Am J Stem Cells 2016;5(1):11-18
www.AJSC.us /ISSN:2160-4150/AJSC0030217

Review Article

Influence of paternal preconception exposures on their offspring: through epigenetics to phenotype

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Received April 10, 2016; Accepted April 28, 2016; Epub May 15, 2016; Published May 30, 2016

Abstract: Historically, research into congenital defects has focused on maternal impacts on the fetal genome during gestation and prenatal periods. However, recent findings have sparked interest in epigenetic alterations of paternal genomes and its effects on offspring. This emergent field focuses on how environmental influences can epigenetically alter gene expression and ultimately change the phenotype and behavior of progeny. There are three primary mechanisms implicated in these changes: DNA methylation, histone modification, and miRNA expression. This paper provides a summary and subsequent review of past research, which highlights the significant impact of environmental factors on paternal germ cells during the lifetime of an individual as well as those of future generations. These findings support the existence of transgenerational epigenetic inheritance of paternal experiences. Specifically, we explore epidemiological and laboratory studies that demonstrate possible links between birth defects and paternal age, environmental factors, and alcohol consumption. Ultimately, our review highlights the clinical importance of these factors as well as the necessity for future research in the field.

Keywords: Transgenerational effects, paternal preconception exposures, epigenetics

Introduction

For much of the previous century, the field of genetics has largely viewed the inheritance of genetic information as uni-directional: from the germ cells to somites but not in reverse. However, this dogma of genetics has recently come under scrutiny. While baseline mutations in the DNA can account for some transmission of phenotypic variance, it cannot account for its entirety. The commonly accepted baseline mutation rate in humans of 2.3×10^{-8} per nucleotide per generation is too low to explain all transgenerational inheritance patterns [1, 2]. For instance, genetically identical organisms such as human twins demonstrate significant phenotypic divergence in only one generation, implying a degree of developmental plasticity that occurs too quickly to be explained by the baseline mutation rate [3, 4]. This suggests that inheritance can occur through another mechanism; epigenetic alterations.

Epigenetics are heritable alterations in gene expression that do not involve changes in the germline DNA sequence. It works primarily through three mechanisms: DNA methylation, histone modification, and microRNA (miRNA) expression. DNA methylation causes gene silencing through methylation of CG dinucleotides that recruit methyl-CG binding proteins, which then block transcription factor binding and further recruit transcriptional corepressors or histone modifying complexes [5]. DNA methylation patterns are usually cleared during embryogenesis and shortly after fertilization, yet some classes of genes can retain their methylation patterns. Two classes of these genes, retrotransposable elements and imprinted genes, are sensitive to environmental exposures and are capable of retaining changes in methylation sequences [5]. Another essential mechanism regulating gene expression and silencing is through histone alterations, which facilitate chromatin transition between the open-euchromatic and closed-heterochromatic states. During spermatogenesis, DNA is stripped of its histones, which are replaced by protamines. Nevertheless, a small amount of

human histones are retained in the fetus and may carry information that is passed onto subsequent generations [6]. By adjusting chromatin states, DNA can pass on structural adaptations of chromosomal regions so as to register, signal, or perpetuate altered activity states [7]. The third mechanism involves short RNA molecules (miRNAs) that latch onto DNA and alter gene expression. Mature miRNAs bind to the 3' untranslated region of a target mRNA and either cause degradation or inhibit protein translation. The system is highly promiscuous; one miRNA can modulate multiple proteins and one protein can be altered by multiple miRNAs. DNA methylation and histone modification influence the expression of miRNAs but miRNAs themselves can also regulate the expression of components in the aforementioned mechanisms. Through their action on chromatin-targeting enzymes, miRNAs subtly alter the epigenome and subsequent gene expression [8].

