Epigenetic Manifestations in Diet-Related Disorders

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Abstract
Epigenetic phenomena are changes in phenotype that are due to resetting of gene expression under the influence of the environment or genetic factors without changing the DNA sequence. Usually this resetting occurs at a certain stage in life and remains fixed thereafter. In humans, evidence for epigenetic involvement in diet-related complex traits and disorders is accumulating. The fetal origins theory indicates that nutrition can influence the later life risk for certain common disorders like the metabolic syndrome. In parent-of-origin effects, the risk for a common disorder like type I diabetes depends on the sex of the parent who transmits genetic risk factors. Interestingly, both dietary and genetic factors can exert their epigenetic influence over several generations. Imprinting, i.e. silencing of one copy of an autosomal pair of genes, can be part of the mechanism pointing to the importance of DNA methylation. In addition, chromatin modifications have been shown to be involved in epigenetic manifestations. The intriguing possibility that diet may influence the direction and extent of epigenetic changes opens new ways for prevention or treatment of common disorders. At the same time, maternal nutrition might be used to actively direct fetal development with consequences for later life performance such as cognitive abilities. More knowledge on those novel applications is needed. This will in part come from novel strategies to map the epigenomic regions, allowing the identification of more genes involved in epigenetics and allowing the study of their response to nutrition.

Introduction
The globally increasing prevalence of obesity and associated conditions like insulin resistance, dyslipidemia and hypertension, together constituting the metabolic syndrome, has profoundly stimulated research into the underlying causal processes. This has revealed some interesting concepts for understanding diet-related traits and disorders. Obviously, the environment plays a role with the sedentary lifestyle, ample availability of food, and the composition of the diet as main factors. On the other hand, genetic background is involved as exemplified in rare cases by carriership of dramatic mutations [1, 2], or more commonly of risk alleles or haplotypes [3]. Although environment and genes can have an absolute impact on risk, in general disease risk is the resultant of their interaction. This interaction is mediated by the regulation of gene expression. An important finding in this respect is that nutrients can act as signaling molecules for gene regulation. Well-known examples are the binding of polyunsaturated fatty acids (PUFAs) or retinoids to the nuclear receptors PPAR and RXR, respectively [4], or the
activation by amino acids of genes with an amino acid responsive element near their promoter [5]. Such interactions are regarded as instantly active and reversible processes, by which an organism can adjust its metabolism to the intake and composition of the diet.

Besides those instantly active, reversible metabolic adjustments in mammals, other mechanisms exist that lead to (re)programming or resetting of the level of gene expression at a certain moment in life to remain thereafter fixed. Those (re)programming events are referred to as epigenetic phenomena. They may have evolved to increase the survival chance of the species by fine-tuning the interaction of the metabolic activity of individuals with an environment that is expected to be stable for a relatively long time, i.e. for years or even generations. The advantage over genetic adaptation by mutation and selection is that epigenetic adjustments can be more easily reversed and as such allow the species to shift between phenotypes depending on the environmental conditions [6]. In this review, I want to bring together some of the observations that demonstrate the existence of epigenetics in humans and the involvement of nutrition therein.

Fetal Origins and Parent-of-Origin Effects

Epigenetic adjustment to long-term food conditions may occur during the fetal stage with the maternal food intake as the level indicator for setting fetal gene expression. This was first brought to the attention by Barker [7, 8] and is now often referred to as the ‘fetal origins’ theory. In fact, evidence for this theory comes from situations in which postnatal conditions do not comply with the expectations for the fetus by maternal sensing, i.e. if the prenatal conditions differ. Then, prenatal setting of gene expression will lead to reduced postnatal fitness and increased risk for diet-related disorders. Famine for instance will lead to nutritional stress in the fetus and may change its metabolism to a thrifty phenotype by resetting the activity of certain genes [9]. This adaptation can be seen as a preparation to a postnatal life under circumstances of poor nutrition. If the prediction is right, the individual will have a higher chance to survive under those conditions. However, if postnatal conditions are opposite and food is readily available, those with the fixed thrifty phenotype will show catch-up growth during the first year of life [10]. During childhood and early adulthood, which is a phase of constant energy demand for growth and reproduction, a thrifty phenotype may not show an adverse influence, but in later life this may lead to disease. It is tempting to speculate that causes of fetal growth retardation other than famine may work through a similar mechanism. Indeed, low birth weight has been reported as a risk condition for later-life obesity [11].

