Do Genes Matter?

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Abstract: In the past two decades our knowledge of genetics has increased substantially with the advent of whole genome sequencing, a better grasp of the genetic factors that may predispose people to certain medical and psychological conditions and, most importantly in the author's estimation, the emergence of epigenetics. This article addresses the question of what our improved understanding of genetics contributes to our understanding of ourselves.

Keywords: genetics, epigenetics, transgenerational inheritance

Genetics Made Simple

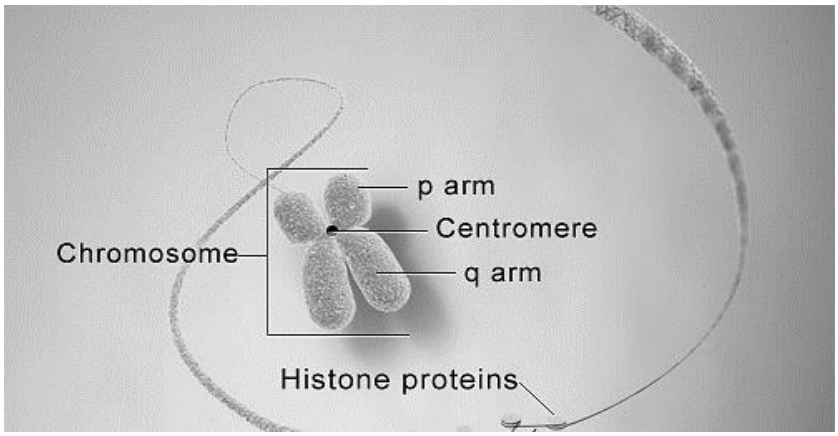
We start with chromosomes. A chromosome is an organized package of DNA found in the nucleus of the cell. Different organisms have different numbers of chromosomes. Humans have 23 pairs of chromosomes.

It is impossible to discuss genetics without the use of scientific jargon. For this I ask your forgiveness and patience. Even if you find some of these terms daunting, please read on. You will get the gist of it. Relax! There is no exam after you have finished the lesson.

- 22 pairs of numbered chromosomes, called autosomes, and one pair of sex chromosomes, X and Y.
- Each parent contributes one chromosome to each pair so that offspring get half of their chromosomes from their mother and half from their father.

Chromosomes are found in the nucleus of each cell. They are made of DNA strands. Sections of chromosomes are called genes and code for protein, that is, everything in our bodies from the neurons in our brain down to the nails on our toes. In order to produce protein, DNA (deoxyribonucleic acid) needs to be "transcribed" into RNA (ribonucleic acid). RNA gets "translated" into protein.

One Chromosome Pair



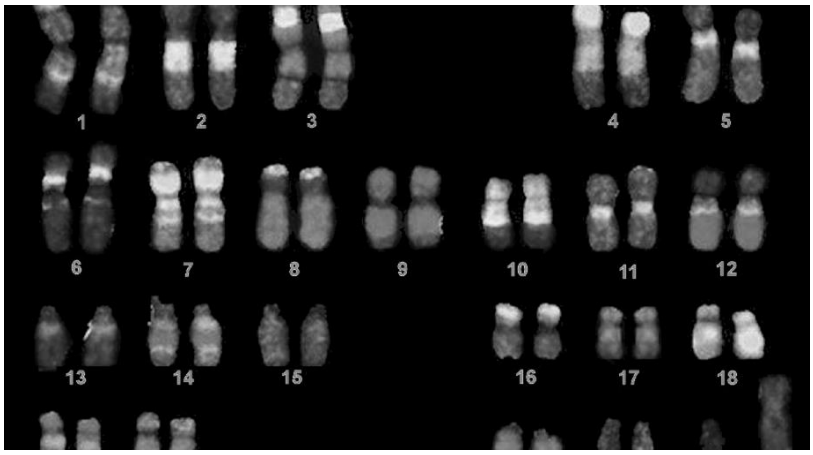
Credit: *Help Me Understand Genetics page: National Library of Medicine (US). Genetics Home Reference [Internet]. Bethesda (MD): The Library; 2016 May 17. What is a chromosome?; [cited 2016 May 23]; Available from: https://ghr.nlm.nih.gov/primer/basics/chromosome*

DNA Sequencing is the process by which the exact order of the 3 billion chemical building blocks called bases is determined. They are

- adenine (A)
- thymine (T)
- cytosine (C)
- guanine (G)

The Human Genome contains an estimated 25,000-35,000 genes as well as the regions (switches) controlling them. A genome is the complete set of DNA in a cell. DNA carries the instructions for building all of the proteins that make each living creature unique. The 25,000 – 35,000 genes make up only 5% of the entire genome. The rest consists of switches. Referring to the Human Genome: it is like you have a 100 page book, and 95 pages are instructions on how to read the other five pages. (Sapolsky, 1994/2001)

46 Human Chromosomes



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Charles Darwin in *On the Origin of Species,* wrote that evolutionary changes take place over many generations and through millions of years of natural selection. Human geneticists have had remarkable success in identifying individual genes with variations that lead to simple Mendelian traits and diseases such as phenylketonuria (PKU), sickle-cell anemia, Tay-Sachs disease, and cystic fibrosis (Botstein & Risch 2003; Risch 2000). Diseases with simple Mendelian patterns of inheritance are uncommon, while most human diseases and traits such as schizophrenia, alcohol dependence, or diabetes, to mention just a few, are complex and multifactorial. Personality development and behavior is considered complex because they are constituted of an array of genetic and psycho-socio-economic-cultural components.

The front cover of TIME Magazine, October 25th, 2004, depicted the serene countenance of a blue phantom-like figure, with long flowing hair, eyes closed, hands folded as if in prayer, a golden double helix of DNA imprinted almost mystically upon her forehead. The title of the cover story was also in gold, "The God Gene**:** Does our DNA compel us to seek a higher power?" The featured article inside that issue hypothesized on the presence of a "god Gene" in our genome (Kluger, Chu, Liston, Sieger, & Williams, 2004).

Stories like this make no sense at all. There is no God Gene, or Anger Gene, or Schizophrenia Gene. It takes many genes to effect a disease or a personality trait. By the same token a different combination of the same genes can create intelligence, musical abilities, foresight, etc. Researchers from the University of Geneva report that genetic variation at a single genomic position impacts multiple, separate genes. If one element changes, the whole system in which this element is embedded will be altered (Watzak et al, 2015).

