

Epigenetics and a New Look on Metabolic Syndrome

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Summary

The incidence of metabolic syndrome increases in the developed countries, therefore biomedical research is focused on the understanding of its etiology. The study of exact mechanisms is very complicated because both genetic and environmental factors contribute to this complex disease. The ability of environmental factors to promote phenotype changes by epigenetic DNA modifications (i.e. DNA methylation, histone modifications) was demonstrated to play an important role in the development and predisposition to particular symptoms of metabolic syndrome. There is no doubt that the early life, such as the fetal and perinatal periods, is critical for metabolic syndrome development and therefore critical for prevention of this disease. Moreover, these changes are visible not only in individuals exposed to environmental factors but also in the subsequent progeny for multiple generations and this phenomenon is called transgenerational inheritance. The knowledge of molecular mechanisms, by which early minor environmental stimuli modify the expression of genetic information, might be the desired key for the understanding of mechanisms leading to the change of phenotype in adulthood. This review provides a short overview of metabolic syndrome epigenetics.

Key words

Metabolic syndrome • Epigenetics • Transgenerational inheritance • Gene-environmental interactions • Obesity • Hypertension

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Introduction

Metabolic syndrome (MetS) represents a specific set of symptoms, which can play an important role in cardiovascular morbidity and mortality. Since mortality on cardiovascular diseases is one of the leading problems worldwide, the study of particular symptoms of MetS is very important. Metabolic syndrome is a progressive phenotype characterized by insulin resistance, abdominal obesity, hypertension, dyslipidemia or Type-2 diabetes. Due to the intensive clinical and experimental research, the therapy of particular symptoms of MetS has been continuously improved. Despite of great effort has been applied during the last decades to the study of the etiology of MetS, its major causes are still unclear. Typical example can be the study of hypertension. More than fifty years ago, Guyton *et al.* (1969) introduced a very complicated scheme for the regulation of circulation. Nowadays this scheme is even more complicated because there is a lot of new information including physiological, biochemical and genetic mechanisms. When the boom of genetic studies has started, many researchers believed that the etiology of many important diseases would be solved very soon. One should ask, is it true? The answer is *No* and the new question is *Why?*

There is a growing evidence that complex interactions among multiple genes and multiple environmental factors play an important role in determining an individual's risk of various common diseases (Kuneš and Zicha 2009). The techniques of

molecular biology and genetics as well as particular knowledge emerging from several genome projects have revolutionized the process of localizing and identifying genes involved in different human diseases (Altshuler *et al.* 2008). Nevertheless, to find the dominant genes for polygenic diseases such as MetS is very difficult (Glazier *et al.* 2002). The interest in the genetic basis of human diseases has spanned the decades and the announcements of discoveries of disease-related genes often suggested that the tests to predict people at future disease risk will soon be available. The progress in the battle against MetS is being accelerated by the availability of total genome information for human, rat, mice and other organisms. However, one should keep in mind that genetic mapping is only the first step for full physiological understanding and clinical application of these results. Moreover, our knowledge from experimental approaches could not always be simply translated into useful clinical applications. This is mainly true for the common complex diseases including particular symptoms of metabolic syndrome (e.g. hypertension, obesity, Type-2 diabetes, etc.). As our understanding of the role of genetics and the use of gene-based markers extend to complex multifactorial diseases, physicians would have to learn how to recognize patients with higher than general risk. Whether and when so called "personalized medicine" will prevail over the traditional medicine is the question of the future. Even if genotype can predict response to a certain drug acting within a narrow therapeutic window, it cannot be assumed that genetic testing will necessarily lead to improved clinical outcomes (Altshuler *et al.* 2008).

Human genome has remained unchanged since the agriculture revolution more than 10,000 years ago when the diet and lifestyle have become progressively more divergent. Accumulating evidence suggested that the discrepancies between our "Paleolithic genome" and "modern" diet and lifestyle could play a significant role in the ongoing epidemics of obesity, hypertension, diabetes, atherosclerosis and other symptoms of metabolic syndrome (Kuneš and Zicha 2009). The main cause was a change in eating habits from natural food to a diet high in fat, sugar and salt. Predominantly the changes of Na:K ratio, lower intake of magnesium as well as the higher consumption of saturated fatty acids played an important role. Recently, nutritional genetics contributed to a better understanding of the mechanisms of diet-related diseases including metabolic syndrome (Lovegrove and Gitau 2008, Phillips 2013). Nutritional genetics is considered as the combination of

