"The Genes in Irritable Bowel Syndrome Research Network"



COST Action BM1106
GENIEUR Final Outcome Brochure

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Preface

Dear Colleagues,

With great pleasure we share the final major outcomes of our GENIEUR (*The Genes in Irritable Bowel Syndrome Research Network Europe*) initiative. GENIEUR was funded from 2012–2016 as part of the *Cooperation in Science and Technology* (COST) program (COST Action BM1106 GENIEUR). Before the Action kicked off in 2012, investigators worldwide had started to perform pilot studies on the genetics of irritable bowel syndrome (IBS). These were mainly single center studies, focusing on single genes in IBS etiology. A major shortcoming was the lack of standardized criteria for case-control recruitment and detailed phenotyping. No network existed to promote the systematic investigation of genetic factors in IBS. To address this problem, the Chair of the Action actively initiated the construction of a pan-European network and founded the COST Action. We realized that joining forces was the only way to collect enough standardized, well-characterized case-control samples and intermediate phenotypes. Since GENIEUR kicked off in 2012, we have received positive feedback from more than 80 groups in 21 countries all over Europe. These include renowned experts and key players in the field of neurogastroenterology, psychiatry, immunology, genetics/epigenetics, and microbiota research. Furthermore, additional groups have expressed an interest in joining the network. During the funding period, GENIEUR has also connected with teams from Chile, the USA, and Australia. We have successfully established a gold standard for case-control recruitment, deep phenotyping, biosampling, and functional characterization. Thereby, GENIEUR has established the basic infrastructure for ongoing and future studies. Key publications from our first collaborative efforts have been published and were recently highlighted in Nature Outlook IBS. Another major focus of our Action was education and training. We established two successful training schools and 20 scientific missions for youngsters in the field. GENIEUR has provided a solid basis on which to foster harmonized multicenter studies in the future.

These achievements would not have been possible without the enthusiasm and commitment of our members. Most importantly, GENIEUR will continue and is currently supported by the *European Society for Neurogastroenterology and Motility* (ESNM). Our mission is to increase awareness and advance knowledge of IBS to improve the diagnosis and treatment of IBS and the quality of life of those who suffer.

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Summary

Irritable bowel syndrome (IBS) is a heterogeneous functional gastrointestinal (GI) disorder, presenting with bowel problems, abdominal pain, and comorbid conditions, including psychiatric (anxiety, depression) and pain (fibromyalgia, migraine) disorders. In the past, only a few scattered centers worldwide have investigated the genetics and the role of microbiota in IBS. These studies were predominantly small case-control cohorts focusing on single genetic variants. This prompted us to establish the COST Action BM1106 GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe) and construct a pan-European interdisciplinary network to identify the epi-/genetic factors underlying IBS. GENIEUR has established a phenotyping tool and standard operation procedures (SOPs) to quantify feasible intermediate traits and to collect patient material (blood, tissue, and stool). These are now new standards in IBS research. GENIEUR is the first interdisciplinary network of its kind worldwide to combine the expertise of clinicians specialized in functional GI disorders, immunology, and psychiatry and basic scientists focusing on epi-/genetics, microbiomics, and phenotypic analysis to tackle molecular causes of IBS. We have successfully set up interdisciplinary endeavors and have created the infrastructure for detailed patient definition in Europe and associated overseas member countries (MC observer) including Australia, Chile, and the USA. This will boost the generation of data on phenotypes, environmental factors, genetics, epigenetics, and microbiota. This data will be used to identify novel IBS subgroups and ultimately homogenize patient entities for subsequent interventional studies.

Involving early career researchers and trainee clinicians was a high priority of this Action. We wanted to support the next generation of experts who will shape the future of research on IBS and comorbid phenotypes within Europe. To support young colleagues and foster collaborations between centers, 20 Short Term Scientific Missions (STSMs) were funded. As a result, various new collaborations were initiated between centers. Furthermore, two training schools (TSs) were organized. These were dedicated to (1) the clinical background (diagnostics and therapy) of IBS and the role of microbiota and nutrition in IBS, and (2) the *state of the art* in genetics, epigenetics, and microbiota research. In addition to original articles, we reported our recommended GENIEUR standards for defining case controls, *state of the art* findings regarding the epi-/genetics of IBS, and the influence of nutrition on microbiota in IBS patients, in three high impact papers. In these papers, we stressed the current flaws and pitfalls in IBS research and conveyed our vision of how these can be improved to unravel the complicated IBS phenotype in the future. Last but not least, we have lobbied for IBS and our efforts have resulted in the UEG LINK Award *'Help EU in IBS'*, and connection to patient support groups. GENIEUR will now continue as *GENIEUR-reloaded* and will be supported by the *European Society of Neurogastroenterology and Motility* (ESNM).

In conclusion, GENIEUR has made pioneering advances in defining new standards and setting the infrastructure to foster international EU/worldwide interdisciplinary multicenter studies. This has raised IBS research to a higher level.

General background

Irritable bowel syndrome (IBS) is a neurogastroenterologic disorder. Gastrointestinal (GI) symptoms of IBS include cramping, abdominal pain, bloating, gas, and changes in bowel habits. Diarrhea-predominant IBS (IBS-D) is characterized by loose and frequent stools, constipation-dominant IBS (IBS-C) is characterized by hard and infrequent stools, and mixed-type IBS (IBS-M) is characterized by mixed bowel habits. IBS is a very heterogeneous condition and has high comorbidity with psychiatric disorders such as anxiety and depression, functional pain syndromes, and fatigue (Enck et al. 2016). Recent data suggests that **85 million European citizens** currently suffer from IBS. This represents a serious burden on the healthcare system with considerable direct (consultations, medications, diagnostics, hospitalization, and comorbid conditions) and indirect (absenteeism, reduced efficiency, and impairment during working time) costs. The total annual societal cost for IBS in Europe is around **43 billion €** (Hillila et al. 2010). Furthermore, understanding IBS is highly important because of the impact it has on patients' daily activities and quality of life (Simren et al. 2006).

Until now, specific diagnostics and adequate treatment have not been available because the pathogenesis of IBS is poorly understood. IBS is a heterogeneous disorder and is undoubtedly caused by multiple factors. Intrinsic (genomics, epigenomics, and microbiota) and environmental (stress, infection, and nutrition) factors act in concert to cause the disease. IBS is often referred to as a 'functional' condition, and is often erroneously interpreted as 'idiopathic' or 'cryptogenetic'. IBS has been recognized as a disorder of the brain–gut axis, which refers to bidirectional communication between the intrinsic GI enteric nervous system and the central nervous system. The brain–gut axis modulates GI homeostasis (motility and secretion), visceral perception, pain modulation, and emotional regulation. Abnormalities in the physiological response to stress mediated by the enteric, central, and autonomic nervous systems impairs the gut barrier and immune response (Enck et al. 2016). Furthermore, genetic and epigenetic studies have revealed an accumulation of impaired mucosal barrier function, changes in neuronal signal transduction, and immune system alterations in IBS patients (Gazouli et al. 2016). In addition, changes in enteric microbiota have recently become obvious in IBS patients (Rajilic-Stojanovic et al. 2015). A significant role of genetic factors in the etiopathogenesis of IBS has been demonstrated in family and twin studies (Saito 2011). The importance of environmental factors such as early/childhood trauma or stress, exposure to certain germs, and changes in the gut microbiota in the clinical manifestation of IBS cannot be ignored. These may represent crucial risk factors for IBS. Consequently, epigenetic changes such as differential DNA methylation or histone modification, (which are heritable but do not change the DNA code) caused by stress-related events (abuse, childhood trauma, infection, and nutrition) could also be involved in IBS (Gazouli et al. 2016).

During the last decade, evidence has accumulated that 'the prototypical IBS patient' does not exist. A variety of pathophysiological changes may manifest as distinct IBS phenotypes. This is underscored by the fact that IBS patients can present with different symptoms. Owing to its heterogeneity, IBS is currently treated with a range of medications, each of which benefits only a small proportion of patients. Specific pathway disturbances can be treated with certain medication, but these disturbances are not present in all patients, therefore the treatments are not universally successful. Epi-/genetic determination of different molecular signatures (SNPs, mRNA/ncRNA/miRNA profiles) in conjunction with microbiota profiles and phenotypic features

will help to define novel subgroups. This will facilitate the development of better diagnostics and novel tailored treatments that specifically target the affected pathways.

Reasons for the Action

Before the COST Action BM1106 kicked off in 2012, genetic research into IBS was poorly advanced. Only small pilot studies with low statistical power had been performed in scattered centers throughout the world. In Europe, only a few centers had started to collect samples for genetic studies and only a small number of pilot studies had been performed. No worldwide or European platform existed that focused on identifying the genetic factors of IBS pathogenesis. The past has taught us that large case-control samples are mandatory to nail down the genetic factors of complex diseases. Furthermore, detailed clinical and phenotypic characterization is a prerequisite for stratifying intermediate phenotypes in association and correlation studies. Only a few studies have addressed the molecular changes during IBS pathogenesis.

IBS is heterogeneous and has a multifactorial origin. Therefore, collecting detailed information on intrinsic and extrinsic factors in addition to detailed phenotypic assessment is the key for research success. Most studies have simply correlated genotypes with the IBS subtypes (IBS-C, IBS-D, and IBS-M) and no systematic approaches have been made that take other crucial factors into account. This called for a pan-European network to provide a common platform for collaboration on the investigation of IBS genetic factors.

A better understanding of the underlying pathomechanism of IBS will potentially improve diagnostics and facilitate the identification of novel drug targets. This will improve IBS therapy and the patient's quality of life.

The COST Action BM1106 GENIEUR

GENIEUR is a unique interdisciplinary network of teams from different research fields, including human molecular genetics, epigenetics, microbiomics, molecular pathology, neurophysiology, neurogastroenterology, immunology, and psychiatry. Together, these teams will identify epi-/genetic factors in IBS and link them to phenotypic traits and potential pathophysiological IBS mechanisms. A prerequisite for this systematic interdisciplinary endeavor is the harmonization of patient recruitment guidelines and tools for detailed phenotypic characterization as well as standard procedures and guidelines for sample collection, and biobanking of patient material (blood, stool, and gut biopsies). The COST Action aimed to collect diverse patient samples and characterize a large number of IBS case-control cohorts for systematic genetic and molecular analyses. The collection of enough samples from around Europe had been hampered by the lack of a respective network. Groups from different European countries had started to collect blood samples for genetic analyses or stool samples for microbiota studies and had performed small pilot studies. However, limited samples were available and recruitment and phenotypic characterization were not standardized between studies. In order to unify projects across Europe, a common platform for collaboration was needed. To speed up sample collection and to join forces, the participating GENIEUR groups established further collaborations with clinical and basic research groups all over Europe. The vision was to strengthen and expand the network during the funding period of the COST Action and to submit research proposals in collaboration for a pan-European endeavor. The aim was to use case-control cohorts for (1) replication studies to confirm preliminary data from pilot studies, and (2) more global genetic approaches using genome-wide

association studies with the latest SNP-chip and next generation sequencing technologies and expression profiling and methylation analyses. The Action aimed to identify the genetic factors contributing to the manifestation of IBS, thereby improving the diagnostics and therapy and quality of life of the patients.

GENIEUR is a unique effort and the only international network worldwide that takes the intrinsic factors (epi-/genetics and microbiota), extrinsic factors (stress, infection, and nutrition), and pathophysiology of IBS into account. Indeed, GENIEUR provides the necessary framework for a global initiative that benefits society and public health.

Scientific objectives

The aim of this Action was to create a pan-European interdisciplinary network that identifies the genetic factors contributing to IBS etiopathogenesis. Creation of a phenotyping tool was planned and intermediate traits (quantitative traits) were to be coined for correlation analyses. The interdisciplinary connection of clinicians specialized in functional GI disorders, immunology, and psychiatry and basic scientists focusing on epi-/genetics, microbiomics, and phenotypic analysis of case-control cohorts in the COST Action were important for its success.

Deliverables

- *The GENIEUR network coined harmonized recruitment guidelines for phenotypic characterization and standard operating procedures (SOPs).*
- *The guidelines, SOPs, and state of the art information/literature have been published in peer reviewed journals and included in a database as a central source of information for participating laboratories and clinics. This repository is accessible from the GENIEUR website as a central source of information in a password-protected manner.*
- *Gold standard case-control recruitment and SOP guidelines of the COST Action will be important to allow clinicians and basic researchers all over the world to perform uniform epi-/genetic studies in functional GI disorders.*
- *Education and training of clinicians, clinical scholars, and basic scientists from different fields stimulated the exchange of methods and techniques at the bench and bedside.*
- *Research proposals were set up within HORIZON 2020.*

Objectives

The COST Action focused on the following secondary objectives:

- Standardization and harmonization of case-control definition and recruitment and IBS patient characterization.
- Establishing a gold standard phenotyping tool for large-scale studies.
- Creation of a database to collect data on phenotypes (standardized by the phenotyping tool), environmental factors (exposure to certain germs, infection, gastroenteritis, microbiome data, and nutrition), and genotypes/epigenetic factors.

- Establishment of a biobank to improve data availability by collecting patient and control material (blood, tissue, and stool) for genetic studies and functional/phenotypical analysis of coined genetic factors.
- Identification of epi-/genetic risk factors of IBS.

To achieve these aims and to share workload, the following working groups (WGs) were set up:

WG 1) Establishment of a gold standard for patient recruitment and characterization
WG 2) Definition of quantitative traits as intermediate phenotypes
WG 3) Genetics: Molecular genetics and epigenetics
WG 4) Microbiomics

Overall achievements of the COST Action

In a series of consecutive meetings (see addendum), GENIEUR network members have developed criteria and recommendations for international IBS biobanking in the form of the COST Action BM1106 GENIEUR protocol. This is a gold standard for patient recruitment and characterization as well as sample assessment. This protocol was accepted and endorsed by all Action members. It was implemented in a multistage process in 18 out of 21 COST Action BM1106 GENIEUR member countries. Establishing the pan-European IBS database is an ongoing, long-term process. Harmonization of sample collection is crucial for the success of future epi-/genetic and microbiota research within the GENIEUR network. Therefore, SOPs have been developed. The GENIEUR protocol has harmonized and standardized the selection and biosampling of controls and IBS patients by groups in several countries. Currently, 11 countries have received Medical Ethics Committee (METC) approval (**Belgium, Bosnia and Herzegovina, Germany, Greece, Italy, Netherlands, Norway, Poland, Romania, Spain, and Sweden**), and in 8 countries METC approval is pending (**Austria, France, Ireland, Israel, Malta, Serbia, Switzerland, and the UK**). Colleagues from Croatia and Hungary have recently joined the network. Furthermore, groups from Portugal and Russia have expressed interest to join GENIEUR. To date, no clinical partners are involved in the GENIEUR member countries Cyprus and Finland, therefore local implementation is pending.

