Non-genetic Transgenerational Inheritance of Acquired Traits in *Drosophila*

Brian Xia and J. Steven de Belle

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71643

Abstract

It is increasingly recognized that acquired traits may be transgenerationally transmitted through non-DNA sequence-based elements, with epigenetics as perhaps the most important mechanism. Here we review examples of non-genetic transgenerational inheritance in *Drosophila*, highlighting transgenerational programming of metabolic status and longevity, one particular histone modification as an evolutionarily conserved underlying mechanism, and important implications of such studies in understanding health and diseases.

Keywords: aging, *Drosophila*, H3K27me3, metabolic state, PRC2, transgenerational epigenetic inheritance

1. Introduction

Epigenetics is the science of non-DNA sequence-based modifications of gene expression and, subsequently, phenotypic variability at both the genomic and organismal levels [1]. Studies over the past several decades have distinguished DNA methylation, histone modification, and non-coding RNA-based processes as the key mechanisms underlying epigenetic regulation. Epigenetic inheritance has been observed across species, including prokaryotes, plants, and animals [2–8], with an epigenetic trait defined as “a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” [9]. Interestingly, certain epigenetically-regulated phenotypes can propagate across multiple generations, leading to the concept of transgenerational epigenetic inheritance (TEI) [4–8]. This emerging concept has triggered numerous debates and revived old controversies in the scientific community as to whether acquired traits may be transmitted across generations. Nonetheless, it has profoundly reshaped our understanding of biology, particularly human diseases, as
| Year | Intervention/treatment (F0 only) | Phenotypic/genomic response | Generation with effect | Authors |
|------|--------------------------------|----------------------------|------------------------|---------|
| 2007 | Tumor suppressor gene mutation | Tumor risk                 | F2 but not F3          | Xing et al. |
| 2009 | Chronic pentylenetetrazole treatment of adult males | Transcriptomic profile in CNS | F2 | Sharma and Singh |
| 2010 | Old age | Memory loss | F2 | Burns and Mery |
| 2012 | Low male availability during mating | Number of offspring (to quantify fitness) | F2 & F3 | Brommer et al. |
| 2013 | Post-eclosion feeding of virgin females with a high-sugar diet | Body composition in larvae | F2 | Buescher et al. |
| 2015 | Gamma radiation in young adult males | Longevity & rate of development | F2 but not F3 | Shameer et al. |
| 2015 | Yeast concentration in diets used to raise larvae through development | Somatic rDNA instability & copy number variation | F2 & up to F60 | Aldrich and Maggert |
| 2016 | Post-eclosion feeding of both virgin males and females with various diets | Longevity & reproduction | F2 & F3 | Xia and de Belle |
| 2016 | Extended olfactory training with young adults | Approach bias to the same trained odors | F2 | Williams |
| 2016 | High fat diet to raise larvae through development | Pupal body weight | F2 | Dew-Budd et al. |
| 2016 | Different food conditions used to raise male larvae and adults | Longevity | F2 | Roussou et al. |
| 2016 | Post-eclosion dietary, genetic and pharmacological treatments of both virgin males and females | Longevity & H3K27me3 levels | F2 | Xia et al. |
| 2017 | Epialleles, as defined by differential levels of H3K27me3 | Eye color | F5 & up to F10 | Ciabrelli et al. |
| 2017 | Grandmaternal age | Embryonic & embryonic to adult viability | F2* | Bloch Qazi et al. |
| 2017 | Genetic manipulation of parental metabolism | Triglyceride levels & transcriptional profile | F2 | Palu et al. |

*Potential transgenerational effects were not clearly-defined and quantified.

Table 1. Primary research papers describing TEI in Drosophila where phenotypic and/or genomic responses were investigated in the F2 or later generations.

Stable epigenetic marks may record environmental challenges through modified gene expression patterns and ensure long-lasting, while reversible responses in the absence of the initial triggering events [10–17]. Importantly, the adaptive and reversible nature of epigenetic regulation may offer exciting therapeutic targets to help prevent or treat most, if not all, chronic diseases, including cardiovascular disease (CVD), diabetes, neurodegenerative diseases, and cancers [10–13, 16, 18–20].
The fruit fly (*Drosophila melanogaster*) offers multiple advantages for assaying TEI, in particular to characterize the underlying epigenetic mechanisms, and to identify gene targets for drug discovery. First, the short rearing period and lifespan of fruit flies facilitate transgenerational experiments over multiple generations within a reasonable time scale. Second, various examples of transgenerational inheritance have been established in *Drosophila* (Table 1) that enable rapid identification and characterization of underlying epigenetic mechanisms. Third, all major epigenetic mechanisms are present in this model system [1], although DNA methylation in flies appears to be different from many other eukaryotic organisms and is present only at very low levels in adults [21, 22]. Importantly, N6-methyladenine may complement the function of DNA methylation in flies [23]. Finally, *Drosophila* has been increasingly used for modeling human diseases and drug discovery [24–28]. The *Drosophila* heart has been used to model several different aspects of human CVDs, including congenital heart disease and cardiomyopathy [29–31]. *Drosophila* is a recently-established model system for obesity and diabetes [26, 32, 33]. It has also been widely used to model cognitive diseases [34, 35], and various cancers [36].

TEI has been thoroughly reviewed, focusing mostly on data obtained from mammals [5, 6, 8, 37–39]. Here, such studies from *Drosophila* are discussed, in particular, to highlight transgenerational programming of metabolic status and longevity, and tri-methylation of histone H3 at lysine 27 (H3K27me3) as an evolutionarily conserved epigenetic mechanism underlying TEI.

2. Transgenerational inheritance at the organismal level

2.1. Metabolism

The current Western diet has been defined by increased consumption of meat products, dairy items, grains, and sugar-infused drinks [40]. Having profound effects on glycemic load, fatty acid composition, macronutrient composition, micronutrient density, acid-base balance, sodium-potassium ratio, and fiber content, this diet may underlie the growing prevalence of chronic diseases in Western society, especially CVD, obesity, diabetes, and dementia [41–44]. Often, multiple conditions manifest themselves simultaneously in afflicted individuals, suggesting shared elements in disease pathology. Obesity and other metabolic disorders, for example, are associated with various secondary disease indications as the underlying cellular and organismal metabolism is fundamental to nearly all necessary biological processes [45]. The prominent role of nutrition and other environmental factors in the development of metabolic disorders offers a promising model to identify and characterize the underlying epigenetic mechanisms, leading to diet optimization and nutrition-responsive therapies to combat chronic diseases (cf. [42]). Thus, nutrition has been studied extensively regarding TEI of diabetes and other metabolic disorders across various animal models [14, 46–48]. Metabolic dysfunctions are often measured through development and glucose/insulin homeostasis after nutritional or dietary interventions including overnutrition, high-fat, low-protein (LP), and high-sugar (HS) diets. Typically, the well-controlled application of dietary manipulations and well-established hallmarks of various metabolic disorders offer a tractable yet indispensable approach to studying TEI in many animal models.
**Drosophila** shares key metabolic pathways and characteristics with vertebrates (cf. [29, 32]). Glucose has been well-studied in the context of metabolic status given its pivotal role in insulin signaling since the 1930s [49]. *Drosophila* utilizes trehalose, the disaccharide of glucose, as its primary form of hemolymph (insect equivalent of blood) sugar [50, 51]. Regulating glycometabolism and maintaining viability in response to shifting external factors [51], trehalose is broken down through the catalyzing activity of trehalase into accessible glucose molecules. Thus, hemolymph trehalose and glucose levels may be quantified to assay glycometabolism in *Drosophila* [29, 32]. As the primary source of body fat from *Drosophila* to humans [52], triglycerides (TAGs) may be quantified for monitoring gluconeogenesis, the metabolic pathway responsible for glucose generation from non-carbohydrate substrates [29, 32]. Both AKT and 4EBP proteins are phosphorylated in response to insulin signaling [53, 54]. AKT is particularly well-characterized as a core component of the PI3K/AKT/mTOR pathway, which is linked to cell cycle regulation, cancer, and longevity [55]. Quantification of phosphorylated-AKT and phosphorylated-4EBP levels has been used to measure insulin sensitivity or resistance [29].

The availability of these assays to characterize both metabolic homeostasis and underlying pathways has supported the use of *Drosophila* to examine TEI of metabolic status after nutritional or genetic manipulations in the founding (F0) generation [32, 56, 57]. Buescher et al. recorded elevated trehalose, glycogen, and TAG levels as well as reduced body weight in adult female F0 flies after feeding on an HS diet for 7 days post-eclosion [32]. Glucose levels were found to be affected by the HS treatment, suggestive of gluconeogenesis dysregulation. Interestingly, trehalose and glucose levels were elevated in the first generation (F1) male larvae, along with a decrease in glycogen levels. Consistently, gene expression analyses demonstrated decreased expression of the genes involved in fat body lipolysis and gluconeogenesis, and increased expression of the ones involved in gut lipolysis, fatty acid synthesis, sugar transport and glycolysis. These results have confirmed the traditional models of insulin signaling, in which impaired insulin sensitivity leads to global increases in circulating blood sugars and decreases in sugar storage. Both glucose and trehalose levels were elevated, with TAG unaffected in the F2 male larvae; trehalose was elevated while TAG was decreased, with glucose unaffected in the F2 female larvae, supporting the existence of gender-dependent differences in transgenerational inheritance of metabolic programming. These results have demonstrated the long-lasting and transgenerational effects of early-life (post-eclosion) nutrition on metabolic status, establishing *Drosophila* as a useful model system to study TEI of nutritional programming of metabolic homeostasis and disorders.