nutrigenomics and nutrigenetics. While, nutrigenomics is establishing the effects of individual food components on gene expression and gene regulation, nutrigenetics, on the other hand, concerns individual differences in the reaction to food based on genetic factors (Fenech *et al.* 2011, Ordovas and Mooser 2004). Thus, nutrigenomics treats food as a major environmental factor in gene-environment interaction with the aim to personalize nutrition. However, not only dietetic changes but also other environmental factors such as stress, lower physical activity, (e.g. total life style), endocrine disruptors, etc. are playing a significant role. As mentioned above, the development of human population throughout the centuries was influenced by some strong environmental factors, which could sensitize our organism to the noxious factors. It is hard to believe that all these environmental factors were able to induce changes of our genome only through mutations. The concept that the DNA sequence alone and the mutations in this sequence are responsible for evolutionary changes and disease development is thus false. It is clear that additional mechanisms are involved and thus epigenetic changes were taken to the account. Mainly the explanation for the adaptation to the environment could be used as an example for epigenetics, especially, if epigenetic transgenerational inheritance exists (Skinner *et al.* 2010, Skinner 2014).

Gene-gene and gene-environmental interaction are important processes for initiation of particular symptoms of metabolic syndrome and their progression. Recent studies have revealed that epigenetic mechanisms including DNA methylation, chromatin modification and remodeling, miRNA regulation and diverse activities of non-coding RNAs may account for a majority of MetS initiation. However, epigenetic processes are not involved only in the pathogenesis of diseases but they are also essential for normal growth and development. Several authors proposed a definition of epigenetics as the molecular factors and processes that are mitotically stable and which regulate the genome expression independently of DNA sequence (Ordovas and Smith 2010, Skinner *et al.* 2010).

Although, a large number of studies suggest that the environment could play a significant role in the disease etiology, the exact mechanisms are still unclear. In this short review we would like to outline some information how the epigenetic events interfere with individual metabolic syndrome symptoms, with a special attention to epigenetic transgenerational inheritance.

Experimental animals as a tool for the understanding of the etiology of metabolic syndrome

Currently, the clinical research tests usually the best conditions for the treatment of already sick patients, whose treatment costs annually hundreds of millions of dollars worldwide. On the contrary, the aim of the experimental research is to understand the pathophysiological mechanisms of many common diseases that could lead to their prevention. Therefore, a lot of specific models have been developed for the different diseases which serve to this purpose.

One should keep in mind that using experimental models has some advantages but it has also some limitations. There is no doubt that the comparison of only two outbred strains is not sufficient. Therefore, most models currently used are well-defined inbred strains of rats and mice besides some rare animal species (e.g. rabbits, ewes, pigs, monkeys, etc.). A big advantage is to use inbred strain, because these individuals are genetically identical. This is very important mainly to study the genetic nature of diseases. The development of particular symptoms of MetS is slow in relation to the longevity of the model species. The main advantage of experimental models is their availability, the possibility of standardization of experimental conditions and genetic characteristics of animals used, as well as the speed and reproducibility of the development of particular symptoms of MetS and its complications. However, it is rather difficult to compare the results in the literature due to not entirely standardized experimental conditions in particular studies.

Numerous experimental models for the study of metabolic syndrome and similar diseases have been developed (Hiraoka-Yamamoto *et al.* 2004, Fellmann *et al.* 2013, de Artinano and Castro 2009). Basically, experimental models can be divided into two groups. Firstly, the model is induced by surgery, dietary or pharmacological intervention, and secondly, the disease occurs as a genetic disorder either spontaneously or as a consequence of environmental noxious stimuli. In particular, genetic models are very useful as they allow to study gene-gene interactions, gene-environmental interactions, etc. Because metabolic syndrome does not constitute a single disease, it is difficult to find one experimental model suitable for the study of this complex pathology. Therefore, one can find numerous models some of which exhibit non-functional leptin pathway or

metabolic disorders induced by high-fat diets. A detailed description of various rat and mice models (SHROB, SHHF, JCR:LA-cp, Zucker, ZDF, Wistar Ottawa Karlsburg W, and Otsuka Long-Evans Tokushima Fatty rats, ob/ob, db/db, agouti yellow and Mc4R KO mice models) can be found in several other reviews (Hiraoka-Yamamoto *et al.* 2004, de Artinano and Castro 2009, Fellmann *et al.* 2013). Moreover, some information about the epigenomic regulation during the development in nonhuman primates and humans could also be interesting (Ganu *et al.* 2012). Recently, some models have been introduced in which the effect of maternal obesity on offspring development can be studied (Bayol *et al.* 2007, Wright *et al.* 2014).