The study protocol and informed consent form have been developed and translated into different languages. The respective documents have been uploaded onto the GENIEUR website repository and are available upon request.

The gold standard of case-control recruitment developed within the COST Action BM1106 GENIEUR network comprises a standardized interview-based case report form that collects information on demographics, clinical data, family, personal and dietary history, use of pharmacological agents, clinical investigations, and other study data. Validated questionnaires assess the severity of intestinal symptoms, non-GI symptoms, general anxiety, GI-specific anxiety, depression, somatization, personality, perceived quality of life, and sexual and physical abuse. Patient-reported outcomes or self-evaluation of symptoms will be quantified using harmonized instruments. Symptoms and psychological profiles will be assessed using the following validated questionnaires:

- **Rome III modular questionnaires for IBS and functional dyspepsia:** The Rome III modular questionnaires are validated and widely used assessment criteria for the diagnosis of functional

GI disorders (http://www.romecriteria.org/translations/). They exist in all validated language versions for all countries involved in GENIEUR.

- **Gastrointestinal Symptom Rating Scale (GSRS):** The GSRS is a validated 15-item questionnaire that determines the perceived severity of upper and lower GI symptoms. This has recently been adapted for specific use in IBS.
- **Visceral Sensitivity Index (VSI):** The VSI consists of 15 items that reliably assess GI symptom-specific anxiety related to pain, diarrhea, constipation, bloating, and a sense of urgency in the upper or lower abdomen. Subjects were asked to rate their level of agreement (strongly agree to strongly disagree) to the 15 statements. The scale has been validated and shown to have good discrimination.
- **Nepean Dyspepsia Index (NDI):** The NDI is a 42-item quality of life scale designed to measure impairment of a subject's ability to engage in and enjoy relevant aspects of their life. In addition, the individual importance of each aspect is assessed. It has been validated in out-patients presenting to general practice with upper GI complaints and in a randomly selected population-based sample. The NDI is a reliable and valid disease-specific index for dyspepsia, measuring symptoms, and health-related quality of life.
- **GAD-7:** The GAD-7 (Generalized Anxiety Disorder 7) is a validated nine-item questionnaire that efficiently screens for generalized anxiety disorder and assesses its severity in clinical practice and research (http://www.phqscreeners.com).
- **Patient Health Questionnaire-9 (PHQ-9):** The PHQ-9 is a validated nine-item depression scale of the Patient Health Questionnaire (http://www.phqscreeners.com).
- **Patient Health Questionnaire-15 (PHQ-15):** The PHQ-15 is a validated 15-item questionnaire assessing somatic symptoms **(**http://www.phqscreeners.com**)**. It is available in all languages used by this consortium.

SOPs for measuring rectal sensitivity using barostat procedures have been developed and implemented in the basic protocol of specialized centers. A number of successful STSMs in participating centers have facilitated the harmonization of protocols for data collection:

- **Rectal sensitivity testing** - balloon distension in the rectum measures rectal sensitivity, which is a validated proxy for general visceral sensitivity.
- **Colonic marker/transit study protocol** - an indirect measure of colonic motility using radiopaque markers.
- **Gut barrier function** - Intestinal permeability is measured by ingesting two different sugars (lactulose and mannitol) and then quantifying these sugars in the urine by HPLC to reflect permeability in different parts of the GI tract.

A manuscript describing the recommendations for patient characterization as part of the COST Action BM1106 GENIEUR has recently been published (Boeckxstaens et al. 2016), see publication list addendum). All SOPs and information on the implemented questionnaires are available on the GENIEUR website.

GENIEUR partners have combined knowledge and expertise in the systematic analysis of available case-control blood, biopsy, and stool samples to identify genetic, epigenetic, and metagenomic factors involved in IBS.

The GENIEUR protocol is the first to collect environmental (stress, infection, and nutrition), intrinsic (genomics, epigenomics, and microbiota), and detailed phenotypic data (e.g. psychophysiological profiling) in a standardized manner from all centers. This approach advances the understanding of IBS pathogenesis **and comorbid conditions**. To date, **IBS research has been limited by the marked symptom heterogeneity and multifactorial pathogenesis of the disease. One shortcoming of current IBS research is the lack of integrative approaches to investigate the different pathophysiological features using unified phenotyping tools.**

More than 1100 samples have been collected according to the GENIEUR protocol for genetic and epigenetic testing during the course of the Action. In addition, various collaborative projects have been expanded and 20 manuscripts have been published following combined efforts of GENIEUR members. A major achievement was an invited review in *Nature Reviews Gastroenterology and Hepatology.* In this review, WG3 (*Genetics: Molecular genetics and epigenetics)* reported the *state of the art* in the field and the future strategy of the GENIEUR network. A comprehensive review of the literature identified the most promising candidate genes, miRNAs, and related networks and pathways. This has been published as a joint WG3 review (Gazouli M. et al., 2016, *Nature Reviews Gastroenterology and Hepatology,* pdf attached in the addendum). Collaborative studies to replicate and identify novel epi-/genetic factors are currently ongoing. In addition, a comprehensive systematic literature search on the microbial risk factors of IBS has been performed by WG4 (*Microbiomics*) members and published in *the American Journal of Gastroenterology*. New studies to identify microbial taxa involved in symptomatic episodes of IBS have been initiated. This thorough publication search has identified 14 phylogenetic groups that are likely markers of IBS-specific dysbiosis. This literature review has been published in a joint WG4 review (Rajilić-Stojanovicć M. et. al. *Am J Gasteroenterol*, 2015, pdf attached in the addendum). Deep phenotyping and data generation on different levels (epi-/genetics and microbiota) has identified novel IBS subgroups and homogenized patient entities for subsequent interventional studies. The majority of previous studies have failed to do this due to the heterogeneity of IBS. **GENIEUR is undertaking pioneering work to set the infrastructure for international EU/worldwide multi-studies on an interdisciplinary level.**

GENIEUR has recently been highlighted in two articles from a Nature-*Outlook* issue on IBS: http://www.nature.com/nature/outlook/ibs/. Three reviews by GENIEUR members were included in this issue in a Nature online collection on IBS: http://www.nature.com/subjects/irritable-bowel-syndrome.

We also aimed to get clinical and basic scientists from additional countries involved in IBS (epi-/genetics and microbiota) research, including **Bosnia-Herzegovina, Serbia, Malta, Cyprus, Finland, Romania, Poland,** and **France**. One of the major spin-offs of the GENIEUR protocol was the United Gastroenterology LINK Award**:** ***H****armonization and impl****E****mentation of minima****L*** *requirement* ***P****rotocols for phenotype-genotype characterizations to facilitate pan-****EU*** *collaboration in* ***IBS****: "Help EU in IBS"*. This is led by the **National Society of the Netherlands** (Ad Masclee) and the co-applying society in **Germany** (Beate Niesler with Viola Andresen and Miriam Goebel-Stengel) in collaboration with Lukas van Oudenhove and Magnus Simrén and the National Societies of **Malta** (Mario Vassallo), **Bosnia and Herzegovina** (Lejla Kapur-Pojskić), **Serbia** (Mirjana Rajilić-Stovanović), and **Norway** (May-Bente Bengtson, Knut-Arne Wensaas). Support is also provided by the ESNM. External expert Ami Sperber (IE) who is head of the Rome Translation Project and chairs the Multinational Committee of the Rome Foundation https://www.ueg.eu/awards-grants/ueg-awards/link-award/.

Additional outputs and achievements

1. Various collaborations between centers in Zürich (CH), Maastricht (NL), Heidelberg (DE), Leuven (BE), and Gothenburg (SE) have been established to collaborate on pilot deep phenotyping of patients according to the standardized SOPs developed by COST Action BM1106 GENIEUR.
2. As part of the GENIEUR collaboration, a novel study on the association between Ehlers-Danlos Syndrome (EDS) and IBS has been established. The above mentioned groups are collaborating on this study to gain novel insight into the etiopathology of IBS. This will identify IBS subgroups that are more amenable for genetic profiling as EDS is a heritable condition.
3. A clinical trial assessing the efficacy of probiotics in IBS is currently underway in Gothenburg (M. Simrén). In another study, deep phenotyping of biobanks of stool, blood, and urine samples from IBS patients is currently underway in Maastricht, Leuven, and Gothenburg. These studies are using GENIEUR protocols.
4. New collaborations on genetics and epigenetics have been established during the course of GENIEUR between centers in Athens (EL), Barcelona (ES), Heidelberg, and the IBS-Net Germany (DE), Cork (IE), Nottingham (UK), and Santiago de Chile (CHL). Furthermore, an ongoing collaboration between Leuven (BE), Gothenburg, Stockholm, the Swedish IBS-Net (SE), and Bologna (IT) has been expanded by Heidelberg (DE) and Athens (EL). Collaborations between Bergen (NO), Leuven (BE), and Los Angeles (CA, USA) have been expanded as well.
5. First outcomes of these collaborations have been published as original articles, are currently under revision for publication, or have been presented as oral presentations or posters at the following national and international conferences: the *United European Gastroenterology Week* (UEGW) 2013, 2014, 2015, and 2016; the neuroGASTRO 2012 and 2015; the *Digestive Disease Week* (DDW) 2014; the *International Society for Serotonin Research Meeting* 2016; the *German Society for Neurogastroenterology and Motility* 2013, 2014, and 2016; and the *German Society of Human Genetics conferences* 2014 and 2016. More recently, abstracts have been accepted as oral and poster presentations at the *World Neurogastroenterology Meeting* FNM 2016.
6. The *PAIN Research Program* (http://uclacns.org/programs/pain-research-program/), which established a repository on brain fMRI studies, is now collaborating with us. This collaboration is represented by Emeran Mayer (UCLA, CA, USA).
7. Daniel Pohl from Switzerland has established the IBSnet Switzerland.
8. Mauro D'Amato (Sweden/Spain) has launched the Bellygenes Initiative.
9. So far, GENIEUR members have applied for European funding from the HORIZON 2020 program (three applications), the JPI program (one application), UEG LINK Award (three applications) and various national, binational, and trinational programs (eleven applications).

Extent of networking among the participants in the Action

GENIEUR has facilitated equal participation and active engagement of all research centers regardless of size and locally available resources. Implementation of the GENIEUR protocol, enrolment of patients, collection of data, and sampling of material is demanding and time consuming work. This was significantly hampered, especially in centers with no financial resources. However, the STSM program has increased the mobility of young investigators and knowledge and exchange of experience between the Action members. All Action activities were balanced in gender representation (40% females involved in key positions, female Chair, 40% female WG coordinators).

Early stage researchers (PhD and MD students) are part of the group of 86 participants. Nine assistant professors, seven senior scientists and seven postdoctoral scientists make up 39% of participants involved in member countries. Associated staff includes young investigators, and more than 60% of those are female. Furthermore, inclusiveness target countries (ITC) (Malta, Poland, and Romania), EU candidate countries (the former Yugoslav Republic of Serbia) and potential EU candidate countries (Bosnia and Herzegovina) are involved in the Action.

Training and education

The involvement of Early Stage Researchers and Trainee Clinicians was a high priority of this Action. The aim was to support the next generation of experts who will promote future research into IBS and other functional gastrointestinal disorders. This is reflected by the high percentage (>40%) of Early Stage Researchers involved in the Action WGs. Short Term Scientific (STSM) fellowships support Young Researchers and Clinical Trainees. During the running time of the Action, 20 STSM fellowships were granted to 11 female and six male scientists at the early/mid stages of their careers (see addendum). Scientists from Bosnia-Herzegovina, Serbia, and Romania were among the STSM-funded individuals. STSMs have initiated and/or expanded new collaborations, allowed long-term partnerships to develop, and facilitated the harmonization of protocols between centers.

We organized two Training Schools (TS) within the Action. One was established in Barcelona in April 2014 in conjunction with the GENIEUR Spring Meeting, where 37 selected trainees (73% female) and members of the COST Action were educated in the clinical background of IBS diagnostics and therapy and the role of microbiota and nutrition (see program on GENIEUR website 'Meetings'/'Training School Barcelona 2014'). The second TS was organized in Heidelberg in March 2016 in collaboration with the Initial Training Network neurogut (www.neurogut.eu) and included 63 selected trainees (60.3% female) and 20 trainers. Trainees and trainers from the Inclusiveness Target Countries Cyprus, Malta, Poland, and Romania, and the EU candidate countries Serbia, and Bosnia and Herzegovina were involved in both TSs. In Heidelberg, the *state of the art* in genetics, epigenetics and microbiota research were taught. For more information see programme on GENIEUR website 'Meetings'/'Training School Heidelberg 2016'.

Dissemination of Action progress

We disseminated the progress and network activities of GENIEUR by providing Action Fact Sheets at major national and international gastroenterology and neurogastroenterology meetings (e.g. *United European Gastroenterology Week* (UEGW) 2013, 2014, 2015, and 2016; the neuroGASTRO

meeting 2012 and 2015, and the *German Society for Neurogastroenterology and Motility* 2013, 2014, and 2016). Furthermore, press releases about GENIEUR have been published in member countries.

Lobbying for IBS research

One of our major missions is lobbying for IBS to raise awareness of the condition and expand the knowledge on contributing factors such as genetics, epigenetics, and microbiota. To improve European funding, we took part in the Stakeholder Consultations for HORIZON 2020: Societal challenge 1: Health demographic change and wellbeing (September 20, 2014). This was a joint action between COST Action BM1106 GENIEUR, the German Society of Gastroenterology and Motility (DGNM), and the European Society of Gastroenterology and Motility (ESNM) (LINK Award: *'Help EU in IBS'*).

Patient and public involvement in research

To continue lobbying for our mission, we have established contact with various patient groups, including the IBS network. They have given advice about the project and have agreed to work with us in the long term.

All patient support groups are hyperlinked on our website:

https://genieur.eu/index.php?main=home&subcat=Links

We have prepared a leaflet in lay language which explains the Action's mission. This has been translated into different languages (see English version in addendum).

https://genieur.eu/ajxp/GENIEUR.EU/Dissemination

The Future: GENIEUR-*reloaded*

The COST Action BM1106 GENIEUR network is currently running with the support of the ESNM. GENIEUR-*reloaded* will continue to widen, broaden, and share knowledge that is relevant to the network's objectives. We will continue to implement the basic recruitment protocol in additional centers, which will eventually include 24 European and three overseas countries. Patient and controls will be recruited and blood, tissue, and stool samples will be collected for genetics/epigenetics and microbiota studies.