Several excellent studies have made use of data from the Dutch Hunger Winter [12–14]. During the winter of 1944–1945, lack of food in the northern part of Holland led to severe malnutrition of the general population. The offspring of women who were pregnant during this period has been the subject of follow-up studies. For example, Roseboom et al. [15] have divided them into three groups based on the moment of conception and thus the trimester in which they suffered from famine. Those conceived 6–9 months before the famine suffered malnutrition only during (part of) the last trimester of pregnancy. Screening the offspring at the age of 50–60 showed considerable increased risk for glucose intolerance. The same was true for individuals suffering famine during the second or first trimester, making glucose intolerance a major consequence of gestational famine. The first trimester seemed to be the most critical period because persons belonging to this subgroup were additionally at increased risk for obesity (women), atherogenic lipid profile, altered blood coagulation, coronary heart disease and others. Apparently, gestational famine, especially during the first trimester, increases the risk for later-life metabolic syndrome [16]. Observations in other populations confirm the later-life influences of gestational famine [17–19]. As yet no sufficient mechanistic explanation has been put forward, but one could imagine that lack of certain nutrients or their metabolites during the critical period alters the expression activity of particular genes to a level that is fixed and remains so throughout later life.

With regard to nutrition-related disorders complying with the fetal origin theory, the effect on the fetal genome is mediated by one of the parents, in this case the mother. Interestingly, comparable parental origin effects have been observed when studying the transmission of risk in multiple case families with multifactorial disorders. This so-called ‘parent-of-origin’ effect is regarded as a strong indicator for epigenetic involvement. We and others have previously observed this phenomenon in clinically registered families with multiple cases of neural tube defects (NTDs) including spina bifida and anencephaly [20, 21]. Starting from the index patient, it was noticed that the second patient was significantly more often found among the maternal relatives than among the paternal relatives. Very likely, not environmental influences but genetic factors are involved here, which confer higher risk to the fetus when transmitted through the mother than through
the father. It seems that somehow the risk factors carry an imprint of the sex of the transmitting parent, a situation referred to as genomic imprinting.

Genomic imprinting exerts its influence by regulating the activity of a gene. This became clear when the genetic causes of patients with the Prader-Willi syndrome were studied. Those patients have failure to thrive in the first semester of life, then switch to hyperphagic behavior leading to severe obesity if food intake is not limited. A de novo deletion at chromosome 15q12 was found in 75% of patients but, peculiarly, the deletion occurred exclusively in the paternal chromosome. Using polymorphic markers, another 20% of patients were observed to have maternal uniparental disomy 15 \[22\]. This led to the hypothesis of a gene on chromosome 15 that was active on the paternal chromosome but inactive on the maternal chromosome. By definition, imprinting is taken as gene silencing so that the as yet unidentified Prader-Willi syndrome gene is said to be subjected to paternal imprinting.

Genomic imprinting in which gene activity depends on the sex of the parent of origin has now been demonstrated for various autosomal genes, a condition that may even show tissue specificity or can be polymorphic \[23\]. Among those are the genes coding for important growth factors like insulin, insulin-like growth factor 2 (IGF2), and the IGF2 receptor. Both insulin and IGF2 have been shown to be subjected to genomic imprinting in the early human embryo. For the insulin gene, imprinting is tissue specific. Only one gene copy is expressed from 6 to 8 weeks after gestation in the yolk sac, which may function as the primitive liver, and in the thymus. Insulin expression is influenced by a VNTR sequence near the promoter \[24\]. In the yolk sac the paternal gene is expressed \[25\], and therefore insulin production largely depends on the repeat sequence in the paternal allele. It has been speculated that this may interfere with the recognition of insulin produced in the thymus as self, increasing the risk for auto-antibodies leading to type I diabetes. Indeed, paternal transmission influencing the risk for IDDM in families has been reported by some, but not by others \[26\].

