Nature, nurture and the perinatal environment in Syndrome X etiology

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Abstract

Nature and nurture are popular and well known concepts in both popular culture and science. The debate between nature and nurture proponents about which of the two is the most important originator of phenotypes has been silent for decades. Instead, a compromise has been reached where everyone has agreed that most if not all phenotypes originate from a combination of nature and nurture influences. In recent years, considerable scientific evidence suggests that during a specific timeframe in very early life, nature and nurture interact with each other by epigenetic mechanisms. Nurture effects on the mother are shown to cause dramatically differing phenotypes in offspring. This paper proposes a model that incorporates the perinatal environment as a bridge between nature and nurture. It will be argued that this model is a better representation of reality than the existing non-dualistic model. The paper will focus on the ways changes in the perinatal environment affect the etiology of Syndrome X. With syndrome X prevalence rapidly rising, attempts to better understand its causes are very important. The first chapter will better define nature and nurture in *traits* and *states* and introduce the nature – PNE – nurture model. The mechanisms of epigenetic changes in gene expression will be examined thereafter. The paper concludes with a chapter providing insight into specific sites of DNA methylation (an important epigenetic mechanism) that have been associated with syndrome X etiology.

Keywords: nature, nurture, syndrome X, metabolic syndrome, epigenetics, DNA methylation

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1. Introduction

"To say that obesity is caused by merely consuming too many calories is like saying that the only cause of the American Revolution was the Boston Tea Party" (Adelle Davis)

The quest to understand why we are the way we are; the "core-business" of both biology and psychology. At its junction, the nature versus nurture debate is found. Most laymen would call it a stand-off, as most newspapers even do today. In scientific circles there has for long been a reigning "cease fire" paradigm. The sheer amount of possible causes for disease, behavior, intelligence and appearance is mind boggling and interdependent effects are almost certain to play a role. Therefore, it was decided, every phenotype is part nature and part nurture but to what extend it is the one or the other is impossible to compute. Technology helps advance knowledge, and releases vast amounts of new data to analyze and try to find meaning in. Since the sequencing of the complete human genome, renewed faith has been put in genetics as the most important explanation for phenotype creation. In various cases, new proof has been found that physiological or psychological *states* of an organism previously thought to be caused by environmental effects originate in specific genes. It is increasingly evident however that in many cases neither the DNA sequence, nor the effect of the environment can explain the generation of a phenotype. Most importantly, it seems that changes brought about in an individual during life by its environment can be inherited by their offspring. Clearly, in this situation even the theory that every trait is a result of combined effects of genes and the environment fails to be true, as the environment *changes* the genes. The time period where this bridge between nature and nurture occurs mostly is during a period very early in life, from fertilization up to a couple of months after birth. This period is known as the perinatal period and the environment to which a fetus is subjected during that time appears to be critical for the development of phenotypes later in life.

In this paper it will be argued that the perinatal environment (PNE) can be said to function as a phenotype mediator situated in between nature and nurture because scientific evidence increasingly suggests that mother's nurture affects offspring's nature during the critical perinatal period. To do so, the paper will be subdivided as follows. Firstly, current definitions for nature and nurture are flawed and inconsequent. The first question is thus, how are nature and nurture defined? How should they be? After that the PNE is introduced as a third phenotype mediator in a conceptual model consisting of nature, PNE and nurture. While this model is a suitable frame for many phenotypes, the scope of this paper will be limited to syndrome X. Perinatal effects on

Syndrome X development are among the best researched and most relevant today. Obesity is an ever growing problem in today's society. What if it could be shown that some simple diet supplements during pregnancy can help to protect children from developing obesity? That would be a groundbreaking discovery. It is common knowledge that alcohol or drug use during pregnancy exerts harmful effects on offspring, but what if the same could be said about foodstuffs with less apparent adverse effects? This paper will propose a model wherein the changes in gene expression acquired during the perinatal period serve as a bridge to unite nature and nurture.

2. Nature and Nurture

This chapter will outline the evolution of the distinction between 'nature' and 'nurture', the problems with ascribing phenotypes to these concepts and provide reasoning to the definitions of both concepts that will be used in this paper.

