Lessons learned: Resolving the enigma of genetic factors in Irritable Bowel Syndrome

Maria Gazouli[1], Mira M Wouters[2], Lejla Kapur-Pojskić[3], May-Bente-Bengtson[4], Eitan Friedman[5], Gordana Nikčević[6], Christina Demetriou[7], Agata Mulak[8], Javier Santos[9] & Beate Niesler[10]

[1] Department of Basic Sciences, Laboratory of Biology - School of Medicine, University of Athens, Athens, Greece
[2] Translational Research Center for Gastrointestinal Disorders (TARGID), University Hospital Leuven, Leuven, Belgium
[3] Institute for Genetic Engineering and Biotechnology, University of Sarajevo, Sarajevo, Bosnia-Herzegovina
[4] Vestfold Hospital Trust, Tønsberg, Department of Internal Medicine, Division of Gastroenterology, Tønsberg, Norway[5] The Suzanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Centre, Tel-Hashomer, Israel
[6] Laboratory for Molecular Biomedicine, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[7] Department of Electron Microscopy / Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
[8] Department of Gastroenterology and Hepatology, Wroclaw Medical University, Wroclaw, Poland
[9] Neuro-immuno-gastroenterology Lab, Digestive Diseases Research Unit. Vall d'Hebron Institut de Recerca. Department of Gastroenterology, Hospital Universitari Vall d'Hebron & Facultat de Medicina, Universitat Autònoma de Barcelona and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain
[10] Institute of Human Genetics, Department of Human Molecular Genetics, University of Heidelberg, Heidelberg, Germany

*Corresponding author:
PD Dr. Beate Niesler
Institute of Human Genetics
Department of Human Molecular Genetics
Im Neuenheimer Feld 366
69120 Heidelberg, Germany
Tel.: 0049-6221-56 35274
Fax.: 0049-6221-56 5155
beate.niesler@med.uni-heidelberg.de

Abstract

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal disorder (FGID) phenotypically characterized by chronic abdominal discomfort, pain and altered defecation patterns. It is postulated that the pathophysiology of IBS is multifactorial, albeit with a significant 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 more recent years, positive associations are still scarce and/or have not been reproduced. Moreover, 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 GENIEUR BM1106 (GENes in Irritable Bowel Syndrome Research Network EURope, www.GENIEUR.eu) was established in order to address these obstacles. In this review, the epi-/genetics Working Group of GENIEUR reports on the current state of the art in the field, highlights fundamental flaws and pitfalls in current IBS epi-/genetics research and provides a vision of how to tackle and improve epi-/genetics approaches in this complex disorder in the future.

Introduction

Irritable bowel syndrome (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 there are no biomarkers available for IBS, it is a symptom based diagnosis made according to the Rome III criteria (www.romecriteria.org). According to the defecation pattern, IBS is subdivided into the following subtypes: diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), a mixed subtype (IBS-M) and un-subtyped 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. IBS patients 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, hospitalisation, diagnostic tests and ineffective treatment lead to a marked economic burden to the healthcare system with an estimated annual total cost of €41 billion in the EU.[8] Indirect costs of IBS related to work absenteeism and lower productivity are also considerable. Our knowledge of the pathophysiology of IBS, mainly due to its multifactorial origin, remains limited and there is a current paucity of efficacious therapies.

Environmental factors such as psychological stress[9], diet, and smoking, infectious gastroenteritis leading to post-infectious IBS (PI-IBS)[10] as well as alterations in the gut microbiota, produce a complex interaction with genetic variants dispersed in the human genome and leading to individual epigenetic prints. These factors may contribute to central and peripheral (neurobiological) intermediate phenotypes (brain

and enteric nervous system (ENS) function) influencing the brain-gut axis and may consequently manifest as central behavioural and gastrointestinal (GI) intermediate phenotypes and thereby predisposing to IBS and its comorbid conditions (Figure 1).[10-12] The role of intrinsic factors (genetics, epigenetics, sex hormones, coping mechanisms) in IBS has been studied to a lesser extent.

IBS heritability: Family and twin studies

Several studies have reported the familial clustering of IBS, thus suggesting an inherited component. [13 14 15 16 17 18 19] 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[20]. Other studies have shown that having a first-degree relative with IBS may be predictive of IBS and increased IBS risk among first-, second-, and third degree relatives, in the absence of an interaction of gender or age at symptoms onset, thereby providing evidence that a family history of IBS is a potential predictor of personal IBS risk [18] with relatives of IBS patients had a two-threefold heightened risk of having IBS. Moreover, familial clustering was observed to be present irrespective of the subtype of IBS and the known environmental risk factors for IBS are also common in IBS families[15].

Twin studies also support the notion that IBS may 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 between 22–57%. [21] There is a higher concordance rate for IBS among monozygotic compared to dizygotic twins. [13 22 23 24] Twin studies using a co-twin control design have revealed restricted fetal growth in the low range of birth weight (<2500 gram) as the common contributor to development of IBS and symptoms of anxiety or depression. These observations have

led to the proposal that dysfunction within the hypothalamic-pituitary-adrenal (HPA) axis may link restricted intrauterine growth with IBS risk as well as the frequently encountered comorbidity between IBS and anxiety and depression.[22, 25] These findings provide the rationale for the exploration of the role of the expression and epigenetic prints of genes involved in the HPA axis, such as the corticotropin releasing factor (CRF) signaling pathways genes, as outlined below. [26 27]

Nevertheless, it should be noted that despite the consistent observation of familial clustering of IBS coupled with the increased prevalence amongst monozygotic compared to dizygotic twins, such factors 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 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. The aim of this review is to summarize the current *state of the art* in the field of genetics and epigenetics research in IBS and its comorbid conditions, to highlight the fundamental flaws and pitfalls in unraveling the molecular mechanisms of IBS and to share our view how to address these difficulties and therefore improve the approaches to better define epi-/genetics mechanisms in this multifactorial disorder in the future.