Applying the concept of gene silencing as the basis for genomic imprinting, the increased risk for NTDs by maternal vs. paternal transmission could be explained by assuming that genetic risk factors for NTDs are subjected to paternal imprinting (fig. 1). If risk alleles are passed on through the paternal line, the risk would be reduced because the expression of the risk alleles is switched off, whereas passage through the maternal line would leave them active to confer the risk to the offspring. The opposite, i.e. maternal imprinting of risk factors, seems to occur in attention deficit/hyperactivity disorder (ADHD). Recently, Goos et al. \[27\] investigated 222 families with at least one other case in addition to the index patient. They found that in 60 families the disease was only found on the maternal side, whereas in 120 families the disorder was reported at the paternal side only. From this, it is likely that the maternal alleles of the imprinted risk genes are silenced leaving the active paternal alleles to confer the risk. ADHD has been suspected to be caused by food components or additives, although definite proof is lacking \[28\]. In this regard, the parental effect is on the genetic liability to develop the disorder, while the diet may be the environmental trigger leading to the full-blown ADHD. An intriguing thought is that reactivation of the maternally imprinted gene copies might alleviate the symptoms of ADHD.

A low-calorie diet was shown to have a beneficial influence on the prevalence and severity of psoriasis, a common skin disorder characterized by hyperproliferation and poor differentiation of epidermal keratinocytes and considered to be a T cell mediated inflammatory disorder \[29\]. As influential components of the diet several substances have been studied including vitamins, antioxidants and unsaturated fatty acids. It is thought that these nutrients have an ameliorating effect through either an antiproliferative or anti-inflammatory action. A parent-of-origin effect in families was reported by Traupe et al. \[30\] who showed that the risk for the offspring is

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Fig. 1. Parent-of-origin effect explained by paternal imprinting. Genetic risk factors (R) that are passed on through the male germ line are silenced, whereas factors passed on through the female germ line remain active and confer the risk to the offspring.
more often transmitted by affected or gene-carrying fathers than by mothers. In addition, significantly more index patients had an affected grandfather than an affected grandmother. An interesting, yet unexplained influence of the sex of the psoriatic parent on birth weight was observed. Children from psoriatic fathers were significantly heavier than children from psoriatic mothers.

Both the fetal origin and the parent-of-origin effects can be regarded as transgenerational phenomena. The question is then whether such effects are limited to two generations. The grandparental effect in psoriasis indicates that this is not the case. Experimental evidence in humans that the environment can have its consequences for the phenotype two generations later has come from a small isolated community in the remote town of Överkalix in the north of Sweden. Using parish registries together with the harvest records, it was found that various parameters of well-being of the present population were related to food availability to their grandparents. In fact, longevity was associated with food availability to the paternal grandfather specifically during his so-called slow growth period before the prepubertal growth peak [31]. This was later confirmed in a more elaborate study [32]. Another investigation in the same population revealed an association between the paternal grandfather’s food access and the grandchild’s risk for cardiovascular and diabetic mortality [33]. Next, Pembrey et al. [34] profoundly studied the mortality risk in relation to the grandparental food supply. They showed that the grandparent/grandchild effect was specific for sex and for grandpaternal age of exposure. Food supply to the paternal grandfather had only an effect on the mortality risk of the grandson, whereas the paternal grandmother’s food supply was linked with the mortality risk of her granddaughter (fig. 2). In the latter case, a strong increase in mortality was associated with poor food supply at the grandmaternal age of 0–3 years. This led to the supposition that food had an effect on the regulation of gene expression in the developing oocytes. The observed sex specificities suggest the involvement of the Y and X chromosomes. In another cohort BMI was found to be associated with the prepubertal age at onset of smoking of the paternal grandfather, an effect only observed in the grandsons [34]. It shows that in humans there may indeed be a general mechanism for sex-specific, male-line transgenerational responses to environmental factors like nutrition. For Mendelian geneticists, this might be a bit difficult to accept because it resembles Lamarckism.

