ratios (260/280 and 230/260). Samples highlighted in red indicate those selected for downstream molecular analyses based on RNA purity thresholds (260/280 ratio 1.8–2.1 and 260/230 ratio >1.5).

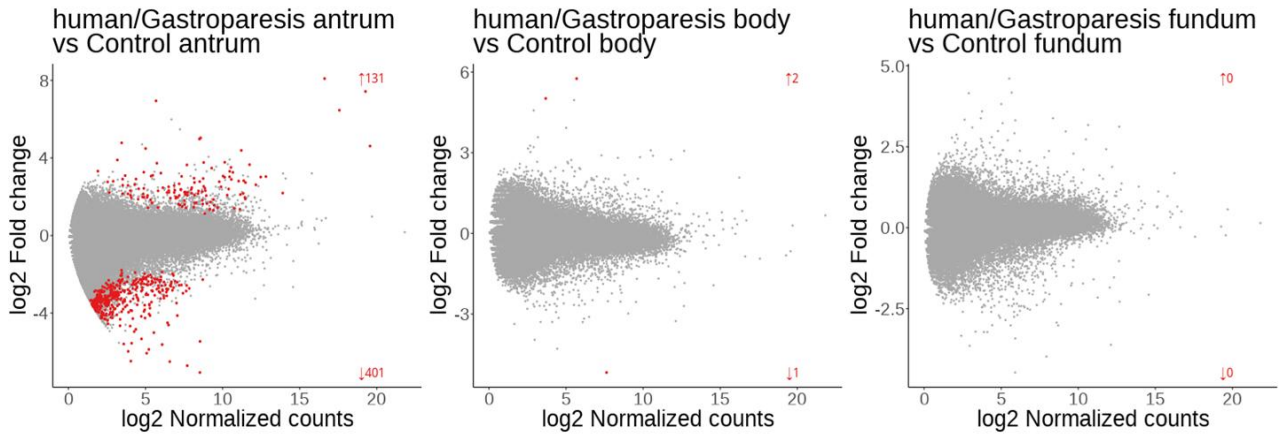
The abbreviations used in the table describe the anatomical origin of each sample. The letter "m" denotes mucosa, while subsequent letters indicate the gastric region: "a" for antrum, "c" for body, and "f" for fundus. The labels "GP" and "Ctrl" refer to samples from GP patients and controls, respectively. For example, the sample labelled "*GP 16 m c*" corresponds to a biopsy from the body of the stomach in the 16th GP patient in the series.

Figure S1. UMAP projection of gastric biopsy transcriptomes from GP patients and controls