According to Michael Meaney (2001), who specializes in biological psychiatry and gene expression at McGill University, Montreal, "At no point in life is the operation of the genome independent of the context in which it functions." In other words, the intracellular environment and the extracellular environment, including hormones and neurotransmitters, regulate gene activity and nutrients derived from food. The extracellular environment in turn is affected by the environment of the individual, for example, by social interactions. And this brings us to the field of epigenetics.

Why Your DNA Is Not Your Destiny

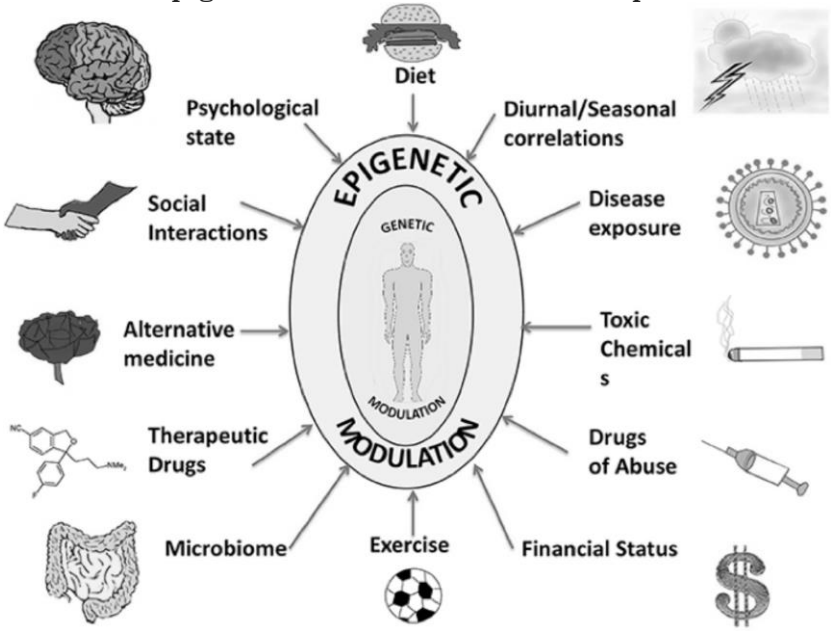
Another cover of TIME Magazine a few years later (in early January 2010) also depicts a double helix of DNA, this time as a giant zipper hanging down across the page, its shiny gold slider opening part way, as if unzipping an actual strand of DNA. This time the cover story reads, "Why Your DNA Isn't Your Destiny: The new science of epigenetics reveals how the choices you make can change your genes - and those of your kids." This time, TIME is on the right track. Epigenetics is the study of changes in gene activity that do not alter the genes themselves but still get passed down to at least one successive generation. These patterns of gene expression are governed by the cellular material — the epigenome — that sits on top of the genome, just outside it (hence the prefix *epi-*, which means above). A central component of epigenetics is methylation, in which a chemical group (methyl) attaches to parts of the DNA -- a process that acts like a dimmer on gene function in response to social and physical environments.

Epigenetic "switches" turn genes on or off, enable them to speak or to remain silent. It is through epigenetic switches that environmental factors like prenatal nutrition, stress, and postnatal maternal behavior can affect gene expression that is passed from one generation to the next. It is important to remember that epigenetics isn't evolution. It doesn't change DNA. Epigenetic changes represent a biological response to an environmental factor. This factor may be positive, life affirming, or negative, life threatening. Epigenetic changes often serve to biologically prepare offspring for an environment into which they will be born. Think of genetics as the hardware and epigenetics as the software in your computer.

Since the advent of epigenetics about 20 years ago, geneticists believed that inheritance of traits is governed by genes and that between each generation the epigenetic marks are erased in cells called primordial gene cells, the precursors to sperm and eggs. This "reprogramming" allows all genes to be read afresh for each new person. However, and this is most important for our purposes, some methylation can "escape" the reprogramming process and can thus be passed on to offspring. Jamie Hackett, at the University of Cambridge, in a trailblazing paper writes, "Our research demonstrates how genes could retain some memory of their past experiences, revealing that one of the big barriers to the theory of epigenetic inheritance - that epigenetic information is erased between generations - should be reassessed." (Hackett et al, 2013).

With a few exceptions, every cell type in a multicellular organism carries the same endowment of genetic instructions encoded in the nucleotide sequence of its DNA genome. Nevertheless, each cell type expresses only those genes required for its specific function. Muscle cells and nerve cells, for instance, implement quite different genetic programs directed by subsets of the genes in the whole genome. Which genes are activated and at which time is largely determined by cell-type-specific chemical modification of the proteins that package the genes in the cell nucleus, the histones. The pattern of modification is determined by the interplay between enzymes that attach or remove the various tags (writers and erasers, respectively), which is in turn controlled by so-called epigenetic signal pathways that respond to changes in the cell's local environment. "Perturbations of these signaling pathways can precipitate the development of diseases, such as cancer or Alzheimer's," says Peter Becker (2015) of the Ludwig-Maximilians-Universitaet in Munich.

Epigenetics across the human lifespan



Credit: "Figure as originally published in Kanherkar RR, Bhatia-Dey N and Csoka AB (2014) Epigenetics across the human lifespan. Front. Cell Dev. Biol. 2:49. doi: 10.3389/fcell.2014.00049".

Just to bring it home with some concrete examples let us consider the characteristics of identical twins. They may already develop differently in the womb and at birth one will be born before the other. All of these factors contribute to differences in their physical and psychological make-up. In scientific terms, one would say they are genetically identical but phenotypically different. Similarly, a caterpillar and a butterfly are the same genetically but depending on their developmental stages their genes are expressed differently so everything about them is changed.

One of the primary objectives of epigenetics is to study data transfer from one generation to the next by zygote (a fertilized ovum). This tiny cell will carry the blueprint for the future of an entire human being. Many questions arise: How is this blueprint constructed? What does it consist of? Is the information limited to just architectural plans for building a body or is it more? If more, then what exactly?

Before we move on to address these questions we should mention two other cellular ways by which information may be exchanged between people that do not involve germ cells. It has recently been discovered that during pregnancy some of the mother's cells pass through the umbilical cord to her unborn child. A number of her baby's cells are transported back to the mother the same way. Surprisingly, several of the mother's cells remain in her child, and vice versa. Also, a few cells from prior pregnancies persist in mothers for many years. This is called Microchimerism (Yan et al, 2005).