Molecular genetics approaches

Despite considerable evidence pointing to the contribution of genetic factors in the pathogenesis of, and predisposition to, IBS, the precise genetic basis remains elusive. Hitherto, the majority of studies have examined the effects of a few single nucleotide

polymorphisms (SNPs) in hypothesis-driven candidate approaches, while two studies performed a hypothesis-free gene-naive genome wide association study (GWAS) (see below).

Candidate gene studies

Genetic variants in the serotonergic system

Based on the hypothesis that disturbances in 5-hydroxytryptamine (5-HT) metabolism and/or signal transduction via the brain-gut axis contribute to altered sensorimotor function in the GI tract, it is not surprising that 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 (SERT) gene *SLC6A4,* has been reported as being associated with IBS-D and IBS-C (Table 1).[31 32 33] The S allele is a functional polymorphism that differentially influences *SLC6A4* transcription, presumably reducing SERT expression and 5-HT re-uptake.[34] Indeed, 5-HT levels have been found to be increased in rectal biopsies of IBS-D patients [33] and 5-HT levels were significantly increased in those who were homozygous for the S allele in comparison to carriers of the long (L) allele. Of note, the S allele is also associated with the disorders that are comorbid with IBS such as depression and anxiety [34 35 36] as well as higher neuroticism/anxiety scores, sympathetic tone, lower parasympathetic tone and cortisol levels.[37] Moreover, S allele carriers and those homozygous for the S allele also have differences in the central processing of visceral pain demonstrating increased amygdala activity in studies applying an emotional paradigm and increased cerebral activity during colorectal distention respectively.[38 39] These findings therefore imply a high susceptibility to negative emotional memory in

S/S carriers providing further support to the biopsychosocial model of IBS in which alterations in psychological state may contribute to enhanced visceral pain perception.

Further evidence for an abnormal serotonergic signaling in IBS and comorbid conditions, is providing by studies examining the role of serotonin type 3 receptors (5-HT_3R) encoded by *HTR3* genes. 5-HT_3Rs influences GI function, in particular, peristalsis and secretion, and are relevant in emotional processing, mood and visceral perception. Moreover 5-HT_3R antagonists, such as ondansetron and alosetron, are beneficial in the therapy of IBS-D.[40 41 42] SNPs in the *HTR3A* (rs1062613), *HTR3C* (rs6766410) and *HTR3E* (rs62625044) genes have been reported to be associated with IBS-D (Table 1).[43 44 45] SNPs rs1062613 and rs62625044 in *HTR3A* and *HTR3E* localize outside the coding region and seem to impair translation of the receptors, thereby causing up-regulation of receptor expression[43], and their role in IBS-D has recently been delineated.[45] Moreover, rs1062613 in *HTR3A* was found to be associated with hypersensitivity in GERD patients and dyspepsia.[46 47] Initially, it was reported to be associated with major depression and 'harm avoidance', an inherited temperamental trait associated with depression and anxiety.[48, 49] In a recent study it has been shown to be correlated with IBS symptom score and anxiety.[50] Furthermore, rs1176744 in *HTR3B* was previously associated with anorexia and depression,[51 52 53] and subsequently with IBS, in particular with an increased anxiety score and alexithymia[20] (Table 1). Of note, in a recent IBS-GWAS, SNPs in *HTR3E* and *SLC6A4* were nominally associated with IBS.[54]

Furthermore, functional magnetic resonance imaging (fMRI) studies have shown decreased amygdala and prefrontal cortex activity in minor allele (T) carriers of rs1062613 in *HTR3A* in an emotional paradigm.[55] In addition, a more recent fMRI

study revealed that carriers of the major allele (C) have increased responsiveness within the amygdala to emotional and non-emotional stimuli, higher anxiety and IBS symptom scores.[50] Furthermore, carriers of rs1176744 in *HTR3B* presented with significantly higher activity of the right amygdala, left insula and left orbitofrontal cortex in a rectal distension paradigm (Table 1). Consequently, *HTR3* variants seem to influence gut-derived responses in brain regions relevant for negative emotion, body recognition, and discrimination of stimulus.[39] In addition, rs1176744 has recently been found to associate with pain catastrophising, a coping style characterized by excessively negative thoughts and emotions in relation to pain.[56]

Taken together, these data support the proposal that disruptions within serotonergic signaling are relevant at least in a subgroup of IBS, presumably IBS-D. However, additional SNPs in genes of the serotonergic such as tryptophan hydroxylase *TPH1* as well as the serotonin receptor genes *HTR2A* and *HTR2C* have been found to be associated with IBS in single studies, albeit yet not replicated in additional cohorts (Table 1).

