

Gene-environment interactions in chronic inflammatory disease

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Chronic inflammatory diseases represent a major challenge for both clinical research and patient care, and evidence indicates that these disorders develop as a result of complex gene-environment interactions. Better understanding of their cause-and-effect relationship is the basis for emerging proposals for therapy and prevention.

Since the end of World War II there has been a substantial increase in the incidence of chronic inflammatory disease that seems to follow a geographical pattern of industrialization and urban living¹. This includes allergic conditions (asthma, allergic rhinoconjunctivitis, food allergies and eczema), as well as autoimmune disorders such as type 1 diabetes, chronic inflammatory bowel disease (IBD) and neurodegenerative disease. Genetics cannot account for the increase in prevalence over time (Fig. 1a), but epidemiological evidence supports the idea of a combination of environmental and genetic risk factors. The nature of these risk factors and the pathogenic mechanisms involved, however, are only just beginning to be understood (Fig. 1b).

The European Science Foundation brought together an international group of experts on subjects ranging from epidemiology to epigenetics for a 5-day meeting (19–22 October 2010) in Barcelona, Spain², intended to help define strategic directions for research into this increasing public health concern. In this first meeting of the Forward Look initiative of the European Science Foundation, discussions focused on the grand challenges that face research into gene-environment interactions in metabolic and immune system-mediated pathologies with inflammatory processes as the key driver of disease initiation and/or perpetuation. Surprisingly, diseases as diverse at the phenotype level as obesity and asthma share similarities at the molecular and cellular levels. Such striking findings shed new light on this area and triggered the organization of an interdisciplinary European Science Foundation initiative. To structure this process, the Barcelona meeting covered intrinsic and extrinsic mechanisms of various chronic diseases together with emerging ideas in disease therapy and prevention.

Intrinsic mechanisms

The increasing incidence of chronic inflammatory disease such as diabetes, asthma, allergy and IBD is widely considered to be due to a combination of environmental and individual risk factors. Scientific findings have highlighted similarities among these conditions at both the genetic level and mechanistic level.

The advent of genome-wide association studies raised the hope that disease risk could be stratified according to individual susceptibility alleles. Such efforts have resulted in the identification of several disease-associated

polymorphisms, which has shown that there are overlapping susceptibility genes in these inflammatory disorders³. A prototypic example is the discovery of polymorphisms in *ORMDL3* in both asthma and IBD^{4,5}.

Despite the identification of many potential susceptibility genes^{6,7}, their individual effects seem to be quite modest. Thus, for many diseases, the alleles that have been identified by genome-wide association studies explain only a very small proportion of disease heritability for both IBD and asthma. Similar observations have been made for type 1 diabetes, for which the most common polymorphisms explain less than 30% of heritable risk^{8,9}, and even in identical twins, a concordance of only about 50% has been observed. The situation in type 2 diabetes is even more striking¹⁰. Having a first-degree relative with type 2 diabetes is associated with a hazard ratio of between 3 and 4 for development of the disease relative to that of people who do not have affected relatives. Despite such distinct heritability, the susceptibility genes identified by genome-wide association studies account for only approximately 1% of the heredity of type 2 diabetes, making them essentially useless for risk prediction^{11,12}. Similar findings have been also obtained for other chronic inflammatory disease, including asthma and allergic eczema, which indicates the multigenicity and complexity of these disorders. Thus, simple genetic explanations for individual susceptibility to chronic inflammatory disease have not been forthcoming.

Such findings highlight a key challenge in defining the genetic factors that determine disease susceptibility in chronic inflammatory disease; that is, the failure of single alleles or even

