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Utility of animal gastrointestinal motility and transit models in functional gastrointestinal disorders

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ABSTRACT

Alteration in the gastrointestinal (GI) motility and transit comprises an important component of the functional gastrointestinal disorders (FGID). Available animal GI motility and transit models are to study symptoms (delayed gastric emptying, constipation, diarrhea) rather than biological markers to develop an effective treatment that targets the underlying mechanism of altered GI motility in patients. Animal data generated from commonly used methods in human like scintigraphy, breath test and wireless motility capsule may directly translate to the clinic. However, species differences in the control mechanism or pharmacological responses of GI motility may compromise the predictive and translational value of the preclinical data to human. In this review we aim to provide a summary on animal models used to mimic GI motility alteration in FGID, and the impact of the species differences in the physiological and pharmacological responses on the translation of animal GI motility and transit data to human.

Introduction

Functional gastrointestinal disorders (FGID) include functional diarrhea, functional constipation, irritable bowel syndrome (IBS) with predominant diarrhea (IBS-D), IBS with predominant constipation (IBS−C), and IBS with mixed bowel habits. Rome IV considers that these disorders exist as a continuum rather than as in isolation and the revised definition of these disorders as"disorders of gut-brain interaction" [1,2]. In addition, these disorders are classified by gastrointestinal (GI) symptoms related to any combination of motility disturbances, visceral hypersensitivity, altered mucosal and immune function, gut microbiota, and/or central nervous system (CNS) processing.

The pathophysiology and etiology of the disturbances in GI motility in FGID is poorly understood, which in part hamper the development of animal model that mimic symptoms of FGID. Therefore, available GI motility models are to study symptoms (delayed gastric emptying, constipation, diarrhea) rather than biological markers to develop an effective treatment that target certain features of altered GI motility seen in patients. Available techniques for the assessment of GI motility and transit are for preclinical test for drug development and for diagnostic purposes in veterinary practice and clinic [3–7]. American and European Neurogastroenterology Societies provided guidelines on the optimal methods for the assessment of gastric emptying, small intestinal and colonic transit in clinical practice [8]. Scintigraphy, breath test and wireless motility capsule are recommended for measurement of regional and whole GI transit.

A variety of *in vitro* and *in vivo* models are used to evaluate GI motility in laboratory animals. A number of tests developed and implemented for clinical diagnosis in human are validated in different animal species with findings relatively comparable to clinic [6,9,10]. However, prior knowledge of species differences is essential for proper interpretation and extrapolation of animal data to human.

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GI anatomy and physiology

There are considerable similarities in the basic structure of the gastrointestinal tract (GIT) in human and laboratory animals, apart from species differences in the anatomy and mechanism controlling the GI function [11–13].

GI motility is an interplay between smooth muscle, enteric nervous system (ENS) embedded in the gut wall and their anatomic connections with the CNS. GI smooth muscle fibers are electrically connected through large numbers of gap junctions [14]. The spontaneous myogenic and rhythmic motor activities of different segment of GIT are triggered by specialized nerve-like pacemaker cells called interstitial cells of Cajal (ICC) located in the ENS [15]. Substantial loss of ICC has been demonstrated in patients with GI motility disorders and diseases like diabetic gastroenteropathy, slow transit constipation, ulcerative colitis, and Crohn's disease [15–18].

The ENS is involved in the regulation of absorption, secretion and motor function of the GI independent of the CNS [19,20]. However, GI functions are modulated by different components of the so-called "gut-brain axis"which is a bidirectional communication system between the CNS and the GI tract. It has been suggested that dysregulation of the gut-brain axis, which encompass the ENS, autonomic nervous system (ANS), CNS and enteroendocrine cells, is involved in the GI disorders [21,22]. In a 12 years prospective study in Australian population by Koloski et al. [23], the bidirectional interaction between gut and CNS was found to account for FGID, including IBS and functional dyspepsia, in patients with higher levels of anxiety and depression at baseline and brain-gut pathway is dominant in inflammatory bowel diseases. In line with the strong connection between CNS and GIT, preclinical and clinical data support the use of psychotropic agents to treat patients with FGIDs (reviewed in Ref. [22]). Interestingly, Rome IV released in May 2016, recognized the bidirectional interaction of CNS and gut, in the new agreed definition of FGID as 'disorders of gut–brain interaction', in which motility disturbance is among group of disorders classified by GI symptoms [1]. The mechanism underlying motility disturbances in FGID is poorly understood, hence animal model that mimic the motility component of FGID is lacking.