Then, Dew-Budd et al. assayed the effects of gender and genetic lineage on transgenerational inheritance of certain metabolic phenotypes after rearing male (F0; paternal ancestry) and female (maternal ancestry) larvae of 10 (to measure pupal body weight) or 3 (metabolic composition and egg size) independent genetic lines on a high-fat diet [57]. Substantial differences in body weight, metabolic composition, or egg size were observed in both F1 and F2 generations between paternal and maternal ancestries or among different F0 genotypes. Interestingly, phenotypic changes in the F0 flies appeared not to be a consistent predictor of these hallmarks in their untreated F1 and F2 descendants. Therefore, “personalized” consideration of ancestral contributions may be needed to understand and prevent metabolic diseases such as obesity and diabetes.
Palu et al. have employed loss-of-function mutants to induce obesity, assayed with elevated TAGs, in F0 parents and then check TAG levels in heterozygous F1 and wild-type F2 offspring [56]. Loss of AKHR (encoding adipokinetic hormone receptor) leads to reduced fat body lipid mobilization and elevated TAG accumulation, as adipokinetic hormone functions analogously to the fasting hormone glucagon in mammals [52]. Mutant AKHR F0 and wild-type flies in reciprocal crosses produced heterozygous F1 offspring. These F1 heterozygotes were then crossed to wild-type females or males to generate four types of genetically distinct wild-type F2 (+/+) progeny, corresponding to mutant AKHR F0 grandpaternal or grandmaternal and heterozygous F1 paternal or maternal ancestors. Both male and female F0 mutants displayed elevated TAG levels, which were then normalized in the F1 heterozygotes, possessing a functional copy of AKHR. Interestingly, this Mendelian model of inheritance was not always followed in the F2 generation with low TAG levels observed in the grandpaternal/maternal group, while normal in the other three groups. Consistently, ACC, encoding a conserved Acetyl-CoA carboxylase that acts as the rate-limiting step in fatty acid synthesis [58], was found to be dysregulated in this particular F2 group. These results suggest that genetic manipulation of parental metabolism can provide an effective approach for studying TEI of metabolic state.

2.2. Aging

Aging has been increasingly recognized as a malleable process and the largest risk factor for most aging-related diseases (ARDs). It is no accident that the rapid increase in life expectancy worldwide is concomitant with the epidemic progression of many of these life-threatening and costly diseases [59, 60]. Recent work has demonstrated that many factors, including environmental conditions (e.g., diet) and genetic mutations, can impact the aging process across species [61–63]. In particular, anti-aging interventions often delay or prevent multiple ARDs in animal models [62–64], stimulating the emerging interdisciplinary field of geroscience to study the connection between aging and diseases, and to develop novel multi-disease preventative and therapeutic interventions by targeting the aging process itself [59, 65]. There are clear practical and ethical complications associated with studying aging and its transgenerational inheritance directly in human populations. The timescale of conducting such longitudinal studies would be unreasonable, at best. The shortage of isogenic replicates (e.g., twins) and imprecise environmental manipulation in human models also pose a significant problem in terms of reproducibility and subsequent mechanistic studies.

Drosophila presents itself as an excellent model to study aging, especially its transgenerational inheritance and the underlying mechanisms, owing to its relatively short lifespan, genetic homology with other models and humans, and suite of enriched investigative tools. Drosophila has an average lifespan of 2–3 months yet undergoes key parallel developmental stages similar to those of humans [25]. Studies on its life cycle have revealed a number of highly conserved pathways involved in organismal development. The Hox genes, for example, which control segment identity during embryonic development, were first identified in Drosophila after observation of mutant flies growing legs in the place of antennae [66]. Hox genes were later found to be conserved in humans and also linked to congenital disorders, including synpolydactyly and hand-foot-genital syndrome [67–69]. In addition, the key aging pathways, including mechanistic target of rapamycin, sirtuin, and insulin/insulin growth factor 1 signaling, are well
Gamma radiation causes DNA damage and mutations, leading to various health dysfunctions and subsequent lifespan reduction [78, 79]. High doses of gamma irradiation were found to decrease longevity in the F0 flies and further propagate to the F1 and F2, but not to the F3 generation [76]. In contrast, low doses extended longevity across the F0–F2 generations, consistent with the concept of hormesis, by which low exposure to harmful agents (irradiation, caloric restriction, heat stress, and free radicals) improves general health and longevity [79–81]. Related studies have revealed several underlying mechanisms including insulin and glucose metabolism, proteasome activity and histone deacetylation [81, 82]. Histone deacetylation may be particularly relevant in this context as an epigenetic modification involved with many biological processes and human diseases, including CVD, metabolic disorders, and cancers [83–85].

Our recent work has established the first animal model of early-life nutrition-mediated programming of longevity and its transgenerational inheritance [74]. Newly-eclosed F0 virgin flies were reared on one of three different diets (low-protein or LP, intermediate-protein, and high-protein) for the first 7 days post-eclosion. Longevity was assayed for males and females, both virgin and mated, across the F0–F2 generations, allowing us to determine the potential impact of gender and mating on transgenerational inheritance of longevity. Our results suggest that early-life nutrition-induced programming effects on longevity may be transmitted to the F1 generation through intergenerational effects and further to the F2 generation through transgenerational effects, independently of gender and mating. The programming effects, although diminishing, were still present in the F3 generation for the low- and intermediate-protein diets. These observations suggest that early-life nutrition may produce long-lasting and transgenerationally heritable effects on the aging process across multiple generations. Notably, these long-lasting programming effects may be derived from both maternal and paternal contributions, as we treated both newly-eclosed F0 males and females to induce potentially maximal alterations. In contrast, a similar treatment was applied only to the females to examine transgenerational programming of metabolic status [32]. Most rodent studies also used either males or females, instead of both [48]. This design would not distinguish potentially different contributions from males and females, something that requires further investigation. Interestingly, transgenerational glucose intolerance in mice (Mus musculus) may be transmitted via the maternal or paternal line through different mechanisms [86, 87], suggesting that transgenerational nutritional programming effects may potentially be additive when induced in both males and females.

A more recent study has demonstrated that distinct dietary manipulations in the larval stage or throughout adulthood may also induce transgenerational programming of longevity [75]. The F2 male offspring were found to be long-lived if F0 male adults were subjected to dietary restriction, but not to starvation, whereas the same outcome was observed if F0 male larvae were exposed to starvation, but not to dietary restriction. The authors also generated two separate
groups of F2 males, from the F1 male (paternal) or female (maternal) offspring of the F0 male larvae exposed to various food media. Extended longevity was observed in both groups of F2 males, but greater extension was seen in the F2 maternal males with one laboratory strain. By contrast, the starvation-induced transgenerational effects were observed only in the F2 paternal males with a different strain. Therefore, cross-generational inheritance of nutrition-mediated longevity changes may be passed through either the male or female line or both, depending on genetic background. Unfortunately, it is unclear whether the observed gender-dependent differences resulted from intergenerational or transgenerational inheritance, as longevity was not assessed in the F0 and F1 generations.

2.3. Fitness

Fitness refers to the reproductive success of an organism over the duration of its lifetime, and has often been linked to genetic regulation. Recent studies, though sparse, have prompted the idea that non-genetic or epigenetic mechanisms may modulate fitness across generations [74, 88–90]. Studying the interplay between genetics and epigenetics through fitness may help us understand various complex traits and disorders [89]. *Drosophila* is particularly suitable for studying TEI of fitness for its rapid maturation following eclosion and high fecundity among model organisms [91].

Brommer et al. have reported that sexual conflict (male availability) may impact the fitness of future progeny up to the F3 generation [90]. Female fitness was quantified by lifetime production of offspring, and male fitness by total offspring produced in a six-day period. For the F0 generation, female flies underwent either a low (one male for 1 day followed by no male for 3 days) or high male (one male for 1 day followed by a different male for 3 days) exposure treatment. This four-day cycle was repeated for the duration of the females’ lifespan to measure lifetime fecundity. The same process was repeated for the F1 and F2 generations, thus producing eight groups of F3 flies with distinct ancestral history. All F3 generation daughters experienced the treatment of high male exposure. All comparisons, when made relative to the low versus high male treatments experienced by the F0 females, provided a measure of transgenerational inheritance of fitness. The results indicated that low male exposure treatment in the F0 females did not affect female fecundity across the F1–F3 generations, but increased male fitness in the F1 generation and decreased male fitness in the F2 and F3 generations.

In the same study where we assayed transgenerational nutrition-mediated programming of longevity (see above), we also explored the transgenerational effects of the same early-life diets on lifetime fecundity (egg production) as a measure of fitness and the potential trade-off between longevity and fecundity [74]. Lifetime fecundity was found to be decreased across the F0–F2 generations after raising the F0 virgin male and female flies on the LP diet for 7 days before their mating, while increased transgenerationally after the same treatment with the intermediate-protein diet. Fecundity was also increased in the F0 and F1 generations after the same treatment with the high-protein diet, but the increasing effect was not seen in the F2 generation. These results demonstrate that early-life dietary changes affect fitness of the same generation and the reproductive success of future generations with certain dietary changes. Interestingly, correlation analyses on longevity and fecundity data revealed no evidence for trade-off between them across the F0–F2 generations. This finding argues
that lab-raised flies, with abundant food supplies at all times, may have evolved to abandon such trade-off constraints through hundreds of generations. Therefore, transgenerational nutritional programming of fitness may be achieved independently of longevity, raising the interesting possibility of elevating both longevity and fitness with proper nutrition across generations.