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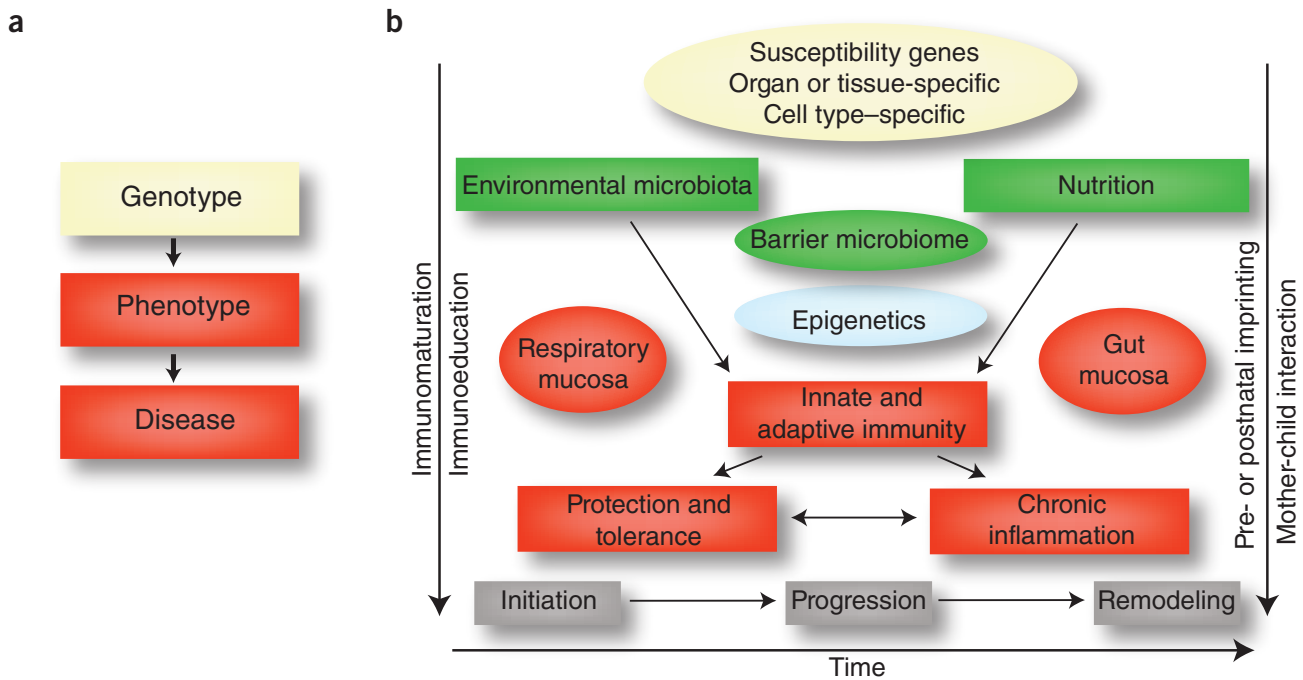


Figure 1 A new paradigm for the development of chronic inflammation. The traditional simplistic paradigm of chronic inflammatory disease development (a) has been revised to a more complex understanding of the cornerstones of these conditions (b). In b, epigenetics (blue) represents a key mechanism linking genetic components (yellow) and the environment. Disease development depends on intimate interaction between intrinsic mechanisms (red) and extrinsic mechanisms (green) in a temporal and spatial way.

allele combinations so far to facilitate distinct risk prediction. It is important to note, however, that the lack of distinct associations with common single-nucleotide polymorphisms does not mean that these genetic differences do not underlie susceptibility. Individual single-nucleotide polymorphisms with small effects may together have metabolic consequences that can be used as biomarkers for risk stratification. Sequencing technology has advanced to such an extent that it can now be considered trivial to analyze 1,500 single-nucleotide polymorphisms at a time. The real challenge is the ability to discern relevant patterns from the data obtained. Furthermore, genetic variation is not only about single-nucleotide polymorphisms. Other factors such as repetitive DNA elements or copy-number variants could also be involved, and these may be much more difficult to address.

As risk alleles are very frequent in the healthy population, epidemiological evidence supports the idea that environmental triggers are involved in the initiation and/or perpetuation of chronic diseases¹³. Epigenetic variables have been recognized as a possible missing piece in the puzzle linking the human genome, the environment and phenotype development (Fig. 1b). Comparison of methylation patterns in people exposed to severe prenatal famine and their unexposed siblings, for example, has shown that transient prenatal exposure to a specific

environmental stimulus leads to persistent and heritable epigenetic changes¹⁴.

A crucial element of epigenetic studies will be the generation of tissue- and cell type-specific data. One example in which evidence has been obtained for the epigenetic regulation of specific cell types is in the differentiation of helper T cells from naive CD4⁺ lymphocytes^{15,16}. These types of cells of the immune response are linked to autoimmunity and allergy, and they may be central to the development of disease chronicity through the generation of a long-lasting cellular memory of earlier environmental exposures. The extent of methylation of the promoter of the interferon- γ gene has been identified as a marker of commitment to the T helper type 1 (T_H1) lineage¹⁷. Thus, in a specific type of cell of the immune response, epigenetic changes can determine important cell fate 'decisions' that are likely to influence disease¹⁸. Given those findings, epigenetic signatures might serve as biomarkers for specific prenatal environments, whereas genome-wide association studies may identify biologically important changes¹⁹.