Genetic variants in genes related to neuronal function

Approximately 60% of IBS patients suffer from increased visceral perception[20] which is potentially mediated by altered neuronal function including sensitisation of nociceptors on afferent nerves, increased nociceptive signaling 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[57]. These data provide evidence that a subset of IBS patients may suffer from *SCN5A*-encoded NaV1.5 ion channelopathy, leading to altered neuronal excitability and therefore potentially contributing to motility disturbances and heightened pain

perception. Moreover, rs2349775 in another gene relevant to neuronal function, neurexophilin 1 (*NXPH1*), was associated with IBS-D.[58] This SNP was previously linked to neuroticism[59], yet its functional relevance in IBS remains elusive. Other potentially relevant variants in neuronal genes reside in the adrenergic receptor genes *ADRA2A* and *2C* [60] [61] [62] as well as in the genes encoding catechol-O-methyltransferase *(COMT),* brain-derived neurotrophic factor *(BDNF),* mu opioids receptor *(OPMR1),* cannabinoid receptor type 1 *(CNR1)* and fatty acid amide hydrolase (*FAAH)* (Table 1).

Genetic variants in genes implicated in intestinal barrier function

Differential expression signatures of genes encoding tight junction proteins (occludin and zonula 1, claudin) have recently provided evidence to support the hypothesis of impaired intestinal barrier function in IBS-D [63] [64] [65] while SNPs in cadherin 1 (*CDH1*, a tight junction protein, also associated with Crohn's disease) is associated with PI-IBS.[66] In addition, rs1783796 in *CDC42* (cell division cycle 42), a small guanosine triphosphate-ase protein that controls the distribution of tight junction proteins, has been linked to IBS-C[58]. Nevertheless, further studies are required to identify and validate SNPs in barrier related genes in order to define subpopulation of IBS patients whose pathogenesis is in part related to intrinsic barrier impairment.

SNPs in genes 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 may predispose to IBS, even without previous GI infection.[67] [68, 69]

To date, SNPs in *TNFSF15* encoding the tumor necrosis factor (TNF) ligand superfamily member TL1A, have been consistently demonstrated to be associated with IBS[68] Associations for *TNFSF15* were shown within the different IBS subtypes: IBS-C,[70] IBS-D[71] and IBS-A.[58] Lately, rs4263839 in *TNFSF15* was nominally associated in the first large IBS-GWAS. [54] *TNFSF15* is implicated in inflammatory bowel disease (IBD) and increased gene product expression has been reported in the intestinal biopsies of patients with IBS as well as Crohn's disease and the skin of pateints with psoriasis.[70 71 72 73 74] TL1A has been shown to enhance immune cell function and cytokine production.[75-77] Consequently, *TNFSF15* risk variants may alter disease susceptibility through differential modulation of pro-inflammatory and/or anti-bacterial responses, thereby contributing to the pathophysiology of a diverse array of immune-mediated human diseases, including IBS.

Various earlier studies suggested SNPs in interleukin-10 (*IL10)* and TNF-alpha (*TNFA)* are associated with IBS.[78] However, the reported findings were inconsistent and not reproducibly replicated (Table 1). A meta-analysis, examining 16 previously analysed SNPs in genes involved in immune response, did not shown an association between any of the tested SNPs, except for *TNFSF15*, thus indicating a minor role for immune-associated genes in IBS.[69]

Whole genome analyses

To date, only two studies have utilised a GWAS approach to delineate the genetic contribution to the pathophysiology of IBS. Firstly, a small pilot GWAS in 172 IBS cases and 1,398 controls reported association on chromosome 10 in the protocadherin 15 gene (*PCDH15*) but this finding could not be confirmed in additional samples.[79] Secondly, using a similar approach, Ek et al.[54] analysed 11 326 Swedish twins including 534 IBS cases and 4932 asymptomatic controls and validated their findings in co-

horts from various centres from Europe, the USA and Australia. One locus, at 7p22.1 encoding for 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, reaching 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 IBS cases compared with controls. However, knowledge gaps remain as to what extent these findings contribute to the symptomatology of IBS and what the precise role of these genes is in IBS intermediate traits, and if these candidate genes are causative or represent another gene or even mechanism that account for the observed signal at 7p22.1.

Epigenetics

Environmental factors such as early or childhood trauma, physical and psychological stress, exposure to pathogens as well as changes in the gut microbiota may play a crucial role in the clinical manifestations of IBS. Epigenetic molecular mechanisms, which include DNA methylation and histone de-acetylation, are implicated in the stress related dysregulation of the HPA axis[80]. The investigation of epigenetic changes in IBS and related disorders remain in their infancy, although several animal studies reported decreased mRNA levels of candidate genes correlating with altered patterns of histone acetylation and DNA methylation at the relevant promoter regions.[80] One preclinical example, applying a stress model reported differential methylation of the genes encoding the glucocorticoid receptors (*Nr3c1*) and corticotropin-releasing factor receptor 2 (*Crfr2*), which inversely correlated with their respective gene expression levels. This study further demonstrated the involvement of central epigenetic

mechanisms in regulating stress-induced visceral hypersensitivity and provides a robust rationale for further work exploring the epigenetic mechanisms that may contribute to IBS-like symptomatology[81]. A recent animal study showed that the susceptibility to stress-triggered visceral hypersensitivity can be transferred across generations, dependent on maternal care[82]. Indeed, cross-fostered pups adapted to the phenotype of the foster mother: pups of normosensitive dams nursed by hypersensitive dams showed visceral hypersensitivity to distension at adult age and vice versa presumably by differential epigenetic prints[82].