Bloch Qazi et al. recently reported the cross-generational effects of grandmaternal and maternal age on offspring viability and development up to the F2 generation [88]. The study, however, appeared not to distinguish between intergenerational (grandmaternal to maternal and maternal to F2 offspring) and transgenerational (grandmaternal to F2) effects. The complicated design with three interacting factors (i.e., grandmaternal age, maternal age, and stress) and subsequent analyses with mixed-model ANOVAs made it challenging to make conclusions about a straightforward transgenerational effect, although the P value was smaller than 0.05 in three of analyses for the “grandmaternal” factor (in the presence of the intergenerational effect or “maternal” factor).

2.4. Memory

Many behavioral traits, including cognitive functions, may be transgenerationally affected by experiences and environmental factors in mammals, most likely through epigenetic mechanisms [92]. Memory is an essential cognitive function which declines during aging and is impaired in most neurodegenerative diseases such as Alzheimer’s disease; it is subjected to various epigenetic regulations, providing novel therapeutic avenues to combat cognitive disorders [12, 93]. Therefore, studying TEI of memory is of immense importance to our understanding of mental health and diseases. A *Drosophila* memory TEI model is established by two recent studies [94, 95] and further corroborated by a similar report in which increased startle responses to the conditioned odor after paternal F0 olfactory fear conditioning was observed in the subsequent adult F1 and F2 mice [96].

A widely-used dual-odor discriminative Pavlovian conditioning assay involves training groups of flies to associate one odor (CS+; conditioned stimulus) with aversive electric or mechanical shocks (US; unconditioned stimulus), and the other odor (CS−) as a non-associative control [97–99]. Aged (25-day-old) flies produced F1 offspring with memory impairment detectable in young adults (3–5 days old), and this impairment was transmitted to the F2 generation [95]. The transgenerational effect was specific to short-term memory (STM; as tested 15 min after training), and appeared to be caused by oxidative stress in both F0 maternal and paternal flies. Although the same authors did not evaluate memory in aged F0 parents, an earlier study [100] demonstrated that aging specially impaired middle-term memory (MTM), which starts to form within 15 min after training and is considered to be an aging-sensitive component of STM [101, 102]. In addition to concluding that offspring cognitive ability may be influenced by parental age [95], these studies collectively argue that aged F0 parents may acquire a loss of oxidative stress-sensitive STM, and this acquired memory loss can be transgenerationally inherited at least to the F2 generation. This new explanation also provides a possible mechanistic direction for future investigation, as MTM formation requires normal function of the...
*amnesiac* (*amn*) gene that encodes a precursor neuropeptide encompassing fly homologs of mammalian pituitary adenylate cyclase activating peptide (PACAP) and growth hormone-releasing hormone (GHRH; see below for further discussion) [103].

In a more recent study, F1 and F2 flies, without any training and prior exposure, displayed selective preference toward the same CS odors which were used during 5 days of discriminative training of F0 parents [94]. This preference was selective for the salient CS odors experienced by the F0 parents but not the specific CS-US association, as the F1 and F2 flies did not differentiate between odors that were originally used to train their F0 parents under an aversive (with electric shocks as US) vs. appetitive (with corn meal and sucrose as US) conditions. Consistently, discriminative conditioning appeared to increase the perceived salience of the CS+ odors [104]. Importantly, the observed odor-selective preference in the F1 flies required normal function of *amn* and preserved function of dorsal paired medial neurons in which *amn* is predominantly expressed [105]. Thus, the *amn* gene may be involved with transgenerational inheritance of acquired loss of STM in aged F0 parents [95] and odor-selective preference from discriminative training in the F0 flies [94]. In agreement with this idea, PACAP and/or GHRH stimulate growth hormone release [106], while down-regulation of growth hormone may be involved with cross-generational toxicity [107]. The *amn* gene also plays an important role in the behavioral response to intoxicating levels of alcohol [108], while alcohol abuse has been known to be transgenerationally heritable [109]. Collectively, these studies support *Drosophila* as a useful model to study transgenerational inheritance of memory impairment triggered by environmental factors (e.g., aging) and behavioral traits acquired from experiences (e.g., training), and epigenetic regulation of *amn*-encoded peptides as one potential underlying mechanism.

### 3. Transgenerational inheritance at the molecular and genomic level

Despite advancement of high-throughput sequencing and the recent surge of research on TEL, there are currently few studies focusing on the transgenerational effects at the molecular and genomic level, and thus the underlying mechanisms remain largely obscure [6, 8, 37–39, 92, 110]. Several recent studies in flies, however, may shed some light on this situation [77, 111–115].

Chronic treatment (7-day feeding and 7-day withdraw) of the F0 males with pentylenetetrazole (PTZ), an FDA-revoked convulsant drug, caused locomotor deficits and long-term alterations in the CNS (central nervous system) transcriptome [116]. A follow-up study from the same group [113] demonstrated that the F0 males (with PTZ treatment) displayed a CNS transcriptomic profile closest to the F2 males; and differentially expressed genes in the F1 males, F1 females, and F2 males showed significant overlap with the PTZ-impacted genes in the F0 males. Interestingly, further clustering analysis of CNS and testis transcriptome profiles and concordant analysis of differentially expressed genes between them implied gametic involvement in the observed transgenerational effect in gene expression. These results suggest that the acquired somatic transcriptomic alteration in F0 PTZ-treated males may be passed via
sperm at least to the F2 generation. This is the first report to study transgenerational inheritance of genome-wide transcriptomic profile as a “phenotype,” acquired through drug treatment in the F0 generation.

In another study, a high-protein diet led to somatic rDNA instability and copy number reduction in F0 parental flies [111]. As the insulin/insulin-like growth factor and TOR signaling pathways regulate ribosome biogenesis and rDNA expression for nutrient availability [117], genetic and pharmacological manipulation of insulin/TOR signaling produced similar effects, corroborating the results from dietary treatment. Importantly, rDNA copy number reduction remained in the F2 generation and was still present in flies maintained on standard food for 6 years. These results suggest that the genome rearrangement in F0 flies acquired through feeding on the high-protein diet occurred in both somatic and germ cells, and was transgenerationally heritable for over 150 generations. This outcome revealed a robust and long-lasting transgenerational consequence of adult diets. In a remarkable recent study, early-life protein restriction in mice induced a linear correlation between growth restriction and DNA methylation at certain rDNA copies that lasted into adulthood [118]. These findings, establishing rDNA as a genomic target of nutritional availability across species, are of obvious importance for human health and diseases, as copy number variations have been linked to many chronic diseases such as schizophrenia and Alzheimer’s disease [119–121].

Another curious study has shown that a dominant and hyperactive mutation in the hopscotch gene (HopTum−l), encoding the Drosophila JAK kinase, caused epigenetic alterations in F0 parental flies that were transgenerationally heritable and thus influenced tumorigenesis in their F1 and F2 offspring [114]. Interestingly, the transcriptional repressor Krueppel, known to repress transcription of the fushi-tarazu gene which encodes a homeodomain protein required for embryonic segment number and cell fate [122], is a HopTum−l enhancer [123]. Krueppel mutations caused increased DNA methylation in the fushi-tarazu promoter region. This effect was transmitted across generations in the presence of HopTum−l [114]. Therefore, DNA methylation may be altered by Krueppel mutations, functioning as heritable epigenetic markings in Drosophila. JAK hyper-activation may then interfere with epigenetic reprogramming, allowing the changed DNA methylation (epimutation) to propagate across generations and influence tumor susceptibility.

4. Polycomb repressive complex 2 (PRC2) mediates H3K27me3 as a conserved epigenetic mechanism underlying transgenerational inheritance

Despite decades of intense studies linking all key types of epigenetic regulation (i.e., DNA methylation, histone modifications and non-coding RNAs) to TEI, direct and convincing experimental evidence in support of underlying mechanisms and governing principles is rare [2–8, 37–39]. The difficulties lie in the time-consuming nature of such studies, and lack of well-established epimutations, clearly-defined phenotypic contributions and stably-inherited epigenetic markings
across multiple generations. Here we highlight two recent persuasive studies in *Drosophila* that have characterized one particular histone modification (H3K27me3) as part of an evolutionarily conserved epigenetic mechanism underlying transgenerational inheritance [77, 112].

H3K27me3 is a repressive methylation mark on histone H3 established by PRC2 through its core catalytic subunit, the H3K27-specific methyltransferase encoded by the E(z) gene in flies [124] and EZH2 in mammals [125]. PRC2 is evolutionarily conserved across species, including unicellular alga (*Chlamydomonas reinhardtii*) and budding yeast (*Cryptococcus neoformans*) [124–127]. Genes marked with higher-than-normal levels of H3K27me3 in human and mouse spermatozoa continue to show repression during gametogenesis, embryogenesis, and development, suggestive of a role of this histone modification during TEI [128–130]. Furthermore, paternal diet affects H3K27me3 marks at specific loci in their offspring, implying that such nutrition-induced epigenetic modifications may be selectively retained across generations in mice [131]. Finally, TEI of longevity has been reported for H3K4me3 in worm (*Caenorhabditis elegans*) [132], and the bivalent chromatin domains covered by H3K27me3 and H3K4me3 marks have been implicated in aging and ARDs in humans [133, 134]. These results collectively suggest that H3K27me3 may function as an evolutionarily conserved epigenetic mechanism underlying transgenerational inheritance. Our recent work and that of Ciabrelli et al. have directly validated the concept in the context of nutrition-mediated longevity programming, transgene expression, and endogenous genetic variation [77, 112]. Further strengthening the idea, H3K27me3 markings have been found to propagate across generations from the maternal (and likely paternal) germline and survive reprogramming events during early embryogenesis in flies [115].

