Molecular Mechanisms of Pediatric Nutrition

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Abstract

Over the last years, major scientific advances allowed to decrypt the human genome with over 22,000 protein-coding genes. We do know some of these genes, but yet only few of their functions and even less of their control and regulation as well as the complex interplay between different genes and their products. Genotyping allows to analyze particular genes, but it cannot predict phenotypes. What can we expect from the recent scientific advances with regard to the needs of the developing child or adult and the intention to prevent disease and/or to improve life quality? We address two particular points in this review: the (direct/indirect) interaction of nutrition with genes of the host and the impact of genetic variations (polymorphisms) on requirements, tolerance or metabolism of nutrition. Over the last 5 years, major research efforts were made to address the potential interaction of nutrition and genes, now named nutrigenomics (interaction of nutrition and genes) and nutrigenetics (impact of gene variants on nutrition and/or their metabolism). We give in this review examples of molecular approaches in the understanding of this bidirectional interaction between nutrition and genes, focusing also on epigenetic imprinting.

Introducing Remarks

At the reference center for disease prevention, year 2039 somewhere in Europe: The parents, both suffering from inflammatory bowel disease (IBD), bring their two healthy children (2 and 5 years old) in your office and ask for advice how to prevent them from developing IBD. They emphasize that several family members are affected by Crohn’s disease, indicating the familial nature of their disease. After a complete physical exam, you check their chip card for their individual genetic and
microbial data. You enter additional key data in the computer which prints out the recommendation for an individual lifestyle and nutritional disease prevention program. Fiction or clinical practice of tomorrow?

Introduction

The scientific progress in various biomedical fields over the last 10 years is impressive. A major breakthrough in this evolution is related to the outstanding progresses in genetics allowing to uncover the human genome with over 22,000 protein-coding genes. However, as often in science, advances open new questions and knowledge becomes more and more complex and difficult. We now have the (very expensive) techniques to analyze the over three billion of base pairs across our 23 pairs of chromosomes. And we do know some of these genes, but yet only few of their functions and even less of their control and regulation as well as the complex interplay between different genes and even more important their products. Genotyping allows to analyze particular genes, but it cannot predict phenotypes.

What can we expect from the recent scientific advances with regard to the needs of the developing child or adult and the intention to prevent disease and/or to improve life quality? One might expect that this new genetic knowledge will help us to better understand the interactions between external, environmental factors, such as nutrition, and the host.

There are two major questions, which we intend to address in the following review:

1. Does nutrition interact (directly/indirectly) with genes of the host?
2. Do genetic variations (polymorphisms) impact on requirements, tolerance or metabolism of nutrition?

If this new biomedical and genetic knowledge (enabling the development of new analytic tools) might help us to elucidate at least one of these questions, we can expect a real ‘revolution’ in the field of nutrition in the near future. In fact, over the last 5 years major research efforts were made to address the potential interaction of nutrition and genes, now named nutrigenomics (interaction of nutrition and genes) and nutrigenetics (impact of gene variants on nutrition and/or their metabolism).

However, the dualistic view of the interaction between nutrition and genes of the host is oversimplistic. At least one third player has to be introduced to complete the picture: the intestinal microflora and the effect of nutrition on the commensal bacteria which in a subsequent step impact on the host. However, this aspect will not be detailed in this review.

That the interaction of nutrition and genetics has an important effect on human well-being and disease development is a quite well established concept [1]. For instance, a particular and specific genetic background is required to develop celiac disease, an immune-mediated inflammatory disease of the
gastrointestinal tract related to the oral intake of gliadin. It is now well established that the DQ2 or DQ8 structure is indispensable for gliadin to bind to the T cell receptor, starting a long and chronic cascade of T cell-mediated inflammation \[2, 3\]. This means that a precise and single genetic factor decides if a host cell can bind and recognize an alimentary antigen, i.e. gliadin, responsible for disease development.