To date, only a small number of microRNA studies have been performed in IBS (Table 2). Notably, recent reports have shown that a subset of patients with IBS-D display altered expression of specific non-coding microRNAs (hsa-miR29a, hsa-miR29b and hsa-miR-199a). Up-regulation of miR-29a/b in the intestinal mucosa decreased levels of glutamine synthetase (*GLUL*),[83] an important regulator of intestinal permeability and homeostasis, and claudin1 (*CLDN1*) as well as NF-kappa-B-repressing factor (*NKRF*). In turn, down regulation of miR-199 correlated with an increased expression of the transient receptor potential vanilloid type 1 (*TRPV1*) going along with increased visceral sensitivity. [84, 85] Therefore, silencing of the miR family miR-29 or applying miR-199a may have important therapeutic implications for selected IBS patients with symptoms 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 IBS patients, compared to healthy controls.[86] Interestingly, hsa-miR-150 has been described to be associated with IBD and pain, whereas hsa-miR-342-3p has been predicted to interact with mRNAs involved in pain signaling, colonic motility, and smooth muscle function. Interestingly, hsa-miR-342-3p has been found to be up-regulated in bladder pain syndrome.[87] In actual studies, hsa-miR-103,

hsa-miR-16, hsa-miR-125b, hsa-miR-338, hsa-miR-502, and hsa-miR-92 expression was found to be diminished in the intestinal mucosa of IBS patients (Martinez, Niesler and Santos, unpublished data). In addition, Niesler's group has identified IBS-D associated variants in the serotonin receptor genes *HTR3E* and more recently *HTR4b*, with SNPs c.*76G>A and c.*61T>C leading to disturbed regulation of hsa-miR-510 and hsa-miR-16, respectively. These two miRNAs have been shown to have impaired binding ability to target regions, leading to a reduction in the translational repression and presumably therefore an increased expression of the target genes.[43 88]

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. Firstly, GWAS are far more informative as they interrogate the whole human genome, irrespective of hypothetic pathway involvement.[54, 79] However, despite the high prevalence of IBS in the population[89] the current number of individuals included in the existing GWAS studies only allows limited statistical power, following adjustments for multiple testing. This GWAS limitation is particularly augmented with respect to IBS, since genetic loci might be sub-type, sex, or clinical phenotype specific[21, 89, 90] and the need for subgroup analyses further reduces statistical power. On the other hand, pathway driven approaches allow for increased statistical power but they are not investigating pathways whose role in IBS has not yet been revealed or suspected.

However, the problem of statistical power and limited subject counts, can in part be addressed by the use of validation and replication data sets[54, 79] as well as restricted analysis of particular sub-phenotypes.[91] This highlights the importance of the

availability of well-characterized case control cohorts for validation/replication studies and sub-phenotyping, especially in cohorts of different ethnic origin.[57] In order to constrain the statistical burden of multiplicity in association studies, one needs to assume an additive penetrance model. Additive, dominant and co-dominant (Pearson 2 degrees of freedom) association tests all show a similar statistical power, and study sample sizes are often too small to identify recessive effects. Allele tests are thus not recommended because their alpha levels depend on Hardy-Weinberg equilibrium.[92] [93] Gene x environment interaction analyses can help delineate the relationships between genetics, environmental factors, and IBS, however, such investigations must rely uniform thorough and detailed data collection as well as on large well phenotyped sample sizes in multi-centre studies.

Pharmacogenetics

A better understanding of the genes that predispose to the development of IBS may pave the way in the future for pharmacogenetic approaches, thereby enabling personalisation of treatment tailored by SNP based prediction of pharmacotherapeutic response. However, a reverse approach might utilize knowledge on individual variation in drug treatment of IBS to generate better insight to IBS underlying pathophysiology.

Serotonergic drug effects

As outlined above, 5-HT is central in the regulation of GI function and may manifest in symptoms such as diarrhea.[94] 5-HT_3 receptor antagonists, such as alosetron and ramosetron, reduce symptoms in IBS-D, retard GI transit, increase fluid absorption and reduce pain. [95] [96] The 5-HTTPLR S/L polymorphism in *SLC6A4* has been shown to correlate with clinical response to alosteron in IBS-D, thereby altering the

risk:benefit ratio with this class of compounds.[97] To what extent *HTR3* SNPs may influence 'setron' response is presently unknown. Treatment by tegaserod, a 5-HT_4 receptor agonist, used for the treatment of IBS-C was also shown to correlate with this *SLC6A4* polymorphism. More specifically, the L/L genotype increases the sensitivity of constipation and causes a lower response to treatment with tegaserod.[98]

Adrenergic and opioidergic drugs

The clinical response to clonidine, an α2-adrenergic agonist, is modulated by gene variations in α2-adrenergic receptor gene *ADRA2A*, such as rs1800544, a SNP reportedly associated with gastric accommodation and rectal sensations of gas bloating and defaecatory urgency.[99] The cannabinoid receptor agonist dronabinol decreases fasting colonic phasic motility and increases colonic compliance, especially in IBS-D and IBS-M patients. However, the effectiveness of dronabinol is correlated with rs806378 in cannabinoid receptor 1 (*CNR1*). [100] [101] This SNP is also associated with colonic transit in IBS-D and sensation rating of gas. [102] Additionally, a variant in the fatty acid amide hydrolase gene *FAAH*, rs324420, and treatment with dronabinol leads to acceleration of proximal colonic motility in patients with IBS-D or IBS-M[103].