Evolutionarily, epigenetics is a necessary part of normal development. Through epigenetics, the organism can alter its patterns of development to prepare for its anticipated environment. Such responses can include both short and long-term adjustments to conditions when the organism is in its early stages of growth. However, these environmental signals may not always be predictive of an individual's future environment. For instance, a murine study found that maternal low protein diet during pregnancy and lactation resulted in increased abdominal adiposity and glucose intolerance of their offspring compared to controls, when both groups were fed high-fat diet postnatally. These impaired metabolic phenotypes were associated with elevated Neuropeptide Y levels, a major determinant of the body's stress response, and up-regulation of the Neuropeptide Y adipogenic pathway in visceral fat [9]. This study indicates that nutrient poor diets during development can lead to an upregulation of stress pathways that cause a higher propensity of obesity because the offspring are expecting a nutrient poor environment. Importantly, the varied developmental pathways triggered by environmental events can occur during critical developmental periods that are often brief. Thus, an environmental influence that sets the characteristics of an individual during these sensitive periods may not be predictive of the overall environment [10]. One of these events may occur transiently, with the event causing adaptation to an environment that does not exist. Therefore, epigenetic modifications can affect individuals adversely if the conditions of preconception and prenatal exposures prove to be incorrect [11]. Armed with an understanding of these epigenetic triggers, clinicians can prevent these negative outcomes from occurring.

The clinical application of epigenetic inheritance patterns has largely been focused on the mother's lifestyle due to her immense prenatal and postnatal investment in her offspring. As epidemiological and laboratory research on maternal inheritance patterns has expanded, the effects a mother can have on a developing embryo has become increasingly recognized and put into clinical practice. For instance, an increase in maternal age is strongly correlated with heightened risk of congenital abnormalities. The CDC lists being a mother over the age of 34 as one of the five main causes of birth defects [12]. Additionally, the nutritional, hormonal, and psychological environment provided by the mother permanently alters organ structure, cellular responses, and gene expression of her offspring [13, 14].

Currently, research is working to understand the father's epigenetic role in promoting survival and adaptive development of his offspring. This new field of inherited paternal epigenetics needs to be organized into clinically applicable recommendations and lifestyle alterations. Our review aims to summarize the existing data in a comprehensive way. Specifically, we will focus on how these epigenetic mechanisms are altered by three different influences: age, environmental factors, and alcohol consumption. Each variable is explored through a combination of epidemiological, animal, and human studies that indicate an inheritance of paternal experiences. However, since the specific mechanisms of action have yet to be discerned, many of these studies can only implicate epigenetic effects as the cause of variation. Furthermore, it may be more important to consider the interplay between maternal and paternal effects as opposed to each in isolation. Future studies will have to be conducted in order to determine the role of paternal influences in mediating epigenetic mechanisms and the interaction of maternal and paternal genomes.

Effects of paternal age on epigenetic disorders

Epidemiological studies

Evidence demonstrates that paternal age has significant influence on offspring phenotype and the chance of acquiring congenital abnormalities. The age of the father has been positively correlated with elevated rates of schizophrenia, autism, and birth defects. Researchers assessed the relationship between schizophrenia and advancing paternal age in a population-based birth cohort. Results found that paternal age was a significant predictor of a schizophrenia diagnosis. Above the age of 25, the relative risk of schizophrenia increased in each 5-year age group with the highest risks being in offspring of men aged 45 and older [15]. Another study assessed a six year cohort of Jewish men aged 40 years or older. The offspring of these men were 5.75 times more likely to have Autism Spectrum Disorders when compared to offspring of men younger than 30 years [16]. In recent years, large scale Danish and US studies have also shown a similar association between paternal age and increased risk of Autism Spectrum Disorders [17]. Additionally, a retrospective cohort study from the 1999-2000 birth registration data in the United States shows that advanced paternal age was associated with increased risks of birth defects including heart defects, tracheo-esophageal fistula, esophageal atresia, other musculoskeletal/integumental anomalies, Down syndrome and other chromosomal anomalies [18].

Animal studies

Epidemiological studies of paternal age effects on offspring have been further supported by laboratory studies using murine subjects. Mice born to older fathers (>120 weeks) showed impairments on a passive-avoidance learning test as well as reduced longevity, lower reproductive success, and delayed sensory-motor development compared to mice sired by younger fathers [19]. In addition, another study revealed that male mice born to fathers older than 10 months were less active in a social-interaction test and less exploratory on a hole-board test compared to males born to fathers of two months of age [20]. This research may reveal links between paternal age and abnormal social interaction in offspring. Current research using rodent models seeks to show further correlation between paternal age and changes in offspring and elucidate the mechanisms governing these effects.