**Nutrient–Gene Interaction**

Theoretically, different ways of nutrient–gene interaction are possible. Nutrients can interact directly with a nuclear receptor and behave like transcription factors, able to induce or repress genes. A good example is the interaction of vitamin A derivates with the retinoic acid (RA) receptor proteins, which can potently control gene expression via so-called RA response elements in the promoter region of distinct genes \[4\]. This interaction can have extremely important functions, since behavior and biological functions of antigen-presenting cells, such as dendritic cells, is markedly influenced by the availability of RA. In the presence of RA, a tolerogenic DC response is obtained within the intestinal mucosa, whereas the absence of RA will cause a potent upregulation of inflammatory responses \[5\]. Other examples are dietary fatty acids or vitamin D which via peroxisome proliferator activate receptors (PPAR) or the vitamin D receptor, respectively, bind directly to the DNA, thereby controlling gene expression. These are examples of short-term signals that directly alter gene transcription. But the effect stops immediately as soon as the specific nutrient is removed.

In contrast to these short lasting and highly specific effects via response elements on gene expression, there is also the possibility to interfere in a long-lasting, sometimes lifelong manner. These sustained effects can be mediated by direct modification of the DNA in form of methylation of CpG motifs or via methylation, acetylation or eventually biotinylation of histones \[6–8\]. Histones are nuclear proteins on which the DNA is wrapped in a very dense manner. This tightly packed DNA is largely inaccessible to transcription; however, after histone modification (methylation or acetylation) these molecules change their tertiary structure. They uncoil or unfold, thereby giving access of transcription factors to previously hidden promoter regions inducing gene expression. Most often histone modification goes along with DNA methylation which occurs at cytosine bases (CpG islands), a mechanism indispensible for genomic stability \[9\]. In the human genome, between 60 and close to 90% of CpG islands are methylated.

Usually, DNA methylation reduces gene expression (gene silencing). We now know the precise mechanism of this gene silencing in that the methylated 5’-CpG-3’ attract capping proteins that hinder the access to the gene for transcription factors (fig. 1). This mechanism of DNA methylation or histone acetylation/methylation is an only recently discovered, but extremely important
mechanism to control gene expression. The knowledge and research in this area which is now called epigenetics is dramatically advancing. The plasticity of the human genome via epigenetic modulation (resulting in the so-called epigenome) is amazing [10]. There are good experimental data to believe that fundamental processes such as cell differentiation, X chromosome inactivation and genetic imprinting are all consequences of epigenetic regulation. Epigenetic modulation does not only result in a postgenetic modification of an individual, but these epigenetic modifications can also be transmitted over generations.

One might ask if alimentation can impact or influence epigenetic phenomena. The clear answer is yes! Via alteration of the levels of alimentary available methyl groups, epigenetic modulation can cause subtle and important, sometimes even lifelong consequences.

One of the nicest examples of epigenetic regulation and the impact of nutrition on this process comes from honeybees (Apis mellifera). The queens of honeybees are characterized by fertility, a markedly larger phenotype with a considerably longer life span (2 years) compared to the majority of bees which are sterile, show the smaller ‘worker’ phenotype and live only a few weeks (fig. 2). What is responsible for the fact that genetically identical larvae end up with so contrasting phenotypes and functions? The only difference between these two is that a few female larvae are fed the so-called ‘royal jelly’, a poorly
defined aliment. These larvae end up with the ‘royal’ phenotype of queens. Very recently, it could be confirmed that the differing nutritional input (royal jelly) results in a higher degree of DNA methylation modifying the expression of genes, such as Apis, implicated in the modulation of epigenetic regulation [11]. One key element seems the activity of DNA methyltransferase Dnmt3 in honeybees. Suppression of Dnmt3 in larvae results in a queen-like phenotype, further emphasizing the importance, but also the plasticity of the system. This observation clearly confirms that environmental factors via epigenetic modification have a major impact on the final adult phenotype.