In this review the appropriateness of the available animal GI models is discussed in relation to the physiological control mechanism and pharmacological responsiveness.

GI motility pattern in animal and human

GI motility pattern in humans, dogs, rats and suncus (*Suncus murinus*), is organized in two distinct patterns in relation to the digestive state of the bowel. i.e. fasted (interdigestive) and fed (digestive) motility pattern [13, 24–26]. The fasted motility pattern referred to as migrating motor complexes (MMC) is characterized by the regular reoccurrence of a period of irregular and low-amplitude contractions (Phase II) followed by a short period of intense burst of regular contractions (Phase III) propagating from the stomach or the duodenum to the ileum. MMC occurs in cycles of approximately every 90–100 min in suncus, dogs, and man [13, 24–26], while in rat it occurs at a much faster rate of 12–15 min [25]. The occurrence of intense propagating Phase III activity in-between meals may provide "house keeper" function to clean the small intestine from digestive derbies. Absence of MMC has been reported in patients with bacterial overgrowth [26], gastroparesis and intestinal pseudo-obstruction [27]. It has been suggested that phase III of MMC is induced by GI hormones motilin in suncus, dog and human, and ghrelin is involved in initiation of phase III in rodents and human [27–29]. The MMC with its alternating periods of cyclic contractions with period devoid of contraction may provides useful preclinical and clinical model for the study of the physiological and pathological motility abnormalities and the pharmacological effects of inhibitors and stimulants of GI motor activity.

After feeding, the fasted contractile pattern immediately changes to a postprandial contraction (PPC), which consists of irregular phasic contractions similar to those in phase II (~160 min) and strong contractions named postprandial giant contractions (PPGC, ~10 min) that occur at the end of PPC [30]. It has been suggested that the gastric contents are mixed and gradually move to the small intestine during the digestive phase (the first half of the PPC with phases II) and are completely propelled into the lower intestine during the discharge phase (the latter half of the PPC with phase III), which provides an emptied stomach in preparation for the next meal [30].

Various studies have demonstrated the important role of 5-HT in the regulation of GI functions as well as its potential role in the pathophysiology and motility disturbances of FGID [31]. Out of the fourteen 5-HT receptors, belonging to seven families [32], 5-HT_3 and 5-HT_4 are mostly studied for their role in GI motility [31–33]. 5-HT increases the amplitude of GI contractions in guinea pig mainly via a cholinergic pathway, and 5-HT_4 receptors distributed in the myenteric plexus are important for enhancing GI motility [34–36]. Species differences were demonstrated in regards to initiation of phase III activity of MMC following intravenous administration of serotonin in human and dog but not in mice and rats [27]. Itoh et al. [37] found that the effect of motilin on phase III activity in dogs was dependent on 5-HT_3 receptor activation which presumably located on the vagal afferents. In human, the prokinetic effect of 5-HT_3 receptor activation is inhibited by 5-HT_3 receptor antagonists such as alosetron, ondansetron, and cilansetron, which reduce colonic and whole-gut transit or motility, increase fluid absorption, and stool consistency or attenuate postprandial dyspepsia-like symptoms (reviewed in Ref. [38]). As 5-HT_3 receptor antagonists reduce GI transit, it is reasonable to assume that a 5-HT_3 receptor agonist would be prokinetic. Preclinical and clinical studies with prucalopride, a 5-HT_4 receptors agonist, showed consistent effect across species; stimulates GI motility in rodents and dog and improve bowel movement in patients with chronic idiopathic constipation (reviewed in Ref. [39]).