To keep the network going, we will:

- Meet during UEGW, ESNM, and FNM as a special interest group.
- Propose epi-/genetics sessions during UEGEW/ESNM/FNM meetings.
- Foster collaborations/grant proposals.
- Support young investigators.
- Keep the GENIEUR website and GENIEUR repository updated and report on new findings.
- Spread news via the website and regular newsletters.
- Disseminate outcomes to the scientific community, patients, and patient support groups in lay language.
- Lobby for IBS. IBS has largely been neglected by funding organizations in the past because it is not life-threatening. However, it presents a huge financial burden on society and deserves attention.
- We aim to increase awareness about IBS and flaws in the current understanding. The lack of evidence-based diagnostics and treatment leads to poor management of the disease. This is frustrating for patients and medical doctors. Patients are often not taken seriously and many cases are dismissed as '*functional*', which is often interpreted as 'idiopathic' or 'cryptogenetic'. In addition, patients are often labelled as 'neurotic' or 'apprehensive' with problems that are all 'in their mind'. However, those who suffer from IBS often report reduced health-related quality of life.

COST (European Cooperation in Science and Technology)

COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

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Literature

Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilic-Stojanovic M, Schemann M et al. 2016. Irritable bowel syndrome. *Nature reviews Disease primers* **2**: 16014.

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Addendum

Parties

Action websites:	www.GENIEUR.eu; http:/www.cost.eu/COST_Actions/bmbs/BM1106

Action details

MoU	4160/11
CSO Approval date	01/12/2011
Start of Action	03/04/2012
End of Action	02/04/2016

Participations

Country	Date	Status
Austria	02/02/2012	Confirmed
Belgium	15/02/2012	Confirmed
Bosnia and Herzegovina	30/11/2012	Confirmed
Cyprus	05/09/2012	Confirmed
Finland	20/09/2012	Confirmed
France	10/01/2012	Confirmed
Germany	13/01/2012	Confirmed
Greece	23/01/2012	Confirmed
Ireland	06/02/2012	Confirmed
Israel	25/01/2012	Confirmed
Italy	13/03/2012	Confirmed
Malta	30/01/2012	Confirmed
Netherlands	21/02/2012	Confirmed
Norway	11/01/2012	Confirmed
Poland	09/02/2012	Confirmed
Romania	06/03/2012	Confirmed
Serbia	28/03/2012	Confirmed
Spain	12/12/2011	Confirmed
Sweden	16/01/2012	Confirmed
Switzerland	18/10/2012	Confirmed
United Kingdom	13/12/2011	Confirmed

Total: 21

COST International Partner Countries

Institution Name	Country
Center for Neurobiology of Stress, UCLA	United States of America
Hospital Clinico Universidad de Chile	Chile
University of Adelaide	Australia

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Sweden	Prof. Greger LINDBERG
Switzerland	Prof. Michael FRIED

COST International Partner Countries

Institution Name	MC Observer
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Hospital Clinico Universidad de Chile	Dr. Caroll Beltrán
University of Adelaide	Dr. Patrick Hughes

Meetings and Conferences

The COST Action BM1106 GENIEUR held meetings twice a year

2012 COST Action BM1106 GENIEUR *Starter Meeting* in Bologna (Italy), 05.–06. 09.2012

2013 COST Action BM1106 GENIEUR *Spring Meeting* in Freising (Germany), 25.–26.03.2013

COST Action BM1106 GENIEUR *Autumn Meeting* in Berlin (Germany), 11.–12.10.2013

2014 COST Action BM1106 GENIEUR *Spring Meeting* in Barcelona (Spain), 11.–12.4.2014

DGVS *Spring Conference Gut Microbiota in Health and Disease* (co-organized by the COST Action BM1106 GENIEUR), 23.–24.Mai 2014 in Berlin (Germany)

COST Action BM1106 GENIEUR *Autumn Meeting* in Vienna (Austria), 17.–18.10.2014

2015 COST Action BM1106 GENIEUR *Autumn Meeting* in Barcelona (Spain), 29.–30.10.2015

2016 GENIEUR *Final Conference* in Heidelberg (Germany), 14.–15.3.2016

2016 *Common Interest Group Meeting* CIG GENIEUR-*reloaded* at UEGW2016 in Vienna (Austria), 18.10.2016

Training Schools

2015 First COST Action BM1106 GENIEUR Training School: 'Assessment of IBS in Clinical Practice and Research: An Introduction for Scientists' in Barcelona (Spain), 10.–12.4.2014

Second COST Action BM1106 GENIEUR Training School: ‚Molecular Methods in IBS Research: Genetics, Epigenetics and Microbiota Research' in Heidelberg, 11.–13.3.2016

Detailed programs of the GENIEUR meetings and training schools can be found on the website: www. GENIEUR.eu

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UK	Prof. Qasim Aziz
UK	Dr. Adam Farmer
USA	Prof. Eamonn Quigley
USA	Prof Emeran A. Mayer

STSM Grantees

Grantee		Host		Date	Topic and value added to the Action
Institution	Country	Institution	Country		
Neda Radovanović, Faculty of Technology and Metallurgy, Belgrade (RS)		Caroline Plugge, Laboratory of Microbiology, Wageningen University, Wageningen (NL)		2013	Characterization of an anaerobic intestinal isolate
Hans Törnblom, University of Gothenburg, Gothenburg, Sweden, (SE)		Lukas van Oudenhove, Translational Research Center for Gastrointestinal Disorders (TARGID) Leuven(BE)		2013	Streamlining phenotyping of patients with IBS
Emilie d'Aldebert, INSERM UMR1043,Toulouse (FR)		Daisy Jonkers, Maastricht University Medical Center, Division of Gastroenterology-Hepatology, Maastricht (NL)		2013	Permeability in a three-dimensional cell culture model
Stefan-Lucian Popa, Iuliu Haţieganu University Cluj Napoca, Cluj Napoca (RO)		Fernando Azpiroz, Laboratory of Neuro-Immuno-Gastroente-rology, Universitàt Autònoma de Barcelona (ES)		2014	Genetic determination of stress susceptibility in IBS
Daniel Pohl, University Hospital Zürich, Zürich (CH), Daniel.Pohl@usz.ch		Magnus Simrén, University of Gothenburg, Gothenburg (SE)		2014	Nutrient challenge testing in IBS
Tim Sloan, The University of Nottingham, Nottingham (UK)		Willem de Vos, University of Helsinki, Helsinki(FI)		2014	Development of IBS microbiome analysis in Nottingham
Irina Vulić, University of Banja Luka, Banja Luka (BA)		Beate Niesler, University of Heidelberg, Heidelberg (DE)		2014	Genetics of neurogastroenterologic disorders
Elhaseen Elamin, Maastricht University, Maastricht (NL)		Nathalie Vergnolle, University of Toulouse, Toulouse (FR)		2014	Isolation and three-dimensional culture of intestinal stem cells
Daniel Pohl,		Qasim Aziz, Wingate		2015	Joint hypermobility syndrome in IBS

University Hospital Zürich, Zürich (CH)	Institute of Neuro-gastroenterology, London (UK)		
Daniel Keszthelyi, Maastricht University Medical Center, Maastricht (NL)	Qasim Aziz, Wingate Institute of Neuro-gastroenterology, London (UK)	2015	Investigation of visceral pain perception in IBS using fMRI
Laura Brockway, Wingate Institute of Neurogastroentero-logy, London (UK)	Ad Masclee, Division of Gastroenterology - Hepatology, Maastricht University Medical Center, Maastricht (NL)	2015	GENIEUR
Miriam Goebel-Stengel, Martin Luther Krankenhaus Berlin, Berlin (DE)	Guillaume Gourcerol, Centre Hospitalier Universitaire de Rouen, Rouen (FR)	2015	Phenotyping IBS patients by means of manometry
Bruno Rodiño-Janeiro, Fundación de investigación del Hospital Universitario de Vall d'Hebron, Barcelona (ES)	Beate Niesler, Genetics of Neurogastroentero-logic Disorders, Heidelberg (DE)	2016	Development of an innovative miRNAs-based diagnostic panel for IBS
Sabrina Berens, Department of General Internal Medicine and Psychosomatics, University of Heidelberg, Heidelberg (DE)	Lukas van Oudenhove, KU Leuven Campus Gasthuisberg, Department of Clinical and Experimental Medicine, Leuven (BE)	2016	Polymorphisms of the serotonergic system in IBS - Psychosocial correlates
Daniel Pohl, University Hospital Zürich, Zürich (CH)	Lukas van Oudenhove, Translational Research Center for Gastrointestinal Disorders (TARGID) Leuven (BE)	2016	Psychological Influence on symptom reporting in IBS
Cristina Pardo-Camacho, Vall d'Hebron Research Institute, Barcelona (ES)	Nathalie Vergnolle, INSERM U1220, Toulouse (FR)	2016	Effects of gluten-related peptides on epithelial proteases and epithelial functions
Eloisa Salvo-Romero, Fundación de investigación del	Gerard Clarke, Department of Psychiatry,	2016	Role of oxytocin in maternal-infant bonding stress-related immune responses

Hospital Universitario de Vall d'Hebron, Barcelona (ES)	Biosciences Institute, Cork (IE)		
Nathalie Weltens, Translational Research Center for Gastrointestinal Disorders, 3000 Leuven (BE)	Qasim Aziz, Wingate Institute of Neurogastroentero-logy, London (UK)	2016	Serotonin transporter genotype and neural mechanisms underlying emotional modulation of visceral pain
Svetlana Bojić, Faculty of Biology, Belgrade (RS)	Chaysavanh Manichanh, Metagenomics Lab, Barcelona (ES)	2016	Validation of oligonucleotide markers for IBS/UC in remission discrimination using deep sequencing data
Lisa Vork, Maastricht University Medical Center, Maastricht (NL)	Hans Törnblom, University of Gothenburg, Gothenburg (SE)	2016	Harmonizing IBS cohort studies; Gothenburg and Maastricht

Invited Speakers

We cordially thank our invited speakers at the meetings for their valuable contribution and support

Participant name	Institution	Country	Event date	Topic and added value to the Action
Thomas G. Schulze	Department of Psychiatry and Psychotherapy, University Medical Center Georg-August-Universität, Göttingen	DE	2013 Spring	Detecting significant genotype-phenotype association rules in bipolar disorder: Market research meets complex genetics Flexible, web-based phenotyping and biobanking meeting highest standards for data protection
Xavier Estivill	Center for Genomic Regulation, Genes and Disease Programme Barcelona	ES	2013 Spring	Genetic and epigenetic studies in complex disorders
Edith Feskens	Wageningen University	NE	2013 Autumn	Dietary assessment in European studies
Jessica Biesiekierski	TARGID, Leuven	BE	2014 Spring	Dietary interventions for FGIDs
Patrick Hughes	Adelaide	AUS	2014 Autumn	Insights into IBS from Peripheral Blood
Caroll Beltrán	Santiago de Chile	CHL	2014 Autumn	IBS Research in Chile

Josef Czimmer	University of Pécs	HU	2014 Autumn	Brain–gut interactions and GI motility. A proposal for admission to GENIEUR from Hungary
Justo Lorenzo Bermejo	IMBI, University of Heidelberg	DE	2014 Autumn	Feasible epigenetics approaches in complex disorders
Jim Kaput	Nestlé Health Science, Lausanne	CH	2014 Autumn	Data mining approaches in complex disorders: Experimental Designs for Systems Nutrition Research
Anita Eliasson	BC Platforms Helsinki	FI	2014 Autumn	Secure data collection, sharing and analysis in collaborative research projects
Annikka Polster	University of Gothenburg	SE	2015 Autumn	Basic Study Protocol – challenges in data collection and implementation
Fermín Mearin Manrique	Barcelona	ES	2016 Spring	Symptom-based criteria to phenotype IBS patients: pros and cons
Emeran Mayer	UCLA, Los Angeles	USA	2016 Spring	Influence of Gut Microbiota on the Brain – Relevance for IBS?

GENIEUR publications

1. Almouzni, G., Altucci, L., Amati, B., Ashley, N., Baulcombe, D., Beaujean, N., Bock, C., Bongcam-Rudloff, E., Bousquet, J., Braun, S., Bressac-de Paillerets, B., Bussemakers, M., Clarke, L., Conesa, A., Estivill, X., Fazeli, A., Grgurevic, N., Gut, I., Heijmans, B.T., Hermouet, S., Houwing-Duistermaat, J., Iacobucci, I., Ilas, J., Kandimalla, R., Krauss-Etschmann, S., Lasko, P., Lehmann, S., Lindroth, A., Majdic, G., Marcotte, E., Martinelli, G., Martinet, N., Meyer, E., Miceli, C., Mills, K., Moreno-Villanueva, M., Morvan, G., Nickel, D., Niesler, B., Nowacki, M., Nowak, J., Ossowski, S., Pelizzola, M., Pochet, R., Potocnik, U., Radwanska, M., Raes, J., Rattray, M., Robinson, M.D., Roelen, B., Sauer, S., Schinzer, D., Slagboom, E., Spector, T., Stunnenberg, H.G., Tiligada, E., Torres-Padilla, M.E., Tsonaka, R., Van Soom, A., Vidakovic, M., Widschwendter, M., 2014. Relationship between genome and epigenome--challenges and requirements for future research. BMC genomics 15, 487.
2. Beckers, A.B., Keszthelyi, D., Fikree, A., Vork, L., Masclee, A., Farmer, A.D., Aziz, Q., 2017. Gastrointestinal disorders in joint hypermobility syndrome/Ehlers-Danlos syndrome hypermobility type: a review for the gastroenterologist. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.
3. Beyder, A., Mazzone, A., Strege, P.R., Tester, D.J., Saito, Y.A., Bernard, C.E., Enders, F.T., Ek, W.E., Schmidt, P.T., Dlugosz, A., Lindberg, G., Karling, P., Ohlsson, B., Gazouli, M., Nardone, G., Cuomo, R., Usai-Satta, P., Galeazzi, F., Neri, M., Portincasa, P., Bellini, M., Barbara, G., Camilleri, M., Locke, G.R., 3rd, Talley, N.J., D'Amato, M., Ackerman, M.J., Farrugia, G., 2014. Loss-of-function of the voltage-gated sodium channel NaV1.5 (channelopathies) in patients with irritable bowel syndrome. Gastroenterology 146, 1659-1668.
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5. Celli, J., Rappold, G., Niesler, B., 2017. The Human Serotonin Type 3 Receptor Gene (HTR3A-E) Allelic Variant Database. Human mutation 38, 137-147.
6. Czogalla, B., Schmitteckert, S., Houghton, L.A., Sayuk, G.S., Camilleri, M., Olivo-Diaz, A., Spiller, R., Wouters, M.M., Boeckxstaens, G., Bermejo, J.L., Niesler, B., 2015. A meta-analysis of immunogenetic Case-Control Association Studies in irritable bowel syndrome. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 27, 717-727.
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10. Gazouli, M., Wouters, M.M., Kapur-Pojskic, L., Bengtson, M.B., Friedman, E., Nikcevic, G., Demetriou, C.A., Mulak, A., Santos, J., Niesler, B., 2016. Lessons learned--resolving the enigma of genetic factors in

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Patient Leaflet

Who are we?