Our most recent study examined E(z)-mediated H3K27me3 as one potential epigenetic mechanism underlying transgenerational inheritance of longevity [77]. It was prompted by our earlier work to establish nutritional programming of longevity and its transgenerational inheritance [74], and by recent studies supporting the notion that PRC2-mediated H3K27me3 may regulate aging across species. H3K27me3 repressive markings and an epigenomic PRC2 signature marked by EZH2 and SUZ12 (another core component of PRC2) binding have been found to be associated with age-associated differentially methylated regions and aging-associated genes in human embryonic stem cells and various other cell lines, implicating this repressive epigenetic marker as a common mechanism of aging in humans [135]. Consistently, Polycomb repression is associated with healthy aging in humans [136], and replicative senescence of stem cells, an *in vitro* aging model [137, 138]. H3K27me3 and H3K4me3 are also the frequent antagonistic partners found on the bivalent chromatin domains which may be implicated in aging and ARDs in humans [133, 134]. In addition, heterozygous mutations of E(z) increase longevity while also reducing H3K27me3 levels in adult flies, suggesting that PRC2-dependent H3K27me3 may regulate aging in *Drosophila* [139]. Interestingly, E(z)-mediated H3K27me3 is required for paternal transmission of obesity through reprogramming of metabolic genes in flies [140], supporting its potential role in transgenerational reprogramming. Finally, UTX-1 (an H3K27-specific histone demethylase) has been shown to regulate aging, and H3K4me3-mediated TEI of longevity has been reported in *C. elegans* [132, 141].

E(z) protein level was significantly upregulated in F0 flies, and back to normal in F2 flies, after post-eclosion treatment of F0 flies with the LP diet [77]. In contrast, the resulting
increase of E(z)-dependent H3K27me3 was seen in the F0 parents and their F2 offspring. Correspondingly, longevity was reduced in both F0 and F2 flies. These results suggest that early-life dietary insults may trigger E(z)-mediated H3K27me3 changes via misregulation of E(z), and consequently nutrition-induced H3K27me3 dysfunction may be transmitted across generations and underlie TEI of nutritional programming of longevity. First, E(z)-mediated H3K27me3 was found to be necessary for TEI of longevity programming, as early-life RNAi-mediated specific knockdown of E(z) only in the F0 parents extended longevity while reducing H3K27me3 activity, and early-life specific inhibition of E(z) enzymatic function with EPZ-6438 (a highly EZH2-selective inhibitor) also extended lifespan while rendering the H3K27me3 level low across generations. Importantly, the effects of RNAi-mediated knockdown on H3K27me3 and longevity were specific, as (I) similar effects were observed with two independent RNAi transgenes, (II) the E(z) protein level was normal in the F2 generation after its knockdown in the F0 parents, and (III) longevity, E(z), and H3K27me3 levels were not affected without heat shock to induce RNAi transgenes. Similarly, the EPZ-6438-induced effects were specific, as (I) EPZ-6438, as a phase II clinical drug, is highly EZH2 selective and considered safe [142], and (II) E(z) protein was unaffected by EPZ-6438 even in the F0 parents. In addition, H3K27me3 was found to be sufficient for TEI of longevity programming, as EPZ-6438 greatly alleviated the longevity-reducing effect of the LP diet, while counterbalancing its upregulation of H3K27me3 across the F0 to F2 generations. Our data have convincingly demonstrated that E(z)-mediated H3K27me3 activity may play a critical role in the general health of an organism and function as one epigenetic mechanism underlying TEI of early-life nutrition-mediated longevity programming. Our findings have also provided the first proof-of-concept for an epigenetic therapy to confer transgenerational health benefits in a model system, manifested through improved longevity.

Another important aspect of our study was early-life rather than adult-oriented interventions. The critical period refers to a time frame in which an organism’s nervous system is especially susceptible to environmental modification. This phenomenon is common to nearly all multicellular model organisms as it primes the organism to environmental stimuli and programs physiological pathways responsible for maintaining general health. Studies have linked abnormalities in the critical period to the development of autism spectrum disorder [143], attention deficit hyperactivity disorder [144], schizophrenia [145, 146], obesity [147], and other ARDs [148]. Indeed, the Developmental Origins of Health and Disease hypothesis (DOHaD) postulates that the current mainstream adult-oriented therapies may be less efficacious than those delivered during the developmental phases of life [149, 150]. Our study has provided direct validation of this concept through the delivery of EPZ-6438 at various time points throughout adult life to alleviate LP-induced longevity reduction. The alleviation effect was found to be greatest, intermediate, or very mild when the drug was delivered within the first 7 days, from day 3–10, or from day 10–17 after eclosion, respectively. The effect was even seen in the F2 generation when the inhibitor was delivered within the first 7 days post-eclosion. These data support the DOHaD approach for studying ARDs in Drosophila and the use of a developmentally appropriate time period for intervention. Our follow-up experiments indicated that early-life administration of EPZ-6438 can also prevent multiple LP-induced ARDs (i.e., cardiomyopathy, type 2 diabetes, and aging-related memory loss) throughout adult life.
This represents a novel proof-of-concept of an early-life multi-disease therapy, leveraging epigenetic reprogramming to provide life-long protection against multiple – possibly all – ARDs (Xia et al., unpublished results).

To study epigenetic phenomena in flies, Ciabrelli et al. employed a transgene inserted in chromosome arm 2L (Fab2L) to establish stable and isogenic epilines that carried distinct epialleles as defined by differential levels of PRC2-dependent H3K27me3 [112]. The Fab2L transgene contains the reporter gene mini-white, whose expression determines red pigmentation in the eye, under the control of Fab-7, a 3.6-kb genomic region that includes a PRE (Polycomb response element). Despite being located on a different chromosome (3R), the endogenous Fab-7 region can affect PRE-responsive repression of the Fab2L transgene through long-range 3D chromatin interactions [151, 152], producing variable mini-white expression-dependent eye colors among individual flies. These epigenetic differences were somatic and not transgenerationally heritable, but enhancing long-range interactions between Fab2L and the endogenous Fab-7 through removal of one copy of Fab-7 induced a plastic epigenetic state, allowing the authors to establish the stable and isogenic epilines with the most repressed (white) or the most derepressed (red) eye phenotypes through 15 generations of selection for eye color.

Their subsequent characterization indicated that (I) these epilines carried either silent or active epialleles of Fab2L, as determined by high or low levels of PRC2-responsive H2K27me3; (II) these epialleles could be stably and dominantly transmitted to naïve flies, with acquired epigenetic states stably maintained at least until the F10 generations through self-crossing; (III) epiallele maintenance required 3D chromatin interactions, with both epialleles fully and specifically reversed to a non-selective state after complete removal of the endogenous Fab-7; (IV) epiallele inheritance also followed the rules of paramutation under natural environment conditions, with environmental factors (e.g., temperature and humidity) affecting the phenotypes of the epialleles; and (V) the paradigm could apply to a naturally occurring phenotype (i.e., antenna-to-leg homeotic transformation [153]) of a spontaneous neomorphic mutation of the homeotic Antennapedia gene. This important work, with well-established stable and isogenic epilines as defined by distinct levels of H3K27me3 markings, has overcome many shortcomings of earlier studies of transgenerational inheritance, such as weak effects fading away within a few generations, ill-defined contributions to the observed phenotypes, and unclear epigenetic markings (cf. [114, 154]). The results have convincingly demonstrated stable transgenerational H3K27me3-mediated inheritance of transgene expression and endogenous genetic variation in fruit flies [112], corroborating our study of establishing the same epigenetic mechanism underlying transgenerational inheritance of nutrition-programmed longevity [77].

In this mode of TEI, PRC2 functions through H3K27me3 repressive markers to acquire specific epigenetic states in response to environmental stimuli or triggers. Alternative states are defined by different levels of H3K27me3 to affect gene expression and epigenetic phenotypes [77, 112, 131]. Polycomb-mediated repression at specific loci and/or long-range chromatin interactions act together to maintain acquired states in cis [112], and distinct levels of H3K27me3, as deposited in the maternal oocytes [155], resist epigenetic reprogramming during early embryogenesis and are transmitted across generations, enabling transgenerational inheritance of acquired states and phenotypes [115]. The acquisition and establishment of epigenetic states may occur...
rapidly during developmentally appropriate time periods [77, 131] or gradually through phenotype selection [112]. Deposit of H3K27me3 appears to be locus-specific in response to environmental factors (cf. [131]). The extent and robustness of its inheritance may be environmental factor- and trait-dependent, with the transgenerational effects upon acquired complex traits (e.g., aging) quickly adapting to further environmental changes and decaying away in a few generations (cf. [77]), or upon simple traits (e.g., transgene expression) being relatively resistant to further environmental modifications and transmitting across many generations (cf. [112]).

5. Conclusion

*Drosophila* as a versatile model organism is profoundly advancing our understanding of TEI and its underlying mechanisms. Short lifespan, well-conserved epigenetic mechanisms, and powerful genetic tools have facilitated TEI studies at molecular, genomic, and organismal levels after various environmental and genetic manipulations (Table 1). Many studies have employed dietary interventions at the larval or early-adult life stages, or throughout adulthood, similar to those in mammals [48, 156]. Early-life nutrition in particular has been linked to adult health and diseases, prompting the increasingly-recognized DOHaD approach for studying various ARDs including CVD, obesity, diabetes, dementia, and certain cancers [4, 150, 156]. Importantly, these existing TEI models have enabled exciting investigations of the underlying molecular and epigenetic mechanisms. Here, we have highlighted PRC2-mediated H3K27me3 markings as an evolutionarily conserved epigenetic mechanism underlying transgenerational inheritance [77, 112, 115].