Bile acids

Genetic variants in the klotho beta gene *KLB* and the fibroblast growth factor receptor 4 gene, *FGFR4* are associated with accelerated colonic transit in patients with IBS-D. These variants also correlate with the colonic transit response to chenodeoxycholic acid in IBS-C,[104] and to colesevelam in IBS-D patients.[105]

Conclusions and future perspective

The delineation of the genetic factors that are involved in the aetiopathophysiology of IBS remains challenging due to the marked heterogeneity of patients' populations assigned to the umbrella diagnosis of IBS. Recent genetics findings support current models of IBS pathogenesis such as altered neuronal signal transduction, impaired immune response and intestinal barrier dysfunction. However, the majority of studies lack the necessary power to apply different predisposing risk models, thereby leading to conflicting or potentially false positive results. In addition, inconsistency of association findings might have been blurred by the different expertise and focus of the recruitment centres (e.g. psychosomatics, hypnotherapy, inflammatory bowel disorders) thereby introducing a centre-specific bias. Furthermore, patients have been recruited applying non-uniform symptom classifications (Rome I/II/III) and comorbidity is rarely adequately assessed. Moreover, control individuals often have not been excluded for IBS and/or comorbid conditions, therefore representing an additional source of bias. This 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 warranted.

Moreover, the currently used symptom-based classification does not guarantee that the groups of patients studied were indeed all afflicted with the same disease and aetiopathogenetic mechanism. In fact, several peripheral mechanisms that contribute to the development of IBS with constipation phenotype may also be involved in other diseases, such as, slow transit constipation, intestinal pseudo-obstruction, and idiopathic megacolon. A diarrhea phenotype may share susceptibility genes to food intolerance, disaccharidase deficiency, bile acid malabsorption, small intestinal bacte-

rial overgrowth, gluten sensitivity, increased mucosal permeability, immune activation, or accelerated colonic transit due to a motility disorder.

It must be stressed that genetic association frequently does not imply phenotype causation and the SNPs associated with IBS may simply implicate a region in the genome where relevant disease causing variants might reside.

Epigenetic findings support the postulation regarding the role 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 impact structure and function of GI tissue as well as the brain-gut crosstalk and stress axis leading to the distinct clinical phenotypes remains unclear. Replication studies in adequately powered independent cohorts of well characterized IBS patients enabling correlation to sub-phenotypes including comorbid conditions are clearly warranted. These are of tremendous importance as these may pave the way for pharmacogenetics approaches in the future.

Based on 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. It is on this premise that a number of multidisciplinary experts from across Europe joined forces to overcome this issue by developing detailed phenotyping strategies in order to allow stratification of genetic data in sub-phenotypes of IBS patients, thereby increasing power and effect size of the associated variants. Furthermore, additional parameters, the collaborative has developed protocols for the measurement of intermediate phenotypes and quantitative traits which are mandatory to dissect epi-/genetic patterns underlying IBS

and subsequently correlate these with symptom complexes. Obviously, this can only be achieved by detailed meticulous phenotypic characterisation of patients based on clinical examination, specific questionnaires that assess GI symptoms and psychiatric comorbidity, personality traits, somatisation status, assessment of physiological parameters and tissue sampling for follow-up of expression changes in candidate genes. In addition, to avoid bias introduced by IBS cases within control populations, such controls should also be evaluated using the same tools as the cases. In conjunction to such detailed phenotyping, family history of FGID and crucial known environmental predisposing factors (stress, infection, nutrition) should be assessed and thereby the identification of distinct patient subgroups will facilitate the stratification of epi-/genetics data in combination with environmental factors. Therefore, the major goal of the COST (COoperation in Science and Technology) Action GENIEUR BM1106 (GENes in Irritable Bowel Syndrome Research Network EURope, www.GENIEUR.eu) is to establish harmonized criteria and a standard protocol in order to recruit a large set of well characterized patients and controls. A further goal of GENIEUR is the establishment and development of an infrastructure that will facilitate future multicenter studies in which not only blood samples for epi-/genetics and stool samples for microbiota studies will be archived but also gut tissue samples from various regions for follow up of epi-/genetics findings.

We also propose to investigate in depth the contribution of copy number variations and rare variants assessed by next generation sequencing applied to the entire exome or genome to the pathogenesis of IBS. The emerging technologies for massive parallel sequencing, combined with reductions in costs coupled with the development of advanced analytical tools, leave little doubt that these new technologies will complement and enhance current efforts focused on improving the field's understanding of

role of genetics of IBS, in a manner similar to what has been achieved in complex GI disorders such as Crohn's disease[106, 107] .

In these studies not only well characterized cohorts but also family and twin studies should be taken into account. Thus the utilisation of hypothesis free approaches may lead to the identification of novel, and as yet un-anticipated, pathophysiological mechanisms across the disorder.

In particular, further exploration of family clustering of FGID might provide useful insights into shared pathogenic pathways and shared environment. The power of this approach was shown in a recent study, where a SNP in *GUCY2C* (guanylate cyclase C, GC-C) which regulates secretion in enterocytes, co-segregated with diarrhea in all affected family members, some of whom were diagnosed with IBS, further underscoring the potential relevance of this signaling pathway.[108]

In addition, functional validation of the effect of any detected genetic variants is essential in order to provide 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 as well as the follow up of expression changes of regulatory variants in relevant tissues.

The outcome of integrative genetics and epigenetics studies may help to define novel IBS subgroups which will facilitate the development of enhanced diagnostics and novel treatment options, specifically targeting towards the affected pathways. The identified genetic variants and epigenetic markers as well as miRNAs may serve as biomarkers for interventional studies and pharmacotherapy in order to individualize therapy in the future and ultimately improve patient outcomes.

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Figure 1. Multiple layers of complexity on genetic, epigenetic and environmental level contribute to the pathogenesis of IBS and comorbid conditions. A diverse array of environmental factors interact with underlying genetic variants in IBS – these predisposing genes lead to an alteration in structure and/or function which itself is represented within myriad of (neurobiological) intermediate phenotypes (central and enteric nervous systems function) and influencing the brain-gut axis. Consequently, it may manifest as central behavioural and GI intermediate phenotypes and thereby predispose to the IBS as well as comorbid conditions.