And for the sake of completeness, we will also mention that blood donations and organ transplants can, similarly, pass information on a cellular level to the recipient. We shall visit these examples in the following pages.

Environmental Epigenetics

In the 1980s, Dr. Lars Olov Bygren, Head of the Department of Social Medicine at the University of Umea, Sweden, wondered "Could parents' experiences early in their lives somehow change the traits they passed to their offspring?" Bygren and other scientists have now amassed historical evidence suggesting that powerful environmental conditions (near death from starvation, for instance) can leave an imprint on the genetic material in eggs and sperm. These genetic imprints can short-circuit evolution and pass along new traits in a single generation (Bygren, Kaati, & Edvinsson, 2001; Kaati, Bygren, & Edvinsson, 2002)

Byrgen's records from a small region in Sweden showed that food availability between the ages of nine and twelve for the paternal grandfather affected the lifespan of his grandchildren. But not in the way you might think.

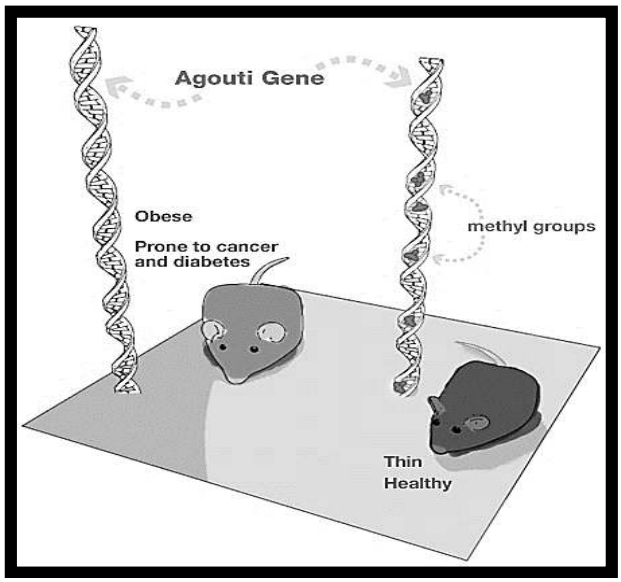
Food abundance for the grandfather was associated with a reduced lifespan for his grandchildren. Early death was the result of either diabetes or heart disease. On the other hand, food shortage for the grandfather was associated with extended lifespan of his grandchildren.

In 1988 a paper was published in *Nature*, one of the very top science journals, by John Cairns, which changed everything (Cairns, Overbaugh, & Miller, 1988). The paper described an experiment in which a particular strain of bacteria E. Coli that could not metabolize lactose, was placed on a lactose medium. Instead of starving, they very quickly underwent genetic changes which allowed them to ingest lactose and thus survive. Cairns reported that at least in some cases, mutations could be specifically directed by selective pressures. So long, "darwinist orthodoxy." Cairns even "brazenly" raised the specter of possible Lamarckian hereditary mechanisms – one could not have gotten more heretic than that in 1988. In the same issue of *Nature*, Franklin Stahl (1988), another one of the founding fathers of bacterial genetics, endorsed these conclusions and ventured his own model of how directed mutations may happen.

Cairns today is Professor of Microbiology at the Radcliffe Infirmary, one of the medical teaching hospitals of Oxford University, and remains a recognized leading authority in mutation genetics. His 1988 article is one of the most highly cited papers in the field, and has spawned an entire new area of study.

One of the crucial experiments supporting epigenetics is one that shows the effect of a mother's diet in shaping the epigenome of her offspring. Both mice and people have a gene called *agouti* (Dolinoy, Huang, & Jirtle, 2007). When a mouse's agouti gene is completely unmethylated (switched off) the mouse has a yellow coat color, is obese, and prone to diabetes and cancer. When the agouti gene is methylated (as it is in normal mice) the coat color is brown and the mouse has a low disease risk. Fat yellow mice and skinny brown ones are genetically identical. The fat yellow mice look different because they have an epigenetic "mutation."

The Agouti Gene



Chemicals and additives that enter our bodies can also affect the epigenome. Bisphenol A (BPA) is a compound used to make polycarbonate plastic. It is in many consumer products including water bottles and tin cans. When pregnant yellow agouti mothers were fed BPA, more yellow, unhealthy babies were born than normal. Exposure to BPA during early development had caused decreased methylation of the agouti gene. However, when BPA-exposed, pregnant yellow agouti mice were fed

methyl-rich foods, the offspring were predominantly brown. The maternal nutrient supplementation had counteracted the negative effects of exposure.

Aberrant DNA methylation in gene promoters is associated with aging and cancer, but the circumstances determining methylation change are unknown. Researchers from the University of Basel investigated the impact of lifestyle modulators of colorectal cancer risk on the stability of gene promoter methylation in the colonic mucosa. Aspirin and hormonal replacement therapy use reduced the methylation rate at these cancer-related genes, whereas smoking and high BMI increased it. They concluded that "lifestyle, including aspirin use, modulates age-associated DNA methylation change in the colonic epithelium and thereby impacts the evolution of cancer methylomes." (Faiza et al., 2014).

Substances known as endocrine disrupting chemicals (EDCs), found in both natural and human-made materials, can pass from one generation to the next. We know that they are toxic. Interestingly, according to a study from The University of Texas at Austin and Washington State University, male and female rats are affected differently by ancestral exposure to vinclozolin, a fungicide commonly used by farmers to treat fruits and vegetables. Female rats whose great grandparents were exposed to vinclozolin become more vulnerable to stress and develop more anxiety. Males who have the same combination of ancestral exposure and stress do not have the same adverse effects. According to one of the lead authors (Gillette, Miller-Crews, Skinner, & Crews, 2014), following exposure to EDCs, what is being passed down from generation to generation is not a change in the genetic code of the animals, but rather a change in the way specific genes are expressed.