The choice of an appropriate animal species to model human responses in preclinical studies should take into account the species differences in functional and molecular pathways controlling GI function that may compromise the translational value of animal data to humans. For example, in human, dog and suncus, GI hormone motilin is involved in the initiation of phase III activity of MMC [13,40]. Consistent with this physiological effect, motilin receptor agonists erythromycin or mitemcinal, exerts phase III-like contraction in human, dogs and suncus [13,41,42] and ameliorate symptoms related to gastroparesis in diabetic patients [43]. In contrast, motilin function and/or receptor gene are lost in rat, mouse and guinea pig [12], therefore preclinical *in vivo* GI motility studies with motilin and motilin receptor agonists are mostly conducted in dog. Schattauer et al. [44] demonstrated significant differences between human and rodent kappa-opioid receptors signaling that may affect the effects mediated by activation of these receptors. In addition, neurokinins (NK) induce potent contractile effects via NK_2 receptors in human intestine [45], whereas NK_1 and NK_3 receptors are the main contributors to smooth muscle contraction in guinea pig and rabbit [46]. In regards to motilin and neurokinins, data suggests that physiological and pharmacological responses in dog seems more comparable to human than that in rodents.

5-HT_3, which is abundant in the GI and CNS is involved in motility and sensory signaling. Holbrook et al. [47] demonstrated five different 5-HT_3 receptor subunits (5-HT_3A-E) in human and

dog compared to only two (5-HT_3A,B) in rodents. The functional significance of this species variation has not been elucidated. Species differences in the role of cholinergic pathway are exemplified by lack of effect of atropine on regular occurrence of MMC in rats [48], compared to potent inhibitory effect on MMC in human [49] and dogs [50].

Taken together, species differences in control mechanism of GI motility and pharmacologically evoked motility responses may compromise the translational value of experimental animal data to human. Therefore, the choice of a representative animal species and model should reveal information relevant to clinical setting and ultimately predictive of effective therapy.

Methods for measurement of GI motility and transit in animals and its predictive value to human

There are several techniques available for the assessment of GI motor functions in laboratory animals and in human for clinical diagnostic purposes [3,5–7]. Rodents (mice, rat and guinea pig) and dog are most commonly used experimental animal in clinical research. A summary of most commonly used techniques is shown in Table 1. The table is for the purpose of comparison and the score given for each technique reflect our view because there is no validated scale for assessing animal techniques. Rao et al. [8] presented a semi-quantitative score for comparison of the various techniques used to assess GI transit in clinic.

Both *in vitro* and *in vivo* models are used to evaluate GI motility in laboratory animals at the cellular (not covered here), tissue and whole animal levels. Animal techniques for study of GI motility and transit are conducted on healthy tissue or animal, using pharmacological tools to interfere or augment a physiological response. Therefore, preclinical models may mimic certain features of the symptomatic alteration in GI motility seen in patients.

In vitro methods to assess GI motility

In vitro studies are made on isolated tissue segment from animal and human or whole organ from GIT of laboratory animal to study physiological and pharmacological responses of smooth muscle and ENS. Vast number of physiological and pharmacological studies were conducted on isolated rodent GI segments. Typically segment of intestine is suspended in organ bath in a longitudinal direction to study the smooth muscle contraction of the longitudinal muscle layer, in spite of the fact that peristalsis is produced by the circular muscle layer [14,51]. Comparison between results from human isolated GI tissues and data obtained from most commonly used isolated rodent tissue preparations revealed marked species-dependent differences in the responses to cannabinoids, 5-$HT_{3,\ 4}$ receptors, histamine receptors, protease-activated receptors and on the effect of morphine on electrically evoked contraction [12,52,53]. Data from *in vitro* studies is limited to the direct myogenic effects and those mediated by ENS without the contribution and interaction with the peripheral and central influences that alter motility. Nonetheless, *in vitro* studies have the advantage to serve as an initial screen for drug effects on smooth muscle and ENS [7].

Much of our understanding about the mechanism underlying colonic motility and transit has arisen from studies on the isolated mouse, rat and guinea pig colon. 5-HT, especially 5-HT_3 and 5-HT_4 receptors, has been mostly studied for its role in the initiation and propagation of colonic migrating motor complexes and its potential use in the treatment of colonic dysmotility [54].

Keating et al. [55] tested fifteen marketed drugs or drugs intended for market at clinically relevant dosing range on colonic peristaltic motor complex (CPMC) activity in a segment of mouse colon, of which eight compounds with validated GI adverse drug reactions as diarrhea and constipation affecting 17% and 100% of patients. When the results of the *in vitro* assay were compared with data obtained at therapeutic plasma concentration using the gold standard *in vivo* charcoal meal transit study in rats, the predictive capacity were considered poor for both *in vitro* (60%) and the *in vivo* model (47%). Some drugs decreased CPMC activity, even if diarrhea was the clinical response to exposure of these drugs.