Figure 2. IBS related pathways based on genetic and epigenetic findings including potential pharmacogenetic targets.

Table 1. Summary genetic association data in IBS.

Table 2. Summary epigenetic data in IBS.

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Bullet 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 are still scarce and/or have not been reproduced.
- Epigenetic and pharmacogenetic approaches are in their infancy.
- A major pitfall is the lack of large homogenized case-control cohorts recruited according to standardized and harmonized criteria.

Table 1

Gene	SNP database number (RS)	*HGVS* nomenclature	Functional variant	3 IBS	IBS-subtype	Association with comorbid disorder or other quantitative traits	Case Group (N)	Control Group (N)	Country of origin	Reference
SNPs in genes in serotonergic pathways										
SLC6A4	rs4795541*	g.28564359_28564360insG	c.-1950_-1949insC	No association			1034	1377	USA, UK, Turkey, China, Korea	31
					LS genotype with IBS-D		54	91	Turkey	32
					SS genotype with IBS-C and LS genotype with IBS-D					
					SS genotype with IBS-D		194	448	USA	33
					SS genotype with IBS-D		190	437	Korea	34
					S allele with M-IBS, but not IBS overall		50	53	USA	35
					SS genotype with IBS-C		151	100	India	36
					SS genotype with IBS-D	Higher 5-HT level in rectal biopsies	150	252	India	37
				No association			33	56	Korea	38
				No association			256	120	USA	39
					LL genotype with IBS-C		81	48	China	40
					LL genotype with IBS-C		87	96	China	41
				LL genotype with the total IBS, IBS-C and IBS-M			99	171	Korea	42
						SS genotype with depression in IBS; LL genotype with decr. in social functioning in IBS	133	-	USA	43
				SS genotype with absence of IBS symptoms		LS/SS with the higher pain rating	122	39	USA	44
				Low frequency of SS genotype in males with IBS			196	92	UK, Germany	45
				NA		S allele with personality disorders in females	NA	374	USA	46

				NA		S allele with higher neuroticism/anxiety scores, greater sympathetic tone, higher cortisol level	NA	120	UK	47
			intron 2 (STin2 VNTR)			Short VNTR with depression in IBS	21 men and 117 women	-	USA	43
				12/10 genotype with IBS			81	48	China	40
	rs25531	g.28564346T>C	c.-1936A>G	G allele with IBS			186	50	USA	48
HTR2A	rs6313	g.47469940G>A	c.102C>T	C allele with a high risk of IBS		TT genotype with more severe pain in IBS	54	107	Turkey	49
	rs6311	g.46897343C>T	c.-1438G>A	A allele with a high risk of IBS		Low frequency of A allele with depressive disorders in IBS	95	-	Poland	50
HTR2C	rs6318	g.114731326C>G	c.68C>G			Low frequency of G allele and GG genotype with anxiety disorders in IBS	95	-	Poland	50
HTR3A	rs1062613	g.113975284T>C	c.-42T>C		With IBS-D		200	100	UK, Germany	51
				C/C genotype with greater IBS symptom scale		C/C genotype with amygdale responsiveness and anxiety ratings	26	29	USA	52
HTR3B	rs1176744	g.113932306A>C	c.386A>C	With IBS		particularly anxiety score and alexithymia	119	229	Japan	20
HTR3C	rs6766410	g.184056974C>A	c.489C>A		With IBS-D		200	100	UK, Germany	51
HTR3E	rs62625044	g.184106769G>A	c.*76G>A		With IBS-D in females		319	295	UK, Germany	53
					T allele with IBS-D and GA genotype with female IBS-D		300	450	China	54
TPH1	rs4537731	g.18047335T>C	c.-6526A>G		With diarrhea in IBS		199	79	USA	55
						With IBS-related cognition and diseases perception	199		USA	56
	rs211105	g.18033757T>G	c.332-383A>C							
	rs684302	g.18038806C>T	c.117+1840G>A			With quality of life in IBS				
	rs1800532	g.18026269G>T (c.606+221C>A)	c.218A>C							
SNPs in genes related to neuronal function										
COMT	rs4680	g.19951271G>A	c.472G>A		158Met allele with IBS-C		645	323	USA	15
				Val/Val genotype with	Val/Val genotype with		70	867	Sweden	57

				IBS overall	increased stool frequency					
BDNF	rs6265	g.27658369C>T	c.196G>A			Met with 'psychiatric' symptoms ratings	645	323	USA	15
OPRM1	rs1799971	g.154360797A>G	c.118A>G	G allele with IBS-M	G allele IBS-D females		645	323	USA	15
CNR1	3'-UTR CNR1		(AAT)n	>10/>10 genotype with IBS			292	298	China	58
							162	423	USA	59
					With IBS-D	Gas (not pain) sensation rating	74	24	USA	60
	rs806378	g.88859551C>T	c.-206-2026G>A			TT genotype with faster colonic transit (at 24 & 48 h) and gas (not pain) sensation rating in IBS-D	74	24	USA	60
					With IBS-D		445	228	USA	60
FAAH	rs324420	g.46870761C>A	c.385C>A	A allele with IBS-M	A allele with IBS-D	A allele with faster colonic transit with IBS-D	270	252	USA	60
SCN5A			p.A997T- Other missense mutations: p.I94V,p.T220I, p.G615E, p.T630M, p.P648L,p.G1158S p.E1780G, p.A1870D	10% of IBS-D are mutation carriers	30% of IBS-C are mutation carriers (loss of function haplotype)		584	1380	USA, Sweden, Italy Greece	61
	rs137854608	g.38651267C>T	c.892G>A	With IBS			49	1500	USA	14
ADRA2A	rs1800544	g.112836503G>C	c.-1252G>C		With IBS-C		256	120	USA	62
					CG/GG genotype with IBS-D		151	100	India	36
				G allele with the total IBS	G allele with the IBS-D		99	171	Korea	42
ADRA2C	rs6846820	g.3766265T>C	c.964–975:Del		With IBS-C		256	120	USA	62
NXPH1	rs2349775	g.8718080G>A	c.55-72558G>A		With IBS-D		935	639	USA, UK, Sweden	63
SNPs in genes related to immune function										
IL10	rs1800896	g.206946897T>C	c.-1117A>G	Low producer genotype with IBS			230	450	UK	64
				Low producer genotype with IBS			111	162	Netherlands	65
					Low producer genotype		45	92	Mexico	66