Leading researchers and expert clinicians promoting research and advancing the treatment of irritable bowel syndrome (IBS) throughout Europe. We have formed an international network supported by the *European Society of Neurogastroenterology and Motility* (ESNM).

What is IBS?

IBS is a frequent disorder affecting 10–15% of the population. The most common IBS symptoms include cramping, abdominal pain, bloating, and gas. Changes in bowel habits also occur - either loose and frequent stools, hard and infrequent stools, or mixed bowel habits. Often, IBS goes along with anxiety and depression as well as pain syndromes and fatigue (Figure 1). Currently the diagnosis of IBS is purely clinical and based upon a characteristic set of symptoms. However, patients repeatedly undergo a variety of tests that are often invasive and that fail to show any abnormality. This is a highly frustrating outcome for patients and doctors alike.

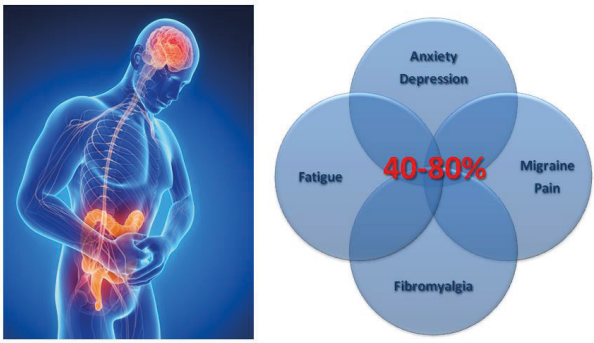

Figure 1. 40–80% of IBS patients suffer from one or more of the following disorders: psychiatric diseases (anxiety, depression), chronic fatigue or pain conditions (migraine, fibromyalgia)

What are the currently known mechanisms involved in IBS?

The current factors suspected of being involved in IBS include: genetics, bacteria in the digestive system, lifestyle, stress and other psychological factors, infections of the gut, environmental factors, and food intake (Figure 2). During the last couple of years, disturbances in communication between the little brain in the gut and the brain via the so called *gut-brain axis*, has impaired the function of nerve cells in the gut and

the brain, disrupted the gut lining, and caused disturbances in the immune system of the gut. However, the causes and consequences are still poorly understood.

Figure 2. Summary of factors influencing the manifestation of IBS: lifestyle: diet/smoking, bacterial infection, stress/trauma, female gender, genes

What is urgently needed for IBS?

IBS is a chronic condition that needs long-term management. However, many of the current treatment modalities are ineffective. The underlying causes of IBS are not fully understood, but are probably complex. This likely explains why some patients do not respond to treatment. Certainly a better understanding of the underlying causes of IBS would contribute to devising better treatment options and decrease the number of invasive tests required.

What is our goal?

The goal of the network is to guide a comprehensive search for the genetic and non-genetic factors that contribute to IBS. Because of the complex nature of IBS, this ambitious goal can only be achieved by analyzing many patients under strict and well-defined guidelines. This will allow us to compare samples and data collected at multiple sites across the 21 countries involved in this collaboration. The outcome of this action will improve international collaborations to unravel the mechanisms of IBS and maximize the potential for better diagnostic tools and superior treatment options.

How can you help?

If you are interested in promoting the goals of our initiative, please contact the Action Chair genieur@yahoo.de

Please find more information on our website @ www.GENIEUR.eu

Lessons learned — resolving the enigma of genetic factors in IBS

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Abstract | IBS is the most prevalent functional gastrointestinal disorder and phenotypically characterized by chronic abdominal discomfort, pain and altered defecation patterns. The pathophysiology of IBS is multifactorial, albeit with a substantial genetic component. To date, studies using various methodologies, ranging from family and twin studies to candidate gene approaches and genome-wide association studies, have identified several genetic variants in the context of IBS. Yet, despite enlarged sample sizes, increased statistical power and meta-analyses in the past 7 years, positive associations are still scarce and/or have not been reproduced. In addition, epigenetic and pharmacogenetic approaches remain in their infancy. A major hurdle is the lack of large homogenized case–control cohorts recruited according to standardized and harmonized criteria. The COST Action BM1106 GENIEUR (GENes in Irritable Bowel Syndrome Research Network EURope) has been established to address these obstacles. In this Review, the (epi)genetic working group of GENIEUR reports on the current state-of-the-art in the field, highlights fundamental flaws and pitfalls in current IBS (epi)genetic research and provides a vision on how to address and improve (epi)genetic approaches in this complex disorder in the future.

IBS affects up to 20% of the Western population[1]. Characteristic symptoms include chronic discomfort and abdominal pain associated with altered bowel habits in the absence of an organic cause[2]. As no biomarkers are available for IBS, a symptom-based diagnosis is made according to the Rome III criteria. On the basis of defecation patterns, IBS is subdivided into the following subtypes: diarrhoea-predominant (also referred to as 'IBS with diarrhoea'; IBS-D), constipation-predominant (or 'IBS with constipation'; IBS-C), a mixed subtype (IBS-M) and unsubtyped IBS (IBS-U)[3]. Anxiety and depression are common comorbidities in IBS, reflecting the complex relationship between visceral sensation and psychological perceptions that are mediated via the brain–gut axis. Patients with IBS also report altered somatic pain perception[4,5], and other somatic pain syndromes such as migraine, fibromyalgia and chronic fatigue syndrome are over-represented[6,7]. Direct medical expenses associated with outpatient visits, hospitalization, diagnostic tests and ineffective treatment lead to a marked economic burden to the health-care system with an estimated annual total cost of €41 billion in the EU[8]. Indirect costs of IBS related to work absenteeism and reduced productivity are also considerable. Our knowledge of the pathophysiology of IBS remains limited, mainly owing to its multifactorial origin, and a paucity of efficacious therapies is evident to date.

Environmental (extrinsic) factors such as psychological stress[9], diet, smoking habits, infectious gastroenteritis that results in post-infectious IBS (PI-IBS)[10] and alterations in the gut microbiota produce a complex interaction with genetic variants dispersed in the human genome (intrinsic factors) and lead to individual epigenetic prints. These factors might contribute to central and peripheral (neurobiological) intermediate phenotypes (affecting brain and enteric nervous system function), influence the brain–gut axis and consequently manifest as central, behavioural and gastrointestinal intermediate phenotypes, thereby predisposing to IBS and its comorbid conditions[10–13] (FIG. 1). The role of intrinsic factors (genetics, epigenetics, sex hormones and coping mechanisms) in IBS has been studied to a lesser extent.

The aim of this Review is to summarize the current state-of-the-art in genetic and epigenetic research in IBS and its comorbid conditions. In addition, fundamental flaws and pitfalls in unravelling the molecular mechanisms of IBS will be highlighted and suggestions will be made on how to address these issues and improve approaches to better define (epi)genetic mechanisms in this multifactorial disorder.

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Key points

- Genetic studies in IBS range from family and twin studies to candidate gene approaches and genome-wide association studies
- Despite enlarged sample sizes, increased statistical power and meta-analyses, positive associations between gene variations and IBS subtypes are still scarce and many have not been reproduced
- Epigenetic and pharmacogenetic approaches are in their infancy
- A major pitfall in IBS research is the lack of large homogenized case–control cohorts recruited according to standardized and harmonized criteria

IBS heritability

A familial clustering of IBS has been reported in several studies, therefore suggesting an inherited component[14–20]. One of the first studies to examine the role of heritability in IBS demonstrated that one-third of patients had a relative with IBS, even in patients without a concurrent psychiatric diagnosis[16]. Waehrens *et al.*[19] have shown that the existence of a first-degree relative with IBS can be predictive of IBS and that an increased risk of IBS is evident among first-degree (OR 1.75), second-degree (OR 1.82) and third-degree relatives (OR 1.11), in the absence of an interaction of gender or age at the onset of symptoms. These results indicate that a family history of IBS is a potential predictor of individual IBS risk[19], with a twofold to threefold risk increase for relatives of patients with IBS. Moreover, familial clustering was present irrespective of the IBS subtype and known environmental risk factors for IBS are also common in families with IBS[16].

Twin studies also support the notion that IBS might be a multifactorial disorder with genetic as well as environmental contributors. To date, at least five twin studies estimated the genetic heritability in IBS as ~22–57%[21]. A higher concordance rate for IBS is apparent among monozygotic twins than among dizygotic twins[14,22–24]. Twin studies using a co-twin control design have revealed restricted fetal growth in the low-birth-weight range (<2,500 g) as common cause for development of IBS and symptoms of anxiety or depression. These observations have led to the proposal that a dysfunction within the hypothalamic–pituitary–adrenal axis might link restricted intrauterine growth with IBS risk and, equally, explain the frequently encountered comorbidity between IBS and anxiety and depression[22,25]. These findings provide a rationale for the exploration of the expression and epigenetic prints of genes involved in the hypothalamic–pituitary–adrenal axis, such as corticotropin-releasing factor signalling pathways genes, as outlined below[26,27].

Nevertheless, it should be noted that despite the consistent observation of familial clustering in IBS combined with the increased prevalence among monozygotic twins (as opposed to dizygotic twins), such phenomena could be explained, at least in part, by shared environmental contributors. Indeed, several studies have demonstrated the importance of social learning as an explanatory factor of IBS development later in life[28,29], although these findings are not universal across the literature[22,23,30]. Based on these lines of converging evidence, we propose that IBS is a multifactorial, complex genetic disorder arising from the interaction between both genetic and environmental factors.

Molecular genetics approaches

Despite considerable evidence pointing to the contribution of genetic factors in the pathogenesis of and predisposition to IBS, the precise genetic cause remains elusive. Hitherto, the majority of studies have examined the effects of a few single nucleotide polymorphisms (SNPs) in hypothesis-driven candidate approaches, whereas two studies performed a hypothesis-free, gene-naive genome-wide association study (GWAS).

Candidate gene studies

Gene variants in the serotonergic system. Owing to the hypothesis that disturbances in the 5-hydroxytryptamine (5-HT, serotonin) metabolism and/or signal transduction via the brain–gut axis contribute to altered sensorimotor function in the gastrointestinal tract, the serotonergic system has been extensively studied. In particular, the homozygous genotype of the short (S) allele of the promoter length polymorphism 5-HT transporter linked polymorphic region (5-HTTLPR), a region upstream of the serotonin reuptake transporter gene *SLC6A4,* has been found to be associated with IBS-D and IBS-C[31–45] (see Supplementary information S1 (table) online). The S allele is a functional polymorphism that differentially influences *SLC6A4* transcription, presumably reducing serotonin reuptake transporter expression and 5-HT reuptake[46]. Indeed, 5-HT levels have been found to be increased in rectal biopsies of patients with IBS-D[37], and 5-HT levels were markedly increased in those who were homozygous for the S allele in comparison with individuals who carry the long (L) allele. Of note, the S allele is also associated with disorders that are comorbid with IBS such as depression, anxiety[46–49], higher neuroticism and/or anxiety scores and sympathetic tone, lower

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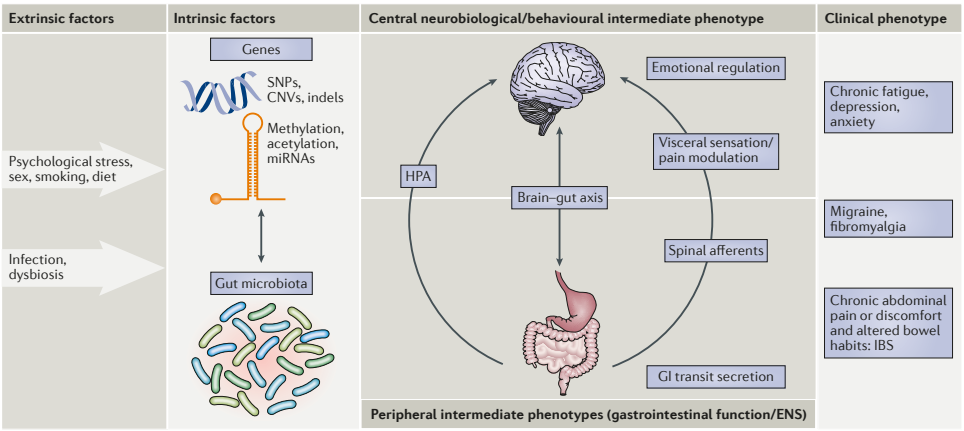

Figure 1 | **Multiple layers of complexity on environmental and genetic or epigenetic levels contribute to the pathogenesis of IBS and comorbid conditions.** A diverse array of environmental factors interact with underlying genetic variants and modify the genome directly and/or its DNA-binding proteins by methylation and/or acetylation, making people susceptible to IBS development. These predisposing genes lead to alterations in structure and function, represented by a variety of (neurobiological) intermediate phenotypes. These phenotypes influence the bidirectional crosstalk via the brain–gut axis, signalling via the HPA axis and spinal afferents from the periphery to the central nervous system.
CNV, copy number variation; ENS, enteric nervous system; GI, gastrointestinal; HPA, hypothalamic–pituitary–adrenal axis; indel, insertion and deletion of nucleotides; miRNA, microRNA; SNP, single nucleotide polymorphism.

parasympathetic tone and higher cortisol levels[44,50]. Moreover, patients who carry an S allele and those homozygous for the S/S genotype also have differences in the central processing of visceral pain, demonstrating increased amygdala activity in studies, which apply an emotional paradigm, and increased cerebral activity during colorectal distention, respectively. These findings therefore imply a high susceptibility to negative emotional memory in patients who carry the S/S genotype providing further support to the biopsychosocial model of IBS, in which alterations in the psychological state might contribute to enhanced visceral pain perception[51,52].

Further evidence for abnormal serotonergic signalling in IBS and comorbid conditions is provided by studies that examine the role of serotonin type 3 receptors (5-HT_3R) encoded by *HTR3* genes. 5-HT_3Rs influence gastrointestinal function, in particular peristalsis and secretion, and are relevant in emotional processing, mood and visceral perception. In addition, 5-HT_3R antagonists, such as alosetron, ramosetron and ondansetron, are beneficial in the therapy of IBS-D[53–57]. SNPs in the *HTR3A* (rs1062613), *HTR3C* (rs6766410) and *HTR3E* (rs62625044) genes have been reported to be associated with IBS-D (see Supplementary information S1 (table))[58–61]. The SNPs rs1062613 and rs62625044 in *HTR3A* and *HTR3E* localize outside the coding region and seem to impair translation of the receptors, thereby causing upregulation of receptor expression[58]. Their role in IBS-D has been confirmed[60,61]. In addition, rs1062613 in *HTR3A* was found to be associated with hypersensitivity in patients with GERD and dyspepsia[62,63]. Initially, it was reported to be associated with major depression and 'harm avoidance', an inherited temperamental trait connected to depression and anxiety and characterized by excessive worrying, cautiousness, apprehensiveness, pessimism, shyness and elevated fatigue[64–66]. In a study published in 2011, the rs1062613 variant has been correlated with the severity of IBS symptoms and anxiety[67]. Furthermore, rs1176744 in *HTR3B* was previously associated with anorexia and depression[68–70], and subsequently with IBS, in particular with an increased anxiety score and alexithymia[71] (see Supplementary information S1 (table)). Of note, in a GWAS that assessed the genetics of IBS in twins in the general population, SNPs in *HTR3E* and *SLC6A4* were nominally associated with IBS[72].