In vivo evaluation methods

In vivo techniques offer direct measurement of smooth muscle contraction or indirect measurement of the motility by assessing

Table 1
Summary of techniques used for the measurement of gastrointestinal motility/transit in laboratory animals.

	In vitro	Marker techniques			Force transducer/ Myoelectrical	Barostat	Scintigraphy	WMC
		Charcoal meal/fecal pallets	Paracetamol/AMN[d], Sulphapyridine	Breath test				
Validation	+ +[a]	++	++	+	+	+	+	+
Standardization	+	+	+	+	+	+	+	+
Specialize facility	+	+	+	++	++	++	++	+++
Animal species	+++	mice, rat, guinea pig	Mice, rat, dog	mice, rat, dog	Suncus, rat, guinea pig, dog,	rat, guinea pig, Dog	Mice, rat, dog	dog
Regional motility/transit	+++[b]	gastric, intestinal	gastric, small intestinal/ orocaecal	Gastric, intestinal	Gastric, intestinal	Gastric	Gastric, intestinal, orocaecal	intestinal
Translation of animal data to human	+	+	+ +	+ +	+ +	+ +	++	++
Animal restraint	NA	+[c]/(+)	+	++	+++	+	+	(+)

NA: Not applicable. WMC: Wireless motility capsule.
Validation (+limited or ++ moderate evidence/data relating test results to clinical data).
Standardization (+several study protocols for testing).
Specialized facility (+test easily run, ++ require moderate or +++ advanced training and custom build facility for radiation hazard or advanced testing).
Translation of animal data to human (+provide preliminary pharmacodynamic data, ++ similar technique in clinic with moderate translational potential).
Animal restraint (+) No or minimal handling stress to obtain test results, + limited or short-term handling/restraint for blood sampling, ++ moderate or +++ long-term handling/restraint for breath sampling or data capture.
[a] : test compound is usually run with control/reference compound.
[b] Taking into account species differences.
[c] Animal should be scarified to secure data.
[d] AMN: Acetaminophen.

the movement of intraluminal content (gastric emptying and intestinal transit). Methods that give direct measurement of smooth muscle contractions include myoelectrical activity, tension or force measured by force transducer, changes in intraluminal pressure measured by intraluminal balloon or multi-lumen catheter and different techniques used to assess the transit of intraluminal contents along the length of GI tract.

Traditional invasive methods for measuring gastrointestinal motility in dogs involve surgical intervention, i.e. implantation of electrodes or pressure strain gauges transducers positioned at several points along the gut [5,7,56]. Electromyography and pressure measurements have been the method of choice to record circular muscle activity and to investigate the propagation of muscle contraction along the GIT. Apart from surgical intervention, motility recordings require animal restrain to capture data. Recent development and implementation of clinically used and less invasive techniques in preclinical studies, like imaging, tracer investigations, scintigraphy and wireless motility capsule investigations become more attractive than the invasive methods because data from these techniques may directly translate to the clinic.

Indirect measurement of pharmacodynamic effects of drugs on GI function has been studied by observation of the faecal pellet number, weight and appearance. Studies in mice and rats showed that compounds with known inhibitory or stimulatory effect induced the expected effects and changes in the number, weight and transit of faecal pellets [57–59]. Interestingly, Rome III and IV subclassification of IBS is based on stool form only not stool frequency to differentiate bowel habits (diarrhea-, constipation dominant and alternating) and is considered as good predictor of intestinal transit time in IBS patients [2,9]. The rodent faecal pellet method is an easy technique and may offer a useful initial marker for the prediction of functional effects such as diarrhea and/or constipation but give limited information on the upper versus lower GI function.

Another common preclinical test in rodents is to study the effect of stress and drugs on the GI transit of a test meal containing non-absorbable markers such as phenol red, barium sulphate, or charcoal (reviewed in Refs. [5,7]). The charcoal meal method is widely used as initial screen in preclinical evaluation of drugs effects on GI transit with findings of variable predictive capacity of detecting drug-induced GI effects in the clinic [7,55,60]. This method gives limited information on the segmental transit because the measurement is made at one time-point and it is a terminal method needing to sacrifice the animal to secure data.