The origin of nature and nurture can be traced all the way back to the *tabula rasa* (clean slate) idea postulated by Aristotle (De Anima, Book 3, chapter 4). He argued that man was born with a 'clean slate', or blank mind and that all knowledge and skill was acquired during life. This was in disagreement with his mentor's postulation that man was born with all (divine) knowledge 'wired into' the soul. When something was learned, one did not actually learn a new fact, but was merely reminded of its existence (Plato, Pheadrus). Thousands of years later, the debate whether disease, appearance and skill was predetermined before birth, or acquired after became known as the 'nature nurture controversy'. The controversy was largely fought out during the first half of the 20th century. Nature proponents based themselves on Mendel's famous experiments on heredity in plants whereas 'behaviorism' as pioneered by, among others, Pavlov and his dogs was the most important case favoring the nurture theory. It is important to note however, that the scientific community has mostly agreed that both nature and nurture are important drivers of phenotype. The debated issue was mainly *to what extend* complex phenotypes can be attributed to nature, and to what extend to nurture. Still, the distinction between the two gave rise to a wide variety of research attempting to link genes to traits such as intelligence, social success and even aggression and homosexuality by nature proponents and theories explaining all these things through sociological experience by nurture proponents. Some nature proponents even went as far as writing:

> *"How much could we increase the general level of health and the average IQ of the next generation of children by denying parenthood to the one per cent or five per cent or ten per cent*

> *of those who are most apt to pass on physical or mental disabilities? Suppose parenthood were denied to all individuals failing to achieve a mental age of eight or ten or twelve years, how much of the improvement in the average IQ of the next generation should be attributed to the increase in native intellectual endowments, how much to the increase in the quality of home care and intellectual stimulation which is a by-product of denying parenthood to the less intelligent, and how much to the joint contribution of better endowments and better care?" (Shuttleworth, 1935)*

and even:

> *"What is the best way of educating the public to the desirability of segregating or sterilizing the one per cent or five per cent or ten per cent of the least fit" (same article)*

This type of reasoning is easily arrived at by theorizing from a strictly nature point of view. The obvious ethical problems with these theories may have made the nurture point of view more readily acceptable by the public. Indeed it has been argued that the tendency to find nurture explanations of traits like learning ability and aggression more likely to be true is based largely on political grounds (Butler, 1995).

2.1.2. Difficulties in determining causality

It is not too difficult to find genes that statistically correlate with the presentation of a certain phenotype, nor is it difficult to link environmental differences to phenotype development (Mossé, 2008) (Kelley, 1926). Few correlations however, are very strong (Plomin, 1994). It is interesting to review some basic and well known examples to establish a clear view of the problems encountered in correlating cause (nature / nurture) with effect.

It is well proven that Down's syndrome is caused by an extra copy of chromosome 21 (Avgidou, 2005). Similarly, Sickle-cell Anemia is certainly the result of certain mutations in the gene encoding Hemoglobin (Galloway, 1988). These are thus clearly nature driven phenotypes. On the other hand, heavy exposure to loud noise produces hearing impairment regardless of genetic makeup. Clearly an entirely nurture cause. In most cases it is not that simple. Smoking tobacco for example, is known to correlate strongly with lung cancer. However, not all smokers get lung cancer[1] and not every case of lung-cancer can be linked to above average exposure to tobacco smoke during life. This suggests that some smokers are more vulnerable to develop lung cancer than others. It is possible that this is caused by nature differences, but it could just as easily be explained by unknown environmental effects (pollution, earlier disease, an unnoticed viral infection etc). A clear example of a phenotype in which both nature and nurture play a role is Parkinson's disease (PD). A 1999 study by the American Medical Association showed that in monozygous twins, PD was present in both siblings 100 percent of the time

[1] Even if they live as long as the non-smokers

when the first sibling was diagnosed with PD before the age of 50. If the first diagnosis was made at an age of over 50, PD was only present in the other sibling in 10 percent of cases (Tanner et al., 1999). Moreover, the same study showed that in heterozygous twins, this large difference does not exist. Instead, the probability of finding PD in both siblings was 16 if the first sibling was diagnosed under 50 and 10 percent when the first diagnosis was made after the age of 50. This shows that PD is a genetically inheritable disease, and that when the inherited form is present, it will manifest before the age of 50. It also shows that besides being inheritable, PD can also be caused by environmental factors. It has already been proved that the disease can actually be caused by environmental factors *alone.* As shown by the existence of chemical compounds that recreate most or all of the characteristics of Parkinson's disease (Tanner, 1999) (Matsui, 2009). Thus it is established that a given phenotype can manifest due to nature or nurture causes. It is not surprising that in most cases a phenotype is determined by a combination of both nature *and* nurture. This non-dualistic view, where both nature and nurture share responsibility for phenotype establishment has been the reigning paradigm for the last 50 or 60 years. Unsurprisingly though, with multiple full human DNA sequences available today, there has been a renewed interest in nature explanations for complex phenotypes. Indeed, it has been possible in recent years to find genetic factors underlying several complex, behavioral phenotypes. Examples include a *"genetically determined . . . deficit in learning from error."* (Klein, 2007) and the invariable finding of loss-of-function mutations in highly anti-social persons (Med Sci (Paris), 2007).