Epigenetic transgenerational inheritance

It was demonstrated that the introduction of angiotensin II type 1 receptor antisense cDNA by a retrovirally mediated delivery system to young SHR prevents the development of hypertension in adult ones (Reaves *et al.* 1999). This protection was transferred to other generations as a result of its genomic integration and germ line transmission. On the other hand, the first example that blood pressure reduction induced by environmental manipulation (early antihypertensive therapy of SHR with captopril) could be transferred to the next generation was reported by Wu and Berecek (1993). This finding prompted Lindpaintner (1993) to ask whether high blood pressure in genetic hypertension is entirely based on the genes or not. He proposed that “conformational changes due to altered neurohumoral milieu” or “chemical modifications such as different DNA methylation pattern” might be a source of this heritability occurring in the absence of any structural alterations of genomic or mitochondrial DNA. The above finding and the resulting hypothesis on the epigenetic mechanisms of transgenerational attenuation of genetic hypertension development were subsequently questioned by Madeddu *et al.* (1995). Using early chronic treatment of SHR with angiotensin AT₁ receptor antagonist they observed that the development of spontaneous hypertension has not been modified in F3 animals whose parents were kept normotensive throughout the entire life.

Nevertheless, 15 years later, two British research groups reported that hypertension induced in rat offspring by maternal protein restriction can be transferred to the next generations (Torrens *et al.* 2008, Harrison and Langley-Evans 2009). The same is also true for

hypertension elicited by maternal global nutrient restriction which is also transmitted to F3 animals (Ponzio *et al.* 2012). Blood pressure elevation observed in F2 or F3 generation is usually more pronounced in male than female offspring and is augmented when the rats are subjected to other nutritional stimuli such as high-fat diet feeding (Harrison and Langley-Evans 2009). Similar transmission of high blood pressure and cardiac hypertrophy to male but not female F2 rats has been demonstrated following intrauterine growth restriction due to uteroplacental insufficiency (Master *et al.* 2014, Gallo *et al.* 2014). The underlying pathophysiological mechanism of such transgenerational programming effects might be the endothelial dysfunction (Torrens *et al.* 2008, Ponzio *et al.* 2012) resulting from decreased NO formation and enhanced superoxide generation (Ponzio *et al.* 2012).

Recently, epigenetic transgenerational inheritance was reported by other authors (Skinner 2014, Nilsson and Skinner 2015). This inheritance was defined as "the germline transmission of epigenetic information between generations in the absence of any direct environmental exposure or genetic manipulations" (Skinner 2014, Skinner 2011). Yao and Jin (2014) have shown that maternal stress or the cumulative effects of recurrent stress influence preterm birth risk and poor health outcomes across three generations. It was demonstrated that prenatal stress is able to program fetal brain development, function of hypothalamic-pituitary-adrenal axis and mental health (Cottrell and Seckl 2009, Raikkonen *et al.* 2012). Molecular mechanisms of early life stress and glucocorticoids could include epigenetic changes mainly through methylation patterns of glucocorticoid receptor (de Rooij *et al.* 2011). In addition to stress condition, nutritional stressors (e.g. high salt, high fat, different chemicals as preservatives, etc.) can also promote the epigenetic transgenerational inheritance and abnormal development of organism and thus the onset of disease (Dunn and Bale 2011, Waterland 2014). Dunn and Bale (2009) reported that maternal high-fat diet exposure in mice resulted in the increase in body size and reduced insulin sensitivity that persisted across two generations via both maternal and paternal lineages. However, since the first generation's primordial germ cells may be affected by gestational exposure, so that the analysis of phenotype transmission into a third generation (F3) is necessary to determine whether stable epigenetic programming has occurred. Therefore, body size and insulin sensitivity of male and female F3 offspring were

determined. It was found that only females displayed the increased body size phenotype and this effect was only transmitted via the paternal lineage. The finding of a paternally transmitted phenotype to F3 female offspring supports a stable germline-based transgenerational mode of inheritance. The authors hypothesized that imprinted genes may be involved in this epigenetic programming (Dunn and Bale 2009).