Translation potential of methods used for measurement of GI transit

In a position paper by the American and European Neurogastroenterology and Motility Societies on the evaluation of gastrointestinal transit in clinical practice, scintigraphy technique, breath tests and wireless motility capsule were recommended for measurement of regional and whole gut transit [8]. These techniques were also tested in laboratory animals to assess GI transit [6].

*Scintigraphic technique*is considered the gold standard method for measurement of GI transit in human (reviewed in Ref. [61]) and has been validated and demonstrated to give measurement in dogs comparable to that in human [62,63]. In dog, ^{99m}Tc and ^{111}In labeled beads were used to investigate GI transit and regional colonic transit, respectively (reviewed in Ref. [4]). Studies on the effect of motilin and prokinetic drugs showed accelerated GI transit and regional colonic transit, findings similar to that in human studies. The gastric emptying of indigestible solid radiopaque marker in the dog is dependent on particle size, density and meal viscosity [4]. Particle size of 1.6 mm pass with digestible food similar to ^{99m}Tc-labeled standard meal [64], compared to particle size > 2 mm which empty with the recurrence of gastric phase III of MMC [4]. Apart from being the gold standard in clinical studies, scintigraphic method requires special facility to manage radiation hazard and limited access to expensive equipment. In addition, stress associated with the restraining of the animal may alter the GI motility [65].

Marker techniques

These techniques are indirect measures for the study of GI transit that involve ingestion of a marker compound that is not absorbed from the stomach but rapidly absorbed in the duodenum or colon. Appearance of the marker in exhaled air (breath test) or in plasma (paracetamol/acetaminophen) is directly related to gastric emptying or GI transit [4,8]. Measurement of gastric emptying using breath test is dependent on rapid absorption of ^{13}C-octanoic acid from the duodenum followed by prompt oxidation in the liver to release $^{13}CO_2$ which can be measured in exhaled air. Thus, the appearance of $^{13}CO_2$ is dependent on the passage of contents from stomach to duodenum. Results from the breath test correspond to those obtained from “gold standard” scintigraphic method [66]. Breath tests offer an attractive method of measuring gastric emptying in small laboratory animals allowing repeated measurements to be made in the same animal to detect both delayed and accelerated gastric emptying induced by meal composition or drugs [67,68]. Lactulose disaccharide is used to study the orocaecal transit time, by taking breath samples to measure increase in hydrogen produced by bacterial fermentation in the colon, using high-resolution mass spectrometer. In animal studies, measurements require animal restraint at multiple time points and analysis by expensive equipment and well trained personnel for hydrogen test. There are still some issues regarding interpretation of breath test results that may be influenced by the type of test meal, the sample size and the lack of standard protocols for breath sampling [69].

Another indirect method for measurement of the effect of food and drugs on GI transit is by monitoring the plasma concentration of absorbable markers paracetamol and sulfapyridine in rats, dogs and human [70–72]. Paracetamol is poorly absorbed in the stomach but rapidly so in the duodenum, whereas sulfapyridine is produced by bacterial metabolism in the colon of orally administered sulfasalazine. Food and drugs that accelerate or inhibit GI motility showed the expected effect on the first appearance and plasma concentration profile of paracetamol and sulfapyridine [4,71,72]. In human, assessment of gastric emptying time by paracetamol absorption test and scintigraphic method produced comparable results [72]. These findings suggest that paracetamol and sulfapyridine absorption test is a simple, non-invasive and inexpensive technique with direct translation potential of GI transit data from animal to human. However, the techniques need standardization to optimize its value for research and clinical studies.

Wireless motility capsule (WMC) technology consists of a single-use, orally ingestible wireless transmitting capsule that sends signals to a portable receiver worn by the individual. Analysis of stored data may shows distinct changes in pH and pressure along the GIT as landmarks to determine gastric emptying, small intestinal, colonic and total GI transit. In human, WMC data showed good agreement with the standard scintigraphy test [10,73,74], whereas preclinical studies demonstrated large discrepancy in measuring gastric emptying time [75,76]. In a recent study by Warrit et al. [77] in 42 dogs of different breeds demonstrated prolonged and wide range in gastric emptying time of 20 h (range, 6.3–119 h), but biologically relevant data for small intestinal transit time of 3.1 h (range, 1.6–5.4 h), large intestinal transit time of 21 h (range,

1–69 h) and total transit time of 52 h (range, 21–151 h).