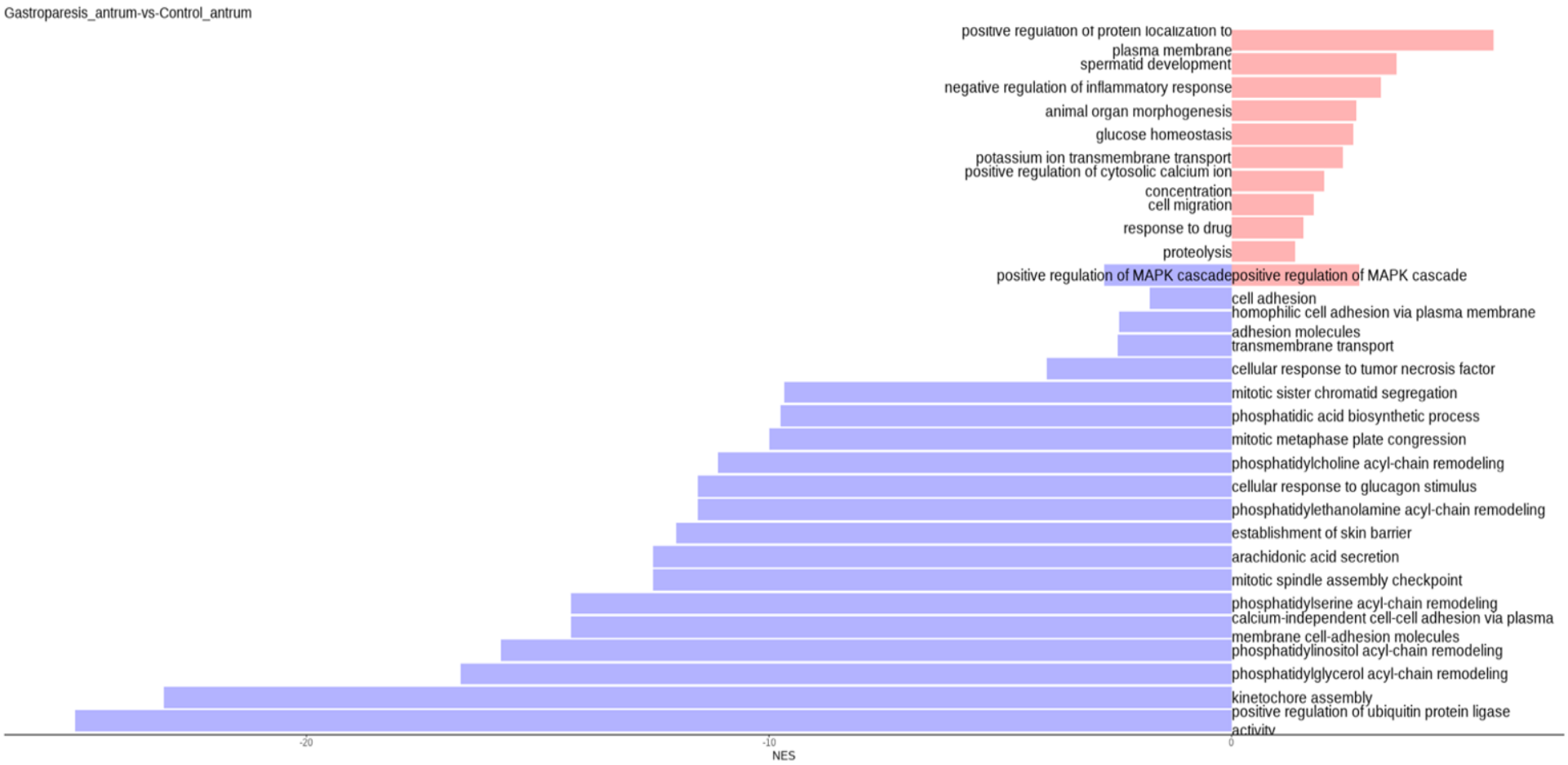
This UMAP plot displays the transcriptional profiles of gastric mucosal samples, with points coloured by anatomical site (antrum, body, fundus) and shaped by group (controls = circles, gastroparesis = triangles). No clear global separation is observed between GP and control samples. In contrast, samples tend to group partially by gastric region, indicating that anatomical site may account for more transcriptional variability than clinical phenotype.

Figure S2. Differential human gene expression in GP versus controls across gastric regions



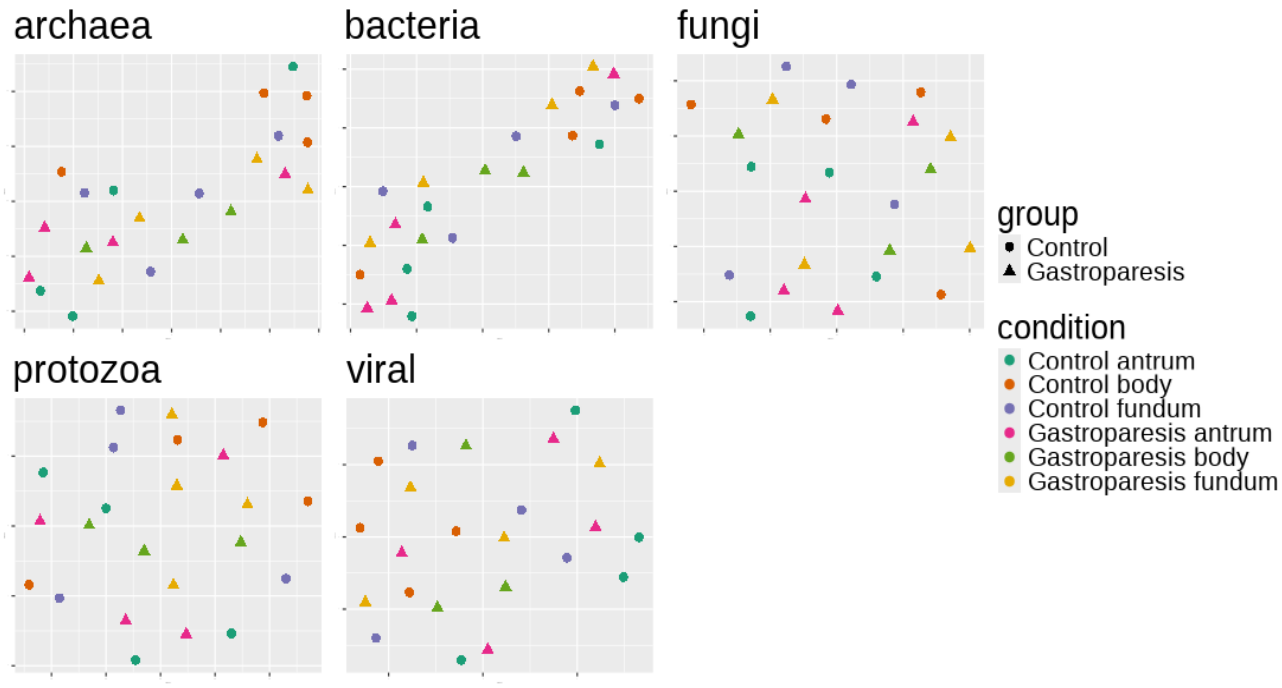
These plots display the differential expression of human genes in GP versus control samples, analyzed separately for the antrum, body, and fundus. Each point represents a gene, with log2 normalized counts on the x-axis and log2 fold change on the y-axis. Significantly dysregulated genes (FDR < 0.05) are shown in red. The number of upregulated and downregulated genes is reported in the upper right and lower right corners, respectively. A marked transcriptional shift is observed in the antrum, with a substantial number of differentially expressed genes (DEGs), whereas the body shows a more limited signature and the fundus displays no significant DEGs.