In 2005, a functional MRI study reported decreased activity in the amygdala and prefrontal cortex during facial recognition tasks in healthy individuals carrying minor alleles (T) of rs1062613 in *HTR3A*, implying a role in emotion and cognition[73]. A more recent functional MRI study published in 2011 revealed that individuals who carry the major allele (C) demonstrate increased responsiveness within the amygdala to emotional and nonemotional stimuli, and also receive higher anxiety and IBS symptom scores[67]. Individuals with the rs1176744 SNP in *HTR3B* also presented with considerably higher activity of the right amygdala, left insula and left orbitofrontal cortex during a rectal distension procedure[74] (see Supplementary information S1 (table)). Consequently, *HTR3* variants seem to influence gut-derived responses in brain regions relevant

for negative emotion, body recognition and stimulus discrimination[74]. The SNP rs1176744 has also been linked to pain catastrophizing, a coping style characterized by excessively negative thoughts and emotions in response to pain[75].

Taken together, these data support the notion that disruptions within serotonergic signalling are relevant in at least a subgroup of IBS, presumably IBS-D. However, additional SNPs in genes of the serotonergic pathway, such as tryptophan hydroxylase 1 (*TPH1*) and the serotonin receptor genes *HTR2A* and *HTR2C,* have been associated with IBS in single studies, albeit not yet replicated in additional cohorts[76–79] (see Supplementary information S1 (table) online).

Gene variants related to neuronal function. Many patients with IBS (~60%) have increased visceral perception[2], which is potentially mediated by altered neuronal function including sensitization of nociceptors on afferent nerves, increased nociceptive signalling at the level of the spinal cord or altered brain function. In fact, associations between SNPs in the voltage-gated sodium channel NaV1.5 gene *SCN5A* and IBS have been reported[80,81]. These data provide evidence that a *SCN5A*-encoded NaV1.5 ion channelopathy might be present in a subset of patients with IBS, leading to altered neuronal excitability and potentially contributing to motility disturbances and heightened pain perception. Similarly, the variant rs2349775 in neurexophilin 1 (*NXPH1*), another gene relevant in neuronal function, was associated with IBS-D[82]. This SNP was previously linked to neuroticism[83], yet its functional relevance in IBS remains elusive. Other potentially relevant variants in neuronal genes reside in the adrenergic receptor genes *ADRA2A* and *ADRA2C*[32,33,84], as well as in the genes encoding catechol-*O*-methyltransferase (*COMT*), brain-derived neurotrophic factor *(BDNF),* opioid receptor mu 1 (*OPRM1*), cannabinoid receptor 1 *(CNR1)* and fatty acid amide hydrolase (*FAAH)*[85–89] (see Supplementary information S1 (table)).

Gene variants affecting intestinal barrier function. Differential expression signatures of genes encoding tight junction proteins (occludin, zonula occludens 1, claudin 1, 2) have provided evidence to support the hypothesis of impaired intestinal barrier function in IBS-D[90–93], whereas SNPs in cadherin 1 (*CDH1*, encodes a tight junction protein also associated with Crohn's disease) are associated with PI-IBS[94]. In addition, rs1783796 in *CDC42* (cell division cycle 42), a small guanosine triphosphatase protein that controls the distribution of tight junction proteins, has been linked to IBS-C[82]. Nevertheless, further studies are required to identify and validate SNPs in barrier-related genes to define the patient subpopulation with IBS whose pathogenesis is in part related to intrinsic barrier impairment.

Gene variants related to immune function. Low-grade immune activation or dysregulation has been proposed as underlying mechanism of IBS, especially following an episode of acute gastroenteritis[12]. SNPs in genes involved in immune modulation and inflammation might predispose to IBS, even without previous gastrointestinal infection[95–97]. To date, SNPs in tumour necrosis factor ligand superfamily member 15 (*TNFSF15*) have been consistently reported as associated with IBS[97]. Associations for *TNFSF15* were shown within the different IBS subtypes: IBS-C[98]; IBS-D[99]; and IBS-A[82]. Lately, rs4263839 in *TNFSF15* was nominally associated in the first large IBS-GWAS[72]. *TNFSF15* is implicated in IBD and increased expression of the gene product has been found in intestinal biopsy samples of patients with IBS or Crohn's disease, and in the skin of patients with psoriasis[98–102]. TNFSF15 has been shown to enhance immune cell function and cytokine production[103–105]. Consequently, *TNFSF15* risk variants might alter disease susceptibility through differential modulation of pro-inflammatory and/or antibacterial responses, thereby contributing to the pathophysiology of a diverse array of immune-mediated human diseases, including IBS.

Various earlier studies suggested that SNPs in *IL10* and *TNF* are associated with IBS[106–114]. However, the reported findings were inconsistent and not reproducibly replicated[96] (see Supplementary information S1 (table)). A meta-analysis, examining 16 previously analysed SNPs in genes involved in the immune response, did not show an association between any of the tested SNPs, except for *TNFSF15*, thus indicating a minor role for immune-associated genes in IBS[96].

Whole-genome analyses

To date, only two studies have utilized a GWAS approach to delineate the genetic contribution to the pathophysiology of IBS. Firstly, a small pilot GWAS was conducted in a cohort of 172 patients with IBS and 1,398 healthy individuals and identified an association of a SNP in the protocadherin-15 gene (*PCDH15*) on chromosome 10 with IBS, but this finding could not be confirmed in additional samples[115]. Secondly, using a similar approach, Ek *et al.*[72] analysed 11,326 Swedish twins, including 534 patients with IBS and 4,932 asymptomatic individuals, and validated their findings in cohorts from various centres from Europe, the USA and Australia. One locus, at 7p22.1 encoding the genes *KDELR2* (KDEL endoplasmic reticulum protein retention receptor 2) and *GRID2IP* (glutamate receptor, ionotropic, delta 2 (Grid2) interacting protein), was significantly associated with IBS risk in the index GWAS and all replication cohorts, achieving a P value of 9.31×10^{-6} in a meta-analysis of all datasets. Expression analysis revealed a trend for increased mucosal *KDLER2* mRNA levels in patients with IBS compared with controls. However, knowledge gaps remain as to what extent these findings contribute to the symptomatology of IBS and to the precise role of these genes in IBS intermediate traits. Likewise, whether these candidate genes are causative or represent another gene or even mechanism at 7p22.1 is yet to be elucidated. Remarkably, SNPs from earlier studies have been confirmed in this GWAS dataset and variants in *ADRA2A, CDC42, COMT, HTR2A, HTR3E, IL1R1, IL4, NXPH1, SCN5A, SLC6A4, TNF* and *TNFSF15* were proven to be nominally associated with IBS[72,76,116].

Epigenetics

Environmental factors such as early or childhood trauma, physical and psychological stress, exposure to pathogens and changes in the gut microbiota might have a crucial role in the clinical manifestations of IBS. Epigenetic molecular mechanisms, which include DNA methylation and histone deacetylation, are implicated in the stress-related dysregulation of the hypothalamic–pituitary–adrenal axis[117]. The elucidation of epigenetic changes in IBS and related disorders remains in its infancy, although in several animal studies decreased mRNA levels of candidate genes were reported that correlated with altered patterns of histone acetylation and DNA methylation at the relevant promoter regions[117]. One preclinical example, in which a psychological stress model was tested on rats, identified differential methylation of the genes encoding the glucocorticoid receptor (*Nr3c1*) and corticotropin-releasing factor (*Crh1*), which inversely correlated with their respective gene expression levels[118]. This study points to a possible involvement of central epigenetic mechanisms in regulating stress-induced visceral hypersensitivity and provides an incentive for further work exploring the epigenetic mechanisms that might contribute to IBS-like symptomatology[118]. In a study performed in rats, the susceptibility to stress-triggered visceral hypersensitivity was successfully transferred across generations, dependent on maternal care[119]. Indeed, cross-fostered pups adapted to the phenotype of the foster mother: pups of normosensitive dams nursed by hypersensitive dams showed visceral hypersensitivity to colorectal distension at adult age and *vice versa*, presumably owing to differential epigenetic prints[119].

To date, only a small number of microRNA (miR) studies have been performed in IBS (see Supplementary information S2 (table)). Notably, Zhou *et al.*[90,120,121] have shown that a subset of patients with IBS-D displays an altered expression of specific non-coding miRs (hsa-miR-29a, hsa-miR-29b and hsa-miR-199a). Upregulation of miR-29a/b in the intestinal mucosa decreased levels of glutamine synthetase[90], an important regulator of intestinal permeability and homeostasis, claudin-1 and NF-κB repressing factor. In turn, downregulation of miR-199 correlated with an increased expression of transient receptor potential cation channel subfamily V member 1 (*TRPV1*), along with increased visceral sensitivity[90,120,121]. Thus, silencing members of the miR family miR-29 or administration of miR-199a might have important therapeutic implications for selected patients with IBS, whose symptoms are caused by increased intestinal permeability or hypersensitivity. Preliminary data also indicate increased levels of circulating hsa-miR-150 and hsa-miR-342-3p in the blood of patients with IBS, compared with healthy individuals[122]. Interestingly, hsa-miR-150 has been described as associated with IBD and pain, whereas hsa-miR-342-3p has been predicted to interact with miRs involved in pain signalling, colonic motility and smooth muscle function[122]. Hsa-miR-342-3p was also upregulated in bladder pain syndrome[123]. Expression levels of hsa-miR-103, hsa-miR-16 and hsa-miR-125b were diminished in the intestinal mucosa of patients with IBS, along with increased levels of claudin-2 and cingulin (C. Martinez, M. Vicario, B. Niesler, J. Santos, unpublished work). In addition, Kapeller *et al.*[58] have identified IBS-D-associated variants in *HTR3E* and *HTR4B*, with SNPs c.*76G>A and c.*61T>C leading to disturbed regulation of hsa-miR-510 and hsa-miR-16, respectively (C. Wohlfarth, C. Martinez, S. Schmitteckert, B. Niesler *et al.*, unpublished work). These two miRs have impaired binding abilities to target regions, leading to a reduction in the translational repression and, presumably, an increased expression of the target genes[58].

Complex genetic data analysis

Genetic and epigenetic data analyses using either hypothesis-free data driven (whole genome) or pathway-driven (gene-specific) approaches have strengths and limitations when applied to IBS (FIG. 2). In general, GWAS are far more informative as they interrogate the whole human genome, irrespective of hypothetical pathway involvement[72,115]. However, despite the high prevalence of IBS in the population, the number of individuals included in existing GWAS only enables limited statistical power, after adjustments for multiple testing. This GWAS limitation is particularly augmented with respect to IBS, as genetic loci might be specific for subtype, sex or clinical phenotype, and the need for subgroup analyses further reduces statistical power[21,72]. By contrast, pathway-driven approaches enable increased statistical power, but they are not investigating pathways for which a role in IBS has not yet been revealed or suspected.

However, the issue of statistical power and limited patient numbers can in part be addressed by the use of validation and replication data sets[72,115], as well as restricted analysis of particular subphenotypes[124]. This established fact highlights the importance of the availability of well-characterized case–control cohorts for validation and/or replication studies and subphenotyping, especially in cohorts of different ethnic origin[125]. To constrain the statistical burden of multiplicity in association studies, an additive penetrance model (a model that assumes each allele contributes to the relative risk) has to be adopted. Genotype-based tests comprise additive, dominant, co-dominant and recessive penetrance models (BOX 1) and demonstrate similar statistical power. Study sample sizes are often too small to identify recessive effects[126]. The allelic association test will be more powerful than the genotypic test as long as the penetrance of the heterozygote genotype is between the penetrances of the two homozygous genotypes. Allelic tests are only suitable in the absence of deviation from Hardy–Weinberg equilibrium[126,127]. For more detailed information, readers are referred to Clarke *et al.*[125] Gene–environment interaction analyses can help to delineate the relationships between genetics, environmental factors and IBS. However, such investigations must rely on uniformly thorough and detailed data collection, as well as on large, well-phenotyped sample sizes in multicentre studies.

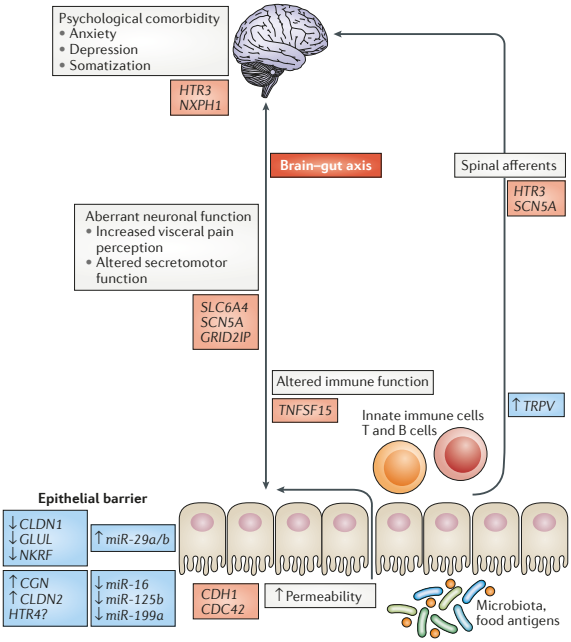

Figure 2 | **IBS-related pathways, based on genetic and epigenetic findings including potential pharmacogenetic targets.** Different pathways might be affected in specific subgroups of patients with IBS: epithelial barrier (permeability) and immune function, neuronal processing and signal transduction via spinal afferents from the periphery to the central nervous system in addition to the bidirectional crosstalk via the brain–gut axis, presumably contributing to psychological conditions such as anxiety, depression and somatization. miR, miRNA.

Pharmacogenetics

A better understanding of the genes that predispose to IBS development might pave the way for pharmacogenetic approaches in the future, thereby enabling personalized treatment tailored to SNP-based predictions of pharmacotherapeutic responses. However, a reverse approach might utilize knowledge on individual variation in drug responses in IBS to generate better insights into the underlying pathophysiology.