Molecular Mechanisms of Epigenetics

The observation that genomic imprinting is part of some epigenetic manifestations has provided an important clue with respect to the molecular mechanism since methylation of DNA is known to be involved. This has been substantiated by generating (conditional) knockouts of methyltransferase genes in the mouse [35]. DNA methylation occurs mainly on the C residues of CpG dinucleotides, which are underrepresented in DNA except at the promoter regions of genes. These areas of apparently clustered CpGs are therefore referred to as CpG islands. Gene activity is usually correlated with hypomethylation of the promoter CpG island, whereas methylation leads to the silencing of the gene. During cell division, the DNA methylation pattern is conserved. However, changes may occur under specific circumstances like during tumorigenesis. A well-studied case of genomic imprinting is that of the mouse IGF2R gene [36]. This gene does not only harbor a CpG island around the promoter, but also a second one in the intron 2. It turned out that the methylation of this second CpG island is implemented or maintained when the gene passes through the maternal germ line. On the other hand, when the gene passes through the paternal line, methylation of the second CpG island is removed. In the early embryos, the maternal and paternal genes are thereby distinguished, directing secondary methylation of the paternal gene promoter leading to the silencing of this gene copy.
Studies on DNA methylation in genomic imprinting have learned that gene activity is strongly influenced by methylation, that for some genes methylation can be removed and reset in the male or female gonads, and that methylation can be set early in life to determine gene activity thereafter. In tumor cells, where DNA methylation can become dysregulated, resetting of promoter DNA methylation is commonly observed and may be part of the microevolution of a tumor [37]. DNA methylation can provide, at least partly, an explanation for the parent-of-origin effects. It is tempting to assume that DNA methylation of crucial genes in the fetus may also underlie (some of) the fetal origin effects.

An important donor of methyl groups is S-adenosyl-methionine, a component of the folic acid metabolism. One could imagine that low folate intake by the mother, as for instance during periods of famine, may result in critical levels of S-adenosyl-methionine and as such may alter the methylation status and activity of genes that in later life contribute to the risk for diet-related disorders. Although folate-dependent DNA methylation provides an interesting working model to explain epigenetic manifestations, other processes are probably involved as well such as the modification of chromatin proteins. Histones are modified in several ways including acetylation, methylation, ubiquitination, phosphorylation and, as more recently detected, also by biotinylation [38–40]. Overall, acetylation of histones has received most attention. The acetylation of lysine-residues in the aminoterminal region of the histones is supposed to lead to the loosening of the binding between the nucleosomes and the DNA, making it more accessible for gene activation and transcription. On the other hand, deacetylation may lead to gene silencing. A special class of histone-deacetylating enzymes, class III enzymes, is the ‘sirtuins’ that for their activity are NAD dependent. In humans most studied is SIRT1, the homologue of yeast Sir2. Targets for SIRT1 are modified in several ways including acetylation, methylation, ubiquitination, phosphorylation and, as more recently detected, also by biotinylation [38–40]. Overall, acetylation of histones has received most attention. The acetylation of lysine-residues in the aminoterminal region of the histones is supposed to lead to the loosening of the binding between the nucleosomes and the DNA, making it more accessible for gene activation and transcription. On the other hand, deacetylation may lead to gene silencing. A special class of histone-deacetylating enzymes, class III enzymes, is the ‘sirtuins’ that for their activity are NAD dependent. In humans most studied is SIRT1, the homologue of yeast Sir2. Targets for SIRT1 are not only Lys9 of histone H3 and Lys16 of histone H4, but also important transcription factors like p53, FOXO and NF-kB [41]. Interestingly, if the NAD level determines the deacetylase activity [42], this can provide a link between the activity of glycolysis and TCA cycle as sensors of food availability and chromatin remodeling with altered gene expression. Although DNA methylation patterns may be passed on from one generation to another as mentioned above for the IGF2R gene imprint, it is not yet clear whether this may also be true for histone modifications [43].