In principle, there are two critical stages in the development of germ cells where epigenetic programming occurs (Messerschmidt *et al.* 2014). These are pre-implantation embryos and primordial germ cells, the precursors for oocytes and spermatozoa (Saitou *et al.* 2012). If epigenetic information is transgenerationally inherited through the germ cells, then the altered epigenetic marks should be observed. However, transgenerational epigenetic inheritance also has the potential to be adaptive and, in some cases, might even respond to the environmental challenges with major implications for heredity, breeding, and evolution. Thus, some critical developmental periods (Zicha and Kuneš 1999) could also play the important role in epigenetic inheritance. Such a critical window for the exposure to hormonal factors, which promote the epigenetic transgenerational phenotype, is gonadal sex determination, which is 6-18 weeks of gestation in humans. Thus the women in the first half of pregnancy would be the population most sensitive to environmentally induced epigenetic transgenerational inheritance. Therefore, the exact age periods for the influence of particular environmental factors eliciting epigenetic transgenerational inheritance remain to be investigated. It was demonstrated that lignans and flavonols (dietary phytoestrogens) are found at high concentrations in the Western diet. The highest flavonols consumption was associated with later breast development suggesting that this consumption may be associated with reproductive end points (Mervish *et al.* 2013).

As already mentioned, environmental noxious factors could be the important players in sensitizing organism to disease development. Several studies analyzed the effect of vinclozolin (i.e. fungicide with anti-androgenic activity) in the late embryonic or early postnatal period on sexual differentiation, gonad formation and reproductive functions in F1-F4 generations (Chang *et al.* 2006, Anway *et al.* 2008, Nilsson *et al.* 2008). When the pregnant rats were treated with vinclozolin on days 8-14 of gestation, i.e. on the

days including the time of embryonic gonadal sex determination, it was demonstrated that some F1-F3 vinclozolin animals had serious abnormalities late in their own pregnancies (Nilsson *et al.* 2008). Interestingly, there are some discrepant results how adult onset diseases can be propagated to the next generation. In the study of Anway *et al.* (2006), this transgenerational spermatogenic defect phenotype (anemia) was transmitted to the next

generation if vinclozolin males were mated with control females and not *vice versa*. On the contrary, Nilsson *et al.* (2008) demonstrated that both female and male rats from vinclozolin-exposed lines can be the transmitters of anemia phenotype. It is clear that further studies are needed and that transgenerational epigenetic phenotype of adult onset disease induction may be different in various diseases.