					with IBS-D					
				GA genotype with IBS			71	140	Iran	67
				A allele and AA genotype with IBS			45	137	Mexico	68, 69
				AA+GA vs GG genotype with a lower IBS risk in Caucasian ethnicity			928	1363	China, India, Iran, Korea, Mexico, Netherlands, UK	70
	rs1800872	g.206946407T>G	c.-627A>C	CC vs GG genotype with a higher risk of IBS in Asian ethnicity			928	1363	China, India, Iran, Korea, Mexico, Netherlands, UK	70
				A allele with IBS			23	20	Mexico	71
	rs1800871	g.206946634A>G	c.-854T>C		CC genotype with IBS-D	-	50	50	Japan	72
TNFSF15	rs4263839	g.117566440A>G	c.210+1643T>C	With IBS	With IBS-C		861	1131	USA, Sweden	73
				With IBS	With IBS-C		2894	3138	USA, UK, Iran, Belgium, India, Netherlands, Mexico, Sweden	74
	rs6478108	g.117558703C>T	c.211-2855G>A		With IBS-A		935	639	USA, UK, Sweden	63
						IBS-D lower MAF frequency	37 IBS-D and 19 IBS -C	25	UK	75
	rs6478109	g.117568766A>G	c.-474T>C							
	rs7848647	g.117569046T>C	c.-754A>G							
	rs4263839	g.117566440A>G,	c.210+1643T>C		With IBS-A		935	639	USA, UK, Sweden	63
IL8	rs2227306	g.74607055C>T	c.65-204C>T		With IBS		45	137	Mexico	68, 69
	rs2227307	g.74606669T>G	c.64+230T>G		With IBS					
IL6	rs1800795	g.22766645C>G	c.-237C>G		C allele with PI-IBS		228	581	Canada	76
				G allele with IBS			71	140	Iran	67
				With IBS			23	20	Mexico	71
IL4	rs2070874	g.132674018C>T	c.-33C>T	CC genotype with IBS			71	140	Iran	67
	rs2243250	g.132673462C>T	c.-589C>T	TT genotype with IBS						
TNFα	rs1800629	g.31543031G>A	c.-308G>A	GA genotype with IBS			111	162	Netherlands	65
	rs361525	g.31543101G>A	c.-238G>A	G allele and GG genotype with IBS			71	140	Iran	67
IL1R	rs2234650	g.102758327C>T	c.-83-12074C>T	C allele with IBS			71	140	Iran	67
TLR9+	rs5743836	g.52260782A>G	c.-1237T>C	T allele with PI-IBS			228	581	Canada	76
	rs352139	g.52258372T>C	c.2848G>A	A allele with PI-IBS						

SNPs in genes involved in barrier integrity										
CDH1	rs16260	g.68737131C>A	c.-160C>A		A allele with PI-IBS		228	581	Canada	76
CDC42	rs17837965	g.22394625A>G	c.-176-5962A>G		With IBS-C		935	639	USA, UK, Sweden	63
SNPs in genes involved in bile acid synthesis, transport and excretion										
KLB	rs17618244	g.39448529G>A	p.R728Q		G allele (Arg728) with IBS-D	Accelerated transit in IBS-D	435	279	USA	77
						G allele with increased bile acids synthesis and excretion	94	30	USA	78
FGFR4	rs351855	g.177093242G>A	c.1097+65G>A,			Colonic tranist in IBS-D	435	279	USA	77
	rs1966265	g.177089630G>A	c.28G>A							

*This SNP represents a polymorphic region consisting of an indel, i.e. either an insertion or a deletion, of 43 or 44 nucleotides, respectively. This SNP is commonly known as the 5-HTTLPR variant of the serotonin transporter *SLC6A4* gene. The deletion allele is referred to as the S allele, the insertion allele is termed as the L allele, and hence the genotypes are usually called the LL, SL, and SS genotypes.