2.1.3. Definitions of the terms nature and nurture

Different definitions for nature and nurture have been coined over the years. Definitions depend on which branch of science is involved and what type of cause and effect is investigated. Surprisingly, there is not much literature devoted to accurately delimitating nature and nurture. The distinction differs and is somewhat arbitrary. The most common and simple definition is to limit nature to genetics and nurture to environmental influences (Petty, 2009). An alternative definition is to assume nature to signify physiological and nurture to consist of sociological influences. Both views have important limitations. Limiting nature to genetics is problematic for an obvious reason. An important step in the forming of an animal's DNA sequence is the recombination process where paternal and maternal chromosomes recombine to form a new DNA sequence, similar to, but distinct of those of either parent. DNA recombination requires proteins and enzymes to function (Alberts et al., 2002 p 1130 - 1135). Environmental effects on

the pre-embryo may affect the concentrations of these compounds and thus influence the DNA sequence and hence, nature, of the offspring. In other words, nurture effects on the mother may cause changes in the nature of the offspring, thus blurring the line between the two (figure 1). Moreover, overwhelming evidence suggests that the gene expression pattern of offspring's DNA (nature) can be permanently altered by environmental factors up to a certain moment post birth (Simmons, 2008) (Jónás, 2009) (Plagemann, 2005). Furthermore, changes in the *epigenome* acquired by nurture effects during life can be transferred to offspring[2], in particular during the perinatal period.

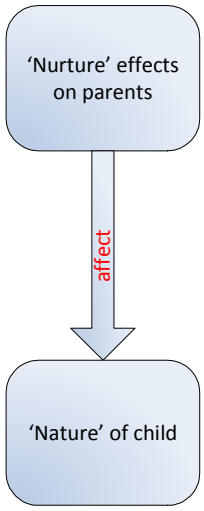


Figure 1

Assuming nature as all physiological, and nurture as all sociological influences poses a problem as well. This would mean that the physiological process of DNA damage by ionizing radiation would need to be classified as a nature influence. This is clearly wrong, as exposure to radiation has nothing to do with the 'innate' quality, or nature, of an animal. Moreover, the DNA changes caused by radiation (or chemical mutagens) are only present in the affected cells and not in the gametes. They are thus not transferred to offspring. It must therefore be concluded that available definitions of nature and nurture are not watertight.

2.1.4. Solving the definition problem

> *"…Some diseases cannot be solely attributable to genetic or environmental continuity or change in the past few decades thus it has become clear that the health and general physiology of people can be affected not only by the interplay of their own genes and conditions of life, but also by the inherited effects of the interplay of genes and environment in their ancestors." (Jablonka, 2004)*

If the problem with the genetics versus the environment explanation is examined, it is evident that the problem only exists in the period between conception and a limited time after birth. The reason for this, is that only then the line between environment and genetics is blurred by the fact that epigenetic changes in gene expression are heritable and acquired for life (thus, incorporated in the nature of the offspring). This timeframe is known as the perinatal period. The environment during the perinatal period can have permanent effects on the nature of offspring. This leads to the conclusion that the problem defining nature and nurture can be solved by introducing the 'perinatal environment' (PNE) as a third mediator of offspring

[2] This is explained in the next chapter on perinatal environmental effects

phenotypes. In unpublished work, G. van Dijk discusses the distinction between 'State' and 'Trait'. *"State phenomena are generally considered a consequence of the disease. For example, severe food restriction by the anorexia patients will lead to reduced thermogenesis, amenorrhea, cardiovascular activity etc. (Bergh and Sodersten, 1996a)."* (Van Dijk). Importantly, the state is reproduced in fasting healthy individuals showing that it does not result from a genetic factor but that instead it is a consequence of a behavioral pattern.

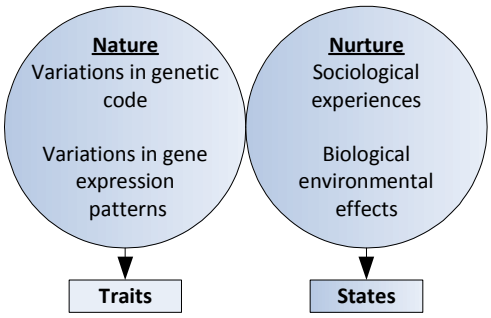
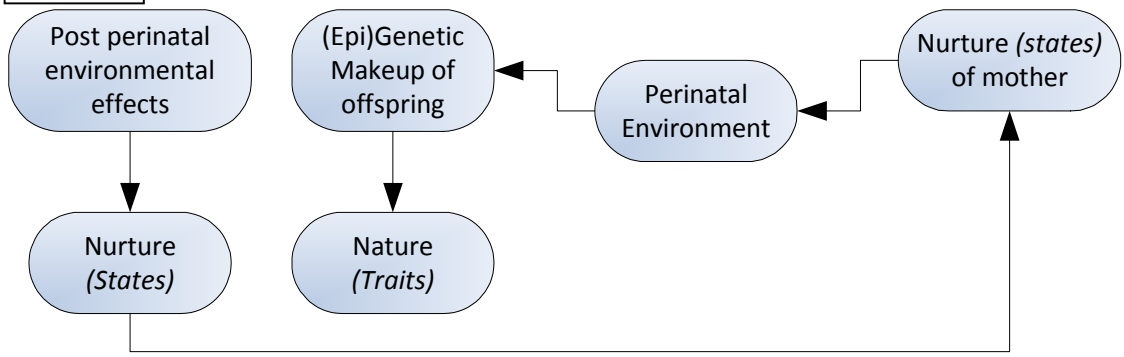