Underlying mechanisms

Although the molecular mechanisms of paternal epigenetic inheritance have not been completely elucidated, research has begun to unearth these mechanisms. Of the methods previously described, the majority of age-dependent research focuses on DNA methylation. Age-dependent alterations of DNA methylation have been observed in both mammalian somatic and germline cells. A rodent study concluded that there were higher rates of methylation in the ribosomal DNA of spermatozoa in older rats compared to younger rats [21]. Serra-Juhé et al. [22] found a correlation between hypermethylation of a gene involved in morphogenesis and isolated heart malformations. In addition, increased hypermethylation of a zinc finger transcription factor was present in mouse fetuses with Down syndrome [23]. DNA methylation patterns are normally stable over short periods but the rate alters with age, which could lead to the abnormal conditions mentioned above. While the data does not establish a causative relationship, it does suggest that the age-associated methylation of male gamete DNA could contribute to the increased incidence of congenital disorders in progeny. However, another study has shown that pregnancy outcomes are significantly improved when sperm DNA methylation exceeds threshold levels [24]. This indicates that further research needs to be conducted to show either a positive or negative correlation between paternal age and offspring congenital abnormalities.

Effects of environmental exposures on paternal epigenetics

Environmental exposures and the epigenome

Studies have shown that certain types of environmental exposures during development can have an epigenetic effect on an individual organism during their lifetime. Although many studies have linked these environmental factors as having effects on progeny, these studies do not provide definitive evidence for the mechanisms of gene transmission. One study

showed that humans subjected to poor availability of food developed phenotypic changes in their offspring that can be linked to epigenetics [25], while others report epigenetic altering effects due to smoking and psychosocial stressors. Although human studies linking environmental effects and epigenetic alterations are limited, many models using mice have been studied and shed light on the importance of understanding the role of environmental factors on the father and future generations.

Effects of diet

Recent research has shown that paternal diet can have transgenerational epigenetic consequences. Specifically, lack of food was associated with marked epigenetic differences. Swedish researchers compared the records from multiple cohorts and analyzed how the diet of ancestors affected future generations. They found that low amounts of dietary resources during the father's pre-adolescence was correlated with a lower chance of cardiovascular mortality in his offspring. The effect held true when the grandfather of that same offspring was subject to decreased amounts of food during his pre-adolescent period. These correlations suggest there are factors passed down paternally over multiple generations via epigenetics because it is unlikely mutations in the genome could manifest so quickly. This study also asserts that children whose grandparents experienced dietary restrictions during their pre-adolescent period were also protected from mortality due to diabetes [25]. Additionally, one study showed that a grandfather's food supply only affected the mortality of his grandsons, suggesting a potential sex-linked relationship [26]. To further elucidate a causative relationship, another study investigated the effect of food deprivation in mice. The male mice were subjected to stressors prior to mating, which ensured that any differences in offspring were due to alterations in the male genome and not gestational or prenatal stress. Among offspring of these stressed fathers, both male and female pups had significantly lower levels of serum glucose when compared to controls [27].

Soubry et al. determined that paternal obesity is linked with hypomethylation at the differentially methylated regions (DMR) of the IGF2 gene. When the IGF2 DMR is hypomethylated, there is increased circulation of IGF2 proteins, which are associated with increased likelihood of obesity [28]. Another study by Soubry et al. shows that the children of obese fathers also had hypomethylation in their MEST, PEG3, and NNAT DMRs. Hypomethylation of these genes can lead to enlargement of adipocytes, changes in metabolic regulation, diabetes, rhabdomyosarcoma, glioma, and obesity [29]. This research provides an important direct connection with epigenetic changes in human studies, which have been lacking. The results of these studies suggest that poor nutrition and obesity in fathers prior to mating can negatively impact future generations.