6. Recommendations

TEI research is a relatively new science. H3K27me3-mediated inheritance is providing a platform to address many important questions about TEI in future studies. What are the signals and underlying molecular mechanisms responding to the initial environmental stimuli? How do these signals trigger an epigenetic process and establish corresponding epigenetic states? How can such specific epigenetic states, likely originating in somatic cells, be transmitted to germ cells to enable transgenerational inheritance? What are the molecular mechanisms that maintain transgenerational inheritance? Is H3K27me3 unique in that it may resist epigenetic reprogramming [115]? Is H3K27me3 a common epigenetic mechanism responsible for non-genetic transgenerational inheritance across species? We anticipate that the *Drosophila* model will continue to broaden our understanding of TEI biology and related human diseases in particular.

Acknowledgements

We thank Drs. Dustin Schones and Wendong Huang (City of Hope Cancer Research Center) for valuable discussion and comments, and Dr. Shouzhen Xia (Dart Neuroscience LLC) for critical reading and editing of our manuscript. This work was supported by internal funding to SD.
Author details

Brian Xia¹* and J. Steven de Belle²
*Address all correspondence to: bxia@mit.edu
1 Massachusetts Institute of Technology, Cambridge, MA, USA
2 Dart Neuroscience LLC, San Diego, CA, USA

References

[1] Allis DC, Jenuwein T, Reinberg D, editors. Epigenetics. New York: CSHL Press; 2007. 500 p
[2] Eaton SA, Jayasooriah N, Buckland ME, Martin DJ, Cropley JE, Suter CM. Roll over Weismann: Extracellular vesicles in the transgenerational transmission of environmental effects. Epigenomics. 2015;7:1165-1171. DOI: 10.2217/epi.15.58
[3] Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nature Reviews. Genetics. 2016;17:733-743. DOI: 10.1038/nrg.2016.106
[4] Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. Environ Epigenet. 2016;2:dvw002. DOI: 10.1093/eep/dvw002
[5] Miska EA, Ferguson-Smith AC. Transgenerational inheritance: Models and mechanisms of non-DNA sequence-based inheritance. Science. 2016;354:59-63. DOI: 10.1126/science.aaf4945
[6] Sharma A. Transgenerational epigenetics: Integrating soma to germline communication with gametic inheritance. Mechanisms of Ageing and Development. 2017;163:15-22. DOI: 10.1016/j.mad.2016.12.015
[7] Jablonka E, Raz G. Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. The Quarterly Review of Biology. 2009;84:131-176
[8] Gapp K, Bohacek J. Epigenetic germline inheritance in mammals: Looking to the past to understand the future. Genes, Brain, and Behavior. 2017. DOI: 10.1111/gbb.12407
[9] Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes & Development. 2009;23:781-783. DOI: 10.1101/gad.1787609
[10] Bay JL, Morton SM, Vickers MH. Realizing the potential of adolescence to prevent transgenerational conditioning of noncommunicable disease risk: Multi-sectoral design frameworks. Healthcare (Basel). 2016;4:E39. DOI: 10.3390/healthcare4030039
[11] Benedetti R, Conte M, Iside C, Altucci L. Epigenetic-based therapy: From single- to multi-target approaches. The International Journal of Biochemistry & Cell Biology. 2015;69:121-131. DOI: 10.1016/j.biocel.2015.10.016
[12] Delgado-Morales R, Agís-Balboa RC, Esteller M, Berdasco M. Epigenetic mechanisms during ageing and neurogenesis as novel therapeutic avenues in human brain disorders. Clinical Epigenetics. 2017;9:67. DOI: 10.1186/s13148-017-0365-z

[13] Li C, Casanueva O. Epigenetic inheritance of proteostasis and ageing. Essays in Biochemistry. 2016;60:191-202. DOI: 10.1042/EBC20160025

[14] Stegemann R, Buchner DA. Transgenerational inheritance of metabolic disease. Seminars in Cell & Developmental Biology. 2015;43:131-140. DOI: 10.1016/j.semcdb.2015.04.007

[15] Sun C, Burgner DP, Porsonby A-L, Saffery R, Huang R-C, Vuillermin PJ, Cheung M, Craig JM. Effects of early-life environment and epigenetics on cardiovascular disease risk in children: Highlighting the role of twin studies. Pediatric Research. 2013;73:523-530. DOI: 10.1038/pr.2013.6

[16] Van der Harst P, de Windt LJ, Chambers JC. Translational perspective on epigenetics in cardiovascular disease. Journal of the American College of Cardiology 2017;70:590-606. DOI: 10.1016/j.jacc.2017.05.067

[17] Vickers MH. Developmental programming and transgenerational transmission of obesity. Annals of Nutrition & Metabolism. 2014;64(Suppl 1):26-34. DOI: 10.1159/000360506

[18] Chen Z, Li S, Subramaniam S, Shyy JY-J, Chien S. Epigenetic regulation: A new frontier for biomedical engineers. Annual Review of Biomedical Engineering. 2017;19:195-219. DOI: 10.1146/annurev-bioeng-071516-044720

[19] Rasool M, Malik A, Naseer MI, Manan A, Ansari S, Begum I, Qazi MH, Pushparaj PN, Abuzenadah AM, Al-Qahtani MH, Kamal MA, Pushparaj PN, Gan SH. The role of epigenetics in personalized medicine: Challenges and opportunities. BMC Medical Genomics. 2015;8(Suppl 1):S5. DOI: 10.1186/1755-8794-8-S1-S5

[20] Yan W, Herman JG, Guo M. Epigenome-based personalized medicine in human cancer. Epigenomics. 2016;8:119-133. DOI: 10.2217/epi.15.84

[21] Capuano F, Mülleder M, Kok R, Blom HJ, Ralser M. Cytosine DNA methylation is found in Drosophila melanogaster but absent in Saccharomyces cerevisiae, Schizosaccharomyces pombe, and other yeast species. Analytical Chemistry. 2014;86:3697-3702. DOI: 10.1021/ac500447w

[22] Panikar CS, Rajpathak SN, Abhyankar V, Deshmukh S, Deobagkar DD. Presence of DNA methyltransferase activity and CpC methylation in Drosophila melanogaster. Molecular Biology Reports. 2015;42:1615-1621. DOI: 10.1007/s11033-015-3931-5

[23] Zhang G, Huang H, Liu D, Cheng Y, Liu X, Zhang W, Yin R, Zhang D, Zhang P, Liu J, Li C, Liu B, Luo Y, Zhu Y, Zhang N, He S, He C, Wang H, Chen D. N6-methyladenine DNA modification in Drosophila. Cell. 2015;161:893-906. DOI: 10.1016/j.cell.2015.04.018

[24] Konsolaki M. Fruitful research: Drug target discovery for neurodegenerative diseases in Drosophila. Expert Opinion on Drug Discovery. 2013;8:1503-1513. DOI: 10.1517/17460441.2013.849691
[25] Pandey UB, Nichols CD. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacological Reviews. 2011;63:411-436. DOI: 10.1124/pr.110.010393

[26] Graham P, Pick L. Drosophila as a model for diabetes and diseases of insulin resistance. Current Topics in Developmental Biology. 2017;121:397-419. DOI: 10.1016/bs.ctdb.2016.07.011

[27] Millburn GH, Crosby MA, Gramates LS, Tweedie S. FlyBase consortium. FlyBase portals to human disease research using Drosophila models. Disease Models & Mechanisms. 2016;9:245-252. DOI: 10.1242/dmm.023317

[28] Ugur B, Chen K, Bellen HJ. Drosophila tools and assays for the study of human diseases. Disease Models & Mechanisms. 2016;9:235-244. DOI: 10.1242/dmm.023762

[29] Na J, Musselman LP, Pendse J, Baranski TJ, Bodmer R, Ocorr K, Cagan R. A Drosophila model of high sugar diet-induced cardiomyopathy. PLoS Genetics. 2013;9:e1003175. DOI: 10.1371/journal.pgen.1003175

[30] Ocorr K, Vogler G, Bodmer R. Methods to assess Drosophila heart development, function and aging. Methods. 2014;68:265-272. DOI: 10.1016/j.ymeth.2014.03.031

[31] Zhu J-Y, Fu Y, Nettleton M, Richman A, Han Z. High throughput in vivo functional validation of candidate congenital heart disease genes in Drosophila. eLife. 2017;6:e22617. DOI: 10.7554/eLife.22617

[32] Buescher JL, Musselman LP, Wilson CA, Lang T, Keleher M, Baranski TJ, Duncan JG. Evidence for transgenerational metabolic programming in Drosophila. Disease Models & Mechanisms. 2013;6:1123-1132. DOI: 10.1242/dmm.011924

[33] Brookheart RT, Duncan JG. Drosophila melanogaster: An emerging model of transgenerational effects of maternal obesity. Molecular and Cellular Endocrinology. 2016;435:20-28. DOI: 10.1016/j.mce.2015.12.003

[34] Akinola OB. Sweet old memories: A review of the experimental models of the association between diabetes, senility and dementia. Metabolic Brain Disease. 2016;31:1003-1010. DOI: 10.1007/s11011-016-9876-0