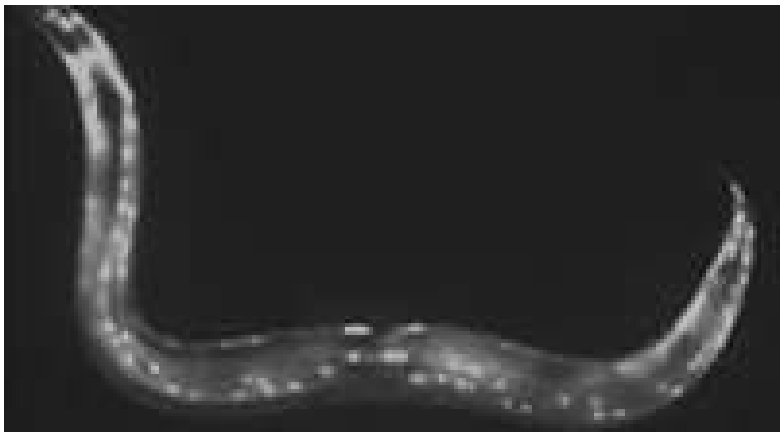
At Emory University researchers (Dias, Maddox,Torsten, & Ressler, 2015) studied the effect of parental olfactory experience on the behavior and neural structures across generations. They gave male lab mice electric shocks every time they were exposed to the smell of acetophenone, a chemical used in perfumes. As a result of this classic conditioning technique the mice became anxious at the mere scent of acetophenone. The mice's noses and brains also adapted accordingly, generating additional M71 neurons - cells receptive to this particular scent—so that they would be extra sensitive to the smell. Their children and grandchildren feared the smell, too, even though they had never been exposed to it.

The mice showed no reaction to other smells and had no fear responses to sounds or different types. To confirm this, the scientists even took sperm from the first set of mice, used in vitro fertilization (IVF) to implant them in females from another lab, raised them in isolation away from any untoward influences, and still found an increased sensitivity to the original scent. It is important to point out that these results did not stem from any socially learned behaviors picked up by hanging around one's anxious parents.

"Our findings provide a framework charged messages regarding fear and anxiety also pass modified brain chemistry to their offspring" (Dias et al, 2015). These experiments suggest that parents in addition to passing emotionally charged messages regarding fear and anxiety also pass modified brain chemistry to their offspring.

Figure #5 Here

C. elegans



Credit: Kapahi Lab Buck Institute for Research on Aging, Novato, CA

C. elegans is a very primitive worm about 1 mm in length that lives in the soil. The worm is conceived as a single cell that undergoes a complex process of development, proceeding through morphogenesis and growth into adulthood. It has a nervous system with a "brain" (the circumpharyngeal nerve ring). It exhibits behavior and is even capable of rudimentary learning. It produces sperm and eggs, mates, and reproduces. All 959 somatic cells of its transparent body are visible with a microscope, and its average life span is a mere 2-3 weeks. Scientists love studying this creature. Between October 1994 and January 1995, 73 scientific articles about C. elegans appeared in international science journals (http://cbs.umn.edu/cgc/what-c-elegans).

Experimenting with C. elegans, scientists at UC Santa Cruz lead by Susan Strome have shown how epigenetic memory can be passed across generations and from cell to cell during development. The study (Gaydos, Wang, & Strome, 2014) focused on epigenetic modification - the methylation of a DNA packaging protein called *histone H3*. Methylation of a particular amino acid (lysine 27) in histone H3 is known to turn off or "repress" genes, and this epigenetic mark is found in all multicellular animals, from the researchers' favorite, the tiny roundworm C. elegans to humans.

Strome's lab created worms with a mutation that knocks out the enzyme responsible for making the methylation mark, then bred them with normal worms. Using fluorescent labels, they were able to track the fates of marked and unmarked chromosomes under the microscope, from egg cells and sperm to the dividing cells of embryos after fertilization. As embryos develop, the cells replicate their chromosomes and divide. The researchers found that when a marked chromosome replicates, the two daughter chromosomes are both marked. Strome noted that all animals use the same enzyme to create the same methylation mark as a signal for gene repression.

Strome commented, "There are dozens of potential epigenetic markers. In studies that document parent-to-child epigenetic inheritance, it's not clear what's being passed on, and understanding it molecularly is very complicated. We have a specific example of epigenetic memory that is passed on, and we can see it in the microscope. It's one piece of the puzzle."

During each life cycle, germ cells C. elegans, the ALG-3/4 Argonaute proteins regulate target genes required for spermatogenesis (production of sperm) and transmit a transgenerational small RNA memory of paternal gene expression. These findings suggest that C. elegans sperm transmit not only the genome but also epigenetic information in the form of Argonaute/small RNA complexes that constitute a memory of gene expression in preceding generations (Conine et al, 2013).

During World War II, from November 1944 to the late spring of 1945 the German army forced the Dutch population to survive on less than a third of their regular caloric intake. After the war scientists investigated the children born to women who were pregnant during this period. These children grew smaller than the Dutch average and their children were also smaller. They also turned out to be more susceptible to diseases of metabolism including diabetes, obesity, and cardiovascular disease. The "hunger winter" studies suggested there was an epigenetic change at work, said Baugh, one of the principal researchers (Jobson et al, 2015).

That starvation early in life can alter an organism for generations to come has been corroborated recently by a new study on roundworms (C. elegans), who else? Duke University biologist Ryan Baugh and his group starved thousands of C. elegans worms, one group for or one day and another for eight days at the first stage of larval development after hatching. When feeding was resumed, the worms that were starved longer grew more slowly and ended up smaller and less fertile. They also proved more susceptible to a second bout of starvation. Their offspring were smaller, fewer, and less fertile. However, these children and grandchildren of famine turned out to be more resistant to starvation. "They have a memory of famine," Baugh said. The scientists responsible for this study were certain that starvation early in life can alter an organism for generations to come due to epigenetic mechanisms (Jobson et al, 2015).

Social Epigenetics

Researchers at the University of Zurich "traumatized" male mice by separating them from the mother at unpredictable times in the first two weeks of life. When these young mice became adults they were more hesitant to enter open spaces and brightly lit areas than mice that had not been separated from their mothers. If they were people, we would say they were neurotic. These behavioral changes were also present in the mice's offspring, which also displayed alterations in metabolism, and in their offspring's offspring (Gapp et al, 2014).

"We were able to demonstrate for the first time that traumatic experiences affect metabolism in the long-term and that these changes are hereditary." said Professor Isabelle Mansuy, who led the study. The question of how epigenetic changes are passed on through sperm continues to be a matter of debate, with one proposed mechanism centering on alterations in the levels of small non-coding RNAs called micro RNAs (miRNAs) in sperm. These miRNAs turn off the expression of specific target genes, potentially causing long-lasting changes in gene expression.