Effects of outside toxicants

Recent studies have reported that paternal exposure to smoking and irradiation may have an epigenetic effect on their offspring's genomes. Increased smoking has been associated with ejaculate containing spermatozoa with significantly damaged DNA. One study measured transcription factors in the sperm of smokers and non-smokers. The study determined that there was a significant difference between transcription factor expression in smokers when compared to nonsmokers, leading to downregulated apoptosis pathways in smoker's spermatozoa [30]. Additionally, it has been demonstrated that cigarette smoke can alter the miRNA within the spermatozoa of smokers, resulting in potentially hazardous epigenetic alterations in cell death and apoptosis pathways [31]. Irradiation has been correlated with decreased viability in murine offspring. Male mice exposed to irradiation exhibited decreased expression of *de novo* methyltransferase, DNA methyltransferase 3a and hypomethylation of both long and short nuclear elements. These epigenetic changes lead to detrimental effects on somatic thymus tissue in the progeny of exposed mice [32]. These studies suggests that many environmental factors besides diet can cause epigenetic changes in offspring via paternal lineage. However, more human studies are needed to address the potential clinical consequences.

Effects of psychosocial stress

Increased levels of paternal psychosocial stress have been shown to negatively impact future generations. Rodgers et al. studied male

mice subjected to various stressors and revealed that offspring of the stressed mice had blunted responses to stress compared to control groups, which can be an indicator of behavioral defects later in life. This study observed that 9 strains of sperm miRNA in fathers stressed by multiple cage changes, fox odor, and light stimuli were expressed at higher levels than those in control groups. This indicates that as the parents became habituated to the stressors, they were able to epigenetically pass on this information to their offspring via miRNA expression [33]. There is accumulating evidence from murine models that not only miRNA expression, but also DNA methylation can pass on stress related information transgenerationally. One test analyzed how paternal exposure to stress affected both DNA methylation in the hippocampus and altered early behavior. Not only did the study reveal reduced stress response based on a geotaxis test, it also observed that DNA methylation patterns were increased in the hippocampus [34]. The accumulation of research has demonstrated a link between psychosocial stress on the father and heritable traits passed on to his offspring via epigenetic mechanisms. However, further research needs to be conducted in human models to demonstrate a causative relationship.

Effects of environmental exposure on parental epigenetics: alcohol

Epigenetic mechanisms play a role in fetal alcohol spectrum disorders (FASDs)

FASDs are a broad array of congenital disorders with major symptoms including reduced birth weight, impaired cognitive function and behavior, and neuropsychological deficits in visual-spatial learning [35]. Studies have shown that paternal alcohol consumption has epigenetic effects on sperm DNA, suggesting a role in the development of congenital disorders in offspring. Up to 75% of children with FASD have biological fathers who are alcoholics, suggesting that preconceptional paternal alcohol consumption negatively impacts their offspring [36]. It has been shown that teratogens such as alcohol significantly reduce the activity of DNA methyltransferases, leading to increased CG hypomethylation and subsequent activation of normally silenced genes [37]. Chronic paternal alcohol consumption alone hypomethylates his offspring's genes even in the absence of maternal alcohol consumption before or during pregnancy [36, 37]. This epigenetic hypomethylation alters gene expression dosages required for normal prenatal development, resulting in offspring with characteristic symptoms of FASDs [37]. Here we examine the effects of paternal alcohol consumption on the prevalence and symptoms of FASDs and related congenital defects.

Effects on birthweight and individual organ weights

A hallmark symptom of FASD is decreased newborn birth weight [38]. Murine studies have shown that offspring from alcohol-treated fathers have a higher prevalence of low birth weights [39]. A study in rats has shown that offspring from alcohol-treated fathers decreased in weight by two or more standard deviations when compared to the average weight of offspring born from controls [36]. This effect was observed in offspring of both acute and long-term alcohol-treated fathers [40-44], which suggests that epigenetic modifications are sensitive to even small amounts of paternal alcohol consumption. In addition to marked decreases in birth weight, studies have shown that alcohol consumption can alter the weight of individual organs. For example, fathers treated with alcohol for several weeks prior to breeding were more likely to produce offspring with increased adrenal weights and decreased spleen weights [45]. This suggests that paternal alcohol consumption may also have an epigenetic impact on the gene expression governing individual organ development. Furthermore, autopsy and brain imaging studies have shown marked reductions in overall brain size, specifically in the cerebellum, basal ganglia, and corpus callosum [35]. This observed physiological effect on brain structures can explain impaired cognitive function displayed by offspring sired by alcohol-treated fathers.