[35] Fernandez-Funez P, de Mena L, Rincon-Limas DE. Modeling the complex pathology of Alzheimer’s disease in Drosophila. Experimental Neurology 2015;274:58-71. DOI: 10.1016/j.expneurol.2015.05.013

[36] Sonoshita M, Cagan RL. Modeling human cancers in Drosophila. Current Topics in Developmental Biology. 2017;121:287-309. DOI: 10.1016/bs.ctdb.2016.07.008

[37] Blake GE, Watson ED. Unravelling the complex mechanisms of transgenerational epigenetic inheritance. Current Opinion in Chemical Biology. 2016;33:101-107. DOI: 10.1016/j.cbpa.2016.06.008

[38] Van Otterdijk SD, Michels KB. Transgenerational epigenetic inheritance in mammals: How good is the evidence? The FASEB Journal. 2016;30:2457-2465. DOI: 10.1096/fj.201500083
[39] Prokopuk L, Western PS, Stringer JM. Transgenerational epigenetic inheritance: Adap-
tation through the germline epigenome? Epigenomics. 2015;7:829-846. DOI: 10.2217/epi.15.36

[40] Halton TL, Willett WC, Liu S, Manson JE, Stampfer MJ, Potato HFB. French fry consump-
tion and risk of type 2 diabetes in women. The American Journal of Clinical Nutrition. 
2006;83:284-290

[41] Hsu TM, Kanoski SE. Blood-brain barrier disruption: Mechanistic links between west-
ern diet consumption and dementia. Frontiers in Aging Neuroscience. 2014;6:88. DOI: 
10.3389/fnagi.2014.00088

[42] Medina-Remón A, Kirwan R, Lamuela-Raventós RM, Estruch R. Dietary patterns and 
the risk of obesity, type 2 diabetes mellitus, cardiovascular diseases, asthma, and neuro-
degenerative diseases. Critical Reviews in Food Science and Nutrition. 2016;1-35. DOI: 
10.1080/10408398.2016.1158690

[43] Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: 
Are they linked? Gut Microbes. 2017;8:130-142. DOI: 10.1080/19490976.2016.1270811

[44] Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O’Keefe JH, Brand-
Miller J. Origins and evolution of the western diet: Health implications for the 21st cen-
tury. The American Journal of Clinical Nutrition. 2005;81:341-354

[45] San Martin R, Brito J, Siques P, León-Velarde F. Obesity as a conditioning factor for high-
altitude diseases. Obesity Facts. 2017;10:363-372. DOI: 10.1159/000477461

[46] Jimenez-Chillaron JC, Ramon-Krauel M, Ribo S, Diaz R. Transgenerational epigen-
etic inheritance of diabetes risk as a consequence of early nutritional imbalances. The 
Proceedings of the Nutrition Society. 2016;75:78-89. DOI: 10.1017/S0029665115004231

[47] Frias AE, Grove KL. Obesity: A transgenerational problem linked to nutrition during 
pregnancy. Seminars in Reproductive Medicine. 2012;30:472-478. DOI: 10.1055/s-0032-
1328875

[48] Aiken CE, Ozanne SE. Transgenerational developmental programming. Human Reproduction 
Update. 2014;20:63-75. DOI: 10.1093/humupd/dmt043

[49] Falta W, Boller R. Insulärer und insulinresistenter diabetes. Klinische Wochenschrift. 
1931;10:438-443. DOI: 10.1007/BF01736348

[50] Becker A, Schlöder P, Steele JE, Wegener G. The regulation of trehalose metabolism in 
insects. Experiientia. 1996;52:433-439

[51] Matsuda H, Yamada T, Yoshida M, Nishimura T. Flies without trehalose. The Journal of 
Biological Chemistry. 2015;290:1244-1255. DOI: 10.1074/jbc.M114.619411

[52] Grönke S, Müller G, Hirsch J, Fellert S, Andreou A, Haase T, Jäckle H, Kühnlein RP. Dual 
lipolytic control of body fat storage and mobilization in Drosophila. PLoS Biology. 2007;5: 
e137. DOI: 10.1371/journal.pbio.0050137
[53] Teleman AA, Hietakangas V, Sayadian AC, Cohen SM. Nutritional control of protein biosynthetic capacity by insulin via Myc in Drosophila. Cell Metabolism. 2008;7:21-32. DOI: 10.1016/j.cmet.2007.11.010

[54] Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harbor Perspectives in Biology. 2014;6:a009191. DOI: 10.1101/cshperspect.a009191

[55] Risso G, Blaustein M, Pozzi B, Mammi P, Srebrow A. Akt/PKB: One kinase, many modifications. The Biochemical Journal. 2015;468:203-214. DOI: 10.1042/BJ20150041

[56] Palu RAS, Praggastis SA, Thummel CS. Parental obesity leads to metabolic changes in the F2 generation in Drosophila. Molecular Metabolism. 2017;6:631-639. DOI: 10.1016/j.molmet.2017.03.012

[57] Dew-Budd K, Jarnigan J, Reed LK. Genetic and sex-specific transgenerational effects of a high fat diet in Drosophila melanogaster. PLoS One. 2016;11:e0160857. DOI: 10.1371/journal.pone.0160857

[58] Parvy J-P, Napal L, Rubin T, Poidevin M, Perrin L, Wicker-Thomas C, Montagne J. Drosophila melanogaster acetyl-CoA-carboxylase sustains a fatty acid-dependent remote signal to waterproof the respiratory system. PLoS Genetics. 2012;8:e1002925. DOI: 10.1371/journal.pgen.1002925

[59] Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T, Sierra F. Geroscience: Linking aging to chronic disease. Cell. 2014;159:709-713. DOI: 10.1016/j.cell.2014.10.039

[60] Partridge L. Intervening in ageing to prevent the diseases of ageing. Trends in Endocrinology and Metabolism. 2014;25:555-557. DOI: 10.1016/j.tem.2014.08.003

[61] Gale CR, Hagenaaars SP, Davies G, Hill WD, Liewald DCM, Cullen B, Penninx BW, International Consortium for Blood Pressure GWAS, CHARGE Consortium Aging and Longevity Group, Boomsma DI, Pell J, AM MI, Smith DJ, Deary IJ, Harris SE. Pleiotropy between neuroticism and physical and mental health: Findings from 108 038 men and women in UK Biobank. Translational Psychiatry. 2016;6:e791. DOI: 10.1038/tp.2016.56

[62] Arriola Apelo SI, Lamming DW. Rapamycin: An inhibiTOR of aging emerges from the soil of Easter Island. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2016;71:841-849. DOI: 10.1093/gerona/glw090

[63] Fontana L, Partridge L. Promoting health and longevity through diet: From model organisms to humans. Cell. 2015;161:106-118. DOI: 10.1016/j.cell.2015.02.020

[64] Gillespie ZE, Pickering J, Eskiw CH. Better living through chemistry: Caloric restriction (CR) and CR mimetics alter genome function to promote increased health and lifespan. Frontiers in Genetics. 2016;7:142. DOI: 10.3389/fgene.2016.00142
[65] Sierra F. The emergence of geroscience as an interdisciplinary approach to the enhancement of health span and life span. Cold Spring Harbor Perspectives in Medicine. 2016;6:a025163. DOI: 10.1101/cshperspect.a025163

[66] Lappin TRJ, Grier DG, Thompson A, Halliday HL. HOX genes: Seductive science, mysterious mechanisms. The Ulster Medical Journal. 2006;75:23-31

[67] Quinonez SC, Innis JW. Human HOX gene disorders. Molecular Genetics and Metabolism. 2014;111:4-15. DOI: 10.1016/j.ymgme.2013.10.012

[68] Innis JW. Role of HOX genes in human development. Current Opinion in Pediatrics. 1997;9:617-622

[69] Goodman FR, Scambler PJ. Human HOX gene mutations. Clinical Genetics. 2001;59:1-11

[70] Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Current Biology. 2004;14:885-890. DOI: 10.1016/j.cub.2004.03.059

[71] Frankel S, Ziafazeli T, Rogina B. dSir2 and longevity in Drosophila. Experimental Gerontology. 2011;46:391-396. DOI: 10.1016/j.exger.2010.08.007

[72] Partridge L, Alic N, Bjedov I, Piper MD. Aging in Drosophila: The role of the insulin/IGF and TOR signalling network. Experimental Gerontology. 2011;46:376-381. DOI: 10.1016/j.exger.2010.09.003

[73] Dietzl G, Chen D, Schnorrer F, Su K-C, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, Couto A, Marra V, Keleman K, Dickson BJ. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448:151-156. DOI: 10.1038/nature05954

[74] Xia B, de Belle JS. Transgenerational programming of longevity and reproduction by post-eclosion dietary manipulation in Drosophila. Aging 2016;8:1115-1134. DOI: 10.18632/aging.100932

[75] Roussou IG, Savakis C, Tavernarakis N, Metaxakis A. Stage dependent nutritional regulation of transgenerational longevity. Nutr Healthy Aging. 2016;4:47-54. DOI: 10.3233/NHA-160012

[76] Shameer PM, Sowmithra K, Harini BP, Chaubey RC, Jha SK, Shetty NJ. Does exposure of male Drosophila melanogaster to acute gamma radiation influence egg to adult development time and longevity of F1-F3 offspring? Entomological Science. 2015;18:368-376. DOI: 10.1111/ens.12120

[77] Xia B, Gerstin E, Schones DE, Huang W, de Belle JS. Transgenerational programming of longevity through E(z)-mediated histone H3K27 trimethylation in Drosophila. Aging 2016;8:2988-3008. DOI: 10.18632/aging.101107