To achieve the goals stated above, however, future studies will need to obtain a reliable catalog of prenatally induced epigenetic signatures associated with adverse events or environmental exposures. Determining how to sample the environmental effect is perhaps the single greatest challenge for research into gene-environment interactions. It will also be important

to obtain robust evidence that these signatures have a causal relationship with the pathogenesis of the disease. As in genomic analyses, obtaining an overview of epigenetic associations will require more detailed information on disease phenotype, including temporal changes from initiation through to chronicity.

A major focus for research into the cellular mechanisms that underlie chronic inflammatory disease is the tissue-environment interface²⁰. It is here, particularly in the mucosa lining the gut and airways, that a careful balance must be maintained between host defense and uncontrolled unresolving inflammation. One area in which substantial advances have been made is in understanding the role of endoplasmic reticulum stress, autophagy and microbial sensing in the pathogenesis of IBD²¹. The unfolded protein response is a pathway from the endoplasmic reticulum to the nucleus that protects cells from stress caused by unfolded or misfolded proteins. Interference with this pathway in mice—for example, by knocking out the gene encoding the transcription factor Xbp1—results in an IBD-like phenotype²². Closer analysis has shown that the gut epithelium lacks Paneth cells and, to a lesser extent, goblet cells. These are the most secretory cell types and therefore are the most susceptible to endoplasmic reticulum stress. One of the biological functions of the Paneth cells is to secrete antimicrobial peptides. Thus, when endoplasmic reticulum stress can-

not be controlled in Paneth cells, they are unable to respond appropriately to their environment or fulfill their role in managing the microbial milieu. Activation of *Xbp1* mediated by Toll-like receptors 2 and 4 links signaling by endoplasmic reticulum stress to microbial sensing, which supports the idea that cellular stress mechanisms are involved in intestinal inflammation^{23,24}. Many genetic risk factors for IBD converge on the Paneth cell. Interestingly, one such risk allele is *Atg16l1*, which encodes a molecule involved in autophagy and is linked to Crohn's disease; an environmental insult is required for the disease phenotype to be expressed²⁵. When susceptible cells are infected with norovirus, they become abnormal and hyperinflammatory, but this is not converted into chronic inflammation. Thus, at least two gene-environment interactions seem to be necessary to initiate disease that then progresses to a chronic inflammatory phenotype. In addition, nutritional factors such as luminal iron have been identified as risk factors in experimental ileitis, in which the targets are inherent endoplasmic reticulum stress mechanisms in the epithelium and also the microbial milieu²⁶. Luminal iron may directly trigger stress-associated apoptosis of epithelial cells through changes in microbial homeostasis. This links endoplasmic reticulum stress and microbial composition to the development of inflammation.

The transcription factor NF- κ B is linked to a wide range of disease processes²⁷. Tissue-specific activation of NF- κ B in the gut epithelium of mice leads to spontaneous colitis²⁸. However, this genetic effect depends on the gut microbiota, as mice reared under germ-free conditions remain healthy. Thus, expression of the disease phenotype is determined by gene-environment interactions, most probably involving a response of the epithelium to the microbial milieu in the gut.

Unequivocal experimental and clinical evidence have linked inflammation, or the inflammatory signaling response in various tissues, to the development of obesity-related alterations such as insulin resistance, glucose intolerance and endothelial dysfunction²⁹. Some fatty acids activate inflammatory pathways directly via pattern-recognition receptors of the innate immune system and, consistent with that, mice deficient in Toll-like receptor 4 are substantially protected from the ability of systemic infusion of lipid to suppress insulin signaling³⁰. Mechanisms related to endoplasmic reticulum stress have emerged as a mechanistic link between inflammation and the development of insulin resistance and glucose intolerance, providing evidence that common cellular mechanisms are shared by metabolic and immune-mediated diseases³¹.