Effects on cognitive behavior and motor ability

Studies have demonstrated the adverse effects of paternal alcohol consumption on the cognitive and motor ability of offspring. For example, one study involved feeding male mice varied liquid alcohol diets containing 25%, 10%, or 0% ethanol-derived calories (EDC). After 7 to 14 weeks of diet treatment, the males were bred

to non-treated females [46]. Offspring were then subjected to a forced swim test, in which offspring of alcohol-sired fathers were more immobile than offspring of fathers receiving 0% EDC. While this may suggest decreased motor ability due to paternal alcohol consumption, this seems to also be a species-specific effect. The same study was conducted on rats, showing opposite results with offspring of alcohol-sired fathers exhibiting increased mobility [46].

One possible explanation for this discrepancy could be the species' specific response to stressful situations. In humans, it is known that children with fetal alcohol syndrome cope poorly with stressful situations, and therefore display hyperresponsiveness to stress [47]. In these situations, stressors cause an increased corticosterone response, resulting in exaggerated reactions to stressful situations. This could explain why the rat offspring of alcohol-treated fathers exhibited increased mobility when forced to swim; the hyperresponsiveness to stress may override the impaired motor function that is normally seen in affected offspring. While these results in mice and rats may seem paradoxical, the mechanisms being affected need to be isolated and examined separately to determine causation.

In addition to this hyperresponsiveness seen in rats, similar studies have also shown that rats sired by alcohol-treated fathers have greater difficulty learning new tasks and have impaired spatial learning skills when subjected to maze tests [48]. Studies also suggest that epigenetic modifications occur in sperm DNA [36], which may be passed onto offspring. Further studies need to be conducted in order to determine the specific mechanism by which these modifications are passed from father to offspring. Understanding these mechanisms would provide more insight into preventative measures against FASDs and similar congenital defects.

Other epigenetic effects of paternal alcohol consumption

Paternal alcohol consumption has been implicated in additional congenital disorders, presumably via epigenetic mechanisms. In an epidemiological study by the Kaiser Foundation, the frequency and severity of certain congenital abnormalities were correlated with paternal alcohol consumption. For example, paternal alcohol consumption was found to be positively associated with ventricular septal defects in newborn children [49]. Separate animal studies have also shown that paternal alcohol consumption can lead to increased susceptibility to *Pseudomonas* infection. The severity of this increased susceptibility was found to be identical to that of animals whose mothers consumed alcohol during pregnancy, suggesting that paternal epigenetic alterations are as crucial to the development of offspring as maternal ones [37]. Overall, these studies imply that early changes in a father's lifestyle can decrease prevalence of congenital disorders in his offspring.

Conclusions

While there have been many studies that correlate maternal influences with congenital disorders, our review shows that paternal influences can cause birth defects via epigenetic mechanisms such as DNA methylation, histone modification, and miRNA expression. These mechanisms may provide the missing link between spontaneous mutation and differences in phenotype transgenerationally. There are strong indicators that paternal age can lead to differential methylation in offspring, potentially leading to heart malformation or other congenital defects. Other factors from the environment such as diet, smoking, and irradiation can lead to diabetes, obesity, cancer, and other diseases in offspring of exposed fathers. The alcohol consumption of the father during his lifetime can lead to FASD in his offspring, as well as cause deficiencies in organ weights in his children. In addition, we found that environmental effects during the lifetime of a father can affect not only his immediate offspring but future generations as well. However, future research should address deficiencies in the current literature. For example, many of these studies fail to take into account the interplay of paternal and maternal factors. The combined effects of both parents may have varying degrees of influence and need to be dissociated to examine the specific role of paternal epigenetics on congenital disorders in offspring [50, 51]. Additionally, these studies were unable to consistently isolate epigenetic inheritance as the sole cause of a specific phenotype. Future data that can elucidate the complex interplay of both maternal and paternal epigenetics can be applied with the goal of finding clinical applications to improve health outcomes in children.

Acknowledgements

This work was supported in part by National Institutes of Health (NIH) grants: 1R01CA123-211, 1R03CA178809, R01CA197964 and 1R-21CA198698 to JK.

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