[78] Tindall KR, Stein J, Hutchinson F. Changes in DNA base sequence induced by gamma-ray mutagenesis of lambda phage and prophage. Genetics. 1988;118:551-560
[79] Moskalev AA, Plyusnina EN, Shaposhnikov MV. Radiation hormesis and radioadaptive response in Drosophila melanogaster flies with different genetic backgrounds: The role of cellular stress-resistance mechanisms. Biogerontology. 2011;12:253-263. DOI: 10.1007/s10522-011-9320-0

[80] Calabrese EJ. Hormesis: A fundamental concept in biology. Microbial Cell. 2014;1:145-149. DOI: 10.15698/mic2014.05.145

[81] Rattan SIS. Hormesis in aging. Ageing Research Reviews. 2008;7:63-78. DOI: 10.1016/j.arr.2007.03.002

[82] Calabrese EJ. Hormetic mechanisms. Critical Reviews in Toxicology. 2013;43:580-606. DOI: 10.3109/10408444.2013.808172

[83] Tang J, Yan H, Zhuang S. Histone deacetylases as targets for treatment of multiple diseases. Clinical Science. 2013;124:651-662. DOI: 10.1042/CS20120504

[84] Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: Implications for disease and therapy. Nature Reviews. Genetics. 2009;10:32-42. DOI: 10.1038/nrg2485

[85] Ceccacci E, Minucci S. Inhibition of histone deacetylases in cancer therapy: Lessons from leukaemia. British Journal of Cancer. 2016;114:605-611. DOI: 10.1038/bjc.2016.36

[86] Ding G-L, Wang F-F, Shu J, Tian S, Jiang Y, Zhang D, Wang N, Luo Q, Zhang Y, Jin F, Leung PCK, Sheng J-Z, Huang H-F. Transgenerational glucose intolerance with Igf2/H19 epigenetic alterations in mouse islet induced by intrauterine hyperglycemia. Diabetes. 2012;61:1133-1142. DOI: 10.2337/db11-1314

[87] Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, Faucette RR, Chow A, Diaz R, Ferguson-Smith A, Patti ME. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes. 2009;58:460-468. DOI: 10.2337/db08-0490

[88] Bloch Qazi MC, Miller PB, Poeschel PM, Phan MH, Thayer JL, Medrano CL. Transgenerational effects of maternal and grandmaternal age on offspring viability and performance in Drosophila melanogaster. Journal of Insect Physiology. 2017;100:43-52. DOI: 10.1016/j.jinsphys.2017.05.007

[89] Bonduriansky R, Crean AJ, Day T. The implications of nongenetic inheritance for evolution in changing environments. Evolutionary Applications. 2012;5:192-201. DOI: 10.1111/j.1752-4571.2011.00213.x

[90] Brommer JE, Fricke C, Edward DA, Chapman T. Interactions between genotype and sexual conflict environment influence transgenerational fitness in Drosophila melanogaster. Evolution. 2012;66:517-531. DOI: 10.1111/j.1558-5646.2011.01449.x

[91] Strømsøaas Ø. Sexual maturity in drosophila. Nature. 1959;183:409-410. DOI: 10.1038/183409

[92] Bohacek J, Mansuy IM. Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. Nature Reviews. Genetics. 2015;16:641-652. DOI: 10.1038/nrg3964
[93] Singh P, Srivas S, Thakur MK. Epigenetic regulation of memory – therapeutic potential for disorders. Current Neuropharmacology. 2017. DOI: 10.2174/1570159X15666170404144522

[94] Williams ZM. Transgenerational influence of sensorimotor training on offspring behavior and its neural basis in Drosophila. Neurobiology of Learning and Memory. 2016;131:166-175. DOI: 10.1016/j.nlm.2016.03.023

[95] Burns JG, Mery F. Transgenerational memory effect of ageing in Drosophila. Journal of Evolutionary Biology. 2010;23:678-686. DOI: 10.1111/j.1420-9101.2010.01932.x

[96] Dias BG, Ressler KJ. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nature Neuroscience. 2014;17:89-96. DOI: 10.1038/nn.3594

[97] Mery F, Kawecki TJ. A cost of long-term memory in Drosophila. Science. 2005;308:1148. DOI: 10.1126/science.1111331

[98] Tully T, Preat T, Boynton SC, Del Vecchio M. Genetic dissection of consolidated memory in Drosophila. Cell. 1994;79:35-47. DOI: 10.1016/0092-8674(94)90398-0

[99] Tully T, Quinn WG. Classical conditioning and retention in normal and mutant Drosophila melanogaster. Journal of Comparative Physiology A. 1985;157:263-277

[100] Tamura T, Chiang A-S, Ito N, Liu H-P, Horiuchi J, Tully T, Saitoe M. Aging specifically impairs amnesiac-dependent memory in Drosophila. Neuron. 2003;40:1003-1011. DOI: 10.1016/S0896-6273(03)00732-3

[101] Ramaswami M. Specifying the age-sensitive component of a short-term memory. Neuron. 2003;40:877-879. DOI: 10.1016/S0896-6273(03)00759-1

[102] Margulies C, Tully T, Dubnau J. Deconstructing memory in Drosophila. Current Biology. 2005;15:R700-R713. DOI: 10.1016/j.cub.2005.08.024

[103] Feany MB, Quinn WG. A neuropeptide gene defined by the Drosophila memory mutant amnesiac. Science. 1995;268:869-873

[104] Xia S, Tully T. Segregation of odor identity and intensity during odor discrimination in Drosophila mushroom body. PLoS Biology. 2007;5:e264. DOI: 10.1371/journal.pbio.0050264

[105] Waddell S, Armstrong JD, Kitamoto T, Kaiser K, Quinn WG. The amnesiac gene product is expressed in two neurons in the Drosophila brain that are critical for memory. Cell. 2000;103:805-813. DOI: 10.1016/S0092-8674(00)00183-5

[106] Montero M, Yon L, Kikuyama S, Dufour S, Vaudry H. Molecular evolution of the growth hormone-releasing hormone/pituitary adenylate cyclase-activating polypeptide gene family: Functional implication in the regulation of growth hormone secretion. Journal of Molecular Endocrinology. 2000;25:157-168

[107] Yu L, Jia Y, Su G, Sun Y, Letcher RJ, Giesy JP, Yu H, Han Z, Liu C. Parental transfer of tris(1,3-dichloro-2-propyl) phosphate and transgenerational inhibition of growth of zebrafish exposed to environmentally relevant concentrations. Environmental Pollution. 2017;220:196-203. DOI: 10.1016/j.envpol.2016.09.039
[108] Moore MS, DeZazzo J, Luk AY, Tully T, Singh CM, Heberlein U. Ethanol intoxication in *Drosophila*: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. Cell. 1998;93:997-1007. DOI: 10.1016/S0092-8674(00)81205-2

[109] Yohn NL, Bartolomei MS, Blendy JA. Multigenerational and transgenerational inheritance of drug exposure: The effects of alcohol, opiates, cocaine, marijuana, and nicotine. Progress in Biophysics and Molecular Biology. 2015;118:21-33. DOI: 10.1016/j.pbiomolbio.2015.03.002

[110] Pembrey M, Saffery R, Bygren LO. Network in epigenetic epidemiology. Human transgenerational responses to early-life experience: Potential impact on development, health and biomedical research. Journal of Medical Genetics. 2014;51:563-572. DOI: 10.1136/jmedgenet-2014-102577

[111] Aldrich JC, Maggert KA. Transgenerational inheritance of diet-induced genome rearrangements in *Drosophila*. PLoS Genetics. 2015;11:e1005148. DOI: 10.1371/journal.pgen.1005148

[112] Ciabrelli F, Comoglio F, Fellous S, Bonev B, Ninova M, Szabo Q, Xuéreb A, Klopp C, Aravin A, Paro R, Bantignies F, Cavalli G. Stable Polycomb-dependent transgenerational inheritance of chromatin states in *Drosophila*. Nature Genetics. 2017;49:876-886. DOI: 10.1038/ng.3848

[113] Sharma A, Singh P. Detection of transgenerational spermatogenic inheritance of adult male acquired CNS gene expression characteristics using a *Drosophila* systems model. PLoS One. 2009;4:e5763. DOI: 10.1371/journal.pone.0005763

[114] Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX. Evidence for transgenerational transmission of epigenetic tumor susceptibility in *Drosophila*. PLoS Genetics. 2007;3:1598-1606. DOI: 10.1371/journal.pgen.0030151

[115] Zenk F, Loeser E, Schiavo R, Kilpert F, Bogdanović O, Iovino N. Germ line-inherited H3K27me3 restricts enhancer function during maternal-to-zygotic transition. Science. 2017;357:212-216. DOI: 10.1126/science.aam5339

[116] Mohammad F, Singh P, Sharma A. A *Drosophila* systems model of pentylenetetrazole induced locomotor plasticity responsive to antiepileptic drugs. BMC Systems Biology. 2009;3:11. DOI: 10.1186/1752-0509-3-11

[117] Grewal SS. Insulin/TOR signaling in growth and homeostasis: A view from the fly world. The International Journal of Biochemistry & Cell Biology. 2009;41:1006-1010. DOI: 10.1016/j.biocel.2008.10.010

[118] Holland ML, Lowe R, Caton PW, Gemma C, Carbajosa G, Danson AF, Carpenter AAM, Loche E, Ozanne SE, Rakyen VK. Early-life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice. Science. 2016;353:495-498. DOI: 10.1126/science.aaf7040