Figure S3. Enriched biological pathways in the antrum of GP patients compared with controls (GSEA analysis)



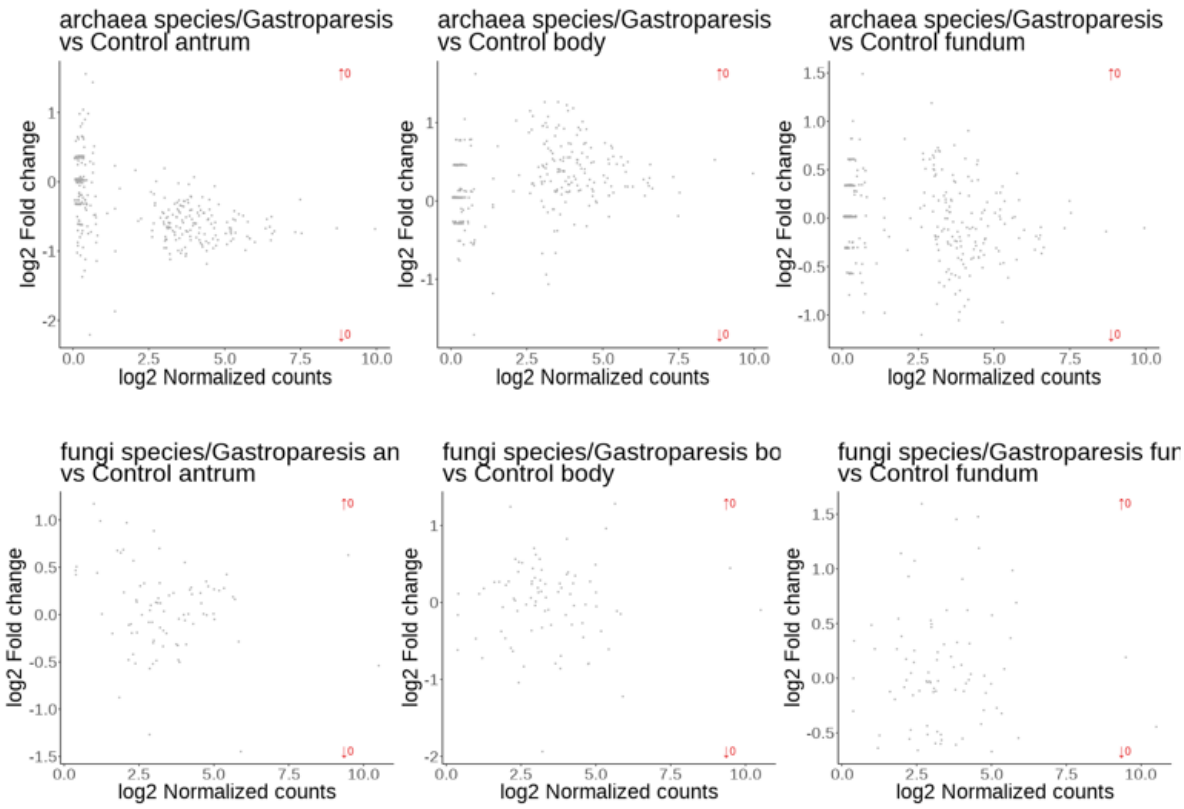
This bar plot shows the top significantly enriched pathways identified by Gene Set Enrichment Analysis (GSEA) in the antral samples of GP patients versus controls. Positive NES (red bars) indicates pathways upregulated in GP, while negative NES (blue bars) indicates downregulated pathways. Upregulated pathways include processes related to MAPK cascade activation, protein localization, inflammatory response, and cell migration, whereas downregulated pathways involve lipid and phospholipid metabolism, membrane transport, mitotic spindle assembly, and cell adhesion mechanisms.

