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Diagnostic yield of ACTG2 and FLNA gene assessment in adult patients with Idiopathic Chronic Intestinal Pseudo-Obstruction and Reduced or Absent Distal Esophageal Contractility

Acronimo: CIPO_GEN

Promoting center: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Sforza 28, 20122 Milano, Italia

Coordinator center: SC Gastroenterologia ed Endoscopia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milano, Italia

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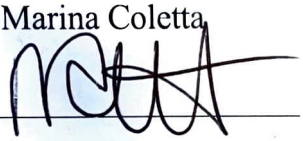
Firma 

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2. List of abbreviations

CIPO: chronic intestinal pseudo-obstruction

MNGIE: Mitochondrial neurogastrointestinal encephalomyopathy

ACTG2: smooth muscle actin gamma 2

FLNA: filamin A

HRM: high resolution manometry

DCI: distal contractile integral

GAD: glutamic acid decarboxylase

ASMA: anti-smooth muscle antibodies

gAChR: ganglionic acetylcholine receptor

3. Responsibilities (Role of the Sponsor and Collaborators)

The sponsor of the study is Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan.

The coordinating center is the Gastroenterology and Endoscopy Unit of the Foundation.

The Principal Investigator (PI) is Dr. Marina Coletta, who will oversee the study.

The following collaborators are affiliated with the same unit and will perform the corresponding roles:

- **Dr. Beatrice Marinoni**: coordinator of all phases of the study, data analysis, manuscript drafting and editing (collaborator of the Gastroenterology and Endoscopy Unit, freelance contract)
- **Dr. Giovanni Aldinio**: coordinator of all phases of the study, data analysis, manuscript drafting and editing (resident physician, University of Milan)

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- **Dr. Beatrice Guzzi**: data manager (scholarship holder at the Gastroenterology and Endoscopy Unit)

External collaborations (biological sample analysis, data analysis, diagnostic procedures, etc.)

Institution	*Operative unit*	*Participant name*	*Role and functions in the study*
Fondazione IRCCS Carlo Besta Neurological Institute	Biotechnologist at Neurology Unit 3	*Dott.ssa Margherita Marchi*	*Biological sample analysis, data analysis, manuscript drafting and editing*

4. Amendments and Other Protocol Changes

Not applicable

5. Timelines

Study status	***Planning***
Start of the study and data collection	October 2025
End of the study and data collection	October 2026
Final report of the study	February 2027

6. Rationale and background

Chronic intestinal pseudo-obstruction (CIPO) is an extremely rare and disabling condition, characterized by recurrent symptoms of intestinal obstruction in the absence of any mechanical occlusive lesion ([1], [2]). CIPO results from the damage of neuronal, muscular, or mesenchymal structures of the gut wall or its extrinsic neuronal control, leading to chronic impairment of tonic and propulsive motor functions in one or more segments of the gut ([1], [2]).

CIPO may be secondary to an underlying disease (Table 1) or it may be idiopathic.

Table 1. Diseases associated with chronic intestinal pseudo-obstruction (CIPO) in adults (2)

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Congenital	Hirschsprung's disease
Mitochondrial diseases	Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)
Neurological diseases	Muscular dystrophy (Myotonic, Duchenne)
	Autonomic dysfunctions
	Multisystem atrophy
Infiltrative	Amyloidosis
Autoimmune	Celiac disease
	Systemic lupus erythematosus
	Anti-synthetase syndrome (myositis, dermatomyositis)
Connective tissue disorders	Systemic sclerosis
Infectious	Borrelia burgdorferi (Lyme disease)
	Strongyloides stercoralis
	Trypanosoma cruzi (Chagas disease)
Paraneoplastic	Small cell lung carcinoma and other cancers
Primary or Idiopathic	Sporadic autoimmune/inflammatory
	Genetic

In the latter group, two distinct subgroups can be identified: one in which CIPO results from an autoimmune or inflammatory response targeting the neural or muscular structures of the gut, and one in which an inherited or de novo genetic mutation disrupts muscle, neural, or interstitial cell function.