Figure 2

Traits are defined as: "*…..trait…. may reflect a heritable genetic variation leading to the phenotype..*" (Van Dijk). If state is extended to include all physiological consequences of behavior and environmental conditions, a suitable definition of nurture is arrived at. Traits, on the other hand are a good definition of nature, when variations in gene expression due to epigenetic changes sustained during the perinatal period are included. The model that is arrived at thus defines nature as organism traits, and nurture as organism states (figure 2). Phenotype variation stems from genetics (nature), environmental (nurture) influences and from the changes in nature that can be caused by nurture effects during the critical perinatal period (the PNE). The model thus consists of three parts; nature – PNE – nurture. The drawing below conceptualizes this model.

Figure 3



The following chapters will explore the evidence supporting the addition of the PNE as a bridge between nature and nurture, in particular with respect to syndrome X.

3. Effects of the perinatal environment

3.1. Epigenetics

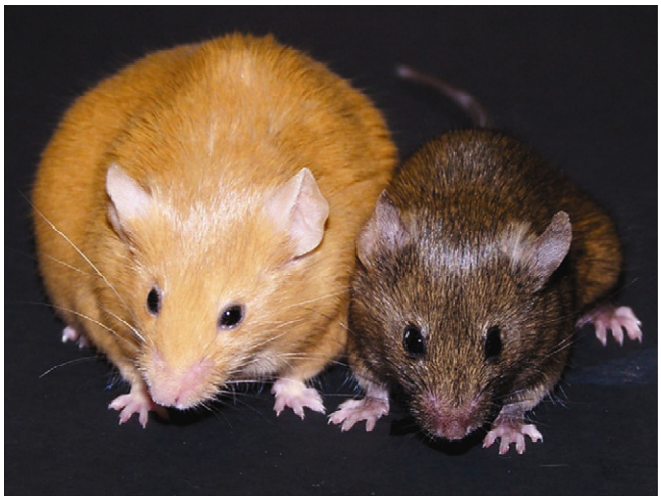


Figure 4

The preceding chapter made mention of the fact that the perinatal environment is able to change the patterns of gene expression in offspring. The most important cause for this is *epigenetics.* Epigenetics studies *heritable* changes in gene expression without changes in the DNA sequence itself (Dolinoy, 2007) (Tamashiro, 2010). It includes among other things DNA methylation and changes in Chromatin packaging. Dolinoy puts it like this: *"Therefore, if the genome is compared to the hardware in a computer, the epigenome is the software that directs the computer's operation*." (Dolinoy, 2007). The importance of differential DNA expression has of course been widely known with regard to cell differentiation. The idea that organism wide expression differences can result in wildly different phenotypes is quite new however. Figure 4 shows the impressive effect epigenetics can have on phenotypes. The two mice are *genetically identical* yellow Agouti mice. The dramatic difference in appearance is caused by supplementation of the brown mouse's mother's diet with methyl donors like folic acid. Besides changing the mouse's color, this also *"reduces the incidence of obesity, diabetes and cancer"* (Dolinoy, 2007). If maternal diet can exert such a profound effect on such complex offspring traits as propensity for obesity, diabetes and cancer, imagine how many other important traits may be influenced by maternal states during pregnancy.

Another interesting example is the way honeybees "produce" a new queen, should the old one be killed or weakened (Kim, 2009). Worker bee larvae are fed a different diet than the destined queen larvae. This produces a wide variety of differences in gene expression causing the large difference in appearance, behavior and reproductive capability between worker bees and queens (Kim, 2009). Lastly, a study on data obtained from humans born during the Dutch hunger winter of 1944-45 showed marked differences in their DNA methylation pattern compared to their unexposed, same sex siblings (Heijmans, 2008).