Serotonergic drug effects

As outlined earlier, 5-HT is central in the regulation of gastrointestinal function and its dysregulation can manifest in symptoms such as diarrhoea[128]. 5-HT$_3$ receptor antagonists, such as alosetron and ramosetron, reduce symptoms in IBS-D, retard gastrointestinal transit, increase fluid absorption and reduce pain[53,54,57]. The 5-HTTLPR L/L genotype in *SLC6A4* has been shown to correlate with clinical responses to alosetron in IBS-D, thereby altering the risk–benefit ratio of this class of compounds[129]. To what extent *HTR3* SNPs can influence so-called –setron'-mediated responses is presently unknown. Administration of tegaserod, a 5-HT$_4$ receptor agonist used for the treatment of IBS-C, also correlates with this *SLC6A4* polymorphism[43]. More specifically, the L/L genotype increases the sensitivity of constipation and results in a decreased response to treatment with tegaserod[43].

Adrenergic and opioidergic drugs

The clinical response to clonidine, an α2-adrenergic agonist, is modulated by genetic variations in *ADRA2A*, such as rs1800544, a SNP reportedly associated with gastric accommodation and rectal sensations of gas bloating and defecatory urgency[130]. The cannabinoid receptor agonist dronabinol decreases fasting colonic phasic motility and increases colonic compliance, especially in patients with IBS-D and IBS-M. However, the effectiveness of dronabinol is correlated with rs806378 in *CNR1* (REFS 131,132). This SNP is also associated with colonic transit in IBS-D and sensation rating of gas[85]. Additionally, a variant in *FAAH*, rs324420, and treatment with dronabinol lead to acceleration of proximal colonic motility in patients with IBS-D or IBS-M[86].

Bile acids

Genetic variants in the genes klotho beta (*KLB*) and fibroblast growth factor receptor 4 (*FGFR4*) are associated with accelerated colonic transit in patients with IBS-D[133–135]. These variants also correlate with the colonic transit response to chenodeoxycholic acid in IBS-C[133], and to colesevelam in patients with IBS-D[134,135].

Drawbacks and future perspectives

The delineation of the genetic factors involved in the aetiopathophysiology of IBS remains challenging, owing to the marked heterogeneity of the patient population assigned to the umbrella diagnosis of IBS (FIG. 1). Inconsistencies among association findings might originate from different expertise and focus of the recruitment centres (for example, psychosomatics, hypnotherapy, IBD), thereby introducing a centre-specific bias. In addition, patients have been recruited by applying non-uniform symptom classifications (such as Rome I, II or III), and comorbidity is rarely adequately assessed. Moreover, control individuals often have not been evaluated for the presence of IBS and/or comorbid conditions, therefore representing an additional source of bias. This concern is reflected by contradictory association findings with overall IBS, and its current subtypes, implying that more extensive studies on larger cohorts and/or better-stratified phenotypes are essential. Another major issue is the symptom-based classification of IBS used to date. This approach does not guarantee that the same disease and aetiopathogenetic mechanism is equally present in every group of patients studied. In fact, several peripheral mechanisms that contribute to IBS development with constipation phenotype might also be involved in other diseases, such as slow-transit constipation, intestinal pseudo-obstruction and idiopathic megacolon[136]. A diarrhoea phenotype can share susceptibility genes with food intolerance, disaccharidase deficiency, bile

acid malabsorption, small intestinal bacterial overgrowth, gluten sensitivity, increased mucosal permeability, immune activation, or accelerated colonic transit due to a motility disorder[137].

A comprehensive understanding of the causes of IBS must take into account extrinsic (environmental) factors and intrinsic (epigenetic and genetic) factors and their interplay. Although still viewed sceptically by some, (epi)genetic research might also open new frontiers in the assessment of the role of the environment. Ideally, microbiota data and stratification for sex should also be considered.

Close collaborations between researchers knowledgeable about (epi)genetics and experts in optimal assessment of environmental factors, measurement of quantitative traits and deep phenotyping are an essential prerequisite for a better understanding of underlying pathological mechanisms that contribute to IBS. Such progress will not only depend on joining forces by merging expertise, but also on the development of novel methods and analytic approaches to capture the complex gene–environment nexus. This strategy could enable researchers to better understand genetic components that make some individuals more vulnerable than others to environmental insults such as stress, infection, dietary factors and consequential epigenetic prints. Ultimately, new models will evolve to explain how the interplay of intrinsic and extrinsic factors influences this vulnerability.

Researchers and clinicians must bear in mind the importance of reliable intermediate traits, which might bring us closer to the molecular pathology of IBS, disturbances in epithelial barrier function, immune response and neuronal signalling in addition to hitherto unknown relevant pathways and networks. A better understanding of the cause-and-effect relationship will ultimately enable emerging proposals for therapy and prevention. According to current collections of research data, one of the most evident shortcomings of contemporaneous research in IBS is the lack of a comprehensive multidisciplinary integrative approach to investigate the different pathophysiological features using unified phenotyping tools. Such an approach seems crucial to facilitate dissection of the various factors involved in the pathogenesis of IBS (FIG. 3). Progress in the field essentially depends on large samples of individuals, who have been deeply phenotyped and for whom (epi)genetic and environmental data are available.

In accordance with this premise, a number of multidisciplinary experts from across Europe joined forces to overcome this issue by developing detailed phenotyping strategies to enable the stratification of genetic data in subphenotypes of patients with IBS, thereby increasing power and effect-size of the associated variants. The collaborative has developed protocols for the measurement of intermediate phenotypes and quantitative traits, such as transit studies, visceral sensation and permeability assays, which are mandatory to dissect (epi)genetic patterns underlying IBS and subsequently correlate these with symptom complexes. Obviously, this aim can only be achieved by meticulous phenotypic characterization of patients based on clinical examination, specific questionnaires that assess gastrointestinal symptoms and psychiatric comorbidity, personality traits, somatisation status, assessment of physiological parameters and tissue sampling for follow-up of expression changes in candidate genes (FIG. 3). In addition, to avoid bias introduced by patients with IBS within control populations, such controls should also be evaluated using the same tools as for diagnosed patients. In conjunction to such detailed phenotyping, family history of functional gastrointestinal disorders and crucial known environmental predisposing factors (stress, infection, nutrition) should be assessed. Using this strategy, the identification of distinct patient subgroups will facilitate the stratification of (epi)genetic data in combination with environmental factors. The major goal of the COST (Cooperation in Science and Technology) Action BM1106 GENIEUR (GENes in Irritable Bowel Syndrome Research Network EURope) is to establish harmonized criteria and a standard protocol to recruit

Box 1 | **Glossary**

Single nucleotide polymorphism (SNP)
A genetic variant consisting of a single base-pair change, usually resulting in two possible allelic entities at this position (biallelic locus)

Genotype
Set of alleles that determines the expression of a particular gene, usually two forms (biallelic)

Allele
Alternative form of a gene at a particular position (locus) on a chromosome

Locus
Location of gene on a chromosome

Dominant penetrance model
One allele is expressed over a second allele at the same locus and contributes to the phenotype

Co-dominant penetrance model
Both alleles contribute to the phenotype

Recessive penetrance model
The homozygous genotype of the minor allele contributes to the phenotype

Additive penetrance model
A genetic model with an increased disease risk of *r* for heterozygous genotypes and 2*r* for homozygous variant genotypes

Penetrance
The risk in a given individual; genotype-specific penetrance reflects the risk of disease regarding the respective genotype

Hardy–Weinberg equilibrium (HWE)
Given a minor allele frequency of *p*, the probability of the three possible genotypes (AA, Aa, aa) at a biallelic locus with major allele A and minor allele a are $(1\text{-}p)^2$, $2p(1\text{-}p)$, p^2

Genome-wide association study (GWAS)
The entire genome is genotyped for several hundred thousand SNPs and genotype or allele frequencies are compared between patients and control groups to find genetic variations associated with disease; as the whole genome is analysed, it is referred to as hypothesis-free approach

Replication cohort
A cohort of patients and controls that is drawn from a population comparable with the original population and that is analysed to test the validity of the initial association

Epigenome
Genome-wide distribution of epigenetic marks, including covalent DNA modifications, and histone modifications influencing genomic functions without altering the DNA sequence

a large set of well-characterized patients and healthy control groups. A further aim of GENIEUR is the establishment and development of an infrastructure that will facilitate future multicentre studies, in which not only blood samples for (epi)genetic and stool samples for microbiota studies will be archived, but also gut tissue samples from various regions for follow-up of (epi) genetic findings.

We also propose an in-depth investigation of the contribution of copy number variations, small indels (insertion and deletion of nucleotides) and rare variants, as assessed by next-generation sequencing and applied to the entire exome or genome, to the pathogenesis of IBS. The emerging technologies for massive parallel sequencing, combined with cost reductions and the development of advanced analytical tools, leave little doubt that these

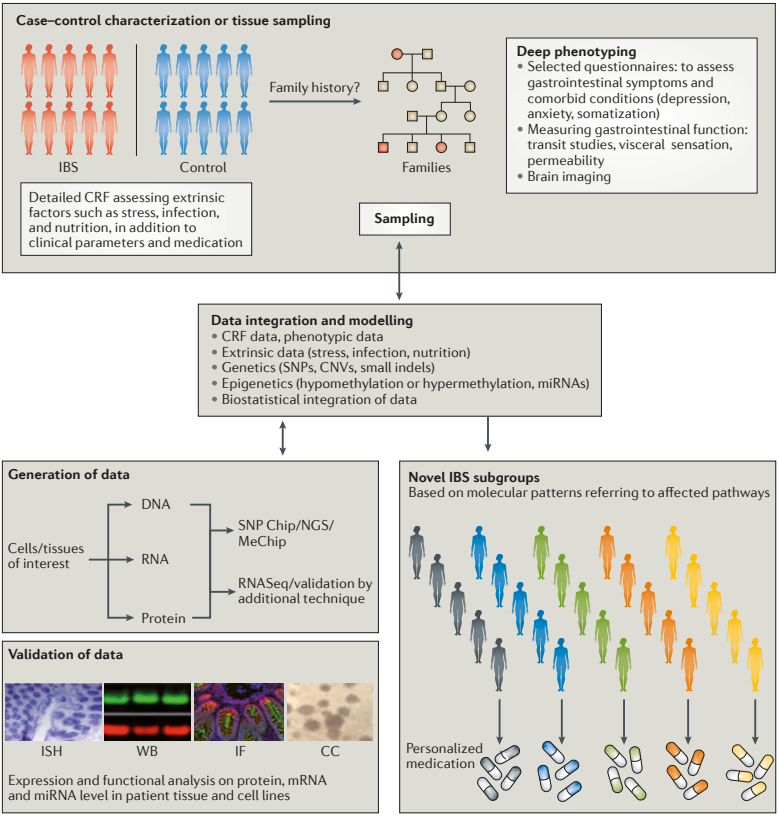

Figure 3 | **Future approach in IBS genetics or epigenetics research.** Patients and healthy individuals will be characterized using the same CRF, assessing extrinsic factors, medications and family history of IBS. Blood samples and gastrointestinal tissue will be sampled. Phenotyping will be performed utilizing established questionnaires to assess gastrointestinal symptoms (Rome III, GSRS, VSI, Nepean), comorbid conditions and somatization (HADS, GAD7, PHQ). In specialized centres, more-detailed measurements of gastrointestinal function might be undertaken (transit, visceral sensation, gut permeability, brain imaging). Biological material will be used for investigation of intrinsic factors in (epi) genetic studies, either candidate-driven approaches or hypothesis-free genome-wide studies using SNP chips or NGS. Gastrointestinal tissue might also be used for complementing epigenetics studies, methylation, acetylation and miRNA studies. If available, complementing studies on gastrointestinal tissue and cell lines might be applied for validation of candidates on mRNA and DNA level. All data will be fed into a central database for biostatistics integration of data and modelling to identify novel IBS subgroups for personalized treatment. CC, cell culture; CNV, copy number variation; CRF, case report form; IF, immunofluorescence; ISH, *in situ* hybridization; miRNA, microRNA; NGS, next-generation sequencing; RNASeq, RNA sequencing; SNP, single nucleotide polymorphism; WB, western blot.

new technologies will complement and enhance current efforts focused on improving the field's understanding of the role of IBS genetics, in a manner similar to what has been achieved for complex gastrointestinal disorders such as Crohn's disease[138,139]. Not only well-characterized cohorts, but also family and twin studies should be taken into account. Thus, the utilization of hypothesis-free approaches might lead to the identification of novel, and as yet un-anticipated, pathophysiological mechanisms across the disorder.

In particular, further exploration of family clustering in functional gastrointestinal disorders might provide useful insights into shared pathogenic pathways and the role of a shared environment. The power of this approach was shown in a study published in 2012, in which a SNP in *GUCY2C* that regulates secretion in enterocytes, co-segregated with diarrhoea in all affected family members, some of whom were diagnosed with IBS, further underscoring the potential relevance of this signalling pathway[140].

In addition, functional validation of the effect of any detected genetic variants is essential in providing an in-depth insight into the potential pathophysiological role of the respective (epi)genetic changes. This effort requires the establishment of appropriate cell culture models and the follow-up of expression changes of regulatory variants in relevant tissues.

Conclusions

The latest genetic findings support existing models of IBS pathogenesis, such as altered neuronal signal transduction, impaired immune response and intestinal barrier dysfunction. Yet it must be stressed that genetic association frequently does not imply phenotype causation and that SNPs associated with IBS might simply implicate a region in the genome where relevant disease-causing variants might reside. To date, the majority of studies lack the necessary power to apply different predisposing risk models and stratification for sex, thereby leading to conflicting or potentially false-positive results. This issue emphasizes the importance of replication studies in harmonized, well-characterized cohorts of appropriate size. A health-centre-specific bias, current patient classification and comorbidity have not been uniformly assessed and a gastrointestinal phenotype might have been missed in control populations. These errors and inaccuracies might in part contribute to conflicting data in IBS genetics.

Epigenetic findings further provide evidence about the influence of neuronal pathways and impairment of intestinal barrier function in the pathogenesis of IBS, and suggest their utility for diagnosis and/or treatment of those affected. However, the manner in which these genetic and epigenetic alterations might affect structure and function of gastrointestinal tissue, the brain–gut crosstalk and stress axis leading to the distinct clinical phenotypes remains unclear. Follow-up studies in patient tissue and cell lines should bring more clarity and help to understand functional consequences. Furthermore, gene–environment studies have rarely been applied to date and should also be taken into account. Undoubtedly, progress in the field essentially depends on large sample sizes of deeply phenotyped people for whom not only (epi)genetic, but also environmental data are available, which have to be generated in interdisciplinary multicentre studies. This approach will enable hypothesis-free genetic and epigenetic studies in adequately powered independent cohorts of individuals, enabling correlation between genotypes and subphenotypes and the inclusion of comorbid conditions and environmental factors.

The outcome of such integrative studies might help to define novel IBS subgroups, which will facilitate the development of enhanced diagnostics, disease prevention and novel treatment options, specifically targeted towards the affected pathways. The identified genetic variants and epigenetic markers (including miRs) could serve as biomarkers for interventional studies and pharmacotherapy to individualize therapy in the future and ultimately improve patient outcomes.