Among the genes associated with CIPO, mutations in the smooth muscle actin gamma 2 (ACTG2) gene are the most common with 139 published cases, 36 of them reaching the adult age ([3] – [27]). More rarely, CIPO has been reported to be associated with mutations in filamin A (FLNA) gene. This gene is located on the X chromosome and accordingly the disease has been reported only in males. 31 cases of CIPO linked to mutations in filamin A (FLNA) gene have been reported so far in pediatric patients ([28] – [37]) and in only one adult patient in which the disease was transmitted from the healthy mother [38].

Both ACTG2 and FLNA mutations impair intestinal smooth muscle cell function, leading to heterogeneous phenotypes of visceral myopathy [39]. The functional impairment of the smooth muscle can be suggested by an abnormal esophageal motility as shown by the genotype/phenotype correlation in patients with ACTG2 and FLNA mutations ([40] – [42]). Kocoshis et al showed that esophageal motility is reduced or absent in children with severe impairment of gut motility due to ACTG2 gene mutations [5]. Topa et al reported that the esophageal distal contractile integral was reduced (DCI <450 mmHg*s*cm)

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in one adult patient with CIPO due to ACTG2 mutation [4]. In the above-mentioned adult patient with CIPO due to FLNA mutation, the esophageal distal contractility was absent (DCI = 0 mmHg*s*cm).
According to these observations it can be hypothesized that at least some of the patients with idiopathic CIPO and ineffective or absent esophageal contractility might underlie a mutation in genes that control smooth muscle function. Thus, the aim of this study will be to assess the diagnostic yield of Sanger sequencing for ACTG2 and FLNA gene mutations (the latter tested only in males) in patients with idiopathic CIPO in which high-resolution esophageal manometry (HRM) shows reduced (DCI <450 mmHg*s*cm) or absent distal esophageal contractility.
The results of this study will allow a better classification of idiopathic cases of CIPO in which the detection of a genetic cause of the disease should prompt genetic counselling and in future genetic therapy. Moreover, the precise definition of a myopathic phenotype of CIPO, that is unrelated to the genes that are known to cause this condition, will allow further studies of whole exome sequencing aimed to identify new gene mutations that may cause the smooth muscle impairment.

7. Research question and objectives

The study object is to assess the diagnostic yield of Sanger sequencing for ACTG2 and FLNA genes evaluation on blood or saliva sample in patients with idiopathic CIPO in which high-resolution esophageal manometry (HRM) shows reduced (DCI <450 mmHg*s*cm) or absent distal esophageal contractility.

8. Methods

8.1 Design of the study

No profit multicenter prospective observational study.

8.1.1 Primary endpoint

The prevalence of mutations of ACTG2 or FLNA genes in a subgroup of patients with idiopathic CIPO with a myopathic phenotype revealed by reduced or absent distal esophageal contractility evaluated with high resolution esophageal manometry

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8.2 Setting

The CIPO-GEN study is a multicenter investigation conducted at one center in Italy and one in the United Kingdom (Nottingham), with the Gastroenterology and Endoscopy Unit of the Policlinico of Milan serving as the coordinating center. Patient recruitment will take place through the participating hospital facilities, using medical records, endoscopic and radiological reports, and manometric evaluations as data sources. The study will begin in October 2025 and conclude in June 2026, with a 12-month enrollment period.

8.2.1 Study population

Patients with primary CIPO enrolled between October 2025 and October 2026.

8.2.2 Inclusion criteria

Patients who simultaneously meet the following criteria will be included:

- Age ≥18 years
- Both male and females
- Primary CIPO (2)
- Reduced (DCI <450 mmHg*s*cm) or absent distal esophageal contractility at HRM.

8.2.3 Exclusion criteria

Patients who do not meet the inclusion criteria and who have the following exclusion criteria will be excluded:

- Diagnosis of secondary CIPO (Table 1)
- Diagnosis of primary CIPO caused by an autoimmune/inflammatory disorder revealed by the presence of anti-Hu, anti-GAD, ASMA or anti-gAChR antibodies.