Another interesting and well-studied observation is the impact of maternal nutrition (before and during pregnancy as well as during suckling) on the phenotype of the pups of so-called agouti mice. The agouti gene is expressed in hair
follicles of mice during a brief stage of hair development and growth. It encodes a paracrine signaling molecule which is responsible for the production of a yellow pigment by specialized pigment-producing cells. In wild-type mice, a yellow band appears on the otherwise brownish hair. In the viable yellow agouti (A^{vy}) mice however, an intracisternal A partial (IAP), a retrotransposon common in the mouse genome, has spontaneously inserted into the agouti gene [12]. The result is a constitutive and permanent expression of agouti in all tissues, due to a cryptic promoter within IAP. Therefore, A^{vy} mice show a yellow coat and they are markedly obese. This can be explained by the ectopic agouti expression and ability of agouti protein to bind antagonistically to the melanocortin-4 receptor in the hypothalamus, thereby causing hyperphagia. For a still unknown reason, insertion of IAP into the agouti gene also causes epigenetic dysregulation, resulting in spontaneous interindividual variability in CpG methylation at the A^{vy} locus. Therefore, within a single litter of genetically identical A^{vy}/a mice, some have a very low level of methylation resulting in a yellow and obese phenotype, whereas those with a high methylation level, repressing agouti, display the normal ‘agouti’ phenotype. This interesting model opened the door for nutritional intervention studies in supplementing high or low levels of methyl donors. Indeed, a supplementation with choline, vitamin B_{12} and folic acid before and during pregnancy clearly shifted the coat color from yellow to brown, along with body fat mass differences (fig. 2). Indeed, Waterland et al. [13] demonstrated that the differences in maternal food supplementation cause a differing methylation status at A^{vy}, which clearly correlated with the definite adult phenotype of the offspring.

These data provide clear evidence that specific and targeted nutritional intervention at a critical time point of development causes a permanent phenotypic change by epigenetic gene regulatory mechanisms.

The interaction of nutrition with genes is not unidirectional, it should also be analyzed the other way round. There are excellent data indicating that genetic variations (polymorphisms) have a major impact on nutritional requirement as well as functions. One well-studied interaction is the requirement and metabolism of folate. The enzyme 5,10 methylenetetrahydrofolate reductase (MHTFR) is a key enzyme in folate metabolism [14, 15]. MHTFR has an important role in supplying methionine, which is important in many metabolic pathways, such as the production of neurotransmitters and the regulation of gene expression. Folate is essential to the efficient functions of this enzyme. MHTFR has a common single nucleotide polymorphism (SNP) at position 677 with a C to T transition, resulting in the conversion of an alanine to a valine (MTHFR Ala 222Val); this SNP results in a thermal labile version of the protein which has a markedly reduced enzyme activity. People with one or two C copies have normal folate metabolisms, whereas homozygous persons (TT) with reduced enzyme activity have elevated plasma homocysteine levels, unless they have an increased folate intake. This allows to compensate the slow enzyme activity by an increased substrate supply. The link between increased homocysteine levels and increased risk for cardiovascular diseases, one of the main causes of mor-
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tality in our society, is well established. In addition, various efforts are under-
way to control or lower homocysteine levels in view of disease prevention. On
the other hand, the observation that approx. 15–30% of the European popula-
tion has the TT genotype raises the question why this polymorphism persisted
over generations and if there is not a distinct evolutionary advantage.

Another important aspect of this gene-nutrition interaction in the folate
metabolism is the observation that the risk of having a child with a neural
tube defect (spina bifida, etc.) is several times increased in pregnant women
with another very common SNP (MTHFD1-G1958A). This risk clearly dif-
fered between mothers with the highest choline intake and mothers with the
lowest choline intake, with the former having a lowered risk for a baby with a
neural tube defect [15].

The list of examples how genetic factors may impact and influence nutri-
tional requirements is getting longer with at least 20 genes that have a poly-
morphism that may confer a specific disadvantage in the view of disease
development, but which may be overcome with a specific dietary modification.
Other well-studied interactions are the effect of mutations in the apolipopro-
tein E protein (e4/e4) or polymorphisms of APOA1 or PPARA and the intake of
lipids or cholesterol and the risk of the development of cardiovascular diseases
[16, 17]. To increase this knowledge and to create a research network, the
European Nutrigenomics Organisation was built up (www.nugo.org) in 2004.