3.1.2. Mechanisms

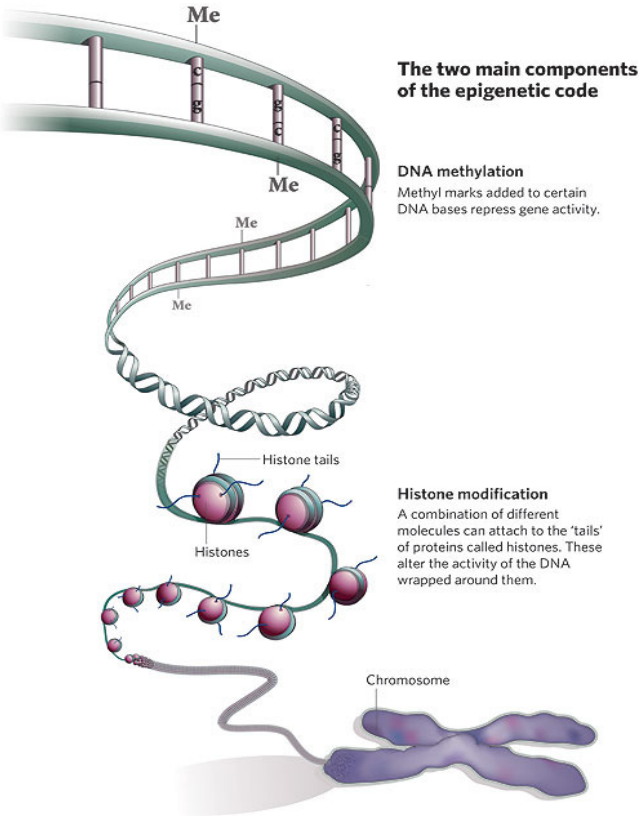


Figure 5, source: http://www.nature.com/nature/journal/v441/n7090/images/441143a-i2.0.jpg

Multiple epigenetic mechanisms are known today. The most important are DNA methylation and Chromatin Packaging (most importantly histone modification) (Dolinoy, 2007) (Gicquel, 2008). DNA methylation occurs at CpG nucleotides. These are simply locations within the DNA were a C nucleotide is followed by a G nucleotide (p is short for the connecting phosphate). The Cytosine ring of the C nucleotide is enzymatically methylated by the transfer of a methyl group from S-Adenosylmethionine (SAM) (Dolinoy, 2007) (Hogarth, 2008). The resulting 5-methylcytosine is distinct from the four unmethylated bases and thus is said to function like a "fifth base" (Dolinoy, 2007). The methyl group is situated in the major groove of the DNA double helix and functions to block access to the DNA by transcription factors. The pattern of DNA methylation is therefore an important modulator of gene expression and can have profound effects on an animal's phenotype. What is particularly interesting is that altered patterns of DNA methylation appear to be heritable, sometimes even skipping a generation (Gicquel, 2008) (Dolinoy, 2007) (Kim, 2009) (Pembrey, 2006).

Chromatin packaging is the coiling of DNA around histone complexes. Several histone species have been discovered and their modification can result in gene silencing or gene activation. Apparently, Histone acetylation is primarily associated with gene activation whereas Histone methylation is associated with gene silencing. The resulting "Histone code" is very complex, because each Histone has multiple Lysine residues in its amino acid sequence and each of these lysines can be mono-, di-, or trimethylated (Dolinoy, 2007).

3.2. The importance of the perinatal period

> *"Nevertheless, it* [the epigenome] *is most vulnerable to environmental factors during embryogenesis because the DNA synthetic rate is high, and the elaborate DNA methylation patterning and chromatin structure required for normal tissue development is established during early development." (Dolinoy, 2007)*

This quote contains the most important reasons for the significance of the perinatal period for DNA expression pattern change. One of the first and most influential theories linking the perinatal environment to later phenotype is what became known as the "Barker hypothesis". Barker postulated that the level of maternal nutrition during the perinatal period causes adaptational changes in the fetus. The offspring is prepared for a life with a similar level of nutrition availability as that during its first growth in the womb (Barker, 1997). It is theorized that adaptation puts offspring at risk of developing disease later in life if the perinatal environment does not correspond to the postnatal environment (Gicquel, 2008). This might explain the apparent link between under nutrition during the perinatal period and susceptibility to develop obesity later in life. The reasoning is that under nutrition programs the child to be more energy efficient and thus logically accumulate energy when normal or high nutrition is provided (Barker, 1992 & 1997) (Eisenmann, 2006). It is very well possible that changes in DNA methylation patterns brought about by maternal nutrition conditions during the perinatal period underlie this adaptation effect (Eisenmann, 2006).

Other studies link perinatal environmental effects with changes in neuromotor competence (Darbra, 2003), testicular cancer (Zhang, 2007), lung structure and function (Wright, 2010) and expression levels of complexins (Zink, 2009). Perinatal effects of under- and over nutrition have certainly been the most investigated. Moreover, with Syndrome X rising to be one of the most deadly and prevalent diseases in the developed world, establishing which perinatal conditions promote it is very important. The next chapter is therefore devoted to applying this thesis' model to syndrome X.

4. Nature and nurture influences on syndrome X

This chapter will start with a brief description of syndrome X. thereafter, studies on the etiology of syndrome X will be placed in the context of the conceptual model.