[119] Usher CL, McCarroll SA. Complex and multi-allelic copy number variation in human disease. Briefings in Functional Genomics. 2015;14:329-338. DOI: 10.1093/bfgp/elv028
Cuccaro D, De Marco EV, Cittadella R, Cavallaro S. Copy number variants in Alzheimer’s disease. *Journal of Alzheimer’s Disease*. 2017;55:37-52. DOI: 10.3233/JAD-160469

Rutkowski TP, Schroeder JP, Gafford GM, Warren ST, Weinshenker D, Caspary T, Mulle JG. Unraveling the genetic architecture of copy number variants associated with schizophrenia and other neuropsychiatric disorders. *Journal of Neuroscience Research*. 2017;95:1144-1160. DOI: 10.1002/jnr.23970

Weiner AJ, Scott MP, Kaufman TC. A molecular analysis of fushi tarazu, a gene in *Drosophila* Melanogaster that encodes a product affecting embryonic segment number and cell fate. *Cell*. 1984;37:843-851

Shi S, Calhoun HC, Xia F, Li J, Le L, Li WX. JAK signaling globally counteracts heterochromatin gene silencing. *Nature Genetics*. 2006;38:1071-1076. DOI: 10.1038/ng1860

Jones RS, Gelbart WM. Genetic analysis of the enhancer of zeste locus and its role in gene regulation in *Drosophila melanogaster*. *Genetics*. 1990;126:185-199

Laible G, Wolf A, Dorn R, Reuter G, Niçlow C, Lebersorger A, Popkin D, Pilus L, Jenuwein T. Mammalian homologues of the Polycomb-group gene enhancer of zeste mediate gene silencing in *Drosophila* heterochromatin and at *S. cerevisiae* telomeres. *The EMBO Journal*. 1997;16:3219-3232. DOI: 10.1093/emboj/16.11.3219

Dumesic PA, Homer CM, Moresco JJ, Pack LR, Shanle EK, Coyle SM, Strahl BD, Fujimori DG, Yates JR 3rd, Madhani HD. Product binding enforces the genomic specificity of a yeast Polycomb repressive complex. *Cell* 2015;160:204-218. DOI: 10.1016/j.cell.2014.11.039

Shaver S, Casas-Mollano JA, Cerny RL, Cerutti H. Origin of the Polycomb repressive complex 2 and gene silencing by an E(z) homolog in the unicellular alga *Chlamydomonas*. *Epigenetics*. 2010;5:301-312. DOI: 10.4161/epi.5.4.11608

Ihara M, Meyer-Ficca ML, Leu NA, Rao S, Li F, Gregory BD, Zalenskaya IA, Schultz RM, Meyer RG. Paternal poly (ADP-ribose) metabolism modulates retention of inheritable sperm histones and early embryonic gene expression. *PLoS Genetics*. 2014;10:e1004317. DOI: 10.1371/journal.pgen.1004317

Erkek S, Hisano M, Liang C-Y, Gill M, Murr R, Dieker J, Schübeler D, van der Vlag J, Stadler MB, Peters AHFM. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nature Structural & Molecular Biology* 2013;20:868-875. DOI: 10.1038/nsmb.2599

Bryczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, Beisel C, Schübeler D, Stadler MB, Peters AH. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nature Structural & Molecular Biology*. 2010;17:679-687. DOI: 10.1038/nsmb.1821

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N, Rando OJ. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell*. 2010;143:1084-1096. DOI: 10.1016/j.cell.2010.12.008
[132] Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, Benayoun BA, Shi Y, Brunet A. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. Nature. 2011;479:365-371. DOI: 10.1038/nature10572

[133] Watson CT, Disanto G, Sandve GK, Breden F, Giovannoni G, Ramagopalan SV. Age-associated hyper-methylated regions in the human brain overlap with bivalent chromatin domains. PLoS One. 2012;7:e43840. DOI: 10.1371/journal.pone.0043840

[134] Rakyan VK, Down TA, Maslau S, Andrew T, Yang T-P, Beyan H, Whittaker P, McCann OT, Finer S, Valdes AM, Leslie RD, Deloukas P, Spector TD. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Research. 2010;20:434-439. DOI: 10.1101/gr.103101.109

[135] Dozmorov MG. Polycomb repressive complex 2 epigenomic signature defines age-associated hypermethylation and gene expression changes. Epigenetics. 2015;10:484-495. DOI: 10.1080/15592294.2015.1040619

[136] Kim S, Welsh DA, Myers L, Cherry KE, Wyckoff J, Jazwinski SM. Non-coding genomic regions possessing enhancer and silencer potential are associated with healthy aging and exceptional survival. Oncotarget. 2015;6:3600-3612. DOI: 10.18632/oncotarget.2877

[137] Wagner W, Ho AD, Zenke M. Different facets of aging in human mesenchymal stem cells. Tissue Engineering. Part B, Reviews. 2010;16:445-453. DOI: 10.1089/ten.TEB.2009.0825

[138] Schellenberg A, Lin Q, Schüeler H, Koch CM, Joussen S, Denecke B, Walenda G, Pallua N, Suschek CV, Zenke M, Wagner W. Replicative senescence of mesenchymal stem cells causes DNA-methylation changes which correlate with repressive histone marks. Aging. 2011;3:873-888. DOI: 10.18632/aging.100391

[139] Siebold AP, Banerjee R, Tie F, Kiss DL, Moskowitz J, Harte PJ. Polycomb repressive complex 2 and Trithorax modulate *Drosophila* longevity and stress resistance. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:169-174. DOI: 10.1073/pnas.0907739107

[140] Öst A, Lempradl A, Casas E, Weigert M, Tiko T, Deniz M, Pantano L, Boenisch U, Itskov PM, Stoeckius M, Ruf M, Rajewsky N, Reuter G, Iovino N, Ribeiro C, Alenius M, Heyne S, Vavouri T, Pospisilik JA. Paternal diet defines offspring chromatin state and intergenerational obesity. Cell. 2014;159:1352-1364. DOI: 10.1016/j.cell.2014.11.005

[141] Maures TJ, Greer EL, Hauswirth AG, Brunet A. The H3K27 demethylase UTX-1 regulates *C. elegans* lifespan in a germline-independent, insulin-dependent manner. Aging Cell. 2011;10:980-990. DOI: 10.1111/j.1474-9726.2011.00738.x

[142] Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, Porter Scott M, Chesworth R, Moyer MP, Copeland RA, Richon VM, Pollock RM, Kuntz KW, Keilhack H. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:7922-7927. DOI: 10.1073/pnas.1303800110
[143] Takahashi T, Yoshimura Y, Hiraishi H, Hasegawa C, Munwue T, Higashida H, Minabe Y, Kikuchi M. Enhanced brain signal variability in children with autism spectrum disorder during early childhood. Human Brain Mapping. 2016;37:1038-1050. DOI: 10.1002/hbm.23089

[144] Halperin JM, Bédard A-CV, Curchack-Lichtin JT. Preventive interventions for ADHD: A neurodevelopmental perspective. Neurotherapeutics. 2012;9:531-541. DOI: 10.1007/s13311-012-0123-z

[145] Iannitelli A, Quartini A, Tirassa P, Bersani G. Schizophrenia and neurogenesis: A stem cell approach. Neuroscience and Biobehavioral Reviews. 2017;80:414-442. DOI: 10.1016/j.neubiorev.2017.06.010

[146] Selemon LD, Zecevic N. Schizophrenia: A tale of two critical periods for prefrontal cortical development. Translational Psychiatry. 2015;5:e623. DOI: 10.1038/tp.2015.115

[147] Gillman MW. Early infancy as a critical period for development of obesity and related conditions. Nestlé Nutrition Workshop Series. Paediatric Programme. 2010;65:13-20; discussion 20. DOI: 10.1159/000281141

[148] Cameron N, Demerath EW. Critical periods in human growth and their relationship to diseases of aging. American Journal of Physical Anthropology. 2002;(Suppl 35):159-184. DOI: 10.1002/ajpa.10183

[149] Hanson MA, Low FM, Gluckman PD. Epigenetic epidemiology: The rebirth of soft inheritance. Annals of Nutrition & Metabolism. 2011;58(Suppl 2):8-15. DOI: 10.1159/000328033

[150] Barker DJP. Developmental origins of adult health and disease. Journal of Epidemiology and Community Health. 2004;58:114-115

[151] Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, Nieuwland M, Simonis M, de Laat W, van Lohuizen M, van Steensel B. Interactions among Polycomb domains are guided by chromosome architecture. PLoS Genetics 2011;7:e1001343. DOI: 10.1371/journal.pgen.1001343

[152] Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, Tixier V, Mas A, Cavalli G. Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. Cell. 2011;144:214-226. DOI: 10.1016/j.cell.2010.12.026

[153] Talbert PB, Garber RL. The Drosophila homeotic mutation Nasobemia (AntpNs) and its revertants: An analysis of mutational reversion. Genetics. 1994;138:709-720

[154] Heard E, Martienssen RA. Transgenerational epigenetic inheritance: Myths and mechanisms. Cell. 2014;157:95-109. DOI: 10.1016/j.cell.2014.02.045

[155] Iovino N, Ciabrelli F, Cavalli G. PRC2 controls Drosophila oocyte cell fate by repressing cell cycle genes. Developmental Cell. 2013;26:431-439. DOI: 10.1016/j.devcel.2013.06.021

[156] Langley-Evans SC. Nutrition in early life and the programming of adult disease: A review. Journal of Human Nutrition and Dietetics. 2015;28(Suppl 1):1-14. DOI: 10.1111/jhn.12212