Use of Nutrients in Epigenetic Treatment

The fact that nutrition can have influence on epigenetic manifestations generates the intriguing possibility that epigenetic processes can be actively influenced via the diet. This idea is powered by the observation that certain drugs are able to modify the activity of enzymes like methyltransferases and histone deacetylases [44]. Some of those are already part of (pre)clinical trials for cancer therapy with the intention to remove the epigenetic blockade of tumor suppressor genes. In nutrition, suggestive results have been obtained with folic acid. As mentioned above, epigenetics is involved in the etiology of NTDs. Women with low folic acid intake, especially those who carry the 667T allele coding for the thermolabile valine variant of the enzyme methylene tetrahydrofolate reductase, are at increased risk for a child with NTD [45]. Although not proven, altered methylation of DNA may underlie this increased risk. A population-based cohort study revealed that up to 65% of NTD occurrences and up to 50% of NTD recurrences can be prevented by periconceptional folic acid supplementation [46]. Similarly, folic acid supplementation is expected to be beneficial in the treatment or prevention of certain types of cancer, for instance colon cancer. Extra folic acid might lead to (re-)methylation and silencing of growth factor genes [47].

The potency of folic acid has also been demonstrated in the rat where a protein-restricted maternal diet activates the hepatic expression of the glucocorticoid and PPAR-α genes in the offspring. Detailed analysis by genomic sequencing learned that the protein-restricted diet led to the hypomethylation of the promoter of the PPAR-α gene persisting in the adult offspring [48]. This effect was counteracted by supplementing the diet with folic acid. Folic acid may also hold promise for ADHD. Survivors of the ALL type of leukemia may develop ADHD. Linkage was found between the polymorphisms in the gene for methylene tetrahydrofolate reductase and inattentive symptoms in these subjects, indicating a link between the folic acid metabolism and ADHD [49]. It would therefore be interesting to compare the incidences of ADHD between societies with fortified cereals and those without.

Knowledge about the fetal origin effects and the conditions that modify them may provide ways to influence the offspring’s future health and capabilities through adjusting maternal nutrition. Attention has been given to the possible role of PUFAs, in particular n–3 fatty acids, in brain development and function. Helland et al. [50] have compared the children from mothers either taking
cod liver oil rich in n–3 PUFAs or corn oil with mainly n–6 PUFAs during pregnancy from 18 weeks onwards and 3 months of lactation. This is the time when synapse formation important for learning is assumed to begin and then continues until puberty. When the children were tested for intelligence at the age of 4 years with the Kaufman Assessment Battery for Children, those from the n–3 PUFA taking mothers scored higher on the parameters assumed to reflect problem solving and information processing. Apparently, providing extra n–3 PUFAs from mid-gestation gives rise to an effect in brain function that persists for years. Giving docosahexaenoic acid (DHA), an n–3 unsaturated fatty acid, as a functional food to pregnant women from 24 weeks until delivery showed a positive effect on problem solving in the child at 9 months [51]. DHA was also found to display some selectivity, because no effect was seen on recognition memory tasks. Other findings point to a beneficial effect of DHA intake on visual acuity at 4 months, but not at six months [52]. Visual acuity is measured by determining the smallest black character that can be identified on a white background, and by the smallest distance at which two symbols can be seen as separate objects. Visual acuity does not only depend on the physical characteristics of the eye, but also takes the sensitivity of the eye nerves and the interpretative faculty of the brain into account. So far, meta-analysis of various studies seems not to support strong effects of infant milk formula with long-chain PUFAs [53], although some results are suggestive.