In the study researchers found increases in several miRNAs in the sperm of traumatized mice. The same changes were also observed in the hippocampus (the area of the brain usually associated with stress sensing) in their offspring. To see whether these alterations could be responsible for the abnormal behavior in the next generation, the scientists isolated RNA from the sperm of traumatized mice and injected it into an ovum that had already been fertilized, excluding any effects due to changes to the DNA of the sperm. They found that the resulting mice developed the same behavioral and metabolic abnormalities as the natural offspring of the traumatized mice, suggesting that these effects were transmitted through miRNA in the sperm. (Gapp et al, 2014).

Overall, these data support the existence of a sensitive period of early gestation when epigenetic programming of the male germ cells can occur, permitting transmission of specific phenotypes into subsequent generations (Morgan & Bale, 2011).

Researchers from the University of Lethbridge in Canada wanted to investigate how preterm births are influenced by stress. They studied pregnancies in four generations of rats and showed that inherited epigenetic effects of stress could affect pregnancies for generations (Yao et al., 2014). Gerlinde Metz senior author of the article says, "In our study we provide new insights into how stress in our mothers, grandmothers and beyond could influence our risk for pregnancy and childbirth complications. The findings have implications outside of pregnancy, in that they suggest that the causes of many complex diseases could be rooted in the experiences of our ancestors."

Most of the research in the field of epigenetics has focused on epigenetic mechanisms involving DNA and certain molecules (methyl groups and acetyl groups) that attach to DNA. McGill researchers and their Swiss collaborators have discovered that proteins known as histones, which have attracted relatively little attention until now, may play an equally crucial role in the process. Histones are part of the content of sperm transmitted at fertilization. Histones are distinct from DNA, although they combine with it during cell formation, acting a bit like a spool around which the DNA winds. The researchers created mice in which they slightly altered the biochemical information on the histones during sperm cell formation. The offspring were adversely affected both in terms of their development, and in terms of their survival

These effects could still be seen two generations later. Keith Siklenka, the lead investigator stated, "These findings are remarkable because they indicate that information other than DNA is involved in heritability. The study highlights the critical role that fathers play in the health of their children and even grand-children." (Siklenka et al, 2015).

Transgenerational Inheritance of Behavioral Traits

In the realm of maternal behavior, it has been well documented that what we would call affectionate maternal care given to female rat pups postnataly predictably results in them engaging in the same high quality care toward their own offspring when they become mothers (Meaney et al, 2007).

In 2011 a University of Delaware group decided to study whether early-life adversity alters gene expression. They exposed male and female infant rats to stressed "abusive" caretakers for 30 minutes daily during the first seven days of life. They induce abuse in mother rats by placing them in an unfamiliar environment with limited space. As a result, caretakers began to step on, drop, drag, actively reject, and roughly handle their infants. This treatment, naturally, elicited distress responses in the infants. (Roth, & Sweatt, 2011a). The maltreated infant rats were found to have significant methylation of Bdnf DNA in the prefrontal cortex, and that the hyper methylated DNA persisted through development and into adulthood. The observed decrease in Bdnf gene expression was in line with previous findings where early life experiences are known to have a lasting impact on this gene; however, these results provided novel evidence regarding an epigenetic basis for such lasting effects (Roth, & Sweatt, 2011b).

Abusive and neglectful caregivers are known to leave children particularly susceptible to cognitive and emotional dysfunction. Indeed, there is a significant association of reported childhood maltreatment and the later diagnosis of adolescent and adulthood schizophrenia, borderline personality disorder, posttraumatic stress disorder, and major depression (Bremner, 2003; Heim & Nemeroff, 2001; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Schore, 2002).

Why are some of us calm and others anxious? Recent research on rats provides a good clue.

Some mother rats spend a lot of time licking, grooming, and nursing their pups. Others seem to ignore their pups. Highly nurtured rat pups tend to grow up to be calm adults, while rat pups who receive little nurturing tend to grow up anxious. The nurturing behavior of a mother rat during the first week of life shapes her pups' epigenome. And the epigenetic pattern that the mother establishes tends to endure, even after the pups become adults. Through a series of cross-fostering studies, the researchers were also able to demonstrate that the epigenetic changes were determined by the mother's behavior during the postnatal period and were not a product of the biological mother's behavioral genome (Weaver et al, 2004). The difference between a calm and an anxious rat is not genetic, it is epigenetic.

These data indicate that higher levels of care-taking behavior during the first week of life modifies gene transcription throughout the lifespan and promotes adult behavior that is characterized by stress resilience and increased maternal care. In this instance, what is true for mice also applies to humans.

Researchers at the University of British Columbia have demonstrated that human infants of mothers with high levels of depression and anxiety during the third trimester have increased methylation of the Nr3c1 gene promoter in cord blood cells (Devlin, Brain, Austin, & Oberlander, 2010).

Patrick McGowan from McGill University, looked at 24 samples of brain tissue taken from autopsies of male suicide victims, half of whom had been abused as children and half of whom had not. They compared these people to a dozen others, who had never been abused and had died suddenly through accidents. He focused his attention on a gene called NR3C1 that affects a person's ability to deal with stress and is part of the fight-flight system. It affects the HPA axis, where HPA stands for the hypothalamus, the pituitary gland, and the adrenal glands. NR3C1 produces a protein called the glucocorticoid receptor, which provides a harbor for cortisone, the so-called "stress hormone." McGowan found that the activity of the NR3C1 gene was much lower in abuse victims who took their own lives, than in either of the other groups. Not surprisingly, some scientists have found a link

between low levels of this receptor and schizophrenia, mood disorders, and suicide. (McGowan et al, 2009).

Delivery by elective cesarean section is increasing dramatically worldwide, and is today the most common surgical procedure in fertile women. Among those born by cesarean section, an increased risk of certain diseases, such as asthma, type 1-diabetes, obesity, celiac disease, and cancer has been noticed. Tomas Ekström, Professor of Molecular Cell Biology at the renowned Karolinska Institute in Sweden investigated epigenetic alterations of 64 healthy, singleton, newborn infants born at term. Ekström compared cord blood taken from elective C-section and vaginal delivery babies. Blood stem cells from infants delivered by C-section were globally more DNA methylated than DNA from infants delivered vaginally. The researchers found specific epigenetic differences between the groups in 343 DNA regions, including genes known to be involved in processes controlling metabolism and immune deficiencies (Almgren et al, 2014).