8.2.4 Description of study object

The CIPO-GEN study aims to investigate possible genetic alterations in the ACTG2 and FLNA genes in patients diagnosed with primary CIPO. The study will be conducted according to standard clinical

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practice. Patients will be appropriately informed about the study's objectives, the planned procedures, and the potential benefits and risks. Each participant will receive a detailed informed consent form, outlining the study rationale, the assessments to be performed (such as blood or saliva collection and, if necessary, high-resolution esophageal manometry), the duration of follow-up, and any associated discomfort. Full compliance with ethical standards and good clinical practice will be ensured, safeguarding the voluntary nature of participation and the right to withdraw from the study at any time without any impact on clinical care.

8.2.5 Scheduled Visits and Assessments

The study includes a single outpatient visit during which the patient's clinical history will be collected, a blood (two 3ml test tubes) sample will be taken, and high-resolution esophageal manometry (HRM) will be performed if indicated. A second outpatient visit will take place three months later to communicate the results of the genetic analysis.

DNA will be extracted from peripheral blood samples or saliva. Genomic DNA targeted regions for ACTG2 and FLNA will be sequenced using the Sanger method.

8.3 Variables

Data collection will include:

- Demographic data (age, sex, date of diagnosis, family history of CIPO, age of symptoms onset, site of gut dilatation, involvement of extra intestinal organs (bladder, heart, blood vessels)
- Clinical presentation and symptoms (constipation, nausea/vomiting, abdominal distension and pain, recurrent episodes of acute intestinal obstruction, urinary retention and need for catheterization, neurological and musculoskeletal symptoms)
- Diagnostic tests (radiological and endoscopic findings, esophageal and anorectal manometry, transit studies, full thickness gut biopsies when available)
- Pharmacological therapies (prokinetics, laxatives, analgesics)
- Surgery (decompressive procedures, ostomies, resections)
- Long-term follow-up (complications, need for parenteral nutrition)

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8.4 Source Documents

The study is based on clinical data from patients who have already been followed on an outpatient basis at the Foundation, as well as previous instrumental investigations (HRM, EGDS).

Data derived from HRM will be stored in digital format and analyzed using dedicated software for trace processing.

8.5 Sample Size

Given the extreme rarity of idiopathic CIPO with reduced or absent distal esophageal contractility, a formal sample size calculation was not performed. Moreover, this study is designed as an exploratory investigation to determine the of mutations of ACTG2 or FLNA genes in this population. Thus, all eligible patients meeting the inclusion criteria and providing informed consent at the participating centers between October 2025 and October 2026 will be enrolled consecutively.

Based on the number of patients usually referred to our units and collaborating centers for CIPO, we expect to enroll approximately 20 subjects during the 12-month recruitment period. However, the final sample size may vary according to actual case availability.

The results will provide preliminary data on the prevalence of ACTG2 and FLNA mutations in this population and may inform the design of future studies.

8.6 Data Management

At the time of enrollment, each participant will be assigned a unique code. Data de-identification will be carried out in such a way that individuals accessing the database will not be able to trace the identity of the subjects. Only local investigators will be able to link the code to the subjects' identities.

The necessary data for the study will be recorded in a dedicated eCRF within a Data Management System validated according to national regulations and provided by the Scientific Directorate of the Foundation. The platform used will be REDCap (Research Electronic Data Capture).

The REDCap Consortium includes over 1,000 institutional partners worldwide (research institutions, universities, ministries, etc.). The consortium supports a secure web-based application (REDCap) designed exclusively to facilitate data collection for research studies. REDCap allows users to quickly and

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securely create and manage online databases and is currently used in more than 110,000 projects by approximately 150,000 users across various research domains.