Extrinsic mechanisms

The prevalence of inflammatory disorders such as IBD, type 1 diabetes and asthma has risen sharply, and the spread of these diseases seems to follow lifestyle factors such as nutrition, hygiene and exposure to environmental microbial components (Figs. 1b and 2). Exogenous variables have a definite effect on the intrinsic mechanisms of chronic inflammatory disorders³². For instance, in the nonobese diabetic (NOD) mouse model of type 1 diabetes, the cumulative incidence of diabetes is higher in female mice than in male mice when they are reared under specific pathogen-free conditions³³. When NOD mice are reared in fully germ-free conditions, however, this sex bias disappears. Furthermore, early exposure to a single commensal bacterial species in NOD mice reared under germ-free conditions confers protection against type 1 diabetes. Thus, differences in exposure to microbiota modify interactions with intrinsic factors such as sex to determine the expression of a pathologic phenotype. Similar evidence has emerged from IBD-related animal models indicating that disease initiation depends on the presence of commensals³⁴. Interestingly, however, the influence of the gut microbiota on the development of disease is not unidirectional; mutualistic crosstalk has been established between the immune system and the microbiome in the maintenance of intestinal homeostasis³⁵. Evidence suggests that the microbiome of NOD mice differs from that found in diabetes-resistant NOR mice³⁶. Exactly how these genotype differences influence the microbial environment remains to be determined, but similar findings have been reported for the development of metabolic syndrome in mice lacking Toll-like receptor 5 (ref. 37). A new classification of 'commensal' and 'facultative pathogenic' microbes of the gut microbiota needs to be invented, as it seems to be variable in the context of genetic host factors.

Sequencing technology has been instrumental in opening up a whole new avenue of research into gene-environment interactions; specifically, the role of the microbiota living on various body surfaces in promoting and protecting against chronic diseases. The reference gene sets for the human gut microbiome that have been generated by collaborative networks such as the MetaHIT Consortium are already being used to identify associations between individual bacterial species and diseases, including IBD and obesity³⁸. Looking to the future, however, it is clear that many challenges must be overcome. The identification of organ-specific core microbiomes (for example, the respiratory and gastrointestinal tracts) and the possibility of identifying disease-relevant alterations seem essential for

addressing therapeutic options in terms of the inherent microbial ecosystem. Additional key issues include the ability to determine functionality when most mucosal commensals cannot be cultured and to mechanistically delineate causality in animal models. Defined microbial ecosystems in disease-relevant animal model systems, including gnotobiology (the study of germ-free or specifically inoculated organisms), will be essential in future efforts to understand microbe-host interactions on normal and susceptible genetic backgrounds. Furthermore, the necessary bioinformatic analyses for such studies will be at least as complex as they are for genome-wide data.

Despite the common perception that the respiratory tract is largely a sterile body compartment, microbial sampling of the airways, including the lungs, has shown that the number of bacteria is similar to that in the upper intestine. The most prevalent species differ according to the anatomical level of the airway (for example, oropharynx and upper lobes of the lungs). Comparison of the microbiota in the same region of the airway of healthy people and patients with respiratory inflammatory disorders such as asthma or chronic obstructive pulmonary disease has also shown distinct differences for both adults and children. For example, patients with respiratory disease have more known respiratory pathogens than do healthy people. In contrast, anaerobic species seem to be much more common in healthy airways (W.O.C., unpublished data). Interestingly, host-associated microbial communities such as those of the human gut, skin and vagina show very strong phylogenetic clustering, much stronger than that of samples from different terrestrial or marine environments³⁹. Thus, each individual area of the body seems to represent a discrete ecological niche that determines which bacteria are selected from an apparently more phylogenetically homogeneous environment. Obtaining a detailed spatial description of the human microbiome represents an important challenge for future research.

Substantial advances have nevertheless been made in understanding the influence of microbial exposure in early life on the subsequent risk of chronic disease. Children born in families living on farms with animals have a much lower risk of allergy and asthma, and this effect seems to be due to the diversity of environmental microbes and fodder components to which they are exposed⁴⁰. Interestingly, protection seems to begin prenatally, with maternal exposure to multiple species of farm animal seeming to have a cumulative effect. Studies of a birth cohort (the EFRAIM cohort) to determine the effect of prenatal microbial exposure are now underway.