Figure S4. UMAP projections of archaeal, bacterial, fungal, protozoal, and viral transcriptomic signatures in GP patients and controls



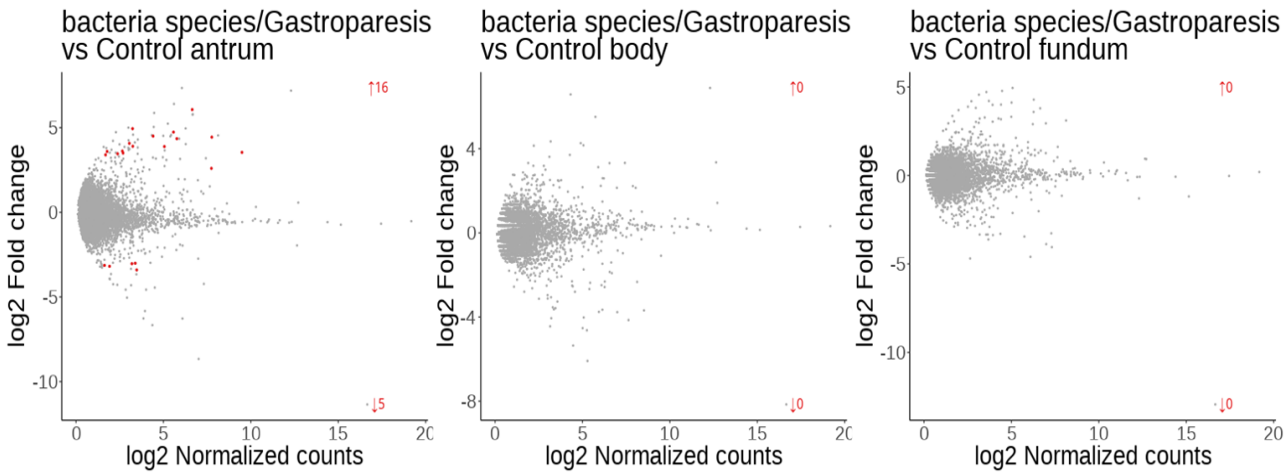
These UMAP plots display the distribution of microbial reads mapped to different kingdoms (archaea, bacteria, fungi, protozoa, and viruses) in gastric mucosal samples. Each point represents an individual biopsy, with shape indicating group (controls = circles, GP = triangles) and color indicating anatomical site (antrum, body, fundus). Across all kingdoms, no distinct clustering by disease status is observed, while partial grouping by gastric region is again detectable.

Figure S5. Differential microbial community composition of archaeal and fungal species in GP versus controls across gastric regions