4.1.1. Syndrome X

Syndrome X, also known as the Metabolic Syndrome or insulin resistance syndrome is a collection of states that makes the affected individual vulnerable for cardiovascular disease and diabetes (Misra, 2007) (Guize, 2008) (Medline Plus). The most important states underlying Syndrome X are insulin resistance, inflammation, obesity and lipotoxicity (Guize, 2008). Basically, Syndrome X is the name given to the collective of the most frequent health problems encountered by obese persons. Clinically, it is defined as:

> *"The AHA/NHLBI 2005 [13] definition is derived from theNCEP—ATP III 2001 definition [12] and requires at least three of the following criteria to be present:*
>
> *• Waist circumference greater than or equal to 102 cm in men and greater or equal to 88 cm in women (W);*
>
> *• Triglycerides greater than or equal to 1.50 g/L or a specific treatment for elevated triglycerides (TG);*
>
> *• high density lipoprotein (HDL) cholesterol less than 0.40 g/L in men and less than 0.50 g/L in women or a specific treatment for reduced HDL cholesterol;*
>
> *• Systolic BP greater than or equal to 130mmHg or diastolic BP greater than or equal to 85mmHg or antihypertensive treatment (BP);*
>
> *• Fasting glucose greater than or equal to 1.00 g/L or drug treatment for elevated glucose (G)." (Guize, 2008)*

4.1.2. States and traits in Syndrome X associated symptoms

Since the Barker Hypotheses, many studies have shown links between PNE factors and obesity and/or diabetes type 2 later in life. In this paragraph the outcomes of some of these studies will be tested against the nature – PNE – nurture model. It will be shown that this model serves as a good explanation for the interaction of the states and traits involved in syndrome X etiology. Studies in mice selected for high voluntary wheel running behavior show that maternal nutrition factors during the perinatal period can cause differences in offspring's energy homeostasis (Jonas, 2009). It was shown that feeding mothers a high fat diet in the perinatal period caused *"increased longitudinal growth, higher adipose tissue mass and elevated insulin and low adiponectin levels."* In the control animals (Jonas, 2009). In contrast, the selected mice's offspring was shown to be resistant to these effects, in fact even responding to the HF perinatal environment with *reduced* insulin levels and higher levels of adiponectin (Jonas, 2009). The observations in the control mice are very easily explained from the model used in this paper.

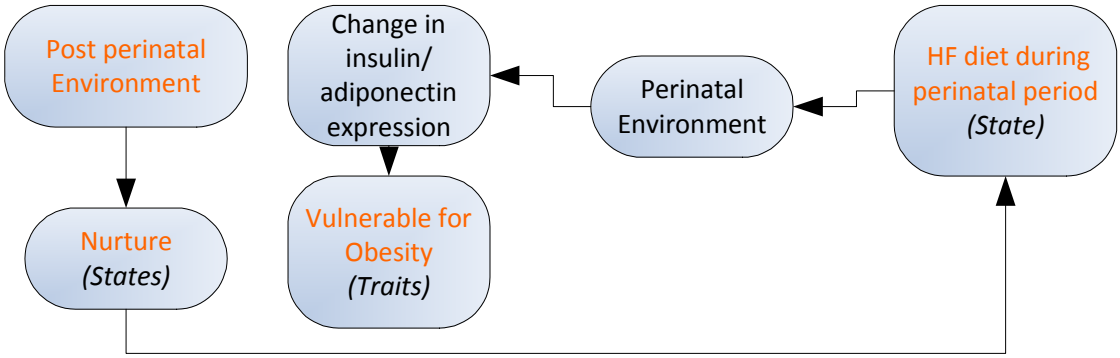


Figure 6

The drawing shows the model for this particular case. The high fat diet fed to the mother during gestation (high fat consumption *state)* creates a perinatal environment that changes the baseline expression levels of important mediators of energy homeostasis (insulin and adiponectin). This change in gene expression causes a *trait* of susceptibility to obesity in the offspring. We may speculate that this has contributed to the fast rise of obesity in humans the last decades.

The Barker hypothesis supposes another route to developing obesity susceptibility. In this case, low birth weight is associated with obesity in later life (Simmons, 2008) (Barker, 1992). It is theorized that *under*nutrition during the perinatal period programs the fetus to be more energy efficient in order to survive under conditions of food scarcity (Orozco-Solis, 2009) (Plagemann, 2005). This is quite a convincing theory because it is likely that such a system would exist for evolutionary purposes. Children born in environments with low nutrient availability would benefit from energy efficient 'power plants' throughout life, as long as the nutrient availability does not change. These children have a tendency to overconsume when nutrients are abundant. Overconsumption that, obviously, leads to obesity. This is known as the 'thrifty phenotype' (Barker, 1992). This would easily fit into the model as well, but gives rise to the paradoxical situation that both over- and undernutrition in the perinatal period cause susceptibility towards Syndrome X in offspring. Several studies have provided data to solve this paradox. It was shown in 1970 that early fetal under nutrition programs offspring for obesity, whereas late fetal underfeeding does not (Ravelli, 1970). At the same time, studies in rats have shown that late perinatal (early post-natal) overfeeding causes obesity in later life as well. It is possible that the Barker observation that low birth-weight correlates with obesity in later life is caused by