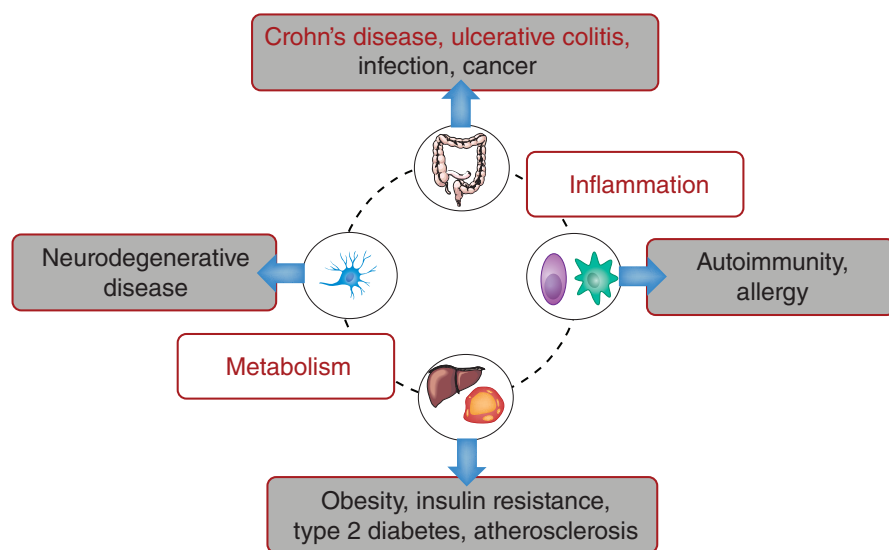


Figure 2 Metabolic and immune-mediated pathologies with inflammatory processes in various target organs as the key driver of disease initiation and/or perpetuation. Extrinsic and intrinsic events (red) lead to the manifestation of disease (black).

Assessment of environmental exposure is limited by the extent to which the effect can be measured. There is a considerable lack of validated methods for more detailed determination of the variables. Sequencing of the human gut microbiome has raised hopes of microbiome-wide association studies, but no such approach can be taken relative to the environment as a whole. Even in a field as apparently straightforward as nutrition, experience has shown that accurate, reliable information about individual food consumption is hard to obtain. The challenge, then, must be to develop paradigms for accurate sampling of the environment. Efforts must be made to determine which environmental factors to sample, as well as when and how to do so.

By combining genetic and epigenetic data with careful collection of environmental and biological samples, well-designed cohort studies are expected to provide important clues about the relationship between intrinsic and extrinsic mechanisms of disease. It may be time, however, to consider extending such approaches to include an embedded mechanistic component. As samples and data are generated, close collaboration with experimental research groups could allow hypotheses to be tested at an earlier stage while the open-minded approach characteristic of epidemiological studies is retained.

Therapy and prevention

The emerging knowledge about the intrinsic and extrinsic mechanisms that underlie chronic inflammatory disease should be translated to the benefit of human and animal health. Although

this represents a great challenge, insight into the response of the host to the environmental effect that determines the transition to disease chronicity will help to identify new therapeutic targets. Furthermore, identification of the exogenous variables that promote or protect against chronic inflammatory disease later in life could raise realistic hopes of prevention.

A knowledge gap between research and clinical development has been identified in several disease areas. In Crohn's disease, for example, many drug developments have targeted T_H1 -mediated immunity, which was thought to have a central role in pathogenesis. However, clarification of the role of the Nod2 pathogen receptor in the gut has emphasized the importance of defects in innate recognition and handling of bacterial components and how this can affect Crohn's disease. In addition, the T_H17 subset of helper T cells has been identified as an important mediator of inflammation in Crohn's disease. The development of T_H17 cells is also linked to both genetic variants and bacteria-derived triggers⁴¹. This situation demonstrates the need to streamline the translation of research for clinical development⁴².

The identification and development of targets leads to the important question of whether the present knowledge of disease phenotypes and individual susceptibility supports the search for a single effective pharmacological target⁴³. One such example is the development of new drugs for the treatment of asthma. Interleukin 13 (IL-13) was identified as a key therapeutic target in asthma on the basis of its ability to influence T_H2 differentiation and airway inflammation and hyper-responsiveness.

The importance of IL-13 was demonstrated by several approaches with allergen-induced T_H2 -driven models of airway inflammation and remodeling. However, the results of human clinical trials have been disappointing. Further subset analysis has identified several major sub-phenotypes of asthma, including ' T_H2 -high' responders, with upregulation of IL-13 targets, more bronchial hyper-responsiveness and a good response to corticosteroid treatment, and, finally, ' T_H2 -low' people. These data indicate that cellular and molecular characterization and definition of phenotypes are necessary for the identification of target-responsive patient subsets⁴⁴.