Conclusions

We are at the starting point to integrate the growing knowledge of genetic
variations and the postgenetic (epigenetic) modulation and plasticity in the field
of the interaction of environment and the host. This is particularly important for
the understanding of developing organisms, prenatally as well as postnatally.
One major environmental factor is nutrition, especially for the developing child.
Research in the field of nutrigenetics and nutrigenomics is at the starting point,
and I am sure that it will ‘explode’ over the next two decades. However, there
are major limitations and key questions that have to be addressed and solved
on a common ground. Who are the drivers of the development of nutrigenomics
and its applications to disease prevention or healthy living and aging? Is it the
private sector or health professionals? How are ethical or confidential aspects
defined and controlled (for example, are life insurances allowed to consider
the risk of persons with an MTHFR ‘TT’ polymorphism, etc.)? The main drivers
should be on one side the societies themselves and on the other side strong
international science foundations elaborating on the clinical value of genetic
risk factors and the effects of specific nutritional targeting in individuals with a
particular (genetic) susceptibility or risks factors. Therefore, in the near future,
individual nutrition advice will enter into clinical routine and will be part of
everyday practice for healthy persons, as well as in the cure of some diseases.
References


Discussion

Dr. Cai: Could you comment about the relationship between diabetes and genetics?

Dr. Ruemmele: As in many diseases, there is also a genetic background in diabetes. But it’s not a monogenetic background which means there is one or a couple of single genes which are responsible for the development of the phenotype which can be seen in some very rare monogenetic causes of congenital diabetes, no the cause of diabetes is multigenetic. Over one or two generations, the genes do not change completely. If you look at the incidence of these diseases over the last 50 years, our genes did not change considerably, however many environmental factors did change. So with regard to genetic aspects for the disease development, you are in the heart of epigenetic modification. It’s some type of lifestyle which changed considerably which impacts on different levels.

Dr. Yang: My question concerns intrauterine growth retardation. As we know, a baby with IUGR is at higher risk to suffer from metabolic diseases in adulthood than a normal baby. Do you think it is because early nutrition has an effect on epigenetic and genetic information?

Dr. Ruemmele: It’s extremely difficult to give a clear answer to this question because I think we would have to redo a huge bench of studies we did in the past
looking on the genetic information and the epigenetic modulation in these high risk group of newborn children. At this time point, we can only speculate on the molecular events. But I think Dr. Koletzko can comment on this.

Dr. B. Koletzko: Please allow me to come back to the question is it genetics or is it substrate supply that matters? In my view it is an interaction of both. A very good example is the effects of the polymorphisms of the fatty acid desaturase 1 and 2 (FADS1 and FADS2) that we first described in 2006 [1]. We found about one quarter of the European population studied to have a low activity of the desaturating enzymes and therefore a low level of essential fatty acid conversion into the long-chain metabolites. This effect has been confirmed in several other studies [2–4]. If one finds effects of these polymorphisms of PUFA metabolism on outcomes such as IQ in breastfed and non-breast-fed populations or as we have described before on the prevalence of atopic dermatitis and rhinitis [1], this represents pretty convincing evidence for an effect of PUFA on these outcomes. George David Smith in Bristol has coined the term Mendelian randomization, proposing that polymorphisms are distributed in the population at random, and unless there is a mechanism that the polymorphisms are directly affecting the end point, then you really can consider effects of polymorphisms on end points as evidence that it’s as close to a randomized clinical trial as you could imagine. Thus, it is justified to conclude from the available observations that PUFA provided with breast milk affect later cognitive development, because breastfeeding has an effect on IQ development if subjects have a certain genotype of PUFA metabolism, whereas breastfeeding has no effect on IQ if subjects have another genotype of PUFA metabolism, assuming that the choice to breastfeed is not related to the FADS polymorphisms. I think the real exciting story here is that we may have infants who have a higher requirement for long-chain PUFA than others, depending on their genotype, to achieve the very same cognitive development outcome or to achieve the same allergy risk. Thus, some people may need different intakes than others. Is that an academic discussion or is it of practical relevance? With respect to folic acid supply perhaps it is of academic interest only. Given that folic acid is so cheap, one could easily provide a sufficient amount of folic acid to every woman of childbearing age, rather than doing genotype testing. With respect to PUFA supply, however, it could be a practically very relevant question, for example if you think of the intensive care situation where today some interventions are made using omega-3 fatty acids enterally or parenterally to downregulate the inflammatory response. Here, one could imagine doing genotype testing before dosing such a targeted clinical intervention.