Considerable research is focused on nutritional intervention of cancer formation or progression. Dashwood and Ho [54] have reported that a single ingestion of 68 g of broccoli sprouts by human subjects induces histone H3 and H4 acetylation in PBMCs and that this might be beneficial for colon and prostate cancer. Broccoli and other cruciferous vegetables contain sulforaphene, an isothiocyanate that inhibits class I and II histone deacetylases. Since it is not unlikely that cancer and fetal epigenetics have mechanistic similarities, such results are interesting in the light of maternal nutrition. In this respect, other nutrients that attract attention are niacin and tryptophane as precursors for NAD synthesis. Also biotin as a major component of egg yolk has recently received more attention since lysine residues in human histones can become biotinylated [40, 55]. It should be noted that histone modifications are in principle reversible. Resetting of gene expression that has to last during lifetime may therefore depend on the maintenance of a particular modifying/demodifying enzyme ratio. As mentioned above, drugs are already being tested for their enzyme modifying potential. More such studies on specific nutrients/diets are expected to follow soon.

Methods to Map the Epigenome

Several techniques have been designed by which more information about the epigenetic regions of the genome, the ‘epigenome’, can be obtained. This will be of utmost importance for the field of nutrition in order to eventually understand the influence of food availability and diet composition on gene expression and phenotype at later life or even for later generations. One of those techniques makes use of antibodies specifically directed against one of the chromatin modifications as, for instance, against acetylated Lys16 of histone H4. From the chromatin that can be immunoprecipitated (ChIP) with the antibody, DNA is isolated and analyzed. Using PCR amplification, one can investigate whether the modified histone is present at any gene of interest and if this situation can be changed by nutritional intervention or drug treatment. Hybridizing the DNA onto specific microarrays (ChIP-on-chip) allows in principle the mapping of all regions in the genome with the modified histone. Some problems like the interference of repetitive sequences with the hybridization still remain to be solved. Therefore, efforts are now being undertaken to speed up the identification of the epigenomic regions by high-throughput sequencing of the immunoprecipitated DNA (http://nihroadmap.nih.gov/epigenomics/).

Another approach to identify genes and genomic regions involved in epigenetic modification is by performing genome-wide DNA methylation profiling. One such approach [56] is based on the use of methylation-sensitive restriction digestion and selection of hypomethylated vs. hypermethylated DNA followed by hybridizing these fractions onto DNA microarrays. Theoretically, in this way a relative methylation status of every region of the genome can be obtained and changes upon drug treatment or diet intervention can be monitored. At this moment, the technique has been applied for only relatively small parts of the genome.

A third approach addresses epigenetics from the angle of gene expression. Imprinting of genes is part of the epigenetic phenomena and, consequently, a broad search for potentially imprinted genes has been started. If a gene is imprinted, one copy of the autosomal gene pair is silenced. If the genes can be distinguished by a polymorphism, only one allele would be represented in the RNA.
This is referred to as mono-allelic expression. Of course, one could start from the genomic methylation profile to select potential candidate genes and test them for mono-allelic expression [57]. In a more direct approach, Gimelbrant et al. [58] analyzed RNA as cDNA on the Affymetrix 500 K SNP array and compared the signals with those from genomic, bi-allelic DNA. Applying this technique to single-cell-derived clones of human B-lymphoblastoid cell lines, they detected monoallelic expression with random silencing of either the paternal or maternal gene copy for more than 300 out of 4,000 genes, a phenomenon resembling X chromosome inactivation in female mammals. Optimizing this approach for the use of RNA from tissue sections would allow the detection of imprinted genes in the various tissues.

In summary it can be stated that in humans epigenetic manifestations with a link to nutrition are observed as fetal origin and parent-of-origin effects and as male-line transgenerational responses to the environment. Mechanistic investigations have revealed the involvement of DNA methylation and chromatin modification. Further studies together with the unraveling of the human epigenome will provide the knowledge for the use of specific nutrients or diets to prevent or treat nutrition-related disorders with epigenetics as part of their etiology. Use of such nutrients during pregnancy may create the possibility to modulate cognitive functions in the offspring. The latter seems to invoke ethical considerations.

References