Infants of both sexes delivered by C-section have been shown to have higher levels of DNA methylation in leucocytes (white blood cells) compared to those delivered vaginally (Schlinzig, Johansson, Gunnar, Ekstrom, & Norman, 2009). Again, these studies indicate that the epigenome of an infant is sensitive to the prenatal environment, and the experience of birth.

Scientists are great at proving in labs what every mother has learned by experience. Take, for example, the study by neurobiologist Regina Sullivan, professor at the NYU School of Medicine, who has by carefully analyzing the genes that were active in infant rat brains when the mother was present or absent, found that several hundred genes were, more or less, active in rat infants experiencing pain than in those that were not. With their mothers present, however, fewer than 100 genes were similarly expressed. Sullivan has successfully demonstrated that a mother comforting her infant in pain alters gene activity in a part of the brain involved in emotions (amygdala) and thus elicits a positive short term behavioral response in her child (Sullivan, 2014).

This is really how attachment and bonding work. Mother's affection changes gene activity in her infant's brain, which leads the infant to develop attachment to the mother. The smiling child's response elicits epigenetic changes in the mother. The repetition of this interaction over time eventually contributes to the development of maternal love and vice versa.

Michael S. Kobor, associate professor of medical genetics, UBC, Vancouver, found higher stress levels reported by mothers during their child's first year correlated with methylation levels on 139 DNA sites in adolescents while higher reported stress by fathers during their child's pre-school years (three-and-a-half to four-and-a-half years old) correlated with methylation levels on 31 DNA sites in adolescence. "To our knowledge, this is the first demonstration, using carefully collected longitudinal data, that parental adversity during a child's first years leads to discernible changes in his or her epigenome, measurable more than a decade later. This literally illustrates a mechanism by which experiences "get under our skin" and stay with us for a long time (Essex et al, 2013).

Whether children grow up in privileged or deprived households has been shown to affect their health as demonstrated in a British study. Marcus Pembrey and his team from the University of Bristol, UK, selected 40 men from a group of 3000 born in 1958 – half born into rich households and half born into poor ones. The researchers chose subjects from the top and bottom 20 per cent according to socioeconomic status, so ensuring they had examples of both extremes (Borghol, 2011).

Focusing on stretches of DNA called promoter regions, which turn genes on or off, the team examined more than 20,000 sites throughout the genome. The patterns were different between the two groups at almost a third of the sites. Most tellingly, methylation levels were drastically different at 1252 sites of the men who came from poor households, but only at 545 sites in men from rich backgrounds. Because the samples were taken in middle age, the researchers couldn't tell exactly when the epigenetic methyl groups were added or subtracted. It is possible that the genes were altered in infancy, childhood, or even adulthood. Yet all the signs point to prenatal or early postnatal life. As Pembrey says, "It's telling us that the epigenetic changes in adult DNA are largely from early life experience."

Moving from the past into the adult present, how we live our lives can have significant effects on how we stay healthy, how we age, and how we develop diseases, including cancer. Take colon cancer, for example. Researchers from the University of Basel found that Aspirin and hormonal replacement

therapy reduced the methylation rate of colon cancer related genes, whereas smoking and high BMI (Body Mass Index) increased it. They concluded, "Lifestyle, including aspirin use, modulates age-associated DNA methylation change in the colonic epithelium and thereby impacts the evolution of cancer methylomes" (Faiza, et al, 2014).

How do relationships impact us on a molecular level? A paper published in *Nature Neuroscience* (Wang, Duclot, Liu, Wang, & Kabbaj, 2013) explored epigenetic changes in the brains of prairie voles. Voles mate for life and share parental responsibilities. This unique behavior makes voles an interesting animal model to study exclusivity, monogamy, and partner preference.

Prairie vole couples express higher levels of oxytocin and vasopressin, the social bonding neurotransmitters, compared to unattached voles. Oxytocin has been described as the "love hormone" and it is excreted together with other hormones such as endorphins and adrenaline in situations that accompany feelings of pleasure and attraction to others. Oxytocin increases male and female social and sexual responsiveness, as well as caretaking in both sexes. Vasopressin, on the other hand, increases male sexual persistence such as courtship. In females, it energizes more aggressive aspects of maternal behavior such as protecting young from harm.

The research team studied vole couples in two different conditions. Some vole couples were housed for 24 hours and permitted to mate and form pair bonds, while others were housed for only 6 hours and were prevented from mating. The voles in this second group were injected with a drug called trichostatin A (TSA). TSA is a histone deacetylase inhibitor. The voles that received the histone-modifying drug formed exclusive pair bonds even though they only interacted for 6 hours and were not allowed to mate.

In follow up experiments, James Burkett of Emory University in Atlanta, GA has shown that blocking oxytocin stopped the animals from consoling each other. "This is the strongest evidence yet that the fundamental building of empathy are conserved in evolution between rodents and humans." (Burkett et al, 2016).

The researchers found that the acetylation of histone proteins resulted in the unfolding of DNA thereby allowing the expression of genes for the production of oxytocin and vasopressin. Thus, a permanent genetic change was brought about by epigenetic factors. This study was the first to demonstrate that epigenetics may play a role in entering loving and enduring relationships.

Researchers at Emory University investigated empathy in voles. Pairs of voles were isolated from each other. Then one of them was exposed to mild shocks. When they were reunited, the voles who hadn't been shocked proceeded to lick their partners sooner and for longer time than those in a control group who were separated but not exposed to shocks. The consoling behavior only took place between voles who were familiar with each other, and not between strangers.

Until recently it was thought that only humans, great apes, and large-brained mammals such as dolphins and elephants were capable of showing consolation behavior towards one another. This latest study is the first time empathy has been identified in rodents.

Transgenerational Transmission of Trauma

Trauma is generally defined as a life-threatening situation. PTSD has long lasting and often debilitating effects.

Over the past five decades, the transgenerational transmission of trauma has been explored in more than 500 articles, of which I shall cite representative studies supporting the concept. The psychiatric literature shows mixed findings regarding this subject.

Anna Freud first described transgenerational trauma. Dorothy Burlingham, an American child psychoanalyst and educator and a lifelong friend and partner of child psychoanalyst Anna Freud, referred to "unconscious messages" passed between mothers and children during the German bombing of London in World War II (Freud & Burlingham, 1942). Margaret Mahler (1968), a child psychoanalyst in the USA observed that in early infancy mother and child function almost as one psychological unit. She held that there is fluidity between a mother's and a child's psychic borders. In the early '80s, a Lakota professor of social work named Maria Yellow Horse Brave Heart coined the

phrase "historical trauma." What she meant was "the cumulative emotional and psychological wounding over the lifespan and across generations" (Brave Heaty MY, 2003, abstract).