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Author contributions

B.N. and M.G. developed the concept, designed, wrote, assembled input data, and edited the manuscript; B.N. and M.M.W. created and revised the figures; A.M., B.N. and L.K.P. summed up the genetics and epigenetics findings in TABLES 1 and 2; M.M.W., L.K.P., M.B.B., E.F., G.N., C.A.D., A.M. and J.S. reviewed the literature, selected the data and wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages.

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The authors declare no competing interests.

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Intestinal Microbiota And Diet in IBS: Causes, Consequences, or Epiphenomena?

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Irritable bowel syndrome (IBS) is a heterogeneous functional disorder with a multifactorial etiology that involves the interplay of both host and environmental factors. Among environmental factors relevant for IBS etiology, the diet stands out given that the majority of IBS patients report their symptoms to be triggered by meals or specific foods. The diet provides substrates for microbial fermentation, and, as the composition of the intestinal microbiota is disturbed in IBS patients, the link between diet, microbiota composition, and microbial fermentation products might have an essential role in IBS etiology. In this review, we summarize current evidence regarding the impact of diet and the intestinal microbiota on IBS symptoms, as well as the reported interactions between diet and the microbiota composition. On the basis of the existing data, we suggest pathways (mechanisms) by which diet components, via the microbial fermentation, could trigger IBS symptoms. Finally, this review provides recommendations for future studies that would enable elucidation of the role of diet and microbiota and how these factors may be (inter)related in the pathophysiology of IBS.

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INTRODUCTION

With a prevalence of approximately 10–15%, irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders in the industrialized world (1). The high prevalence together with the reduced quality of life and associated co-morbidities in patients suffering from IBS put a significant negative burden on both patients and society (2). IBS is a heterogeneous functional disorder and commonly subtyped according to the prevailing bowel habit into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS with both constipation and diarrhea and unsubtyped IBS with neither constipation nor diarrhea using the Rome III criteria (3).

Although the etiology of IBS is incompletely understood, it is generally regarded as a multifactorial disorder involving the interplay of both host and environmental factors, including diet. The host factors include central factors, such as aberrant stress responses, psychiatric co-morbidity, and cognitive dysfunctions, whereas intestinal functions are also involved, e.g., dysmotility, visceral hypersensitivity, low-grade immune activation, altered barrier function, and the intestinal microbiota composition (4).

During recent years, perturbations in the intestinal microbiota are increasingly being linked to the pathophysiology of IBS. The increased risk of new onset IBS after an episode of gastroenteritis (5) and the association with prior antibiotic use (6) support the importance of the intestinal microbiota in IBS. Numerous studies have demonstrated altered microbial profiles in (specific subgroups of) IBS patients compared with healthy individuals (7–11). The intestinal microbiota strongly interacts with exogenous factors, in particular diet, which may also directly or indirectly provoke IBS symptoms.

In this review, we describe the current evidence regarding the impact of diet and the intestinal microbiota and their (inter)relation on the pathophysiology of IBS.

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THE IMPACT OF DIET ON THE INTESTINAL MICROBIOTA

The fetal intestine is thought to be sterile, although some recent studies indicate that colonization may already start before delivery, by bacterial transmission through the placental barrier (12,13). The colonization during and upon delivery involves a succession of microbial populations, a seemingly chaotic process strongly influenced by birth mode and infant feeding. Vaginally born infants are initially colonized by maternal fecal and vaginal microbes, such as *Bifidobacterium*, *Bacteroides*, *Prevotella,* and *Lactobacillus* spp., whereas infants born by cesarean section are inoculated by typical skin bacteria and bacteria from the hospital's environment (14,15).

The strong impact of diet on the indigenous microbial communities can already be observed even before weaning. Compared with formula-fed infants, the microbiota of breastfed infants is less diverse and more dominated by *Bifidobacterium* spp. (16–18). This is generally attributed to unique bioactive compounds such as human milk oligosaccharides that serve as metabolic substrates for a limited number of microbes (19), although breast milk itself also harbors a transferrable microbiota (20). A rapid diversification of the infants' intestinal microbiota and shift toward an adult-like microbiota occurs with the introduction of solid foods (21,22); yet, the maturation of the microbiota appears to continue in parallel to physiological development of, for example, the gastrointestinal tract and the central nervous system, throughout childhood and adolescence (23,24).

Once established, the intestinal microbiota appears to be relatively stable within individuals over time (25), whereas high variability between individuals is observed (26–29).

Pronounced differences in the composition and functional capacity of the human microbiota between geographically distant populations point toward an important role of the diet in these inter-individual variations (26,30–32). The microbiota in non-Western populations, consuming a diet high in plant-derived carbohydrates, has consistently been shown to be more diverse and enriched in *Prevotella* spp. at the expense of *Bacteroides* spp. as compared with Western populations consuming a diet high in animal protein, sugar, starch, and fat (reviewed in (33)). It remains to be determined to what extent the microbiota composition is shaped by the diet, as the impact of genetics, ethnic, and cultural factors, such as hygiene and living conditions, cannot be ruled out in such studies comparing distinct populations.

Nevertheless, habitual long-term dietary patterns have also been directly linked to intestinal microbial enterotypes. Especially, protein and animal fat intake has been associated with the *Bacteroides* enterotype, whereas a high carbohydrate intake was associated with the *Prevotella* enterotype (34). The effect of short-term dietary interventions on the microbiota composition appears to be only modest (34,35), unless the intervention comprises an extreme switch in diet (36–38). David *et al.* (37) demonstrated that even within days after the transition to a diet entirely composed of animal or plant products, changes in microbial structure, metabolic activity, and gene expression can be observed. In subjects receiving the animal-based high fat and protein diet, the abundance of bile-tolerant bacteria (*Alistipes, Bilophila* and *Bacteroides*) together with branched short-chain fatty acids indicative of amino-acid fermentation increased, whereas levels of bacteria metabolizing dietary plant polysaccharides (*Eubacterium rectale, Roseburia, Ruminococcus bromii*) decreased. In agreement, switching to a diet high in resistant starch has been shown to increase *Ruminococcus bromii* and *Eubacterium rectale* related species (39), which are known for their saccharolytic properties. Altogether, these studies indicate that the intestinal microbial community structure as well as its function and metabolic output are influenced by our diet, especially in the case of clearly distinct dietary patterns.

INFLUENCE OF DIET ON IBS

Surveys on perceived food intolerance show that 64–89% of IBS patients report their symptoms to be triggered by meals or specific foods (40–42) The majority indicate that they limit or exclude certain foods without professional counseling (40,42), thereby increasing the risk of inadequate dietary intake. Monsbakken *et al.* (40) found indications for inadequate dietary intake in 12% of IBS patients studied, whereas others found adequate or even increased intake of some nutrients (43–45). Overall, data on habitual dietary intake are limited and may depend on the study population included. Meal-related symptom aggravation is further supported by studies showing a postprandial worsening of pain using symptom diaries for 6 weeks (46) or rectal barostat (47,48).

Foods often reported to provoke symptoms include wheat/grains, vegetables, milk products, fatty foods, spicy foods, coffee, and alcohol (40–42,49) and are especially reported to be associated with abdominal pain and gas problems (41,42). Overall, the percentage of responders on exclusion diets varies from 15 to 71% (50), but it has to be noted that most studies suffer from major methodological limitations and only a minority of subjects was found to react positively to double-blind food challenges (51–53).

Although placebo and possibly nocebo effects have to be considered in IBS, possible physiological mechanisms of (perceived) food intolerance include an exaggerated sensory and motor response and/or incomplete absorption, which may lead to symptoms in a susceptible host. High fat intake, for example, is associated with an exaggerated colonic motor response to eating and increased visceral sensitivity in IBS patients, whereas duodenal lipid infusions delay small intestinal transit. Randomized controlled trials adjusting fat intake are, however, limited (54). Poorly absorbed carbohydrates, like lactose, fructose, and galacto- or fructo-oligosaccharides (fructans), can result in luminal distension by osmotic effects and increased gas production due to microbial fermentation. Although this is a normal physiological phenomenon, it can result in symptoms in subjects with an altered microbiota, increased visceral sensitivity, and/or abnormal gas handling. A recent study by Yang *et al.* (55) found increased mucosal mast cell numbers, serum tumor necrosis factor-α, rectal sensitivity, and anxiety in lactose intolerant IBS-D patients, supporting that neuro-immune modulation of visceral function is a potential underlying mechanism. Perceived intolerance to lactose, fructose, and fructans is frequently

reported and uncontrolled studies point to symptom improvement after removal of milk containing products or wheat from the diet (42). However, these studies should be interpreted with caution because of a large placebo and nocebo response in IBS. The overall evidence for an increased incidence of lactose intolerance in IBS patients was found to be weak and to be moderate—weak for the benefit of low lactose intake in these subjects (56).

Evolving from previous studies on lactose and fructose intolerance, a more generalized approach of intolerance to poorly absorbed and rapidly fermented carbohydrates has been introduced: FODMAPS, including fermentable oligo-, di- and monosaccharides and polyols. Both retrospective and prospective open studies have shown reduced symptoms after introduction of a low-FODMAP diet in IBS patients with suspected or proven fructose or lactose malabsorption (57,58). These findings are confirmed by randomized controlled trials, showing a significant improvement of overall symptoms, abdominal pain and bloating, in patients with bloating and/or diarrhea (59) and in small (n = 15–30) unselected IBS patient groups (60,61). One study showed recurrence of symptoms on re-challenge with fructose and/or fructans (62). Involvement of gas production is supported by findings that IBS patients produced more hydrogen relative to healthy controls and lacked an increased methane production on a high vs. low-FODMAP diet (60). However, it should be noted that the long-term benefit of a low-FODMAP approach in large (unselected) patient groups is unclear, especially considering its invasiveness, which requires strict guidance by a dietician, and the potential risks of, for example, reduced fiber intake and changes in microbiota composition and activity (59,63). Furthermore, randomized controlled trials are needed to prove whether it is superior to other dietary interventions (e.g., lactose or fructose reduction) or general dietary advice (e.g., the NICE guidelines). Finally, the FODMAP levels vary between studies, countries, and products, which should be taken into account when interpreting study results and considering low-FODMAP diets.

Foods can also evoke symptom onset by immune activation or altered neuro-endocrine responses (42). Food allergy or intolerance associated with IgE-mediated immune responses is uncommon and evidence for IgG/IgG4-mediated hypersensitivity is inconclusive (42,64). However, in subsets of IBS patients, increased numbers of T-lymphocytes, mast cells, eosinophils, and/or enteroendocrine cells have been found (65–67), but their exact role in food intolerance and symptom generation is unclear.

Interest in the role of gluten intolerance (and the possible benefit of gluten-free diets) is increasing. A systematic meta-analysis found a pooled prevalence for celiac disease up to 4% among IBS patients (68). The prevalence of non-celiac gluten sensitivity among unselected IBS patients is unknown and complicated by clear diagnostic criteria and overlap in symptoms. Although symptom reduction after a gluten-free diet (69) and worsening of symptoms after a gluten challenge (70) have been reported, the recent study Biesiekierski *et al.* (71) found no symptom induction by giving pure gluten to IBS patients following a gluten- and FODMAP-free diet. These findings suggest that not gluten but fructans and/or other components might contribute to symptoms in perceived "wheat" intolerance. Furthermore, Carroccio *et al.* (72) showed that the majority of IBS patients diagnosed with wheat sensitivity had multiple food sensitivities.

Dietary supplementation studies focus on probiotics, prebiotics, synbiotics, and fiber intake. A recent meta-analysis on the use of probiotics shows significant effects on subjective global symptom improvement, but study heterogeneity is statistically significant and no conclusions can be drawn on which individual species or strains are most beneficial (73). Studies on prebiotics and synbiotics are limited and insufficient to draw conclusions. Evidence for the benefit of dietary fiber was found for soluble fiber intake only (74), but it should be noted that overall study quality was moderate and more studies are needed on its effects in IBS subgroups.

Although several food components are associated with symptom generation, detailed analyses on the effect of food intake on symptom generation and underlying mechanisms are limited. In addition, many studies suffer from methodological limitations, in part inherent to the complexity of food research.

MICROBIOTA ALTERATIONS IN IBS

Following the first comprehensive analysis reporting a distinctive intestinal microbiota composition in IBS patients (75), several studies have identified differences between the microbiota of IBS patients, or subgroups thereof, and healthy controls (reviewed in (7)). Recently, clinical guidance regarding the modulation of intestinal microbiota in IBS was provided by the Rome Team Working Group (76), which concluded that there is good evidence supporting the concept that the intestinal microbiota is perturbed in patients with IBS. However, despite a growing consensus regarding an association between the intestinal microbiota and IBS, results of current studies lack general consensus and a specific microbial signature in IBS remains elusive (**Figure 1**). Lack of detailed phenotypic characterization of patients, small sample sizes, and the cross-sectional study designs (providing only a single snapshot of the microbiota composition) in the majority of studies may all contribute to the fact that the markers of the IBS microbiota reported in several studies (marked in bold on **Figure 1**) are not reproducibly detected in all cohorts. Moreover, exogenous factors, including diet, are most often not taken into account, despite the demonstrated effect of diet on the microbiota and the potential alterations in diet associated with IBS. Finally, it is noteworthy that Jeffrey and colleagues identified distinct subsets of IBS patients, not corresponding to the traditional IBS subtypes, with an altered or normal-like microbiota composition (8), indicating that the disturbed microbiota might be relevant for the pathology of part of the IBS patients. Moreover, those with a normal-like microbiota had more adverse psychological factors, suggesting that central factors may predominate over microbiological factors in some but not all IBS patients.