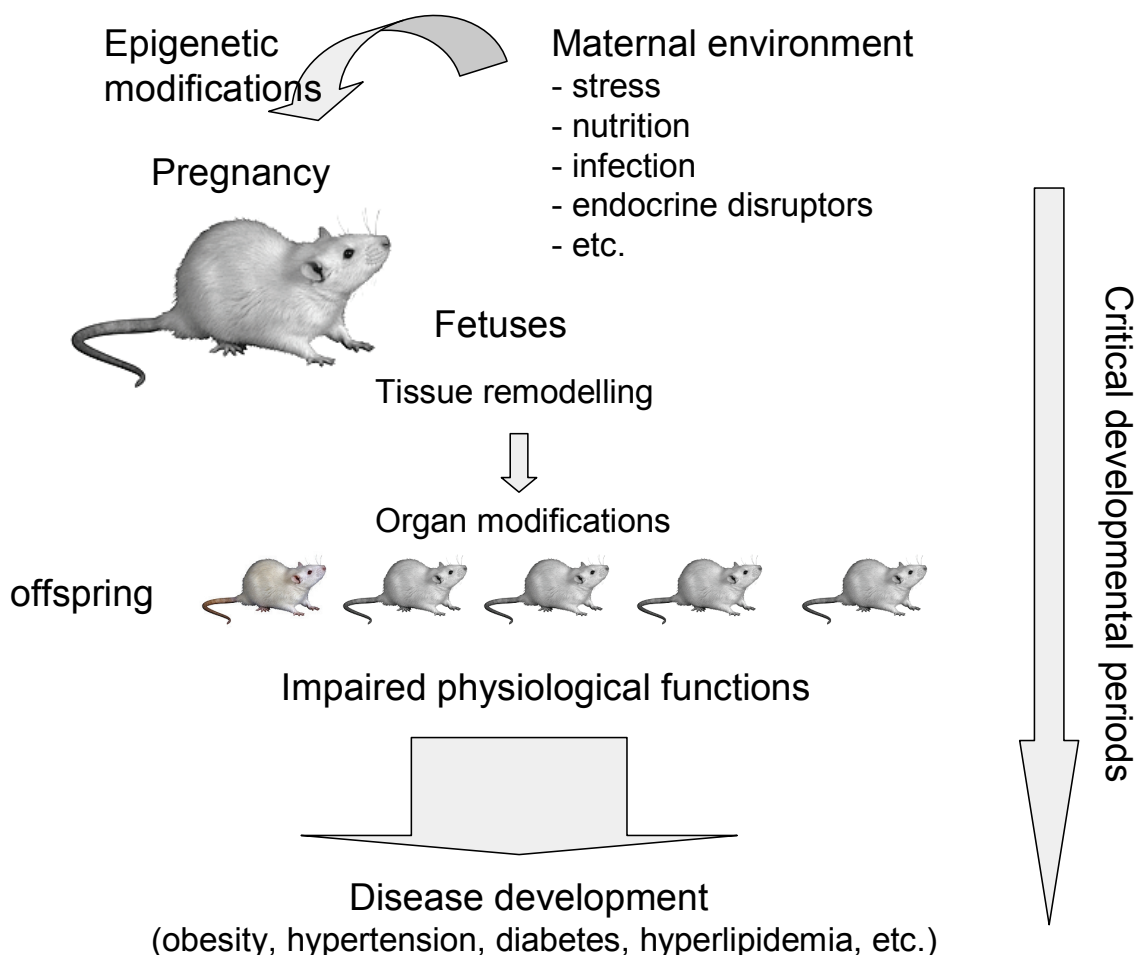


Fig. 1. The effect of environmental factors on neonatal origin of disease. Variation in maternal environment has the impact on embryo and fetus development through tissues and organ modifications.

Epigenetics and metabolic syndrome

According to the “programming hypothesis”, the impairment of intrauterine environment by many environmental factors deprives the fetus of its optimal development leading to the cardiovascular complications in later life (Barker and Osmond 1988, Huxley *et al.* 2000, de Boo and Harding 2006, Eriksson 2006). However, this fetal programming hypothesis in relation to

adult disease is still controversial regarding the cause and mechanisms underlying this phenomenon. A general scheme expects the changes in maternal intrauterine environment leading to the tissue remodeling in fetuses and the impairment of physiological functions (Fig. 1). For example, the changes in maternal nutrition (under- or over-nutrition) are leading to abnormal fetal nutrition, which directly or indirectly influence growth and maturation of various organs (Kuneš and Zicha 2009).

The “thrifty” phenotype hypothesis proposes that humans poor nutrition in the early life results in poor fetal growth and increased risk of metabolic syndrome and cardiovascular diseases (Vaag *et al.* 2012, Hales and Barker 2013). The offspring of laboratory rats in which maternal nutrition was restricted during early life exhibit many correlates with the situation in humans (Bertram *et al.* 2001). One of the most cited example of maternal malnutrition in humans is a cohort of so-called “Dutch Famine” (Tobi *et al.* 2014, Tobi *et al.* 2012). Individuals who were prenatally exposed to famine during 1944-1945, when the caloric intake from protein, fat and carbohydrate was reduced, had less DNA methylation of the imprinted *Igf2* gene compared with their unexposed sex-matched siblings. A higher incidence of chronic somatic diseases, such as obesity (Ravelli *et al.* 1999), glucose intolerance (Ravelli *et al.* 1998), hypertension (Roseboom *et al.* 1999) and other, was demonstrated in these babies in the later life. However, it was demonstrated that the timing of malnutrition challenges during the development determines the magnitude of offspring’s metabolic and cardiovascular phenotype. The exposure to Dutch Famine during mid- and late-gestation period reduced birth weight and glucose tolerance in adulthood which was worsened with obesity (Ravelli *et al.* 1998). On the other hand, early gestation famine exposure did not affect birth weight but increased coronary heart disease prevalence and atherogenic risk (Painter *et al.* 2006). These results suggested that critical developmental period theory (Kuneš and Zicha 2006) should be taken in to account even in epigenetic changes. In sheep, it was demonstrated that the undernutrition either during the first month of gestation or immediately after weaning induces sex-specific changes in metabolic and cardiovascular systems in adulthood (Poore *et al.* 2014). Moreover, the results suggested that epigenetic changes were tissue-dependent.