compensatory 'catch-up' feeding of underweight babies (thus over nutrition during the late perinatal period). This was confirmed by large epidemiological studies (Stettler, 2002). Stettler showed that later life obesity correlated strongly with rapid neonatal weight gain, independent of birth weight and weight at age 1 (Stettler, 2002) (Plagemann, 2005). The final observation in this chapter that needs to be explained is the fact that in the Jonas Study mice selected for high voluntary wheel running showed resistance to perinatally induced obesity. It is most likely that genetics are responsible for this difference. In fact, it has been shown that changes in the gene *for peroxisome proliferator-activated receptor-gamma 2 isoform* (PPAR-γ2) can cause a genetic predisposition to develop obesity. Carriers of one allele were resistant to obesity development induced by a high fat diet, whereas carriers of a different allele were not (Andreassi, 2009). While expression of this gene has not been studied in animals that were over- or underfed perinatally, It is a likely hypothesis that genetic predispositions exists that cause vulnerability for the perinatally acquired changes in gene expression that result in increased susceptibility for Syndrome X disease states in adult life.

4.2. Mechanism

4.2.1. Plagemann's mechanism of perinatal programming for Syndrome X susceptibility

An excellent study by Plagemann et al. in 2005 resulted in strong evidence in favor of an epigenetic mechanism for obesity development resulting from elevated levels of perinatal insulin (Plagemann, 2005). The researchers exploited the fact that insulin blocking components of the Blood Brain Barrier (BBB) are not fully developed during early fetal life. Thus, excess insulin can leak into the hypothalamus during the perinatal period. Offspring exposed to high insulin levels during the perinatal period exhibited lifelong hyperinsulinaemia, impaired glucose tolerance, hyperphagia (overeating) and obesity starting as early as 3 weeks after birth (Plagemann, 2005). The really interesting part is that this *perinatally acquired* obesity *trait* was transferred to following generations. The reason is surprisingly obvious. The original offspring was programmed for obesity by perinatal hyperinsulinism. Since these obese mice exhibited hyperinsulinism themselves as a result of the programming, they automatically exposed their offspring to perinatal hyperinsulism as well, thus transmitting the programming down generations (Plagemann, 2005). These findings strongly suggest an epigenetic mechanism for insulin mediated perinatal programming, as changed insulin levels are probably caused by

changes in gene expression[3]. Indeed, Plagemann et al. found convincing evidence that in programmed rats a lasting *"Malorganization of the hypothalamic NPY[4] system"* is present consisting of *"Persistently increased numbers of neurons expressing NPY in the ARC[5]"*. This malorganization seems to result in *"persisting hypothalamic resistance, in terms of increased thresholds, to the circulating satiety signals insulin and leptin…."* (Plagemann, 2005). As stated in paragraph 4.1.1, insulin resistance is one of the defining characteristics of Syndrome X. Plagemann thus convincingly shows that perinatal programming can result in lasting and heritable changes in an animal's trait for syndrome X susceptibility.

4.2.2. DNA methylation sites associated with Syndrome X

Figure 4 showed the dramatic difference in appearance between two genetically identical mice. Remember that the difference was caused by supplementing the brown mouse's mother's diet with methyl donors during the perinatal period. This is one of the strongest cases evidencing the role of epigenetics in syndrome X. The study showed that the changes were associated with methylation at six distinct CpG nucleotides (Dolinoy, 2006). These sites were situated in the promotor region for the murine Agouti gene, a gene encoding a paracrine signaling molecule that promotes production of a yellow pigment in fur. Normally, it is only expressed during a specific period in the hair growth process, thus creating a yellow band in each hair. By insertion of a DNA fragment upstream of the Agouti gene by ways of a retrovirus, a mutant mouse is created that over expresses Agouti. This mouse is characterized by yellow fur, obesity and tumor growth (Dolinoy, 2006). It was shown that diet supplementation of mothers with methyl donating agents (in this case, genistein) silenced the Agouti gene by methylation of CpG nucleotides upstream of the gene (Dolinoy, 2006) (Cooney, 2002). Secondly, a recent study in humans showed that DNA methylation in the promoter region for the TFAM gene is inversely correlated with syndrome X states in adolescents (Gemma, 2010). In this case it is not clear whether the change in methylation pattern has been acquired in the perinatal period or later, but still it provides more evidence that DNA methylation has a starring role in the etiology of syndrome X. Further evidence for epigenetic effects in genes coding for compounds associated with energy metabolism are summarized in the following quote:

[3] But not necessarily of the insulin gene of course
[4] Neuropeptide Y
[5] Arcuate Nucleus

> *"Additional genes involved in energy homeostasis have been found to be regulated by DNA methylation and/or histone modifications. Studies in vitro have demonstrated that DNA methylation regulates the expression of leptin (Melzner, 2002), SOCS3(Stoger, 2008) (Campion, 2009), and glucose transporter (GLUT)-4 (Yokomori, 1999). Examples of in vivo epigenetic regulation include: leptin (Milagro, 2009), peroxisome proliferator-activated receptor (PPAR)-α (Lillycrop, 2005, 2007, 2008), PPAR-γ (Fujiki, 2009), POMC (Newell-Price, 2001), 11β-hydroxysteroid dehydrogenase (HSD)-2 (Alikhani, 2004) (Friso, 2008), and corticotrophin releasing hormone (Mueller, 2008) (McGill, 2006). Thus, epigenetic modulation of multiple genes encoding peptides involved in energy balance has been demonstrated. While the specific factors responsible for diet mediated epigenetic changes remain to be identified, it is reasonable to hypothesize that similar alterations may occur in offspring exposed to high fat diet." (Tamashiro, 2010)*

It is thus clear that in recent years a variety of DNA methylation sites have been linked to syndrome X occurrence. Obviously, genetics play a role as well, and so does the post birth environment. These are the nature and nurture components of the nature-PNE-nurture model. It would seem clear however, that epigenetic changes caused by factors of the PNE are clearly an important third cause of phenotype variation and syndrome X etiology.

5. Conclusion

To conclude this paper, remember the thesis stated in the introduction. *In this paper it will be argued that the perinatal environment can be said to function as a phenotype mediator situated in between nature and nurture because scientific evidence increasingly suggests that mother's nurture affects offspring's nature during the critical perinatal period.* The preceding chapters have first shown the reasoning used to logically arrive at the three part nature – PNE – nurture model. Thereafter the most important mechanisms of epigenetic change have been discussed and the evidence for PNE effects in the etiology and inheritance of syndrome X has been reviewed. It is self evident from the discussed results that the PNE is an important cause for phenotype differences in later life. The effects of maternal diet on the agouti mice (figure 4 & paragraph 4.2.2.) are the most dramatic proof of this. Recently many specific DNA methylation sites have been statistically connected to various phenotype differences, thus supporting the hypothesis that an epigenetic mechanism underlies the perinatally acquired changes in nature. Most importantly, this paper better defines nature and nurture and shows that they can be successfully incorporated in a single model that hitherto complies with all available empirical data. This three part model is more specific and therefore a better reflection of reality than the non-dualistic paradigm that assumes every trait to consist of "some nature and some nurture". Moreover, considering the speed at which new epigenetic influences on phenotype are found, it is about time to give epigenetics due credit by creating room for it in models for development.

6. Discussion

First of all, it is important to realize that this paper *does not* argue that the PNE is the most important phenotype mediator. Genetic variation is still, and will most likely always remain a very important factor as well. The same is true for the environment encountered after the perinatal period. It has already been shown in various studies that epigenetic changes are acquired throughout life[6]. Regarding behavioral phenotypes the sociological environment is certainly an important development variable as well. To what extend the three factors contribute to the final phenotype can only be computed with a certain statistical degree of confidence, and even then there may be unknown unknowns affecting the system that cause type 2 statistical errors that are impossible to detect. Of course, the same is true when linking specific genes to specific traits. This is a scientific problem that always occurs when attempting to confirm a hypothesis through experimental evidence, even, or maybe especially with high fidelity statistical methods. This problem has been described extensively by Karl Popper in his famous work "Conjectures and Refutations". A theory (conjecture) can never be proved to be true; it can only be proved to be false. An unlimited amount of observations that confirms the theory does not prove it to be true, but only one observation is necessary to permanently prove a theory to be false.

The model proposed in this paper is affected by the same problem as well. It is only true as long as it is not refuted. It is the author's opinion however, that technology and the scientific data obtained by using evolving high-tech research methods have caught up with the reigning non-dualistic nature-nurture paradigm. The *persistent* and *heritable* changes in gene expression that are caused by the PNE cannot be sufficiently explained by this paradigm. Its viability has been refuted by empirical evidence because it does not incorporate a critical bridging factor. The effect of mother's nurture on offspring's nature through epigenetic changes by PNE effects were an unknown unknown in the system causing statistical errors that were impossible to detect. The question that only time can answer is how long it will take before a new unknown player is discovered.

[6] Of course the heritability of these is in fact an obvious case where the PNE bridge between nature and nurture is important

7. References

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