Several recent comprehensive studies of the microbiota in IBS have reported an increase in the relative abundance of *Firmicutes*, mainly *Clostridium* cluster XIVa and *Ruminococcaceae*, together with a reduction in the relative abundance of *Bacteroidetes* (7–9,77). Bifidobacteria have been shown to be depleted in both

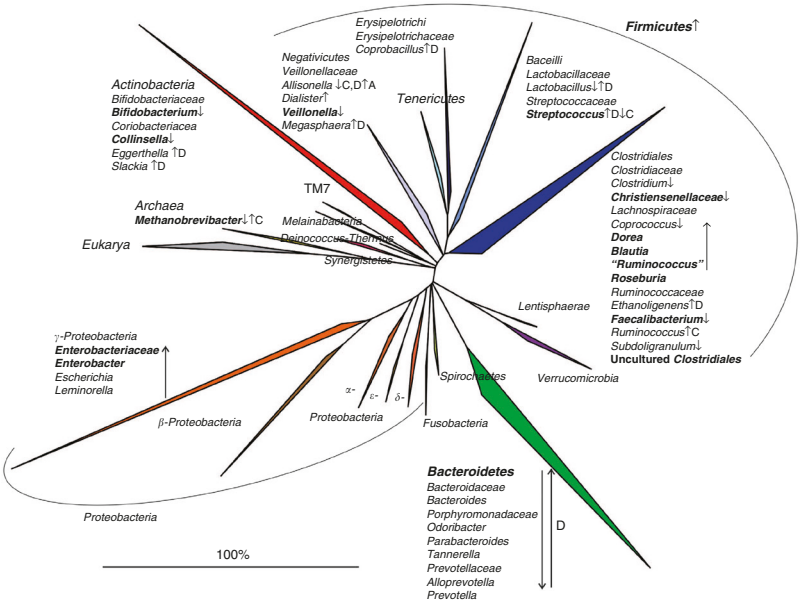

Figure 1. Phylogenetic tree of the human intestinal microbiota with indicated microbial groups that are significantly altered in irritable bowel syndrome (IBS) patients relative to controls. The increase and decrease in microbial groups in IBS patients are marked by arrows facing up and down, respectively, and in bold if reproduced by at least two independent studies. If microbial groups are altered only in diarrhea or constipation predominant IBS patients, letter D or C in superscript follows the arrows, respectively. The figure is generated on the basis of the data published in (8–11,75,77–79,82,85,98,116).

fecal and mucosal samples of IBS patients (9,78–81), which adds to the trend toward a modest beneficial effect of *Bifidobacterium* supplementation, in terms of the improvement of global IBS symptoms and pain scores as reported in a meta-analysis of probiotic trials.(73) A lower diversity and a higher instability of the (*Firmicutes* fraction or function of the) microbiota in IBS patients relative to controls have been reported (82–85), although these findings need confirmation in studies with sufficient depth of the microbiota analysis.

Most if not all studies that compare microbial profiles in IBS patients and healthy controls are associative and cannot distinguish cause from consequence. However, evidence of a potential causal role for the GI microbiota is supported by a fecal transplantation experiment in which visceral hypersensitivity, presumed to underpin abdominal pain in a subgroup of IBS patients, could be transferred via the microbiota of IBS patients to previously germ-free rats (86). Furthermore, germ-free mice colonized with the microbiota from IBS-D patients have been shown to exhibit a faster GI transit and impaired intestinal permeability as compared with mice gavaged with the microbiota from healthy humans (87). The mechanisms through which the microbiota exerts these effects are not fully understood, but the fact that supernatants from colon biopsies of IBS patients, with increased levels of histamine and proteases, excite human submucosal neurons could indicate a brain–gut axis connection (88). Furthermore, an increase in fecal serine proteases has been linked with symptom development in IBS patients (89,90). Although the origin of both histamine and proteases could be both human and microbial, it is noteworthy that some of the *Firmicutes* bacteria that were found to be increased in abundance in IBS patients (8,9) are known to secrete large amounts of extracellular proteases (91). Alternatively, the predominantly endogenous fecal proteases might not be degraded by microbes because of the accelerated transit (as seen in IBS-D patients) or because of the disturbed metabolic activity of the microbiota (92). Serine protease inhibitors, the antagonists of serine proteases, are produced by many bacteria, including bifidobacteria (93), which may contribute to the possible benefit of bifidobacterial supplementation.

Dysregulated intestinal immune function, chronic low-grade mucosal inflammation, and increased mucosal permeability and barrier dysfunction have all been suggested as putative pathogenic mechanisms in IBS, in which the intestinal microbiota might have a role (reviewed in (76,94)). Moreover, the bidirectional interactions between the intestines and the central nervous system, which have an important role in the pathogenesis of IBS,

have suggested to be modulated by the microbiota (reviewed in (95)). Yet, there is a lack of data on the exact mechanisms through which the host-microbiota interactions underlie pathophysiology and generate symptoms. Identifying these mechanisms is further complicated by the fact that the majority of GI microbes remain uncharacterized (96). Indeed, most putative microbial markers of IBS are among uncultured bacteria. For example, uncultured bacteria related to *Ruminococcus torques* are significantly enriched in IBS patients in several cohorts (9,11,75), and their abundance positively correlates with bowel symptoms (9,10,97). It is intriguing that this bacterial group has been shown to be suppressed following a multispecies probiotic treatment that alleviated IBS symptoms (98). Similarly, uncultured bacteria within the *Clostridiales* order are reproducibly detected in significantly depleted abundance in IBS (9,10) but also in ulcerative colitis (99). Focus on the function of these uncultured bacteria should be of major interest for future studies.

MICROBIOTA–DIET INTERACTIONS IN IBS

Intestinal microbes have an important role in the digestion of dietary components, resulting in metabolites that may directly or indirectly contribute to IBS symptoms (**Figure 2**).

Colonic fermentation of carbohydrates mainly results in short-chain fatty acids (SCFAs, including acetate, propionate, and butyrate) and variable amounts of hydrogen and carbon dioxide. Several low-abundant microbial groups can subsequently dispose the produced hydrogen into acetate, hydrogen sulfide, and methane. The fermentation of protein residues, primarily in the distal colon, produces a variety of metabolites, including ammonia, organic acids, heterocyclic amides, and phenolic and indolic compounds, that are toxic and detrimental to gut health (100). Moreover, fat and digested proteins increase the excretion of bile acids that are subject to extensive bacterial transformation in the intestinal tract. This could be of particular relevance as bile acids inhibit many bacteria and may modulate microbiota composition and also have the potential to affect major pathophysiological factors in IBS, including GI motility, secretion, and immune function (101,102). Indeed, in a recent large-scale study, increased colonic bile acid exposure was demonstrated in a subset of, predominantly non-constipated, IBS patients correlating with diarrhea and accelerated colonic transit (101).

An association between the intestinal microbiota and protein metabolism has been established by a significant correlation between the abundance of several post-infectious (PI)-IBS microbial markers and host amino-acid metabolism (10). Among harmful products of protein fermentation (100,103), hydrogen sulfide might be relevant for compromising intestinal health as it directly impairs epithelial metabolism (104) and acts as a gut–brain signaling molecule (105). Hydrogen sulfide can be converted to thiosulfate and further oxidized to tetrathionate during inflammation. The latter supports the growth of *Salmonella* (106) and other tetrathionate utilizing pathogens from the Gamma-Proteobacteria class (107), many of which have been associated with bowel symptoms of IBS patients (9,10,82).

FODMAPS, indigestible carbohydrates, can be fermented by intestinal microbes, resulting in increased gas production and an osmotic effect that can provoke IBS symptoms in a sensitive host (108), although similar changes may go unperceived in normal subjects (109). Carbohydrate utilizing GI bacteria such as *Dorea* spp., major gas producing bacteria in the human intestine (110), were reported to be significantly increased in abundance in IBS patients (9,11). Overproduction of gas, specifically hydrogen, has been associated with IBS symptoms (111), especially abdominal pain and flatulence. In patients with IBS-D, whose colon was found to be less able to accommodate the increased intestinal volume (112), overproduction of gas could be a trigger for increased colonic wall tension. In general, hydrogen produced in the gut is most efficiently removed by the methanogenic archaea (113) that appear to be depleted in IBS-D patients (9,10) and increased in IBS with constipation patients (reviewed in (114)) Although there is substantial evidence for implication of methane in IBS symptomology, most data are based upon methane breath testing. Hence, future studies, especially those addressing the diet-microbiota connection in IBS, should quantify methanogens to provide more direct evidence.

Alternative pathway for gas elimination is the conversion into acetate by acetogenic bacteria, *Blautia* spp. (115). *Blautia* spp. are highly prevalent and dominant intestinal bacteria, which have been found to be elevated in IBS patients (9,79). Intriguingly, the major fermentation end-product—acetate—has been reported as significantly increased in IBS patients (116), although this was not confirmed in another study (117). This may relate to the fact that most studies measure stool concentrations without taking the total stool volume into account, thereby potentially concealing overall increased acetate production as a result of dilution due to increased stool volume.

SCFAs produced upon the microbial fermentation of undigested carbohydrates and reduced levels thereof may also contribute to symptom generation in IBS. SCFAs have some documented health benefits, in particular butyrate, which is an important energy source for colonocytes, and among others inhibits inflammation and enhances barrier function (118). Moreover, intraluminal administration of butyrate into the distal colon has been shown to decrease visceral sensitivity in healthy humans (119). However, excessive intake of fermentable carbohydrates and high SCFA levels can also increase the osmotic load and thereby lead to diarrhea (120).

Data on SCFAs in IBS are limited and inconsistent, showing no alterations, increased or decreased levels of SCFAs relative to controls (61,116,117,121). Furthermore, altered SCFAs profiles might merely reflect diet rather than a feature of the condition. Given that the major products of FODMAPs fermentation are SCFAs, and that some major microbial SCFA-producers may be altered in IBS, the connection between FODMAPs, SCFAs, microbiota, and IBS symptoms calls for further elucidation.

Few studies have examined the impact of dietary interventions, in particular FODMAP restriction, on the microbiota in IBS patients. A low-FODMAP diet has been linked to reduced bifidobacterial counts (59), which seems a paradox given their potential health benefit. A recent study reported a reduction in total bac-

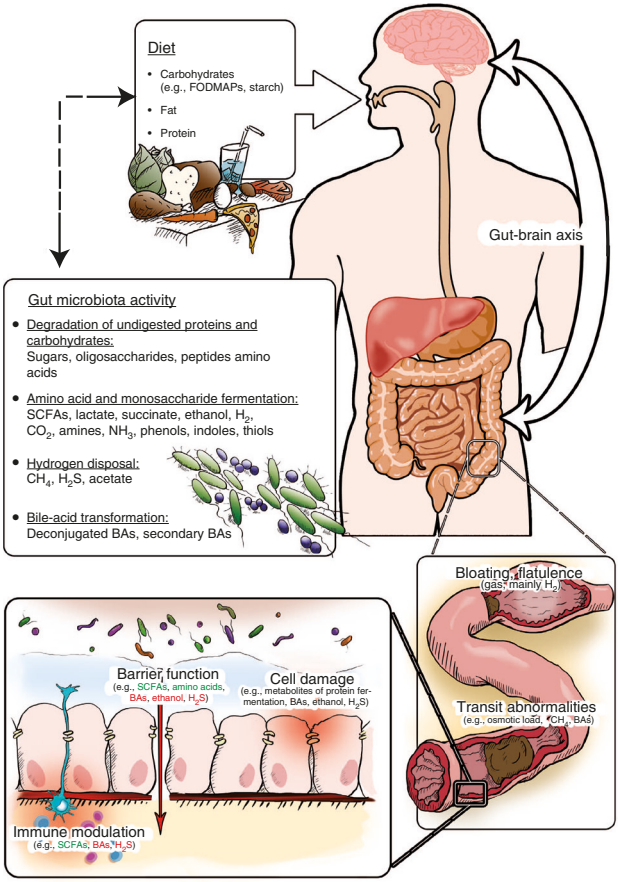

Figure 2. Non-exclusive listing of dietary metabolites that can be regulated by microbial activity and contribute to irritable bowel syndrome (IBS) symptoms. The impact of metabolites affecting different domains of intestinal health is depicted; however, it should be noted that the effects of some metabolites depend on their concentration or further conversion. Note: for immune modulation and barrier function, metabolites with positive and negative effects are colored in green and red, respectively. SCFAs, short-chain fatty acids; BAs, bile acids.

terial abundance following the introduction of a low-FODMAP diet, as well as a reduction in absolute counts but not relative abundance of specific bacterial groups, including bifidobacteria as compared with habitual diet (63). However, as compared with a diet high in FODMAP content, the low-FODMAP diet appeared to be associated with marked lower relative abundances of butyrate-producing bacteria and *A. muciniphila*, and a significantly higher abundance of *R. torques*, adding more paradoxical data on the effects of low-FODMAP diet (63).

Extensive analyses of microbiota composition, functionality, and fermentation products in relation to FODMAP restriction and symptom generation are currently, however, lacking.

The direct impact of diet-microbiota interactions on IBS symptoms has been demonstrated in an elegant study on the impact of a diet rich in fermentable substrates. The introduction of this flatulogenic diet significantly increased the gas volume and the number of gas evacuations, as well as abdominal symptoms and digestive discomfort in both healthy subjects and flatulent

patients (122). Yet, upon dietary challenge, patients' microbiota developed instability in composition, exhibiting variations in the *Bacteroidetes* and *Firmicutes* phyla and reduction in microbial diversity, whereas healthy subjects' microbiota remained stable. Hence, the extent of dysbiosis in IBS patients likely depends on their diet. In a small study among children with IBS, the introduction of a low fermentable substrate diet significantly decreased abdominal pain severity and frequency in some but not all children. Responders to this dietary intervention appeared to differ in intestinal microbiota composition at baseline from nonresponders, indicating that the efficacy of dietary interventions might be influenced by the patient's microbiota (123). To prove the validity, these pilot results will have to be replicated in larger studies.

CONCLUSION, RECOMMENDATIONS FOR FUTURE RESEARCH

Both experimental and observational studies provide good evidence to conclude that microbial alterations frequently observed in IBS patients potentially drive or perpetuate gastrointestinal symptoms. Yet, the strong interaction between distinct dietary patterns and the intestinal microbial communities is likely one of the explanations for the inconclusive findings among studies comparing the microbiota composition in IBS patients and healthy subjects.

More well-designed studies on the effect of food intake on symptom generation and the underlying mechanisms are warranted. The potential benefit of FODMAP restriction seems promising but requires further study and dietary guidance is essential given the complexity and risk of inadequate nutrient intake. In addition, more evidence on long-term outcome and well-performed challenge or re-introduction trials are warranted as important adaptions may occur. The incorporation of microbiota characterization in such studies would both provide insight into the impact of these dietary interventions on the microbiota, as well as offer the opportunity to examine whether the microbiota can predict response.

Given that IBS patients comprise a heterogeneous group both in the type of bowel symptoms and food components to which they might respond, and that the microbiota is extremely complex and variable between individuals, it is clear that defining the correlations between IBS, microbiota, and diet is not an easy task. However, high-throughput molecular methods enable reproducible and in-depth analysis of the intestinal microbiota, providing the necessary tool for testing these correlations. The major challenge remains to include the conscientious collection of dietary information in future studies in this field. Moreover, future studies should include multiple microbiota analysis at various time points in a large number of well-phenotyped patients to increase our insight in the contribution of intestinal microbiota perturbations to the diverse and fluctuating symptom pattern in IBS patients.

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CONFLICT OF INTEREST

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COST Action BM1106 GENIEUR Autumn Meeting in Vienna (Austria), 17.–18.10.2014

GENIEUR Autumn Meeting in Barcelona (Spain), 29.–30.10.2015

Second COST Action BM1106 GENIEUR Training School Heidelberg (Germany), March 2016