Other situation occurred when over-nutrition was applied during pregnancy. In the rat, a maternal high-fat diet (HFD) exposure increased body weight and elevated plasma insulin, glucose and triglyceride levels (Srinivasan *et al.* 2006). In addition, HFD alters the quality of milk, in which protein, cholesterol and triglyceride levels were increased (Franco *et al.* 2012) as well as leptin concentration (Sun *et al.* 2012), suggesting the effect on offspring obesity. The similar effects can be seen in the mouse (Ashino *et al.* 2012, Dunn and Bale 2009). Thus, both maternal obesity and malnutrition predispose offspring to develop a metabolic syndrome. If

any changes are transferred to subsequent generation(s), a vicious cycle is running up. Gallou-Kabani *et al.* (2010) fed pregnant mice with high-fat diet from 0.5 to 15.5 days *post coitum*. They found that this regimen triggered the deregulation of imprinted genes among which the *Igf2r* cluster was particularly significant. They analyzed the DNA methylation on the imprinting control region of this locus and they found that it was sex- and diet-specific. In mice it was also demonstrated that the intrauterine exposure to maternal apoE-deficiency can play a key role in the susceptibility for neointimal lesion formation in the adult offspring (Alkemade *et al.* 2010) and that subsequent postnatal induction of hypercholesterolemia induces epigenetic changes that modulate gene expression pattern of the vasculature. The results have shown that both *in utero* programming and diet-induced hypercholesterolemia affect histone methylation modifications and expression of accompanying lysine methyltransferases in vascular endothelial and smooth muscle cells. Differences in histone triple-methylation modifications in these cells revealed that the offspring from apoE^{-/-} mothers had significantly different responses to a high cholesterol diet when compared with offspring from wild-type mothers (Alkemade *et al.* 2010).

Conclusions

Metabolic syndrome with its particular symptoms contributes to mortality and morbidity on cardiovascular diseases. Moreover, we are afraid that the situation is not improving but rather is rapidly worsening due to our progressive lifestyle changes, e.g. increased stress, decreased physical activity, increased incidence of obesity, endocrine disruptors and other negative factors. This growing global problem needs to find simple strategies to identify individuals and populations that are most at risk. Even after several decades of intensive research the etiology of particular symptoms of metabolic syndrome is still not clearly known. The problem is mainly with essential hypertension and Type-2 diabetes which are diagnosed too late due to their discrete development. Thus, the disease is treated but it is not prevented. It is still the problem to specify a single unifying pathophysiological marker for all symptoms of metabolic syndrome, which could be used for a prompt diagnosis. The main complication is that these symptoms are polygenic traits with multifactorial etiology, involving gene-gene and gene-environment interactions and epigenetics.

There is no doubt that the early life, such as the fetal and perinatal periods, is critical for metabolic syndrome development and therefore critical for the prevention of this disease. However, critical developmental periods for some symptoms of metabolic syndrome (hypertension, obesity, Type-2 diabetes) were precisely recognized only in the experimental animals but still not fully in humans (Kuneš *et al.* 2012). The hypothesis of the fetal origins of adult diseases in humans, so called “fetal programming”, was put forward by Barker and coworkers (Barker and Osmond 1988, Barker 2000), who suggested that environmental factors, mainly nutrition, can lead to permanent metabolic and structural changes in the fetus and thus increasing the risk of many diseases in adulthood. If we want to use such information for the prevention of the disease such as metabolic syndrome, we need to progress beyond epidemiological associations to greater understanding of the underlying cellular and molecular processes. The knowledge of molecular mechanisms, by which early minor environmental stimuli modify the expression of genetic information, might be the desired key for the understanding of mechanisms leading to the change of phenotype in adulthood (Kuneš and Zicha 2006).

Recently, it was clearly recognized that epigenetic influences are very important in the formation of phenotypes and that these effects could be under certain conditions transgenerationally transmitted (Guerrero-Bosagna and Skinner 2014). This is in the case when environmental factors promote permanent changes

of the gamete epigenome. It is true that phenotypic changes cannot be explained solely by the changes in DNA sequence. It was demonstrated that gene-environmental interactions (e.g. nutrient exposure and genetic background) can have a dramatic impact on the development of the metabolic syndrome. However, it is evident that we are just at the beginning of understanding the substantial contributions of epigenetics to human diseases including metabolic syndrome. The assumption that the clarification of the human, rat and mice genome will reveal the causes of the most diseases was false. It is evident that especially in complex traits it is not so easy to find the responsible cause(s). This is true for all symptoms of metabolic syndrome and other social important diseases. We believe that numerous small changes, rather than big ones, throughout the complicated system of regulations will probably play a significant role. These changes might be related to the modifications at the level of genes, proteins, enzymes, etc., and thus contribute to the disease development.

Conflict of Interest

There is no conflict of interest.

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