For this study, REDCap will ensure:

a) user-level identification, with role-based access restrictions

b) real-time validation and data integrity checks

c) patient de-identification prior to data export

d) centralized data archiving with daily backups, on a secure server within the Foundation's IT infrastructure

8.7 Data Analysis

The statistical analysis will primarily be descriptive. Prevalence will be calculated as the proportion of patients with at least one detected mutation over the total number of enrolled patients. Continuous variables will be summarized using mean and standard deviation (SD) or median and interquartile range (IQR), depending on the distribution. Categorical variables will be presented as absolute frequencies and percentages.

8.8 Quality Control

To ensure adequate quality control of the study, the investigator will allow, if requested, direct access to all relevant documentation and dedicate time to discussing study results. In addition, Regulatory Authorities may conduct inspections. In such cases, the investigator must authorize inspectors to access all pertinent documentation and dedicate time and staff to discuss the monitoring outcomes and any other aspects of the study. For all manual data entries, the presence of two operators will be ensured to minimize potential errors.

8.9 Study Limitations

The main limitations of the study include the small sample size, which may limit the generalizability of the results and reduce the statistical power to detect smaller effects. Moreover, the non-randomized and uncontrolled nature of patient enrollment may introduce selection bias.

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9. Protection of Study Subjects

The study will be conducted in accordance with Good Clinical Practice (GCP) guidelines, the ethical principles of the Declaration of Helsinki, and current regulations on observational studies.

The observational study and related documentation will be submitted to the competent Ethics Committee.

The study will begin only after receiving all required authorizations in accordance with the institution's internal procedures.

The Ethics Committee must also approve any protocol amendments and any recruitment advertising, in compliance with local regulations.

9.1 Subject Information Sheet and Consent Form for Personal Data Processing

It is the responsibility of the physicians to obtain consent to the information sheet through a signed and dated privacy consent form from each patient before data collection begins. The signature certifies the patient's understanding of the consent and the information contained therein. Additionally, the investigator must sign and date the informed consent form.

Signed documents must be archived by the investigator, and a copy must be provided to the patient.

The study supervisor ensures that the data of enrolled patients will be stored, archived, and processed in full compliance with privacy regulations in accordance with Article 13 of Regulation (EU) 2016/679, current national privacy legislation, the Code of Ethics for data processing for statistical and scientific purposes, and the "Guidelines for the processing of personal data in the context of clinical drug trials" published in the Official Gazette No. 190 of August 14, 2008.

Patient data will be collected in a pseudo-anonymized format and will only be accessible to internal and external personnel who are specifically authorized and bound by a confidentiality obligation regarding any information learned during the study.

The study supervisor also guarantees that the minimum-security measures prescribed by the aforementioned regulations have been implemented for data processing using both electronic and non-electronic means to prevent any unlawful data processing.

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9.2 Insurance Coverage

Given the observational nature of the proposed studies, no additional insurance policies are required beyond those already in place for standard clinical practice.

10. Dissemination and Communication Plan for Study Results

The study's scientific supervisor commits to preparing a final report and to making the results publicly available at the end of the study. The data will be published anonymously and, when required, presented in aggregated form.

11. Publications and intellectual property rights on the study results

Publications:

The Foundation, as the Sponsor, shall ensure the dissemination and publication of the study results, including negative results, without any restrictions, and shall guarantee the collaborating center visibility proportional to its actual participation.
Any journal or scientific publication containing the results and data of the study must indicate the role and participation of the Centers and the Foundation, in proportion to their actual contribution to the study and the role played by each party.
Data will be published in aggregate or otherwise anonymized form, so as not to allow in any way the identification of the individual to whom the data refer.

Intellectual Property Rights:

The Parties acknowledge that, for the purpose of conducting the collaboration within the framework of the study, data, information, know-how, and inventions (whether patentable or not) owned by each party may be used and shared. Each party shall remain the exclusive owner of such assets, even if it grants the other party a non-exclusive and free-of-charge right of access and use, solely for the purpose of carrying out the activities covered by the study and only for its duration.
It is understood that this right of use does not include the right to sublicense to third parties.
In accordance with applicable laws, the data and results generated within the framework of the

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Study shall be the property of the Sponsor, unless otherwise agreed between the Sponsor and the Centers.

12. Funding

No funding.

13. References

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