This large discrepancy in gastric emptying data between WMC and other techniques using smaller labeled particles or contrast fluid is most likely due to the relatively large size of the capsule (13 × 27 mm). Dog pylorus is more restrictive than the human pylorus allowing the passage of particles < 2 mm in diameter [4,63], leading to prolonged gastric residence of large size WMC [10,75,76]. The WMC may pass through the pylorus after 7.5 h after feeding, which correspond to the estimated time (5–13.3 h) needed for the recurrence of gastric phase III activity of MMC following a meal in dog [77–79]. Therefore, gastric emptying after a meal measured by WMC may not represent natural gastric emptying of food but rather than emptying of a large indigestible particle [77].

In a position paper released 2011 [8], American and European Neurogastroenterology and Motility Societies, data obtained using WMC test showed good agreement with the gold standard scintigraphy technique and was considered suitable for measurement of regional and whole GI transit in clinical practice.

WMC system enable long-term ambulatory recording of GI motility transit in dogs free in their environment, thus circumvent limitations using other techniques. Apart from the large size of the capsule as a major limitation in dog, wireless motility devices in general appear to be promising technology in preclinical studies which merits further development and evaluation in the light of its direct translational potential of the motility and transit data from dog studies to human.

Imaging technology offers another non-invasive method for the assessment of GI function using radio-opaque substances or impregnated spheres (e.g. barium sulphate), ultrasonography and MRI [3,5,80]. These techniques are not widely used in preclinical studies due to limitations for its use that may involve radiation hazard and/or access to expensive equipment, time consuming, limited availability, and require restraint of the animal at selected time points to detect transit or visualise GIT wall motion. These techniques may gain more use in future animal studies.

Animal disease models of human GI motility disorder

Several animal models for human GI motility disorders have been reported to date, most of which were developed for Parkinson's disease [81], Hirschsprung disease [82], irritable bowel syndrome [83], and inflammatory bowel disease [84]. Regarding GI dysmotility within the symptoms of these diseases, the mainly used animal as a disease model is rodents. However, current models have not comprised all symptoms, especially GI motility disorders observed in patients. Research to date on human GI motility disorder has not made much progress due to some restrictions. For example, mice models are too small to measure GI motility and have different structures and contractile patterns than those of humans. Thus, to understand how GI motility dysfunctions occur, studies with other mammals than mice (e.g. marmoset, suncus, dog and guinea pig) are needed in parallel with a murine study since mice are useful as an initial screening tool to examine potential pathogenic candidates. Recent developments in CRISPR-Cas9 gene editing techniques used to manufacture genetically modified animal models have become more convenient and efficient, leading to the development of new genetic animal models of human disorders [85]. Combination studies of new animal models would give us novel findings linked to human GI motility disorder.

Summary

Available *in vitro* and *in vivo* animal models are to study alterations in GI motility and transit rather than biological markers to develop an effective treatment that targets certain features of altered GI motility seen in FGID. Prior knowledge of the species differences in the control mechanism and pharmacological responses of GI motility are essential for proper interpretation of data from different animal species and experimental models. Animal data generated from commonly used methods in clinical settings like scintigraphy, breath test and wireless motility capsule studies has the potential to directly translate to clinic. Larger study groups for preclinical motility and transit studies are necessary to detect significant differences and to assess the accuracy of the available methods to predict the clinical outcome. In preclinical studies, there is a need to standardized study protocol and to identify a representative animal species and technique that mimics the human condition and ultimately to detect significant alterations in GI motility and transit.

Practice points

- The mechanism underlying motility disorders in FGID is poorly understood, hence animal model that mimic the motility component of FGID is lacking.
- Techniques used for clinical diagnosis of FGID like scintigraphy, breath test and WMC have been demonstrated to give relatively comparable measurement in laboratory animals.
- WMC is a promising technique that needs further development to produce biologically relevant preclinical data on gastric emptying.
- Animal studies are essential for screening the primary and off target pharmacodynamics effects of drug candidates.

Research agenda

- Identify the optimal animal model and technique that mimic the alteration in motility and transit in FGID patients.
- Develop standardized study protocols for GI motility and transit techniques to optimize the extrapolation of animal data to the clinic.
- Study motility disturbances in combination with other symptoms of FGID in animal disease model.

Conflict of interest statement

None.

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* Papers of particular interest.