In the past psychologists and psychiatrists thought of transgenerational transmission of trauma in terms of a purely psychological phenomenon. According to this theory, starting at conception the mother's anxiety, unconscious fantasies, perceptions, and expectations are passed to the child's mind and body (Volkan, 1998) by verbal or non-verbal clues. Parents who have experienced trauma may constantly talk about it or, as is more often the case, never talk about it. We are beginning to realize that living with a person (survivor or veteran) who suffers from PSTD can be traumatizing. The children in such families experience their own PSTD by "walking on egg shells" around the PTSD parent and wondering what they are hiding.

Today, we realize that the spoken or unspoken messages of PTSD parents may impact the child both on a biological as well as on a psychological level, namely, affecting their epigenome.

Rodent and non-human primate studies show that early trauma produces lasting changes in neural function and behavior (Korosi & Buram, 2009; Pryce & Feldon, 2003; Sanchez, 2006). One mediator of neural function and plasticity that has received much attention is the brain-derived neurotrophic factor (Bdnf) protein, and indeed changes in Bdnf gene activity continues to be hypothesized as a candidate molecular mechanism through which early-life experiences persistently modifies brain structure and function (Branchi, Francia, & Alleva, 2004; Casey et al, 2009; Fumagali, Molteni, Racagni, & Riva, 2007).

Are there particular genes that are affected by trauma? Rachel Yehuda, Professor of Psychiatry and Neuroscience and the Director of the Traumatic Stress Studies Division at the Mount Sinai School of Medicine, has undertaken to study this question. Such knowledge could elevate interventions to an even higher level of precision than genetic screening. To be effective, we need to discover what can and what cannot be restored to normal. This research, however, is not easy, because one runs the risk of trying to reverse something that actually helps a body adapt - of mistaking resilience for pathology.

Yehuda is best known for her research on the children of Holocaust survivors. She found that they were three times more likely to develop post-traumatic stress disorder if they were exposed to a traumatic event than demographically similar Jewish persons whose parents did not survive the Holocaust. Furthermore, Holocaust offspring exhibited the same neuroendocrine or hormonal abnormalities that were observed in Holocaust survivors and persons with post-traumatic stress disorder (Yehuda, et al 2014).

Yehuda and her colleagues performed a longitudinal study on 38 women who were pregnant on 9/11 and were either at or near the World Trade Centre at the time of the attack, some of whom went on to develop PTSD. The researchers took samples of saliva from them and measured levels of the stress hormone cortisol. They found that those women who had developed PTSD following exposure to the attacks had significantly lower cortisol levels in their saliva than those who were similarly exposed but did not develop PTSD. About a year later, the researchers measured cortisol levels in the children, and found that those born to the women who had developed PTSD had lower levels of cortisol than the others. Reduced cortisol levels were most apparent in those children whose mothers were in the third trimester of pregnancy when they were exposed to the attack (Yehuda et al, 2005).

The children of women who were traumatized as a result of 9/11 subsequently exhibited an increased distress response when shown novel stimuli. Again, this was related to the stage of pregnancy – those with the largest distress response were the ones born to mothers who were in their second or third trimester when exposed to the World Trade Centre attacks.

Yehuda is currently trying to identify genetic variations and epigenetic markers associated with PTSD in combat veterans. Yehuda's work establishes low cortisol levels as a risk factor for developing PTSD and, when taken together with the animal studies, suggests that traumatic experiences can leave epigenetic marks that alter the stress response in offspring. (Yehuda et al, 2005; Yehuda et al, 2013; Sarapas et al, 2011).

Interpersonal Epigenetics

Using mice as a model to study human breast cancer, researchers have demonstrated that a negative social environment (in this case, isolation) causes increased tumor growth. The social environment can alter the level of gene expression in a wide variety of tissues including the brain. The findings also support previous epidemiologic studies suggesting that social isolation increases the mortality of chronic diseases, as well as clinical studies revealing that social support improves the outcomes of cancer patients (Conzen, 2009).

In one of the most creative experiments that I have come across, Gene Robinson, Director of the Institute of Genomic Biology, University of Illinois in what he called Kidnapping-and-Cross-Fostering Study plucked about 250 of the youngest bees from two African hives and two European hives, and painted marks on the bees' tiny backs. Then he and his team switched each set of newborns placing them into the hive of the other subspecies. The move between hives made the bees act differently. But it also made their genes work differently, and on a broad scale. European honeybees raised among more aggressive African killer bees, not only became as belligerent as their new hive mates -- they came to genetically resemble them. And vice versa (Zayed & Robinson, 2012). Michael S. Kobor, a UBC associate professor of medical genetics, University of Wisconsin, found higher stress levels reported by mothers during their child's first year correlated with methylation levels on 139 DNA sites in adolescents, while higher reported stress by fathers during their child's pre-school years (three-and-a-half to four-and-a-half years old) correlated with methylation levels on 31 DNA sites in adolescence. "To our knowledge, this is the first demonstration, using carefully collected longitudinal data, that parental adversity during a child's first years leads to discernible changes in his or her 'epigenome,' measurable more than a decade later. This literally illustrates a mechanism by which experiences 'get under the skin' to stay with us for a long time." (Essex et al, 2013).

A variety of studies, many using long-term medical records from large populations, have found that certain experiences affect future descendants' health risks. Most recently, a new study by Rachel Yehuda, PhD, Professor of Psychiatry and Neuroscience and Director of the Traumatic Stress Studies Division at the Mount Sinai School of Medicine, New York, looked at the descendants of the Holocaust survivors (Yehuda et al., 2014). Like their parents, many have low levels of cortisol, particularly if their mothers had PTSD. Yet unlike their parents, they have higher than normal levels of the cortisol-busting enzyme. Yehuda and her colleagues theorize that this adaptation happened in utero as a result of epigenetic changes.

David Clayton, a neurobiologist also at University of Illinois, found that if a male zebra finch heard another male zebra finch sing nearby, a particular gene in the bird's forebrain would "re-up" and it would do so differently depending on whether the other finch was strange and threatening, or familiar and safe. Songbirds demonstrate massive, widespread changes in gene expression in just 15 minutes. This startlingly quick gene expression response to the social world is a phenomenon we are just beginning to understand. We are learning that brain responses to social stimuli can be massive, involving hundreds, even thousands of genes. Social experiences can lead to changes in brain gene expression and behavior (Clayton & London, 2014).