Dr. Shenoi: I was just wondering whether anybody has studied the genetics of the bacteria in certain disease like Crohn’s disease because there is an interaction between food bacteria and the illness.

Dr. Ruemmele: There is a huge literature on the composition of the bacteria and the change in the gut. There are few papers analyzing the genetic variance between the bacteria because at the moment the question is to identify the different bacteria which are normally implanted in the GI tract and what are the quantitative and perhaps more important qualitative changes that occur in IBD patients. So I think the answer will come in the next years, but at the moment there is no particular genotyping of a single strain or different groups of strains which look different, if this was your question, in Crohn’s disease patients. But we know a lot on the genotyping of patients. On the host side, there are susceptibility factors which are clearly defined and which contribute to the risk to develop the disease, good examples are mutations in nod2, IL23 receptor polymorphisms, autophagy genes etc.

Dr. Cooper: I was just wondering whether identical twins might be a fertile ground for the hypotheses generated in this field. Has any work been done in this area?
Dr. Ruemmele: There is literature on epigenetic analysis in identical twins. The risk to develop Crohn’s disease from a genetic point of view between identical twins is 50%, it’s not 100 or close to 100; but between brothers or non-identical twins this risks is lower with 5–10%. So genetic factors contribute to a high degree to the risk of disease development. But there is a lot of space for other modifications, which impact on disease development. These factors are considered to be environmental, or exogenous factors, opposed to the endogenous genetic factors. An indeed there are good theoretical arguments to believe that epigenetic modifications can contribute to disease onset or perpetuation. So indeed it’s an ideal situation to compare the impact of the environment between two individuals who have the same genotype. The genetic studies on twins and epigenetic studies are of major interest to track down some effects.

Dr. S. Koletzko: A polymorphism which is involved in the metabolism of fatty acids with potential beneficial functional effects may cluster over generations in certain populations. One example is the lactase persistence gene in north-eastern Europe. Since a low socioeconomic status is related to a low breastfeeding rate, it could be that in the group with the lowest socioeconomic status there is an enrichment of certain polymorphism and not a random distribution.

Dr. B. Koletzko: As far as I recall, the investigators found a direct effect on the association of polymorphisms with IQ. They found no association of the polymorphism with breastfeeding, and they found an interaction between breastfeeding, polymorphism and IQ.

Dr. Dhansay: You said that vitamin A may be pro- or anti-inflammatory. Based on that statement can you make any recommendations for vitamin A supplementation especially in developing countries?

Dr. Ruemmele: Vitamin A is very interesting to look at. The local concentration of Vitamin A such as in the intestinal mucosa predefines the way dendritic cells drive T cell responses. However, it is important to strengthen that this very powerful effect of Vitamin A on T cell responses reflects is dependent on local, ie tissue concentration of the vitamin, it does not necessarily reflect the intake, or the total concentration of vitamin A or stocks in your organism, it’s a local effect, it’s very local. It is interesting to underline that vitamin A orients immune responses towards tolerogenic or anti-inflammatory responses and thereby avoids proinflammatory responses. If under the same condition experimental conditions you induce a T cell response, now vitamin A levels are low, you can shift towards a proinflammatory answer. I am not sure that based on this fundamental observation for inflammation and regulation of inflammation we should make any recommendations on the level of supplementation of vitamin A, particularly in developing countries or under special conditions. Before we can do this, we need further analyses on the tissue level, and I do not want to make any new recommendation on vitamin A intake other than those existing today.

References