Table 2

miRNA	Tissue	Target gene	Patients/Controls	Disorder	Quantitative Trait	Reference
hsa-miR29a↑	duodenum/colon	*GLUL* ↓	19 IBS-D/10	IBS	Permeability ↑	111
hsa-miR 29b↑	duodenum/colon	*CLDN1* ↓	183/36	IBS-D	Permeability ↑	112
hsa-miR 29b↑	duodenum/colon	*NKRF* ↓	183/36	IBS-D	Permeability ↑	112
hsa-miR-150 ↑	blood	*AKT2**	12/31	IBS	n/a	113
hsa-miR-342-3p ↑	blood	unknown	12/31	IBS	n/a	113
hsa-miR-199a↓	duodenum/colon	*TRPV1* ↑	45/40	IBS-D	Pain perception ↑	114

AKT2 protein kinase , *CLDN1* claudin1, *GLUL* glutamine synthetase, IBS-D diarrhea-predominant irritable bowel syndrome, n/a not applicable, *NKRF* NF-kappa-B-repressing factor, *TRPV1* transient receptor potential vanilloid type 1, * *in silico* prediction - not validated, ↓ down regulated, ↑ up regulated

Central neurobiological intermediate phenotype

Behavioural intermediate phenotype

Clinical phenotype

Genes

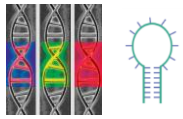
Psychological stress

Sex

Smoking

Epigenetics

Diet



Brain-gut axis

Emotional regulation

Chronic fatigue, depression, anxiety

Visceral sensation/ Pain modulation

Migraine, fibromylagia

Gut microbiota

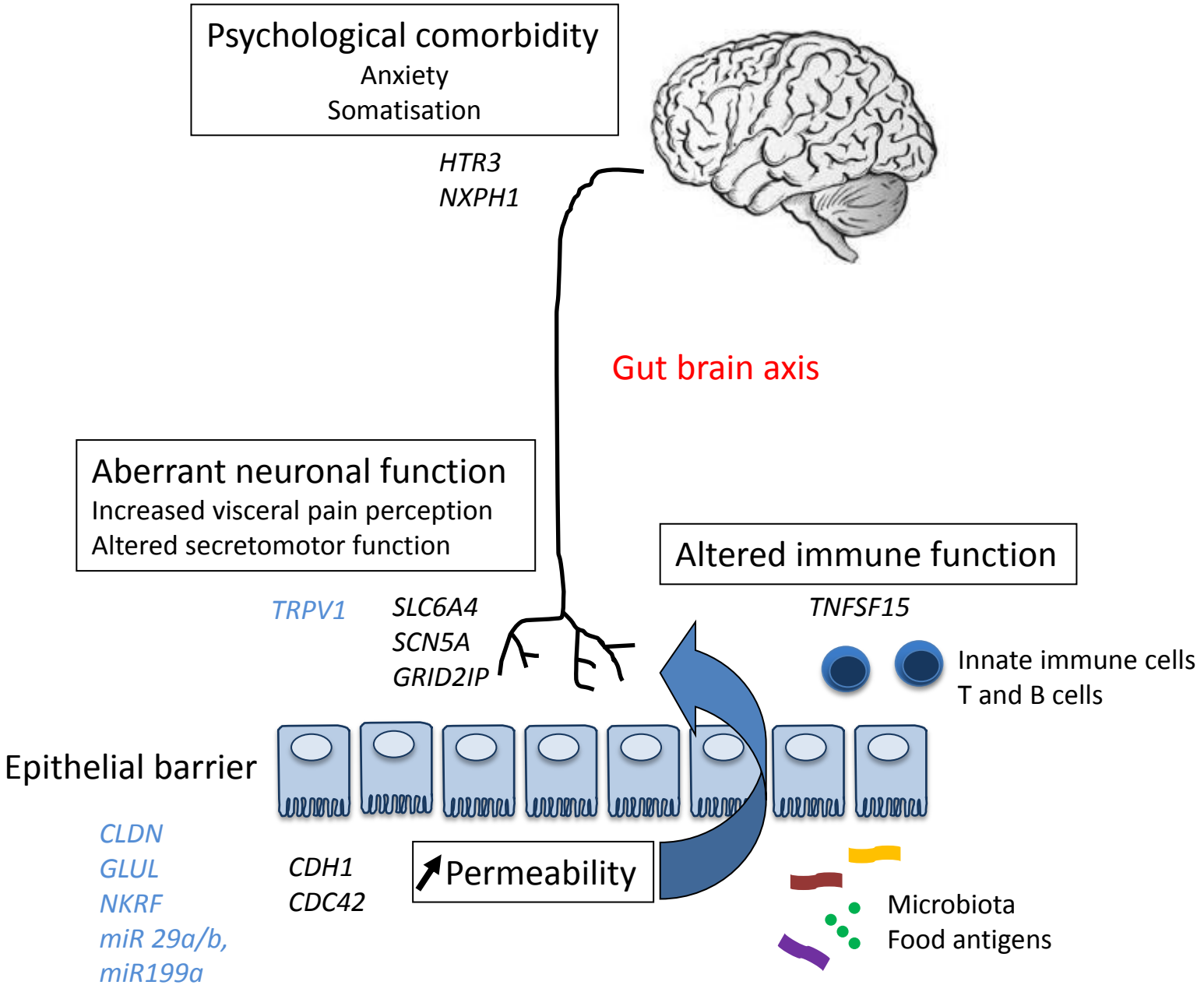
Infection/ dysbiosis

GI transit secretion

Chronic abdominal **pain/discomfort** and altered bowel habits: IBS

Peripheral intermediate phenotypes (ENS)

Peripheral intermediate phenotypes (GI function)


Psychological comorbidity
Anxiety
Somatisation
HTR3
NXPH1
Gut brain axis
Aberrant neuronal function
Increased visceral pain perception
Altered secretomotor function
TRPV1
SLC6A4
SCN5A
GRID2IP
Altered immune function
TNFSF15
Innate immune cells
T and B cells
Epithelial barrier
CLDN
GLUL
NKRF
miR 29a/b,
miR199a
CDH1
CDC42
Permeability
Microbiota
Food antigens