A study by Rachel Yehuda, whose research on PTSD we are already familiar with, is the first researcher to have demonstrated an epigenetic alteration associated with treatment response to psychotherapy in veterans. This study represents an important initial step in establishing relevant molecular markers for PTSD therapies. The data suggest that psychotherapy that brings about substantial symptom change constitutes a form of "environmental regulation" that may alter the epigenetic state (Yehuda et al, 2013).

According to a new study form the University of Helsinki listening to classical music, W.A. Mozart's violin concert Nr 3, G-major, K.216 that lasts 20 minutes, enhanced the activity of genes involved in dopamine secretion and transport, synaptic neurotransmission, learning and memory, and down-regulated the genes mediating the destruction of neurons, referring to a neuroprotective role of music. Several of the up-regulated genes were known to be responsible for song learning and singing in songbirds, suggesting a common evolutionary background of sound perception across species. Unfortunately for those who are not inclined to listen to classical music, "the effect was only detectable in musically experienced participants, suggesting the importance of familiarity and experience in mediating music-induced effects" (Kanduri et al., 2015).

Neurobiologist Regina Sullivan, professor at the NYU School of Medicine, has shown that a mother comforting her infant in pain alters gene activity in a part of the brain involved in emotions (amygdala) which then modifies critical neural circuitry and thus elicits a positive behavioral response in her child (Sullivan, 2014). This is really how bonding works. Mother's affection changes gene activity in her infant's brain, which leads the infant to develop attachment to the mother. The repetition of this interaction over time eventually contributes to the development of maternal love and vice versa.

And now for some really good news. A study by Rachel Yehuda and colleagues, whose research on the children of Holocaust survivors we are already familiar with, has shown in another study that psychotherapy with veterans resulted in substantial symptom change along with changes on an epigenetic level. In other words, just like in the example above, psychosocial interactions constitute a form of "environmental regulation" that may alter the epigenetic state of the organism (Yehuda et al., 2013).

Intrapersonal Epigenetics

We have known the effect the mind has on the body for a long time. In medical parlance it has been referred to as psychosomatic medicine. Now a new study by Harvard researchers reveals the biological mechanism underlying such effects. The researchers divided 84 hotel maids into two groups. One group was told that the work they do is good exercise and satisfies the Surgeon General's recommendations for an active lifestyle. The other group (control) was not given this information. Although actual behavior did not change, four weeks after the start of the experiment, the informed group perceived themselves to be getting significantly more exercise than before. As a result, compared with the control group, they showed a decrease in weight, blood pressure, body fat, waist-to-hip ratio, and body mass index. "It wasn't that they started working harder. Their bodies actually changed their functioning in response to changed perceptions." (Crum & Langer, 2007).

Now consider the following study on meditation. Meditating for eight hours on mindfulness, altruistic love, and compassion induces major epigenetic modifications, according to a study undertaken at Richard Davidson's laboratory in Wisconsin, in collaboration with the Spanish geneticist Perla Kaliman. Compared to a control group that did not meditate but engaged in leisure activities in the same environment, the researchers found reduced expression of histone deacetylase genes, alterations in global modification of histones and decreased expression of pro-inflammatory genes in the meditators. What that means in simple language is that the people who meditated were more resilient to infections and diseases than the control group. "We can glimpse here the possibility of an epigenetic transformation of a person by way of self-regulation rather than the environment," said Perla Kaliman, (Kaliman et al, 2014) the lead investigator.

A good way to enhance your cognitive abilities is by listening to classical music. According to a new study form the University of Helsinki listening to Mozart's violin concerto No. 3, G-major, that lasts 20 minutes, amplified the activity of genes involved in dopamine secretion and transport, synaptic neurotransmission, learning and memory, and down-regulated the genes mediating the destruction of neurons, which is all for the good. Unfortunately, for those not inclined to listen to classical music, "the effect was only detectable in musically experienced participants, suggesting the importance of familiarity and experience in mediating music-induced effects" (Kanduri et al, 2015).

Summary

It is evident from our survey of recent discoveries in epigenetics how nature (genes) and nurture (the environment) work in concert. It is not one or the other that is responsible for a disease or personality trait. Many scientific papers in the past described a disease as 60 per cent or 70 per cent or whatever per cent genetic. In fact, no such numbers have ever been scientifically established. The only thing we know for sure is that we are the product of a dynamic interaction between of these forces, that nothing about us is written in stone and, therefore, as long as we breathe, we are a work in progress, constantly changing. Epigenetic modifications are dynamic and potentially reversible processes occurring throughout lifetime.

Converging and replicated evidence from experimental and clinical studies indicate that, before their offspring is even conceived, a parent's life experiences involving food, drugs, exposure to toxic products and stress can affect the development and health not only of their children, but even of their grandchildren. Thus, an epigenetic hypothesis for environmental contributions to health continues to gain traction (Costa et al, 2009; McGowan et al, 2009; McGowan & Szyf, 2010; Roth, Lubin, Funk, & Sweatt, 2009; Tsankova, Renthal, Kumar, & Nestler, 2007).

Do genes matter? Absolutely. But so do the physical, psychological, and social environments, not only from birth on but extending back to the nine months of womb life and, in many ways, several generations further back. In light of all of this, let me offer you some very practical, down to earth recommendations for genome health.

Three Simple Steps

1. Eat plenty of cruciferous vegetables such as broccoli and cauliflower. They contain antioxidants that enhance our tumor fighting genes and that way lower our risk of developing cancer.
2. Exercise regularly. Exercise has been shown to prevent stem cells from turning into fat cells.
3. Do not suffer silently of stress, anxiety and depression. They affect negatively the epigenome. These conditions need to be dealt with as soon as they arise.

Key Take Away

- An individual's adult health is heavily influenced by early prenatal physiological factors affecting the mother such as food, pollution, and radiation.
- The unborn child will adjust as best it can to the external environment he/she is going to encounter upon birth by way of epigenetic changes.
- At least parts of the changed genetic code can be passed on to future generations.
- Genes don't make you who you are. Gene expression does. And gene expression varies depending on the life you live.
- Gene activity cranks up or spins down in response to changes in your environment.
- Our social lives, our interactions with others and ourselves can change our gene expression with a rapidity, breadth, and depth previously unknown.

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