In the future, therefore, much more information will be needed about the range of phenotypes in disease classification. This will be achieved by 'deep phenotyping' of well-defined patient groups. In parallel, experimental models used to identify putative cellular and molecular signaling cascades amenable to therapeutic intervention must adequately reflect the disease phenotype. Thus, both patient-selection criteria and choice of animal models will depend on improved classification of chronic inflammatory diseases⁴⁵.

Probiotics are viable bacterial strains with reputed health-promoting activity; these have received great attention as a possible means of preventing chronic inflammatory disease. In contrast to the substantial data supporting the efficacy of probiotics in the treatment of certain intestinal infections, there is inconsistent support for the efficacy of probiotics in preventing chronic inflammatory disease such as allergies^{46,47}. As with clinical trials of new asthma therapies, the challenge may be to define the correct biological variables. Furthermore, it is now well appreciated that all probiotic strains even of a given species are unique, whereas most of the results so far have been obtained with distinct strains. At present, a large number of bacteria are being marketed without any distinct demonstration of beneficial health effects. Hopefully, new regulations will require demonstration of such effects, as well as the proven safety of probiotic strains. A major challenge will be to identify changes in the host microbiota associated with disease. The relationship between different strains of bacteria and their interactions with the gut epithelium is important in this⁴⁸. A further approach represents the development of recombinant therapeutics such as genetically engineered *Lactococcus lactis* expressing the tolerogenic cytokine IL-10. Experiments in mouse models of ulcerative colitis have shown downregulation of ongoing inflammation with this approach. Another potential therapeutic target is type 1 diabetes. Preliminary evidence obtained from recent-

onset diabetes in NOD mice suggests that the combination of this recombinant microbial strain and proinsulin mediates an effective decrease in the dose of antibody to CD3 used for treatment, thereby limiting the potential toxicity associated with this antibody⁴⁹. A further challenge is consistent dosing. Carefully controlled studies are needed to identify the biological variables that determine viability and, therefore, dosage control in both genetically modified and naturally occurring probiotics.

Breastfeeding represents a natural way of modulating the cellular and molecular homeostasis of offspring through the maternal environment. Many cellular and molecular components have been identified that not only provide passive protection but also directly affect the immunological and metabolic maturation of the infant. Evidence from several clinical studies has shown that breastfeeding is associated with a lower risk of obesity later in life. The risk of obesity continues to decrease with longer periods of breastfeeding, up to around 7 months. Breastfeeding is also associated with slower weight gain. In contrast, rapid weight gain carries a two- to threefold greater risk of obesity at school age and in adulthood. There are preliminary indications that this protective effect of breastfeeding is linked to the profile of the gut microbiota, which is perhaps influenced by maternal transfer of bacteria. Additional data suggest that probiotics given to infants can have a preventative effect on the development of obesity. In addition to the microbiota, protein intake might also have an important role, as it is much higher in formula-fed infants than breastfed infants. Lower protein intake in the first 12 months of life is significantly correlated with a lower body mass index over a 24-month follow-up period. This is an important example indicating the relevance of the nutritional composition of breast milk^{50,51}.

Future research will determine the extent to which dietary modification can directly or indirectly influence the risk of developing diseases related to obesity, such as type 2 diabetes, cardiovascular disease and T_H2-mediated allergy⁵². It will be important to better understand the effect nutritional and environmental variables exert on the development of the gut microbiota and the immune system. Furthermore, in taking into account the role of genetic susceptibility, it will be important to determine who is likely to benefit from specific intervention in these disease areas.

Effective translational research

The hypothesis-generating research characterized by genome- and metagenome-wide association studies yields large amounts of data that must be confirmed in hypothesis-testing exper-

imental animal models (Fig. 2). Such animal model systems should consider the intrinsic and extrinsic mechanisms of disease development mentioned above. This could be achieved by assembly of the various modules of intrinsic and extrinsic disease modifiers, leading to well-defined (sub)phenotypes. This approach could be called 'synthetic microbiology'. A key challenge for the future, then, will be to obtain maximum benefit from the approaches and increase the likelihood of identifying effective treatment and prevention strategies. This calls for unbiased, high-throughput translational research in which the right disease targets are analyzed in the most appropriate model and potential treatments are tested in the right patients by clinical trials.

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