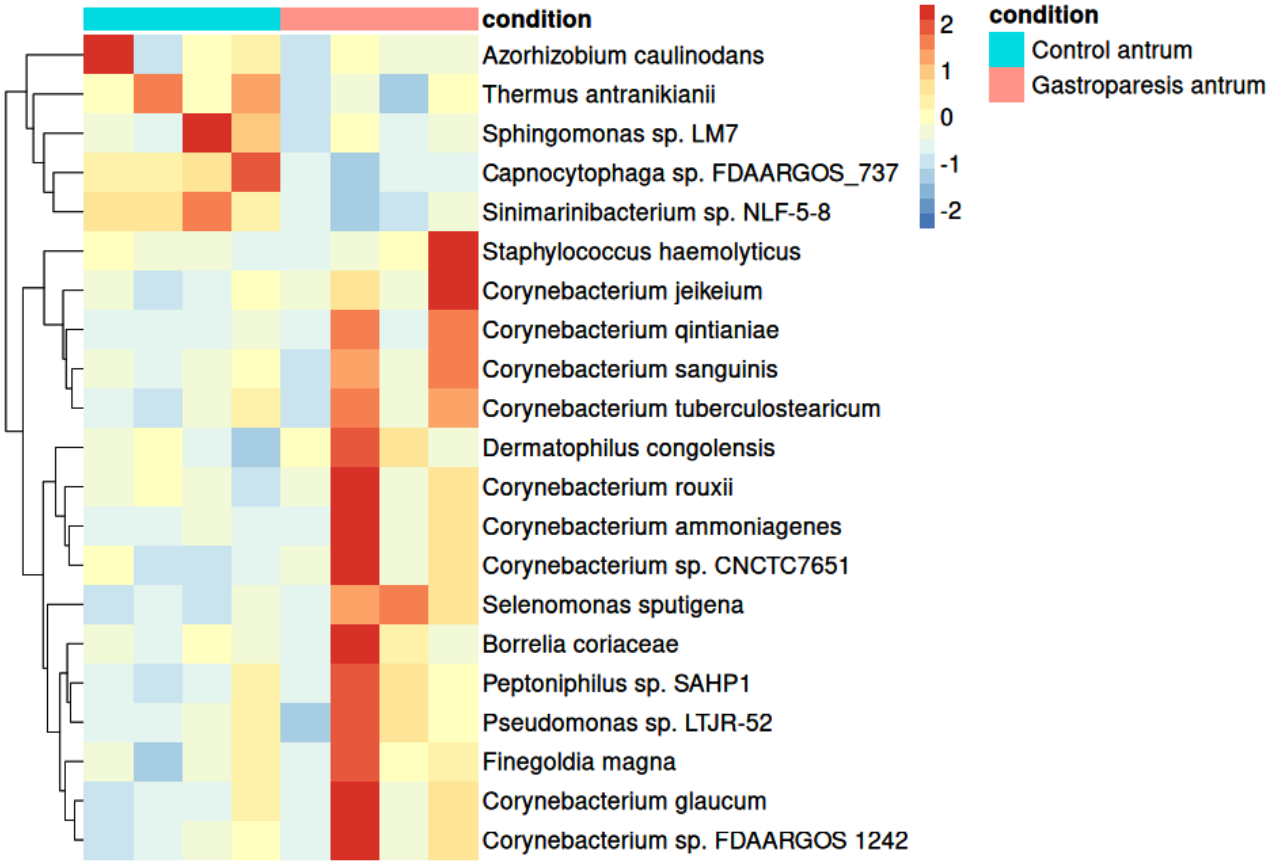
These plots show the differential analysis of archaeal (top panels) and fungal (bottom panels) species in GP and control samples from the antrum, body, and fundus. Each point represents a detected species, with log2 normalized counts on the x-axis and log2 fold change on the y-axis. No species met the significance threshold (FDR < 0.05), as indicated by the absence of highlighted points.

Figure S6. Differential changes in bacterial community composition in GP versus controls across gastric regions



These plots display differences in bacterial community composition between GP patients and controls, analyzed separately in the antrum, body, and fundus. Each point represents an individual bacterial species, with log2 normalized counts on the x-axis and log2 fold change on the y-axis. Significantly dysregulated species (FDR < 0.05) are shown in red. A distinct bacterial shift is detectable only in the antrum, where 16 species are increased and 5 are decreased in GP patients. In contrast, no species reach significance in the body or fundus.

Figure S7. Heatmap of differentially abundant bacterial species in the antrum of GP patients versus controls



This heatmap shows the species-level composition patterns of the bacterial taxa that were significantly altered in the antrum of GP patients compared with controls. Rows represent individual species and columns represent samples, with color intensity indicating scaled expression values (red = higher, blue = lower). Hierarchical clustering reveals a distinct bacterial signature in gastroparesis, characterized by an over-representation of multiple *Corynebacterium* species, *Peptoniphilus*, *Finegoldia* magna, and *Selenomonas sputigena.*