***Chapter Nine***

**Gene Editing and Synthetic Biology**

**Introduction**

In a 1989 interview, James Watson, one of the founders of the structure of DNA, said, “We used to think that our fate was in the stars. Now we know in large measure, our fate is in our genes.”[[1]](#footnote-1) With that knowledge, an intrinsic human desire to control and change our fate developed through biotechnology that manipulated the human genome.

The overall objective of molecular genetics is to better understand how genes and regulatory elements of the genome function in response to various developmental and environmental cues. Rapid advances in mathematical, computational, and molecular biology, along with new technologies in DNA microarrays, will revolutionize genomics in the next few decades. Out of the almost 9 million known species, only several hundred complete genomes from different organisms have been sequenced.[[2]](#footnote-2) As of 2014, about 290,000 genomes from different human volunteers have been completely sequenced[[3]](#footnote-3).

**Prelude to Genetics and Disease**

The term genetics, coined by William Bateson in the early 1900s, comes from the Greek term “to generate” and is the science of biological heredity and variation. In 1866, Gregor Mendel, an Austrian monk, published the results of his decade-long investigations on the inheritance of "factors" in pea plants. He suggested that every cell contains pairs of “factors” and that each pair determined a specific trait. The members of each pair segregated from each other in the process of sex-cell formation so that a gamete contained one member of each pair. The segregation of one pair was independent from the segregation of all other pairs of factors. It wasn’t until 1909 that a Danish botanist, Wilhelm Johannsen, introduced the word "gene" to characterize Mendel’s "factors."

As a prelude to any discussion of genetics and disease, it is important to highlight several basic principles of genetics and its relation to disease. The first principle is that *genetics* refers to the study of specific, individual genes and their role in inheritance. Chromosomes contain the majority of a cell’s genetic information and are composed of nucleic acids and proteins. DNA sequences can also be divided into genes, where each gene encodes the information necessary to synthesize one or several proteins. The location of a gene on a chromosome region is called a locus. In humans there are about 20,000 genes. At any given gene locus, DNA sequences may differ from one individual to another in some small ways. These different DNA sequences within a gene locus are termed alleles. In most animal and plant populations, 10-20% of the genes are composed of multiple alleles. There are several processes by which different alleles develop. One process is through mutations such as a point mutation, where one nucleotide is replaced by another. Another process involves a section of DNA eliminated or translocated from one chromosome location to another on the same or different chromosome.

The second principle is that every human being has mutations in their genes and these mutations can occur at almost any location within the whole genome. This means that there are no individuals with “perfect” human genomes. Some of these changes have no major phenotypic expression in that organism. Other mutations can lead to disabling conditions, specific disease states, or death. Conventional wisdom holds that all the cells within a given organism carry the same genome and that phenotypes are due to variation in gene expression. This is not entirely true. Somatic mutations frequently occur after fertilization and get passed on with each round of mitosis, leaving a trail of base pair changes that varies from cell to cell. Each cell has a set of mutations that is unique to that cell.

The third principle relates to the heterogeneity of response to genetic mutations and the consequences of genetic changes. Cells have a remarkable ability to edit their DNA in order to ensure that mutations do not occur at a high frequency. Even when mutations do occur, these changes have no perceptible effects because the genetic code is redundant. A point mutation that has no effect at all on the expression of the protein it codes for is called a silent mutation. Changes in the DNA sequence that **do not** have profound effects on protein function occur if the changes do not dramatically change the three dimensional structure of the protein.

Genes also vary a great deal with respect to how much they can be mutated before harmful changes occur in the organism. Some genes, such as those that encode the basic components of metabolism, replication, transcription and translation machinery, are hard to mutate without harming an organism. We see very little variation in those gene sequences from one organism to another. Such genes are said to be conserved. In contrast, individuals with genes responsible for cystic fibrosis express a wide range of disease severity because there are many types of mutations in the gene encoding the transporter protein involved in the disease. Thus, **allelic heterogeneity** implies that there are many places on the gene that can be mutated and that not all mutations have the same impact on phenotypic or disease expression.

 Some changes in alleles or DNA sequences can be favorable and promote a healthier life. As an example, there are several alleles that encode for the protein apoE, which is a ligand for the LDL receptor. It is a critical membrane protein in cholesterol regulation. In Italy there is a community near Milan whose residents are less likely to develop atherosclerosis because of a fortunate mutation in one of their forbearers. Their apoE isoform, referred to as apoE2, appears to protect them from developing atherosclerosis. In addition, the expression of this type of apoE also has been shown to be an important determinant of Alzheimer’s disease. Individuals with the apoE4 isoform have a higher rate of atherosclerosis, heart disease, and Alzheimer’s disease.

 The term *genomics* refers to the study of an organism's entire genetic makeup, which is called a genome. The study of genomics includes understanding how the genome interacts with environmental or non-genetic factors, such as a person's lifestyle. This new area of science has the potential to improve our understanding of complex diseases such as diabetes, heart disease, and asthma, as well as to improve medical treatment.

The fourth principle is that epigenetics regulate genes and their functions. Epigenetics involve methylation or acetylation of either nucleotides or DNA associated proteins, such as histones. Through the processes of methylation and acetylation, the contraction and expansion of DNA can be controlled. When an acetyl group is added to the lysine region of the histone, the chromatin that is wound around the histone becomes uncondensed. This unraveling leads to the expression of the gene. When a methyl group is added to the lysine region of the histone, the DNA can either become condensed or uncondensed. This depends on which lysine on the histone the methyl group associates with. As expected the modifications of histones can either result in the expression or silencing of a gene. If you think of our DNA as an immense piano keyboard and our genes as keys -- each key symbolizing a segment of DNA responsible for a particular note, or trait, and all the keys combining to make us who we are -- then epigenetic processes determine when and how each key can be struck, changing the tune being played.

The final principle in basic genetics is that the understanding of any genetic process or phenotype will often require a complete understanding of how each region of the DNA operates within the whole human genome. For example, there are genes that increase the chances of getting lung cancer in smokers, and yet, there are many heavy smokers whose genetic makeup enables them to never come down with lung cancer.

**Parental Genes Impact the Health of the Offspring**

On a basic level, each parent donates one chromosome and consequently one gene to the child. Over 60% of the genes have the ability to undergo alternative splicing in order to form several protein products. This accounts for the excess of 200,000 different proteins expressed in human beings and encoded within about 20,000 genes. The nature of each contributed gene influences the health of the child in various ways. If a genetic disease is inherited in a dominant manner, such as Huntington’s disease, then one parent donating this mutated gene to the fertilized egg will result in a child who will eventually be stricken with the fatal disease. Statistically, each child in such a family has a fifty percent chance of inheriting the gene for Huntington’s disease.

 Most genetic diseases, however, are recessive disorders. To be affected by a recessive disorder requires that an individual possess two abnormal or mutated copies of a gene. Therefore, each parent must donate one copy of the abnormal gene to the child. Cystic fibrosis and Tay-Sachs are examples of recessive disorders. A person who obtains only one abnormal copy of a gene for a recessive condition is known as a carrier. In general, a carrier of a genetic condition will not develop the disease and should not have any health-associated abnormalities due to the presence of a recessive gene.

 Many human diseases, such as heart disease, Alzheimer’s disease and cancer, are influenced by multiple genes in a complex fashion. It should be noted that being genetically predisposed to a disease does not necessarily mean that an individual will suffer from the disease in question. It simply means that there is an increased risk of developing the disease. Of great concern is a woman who tests positive for a genetic mutation in BRCA1. Women with this mutation may have a 55 to 85 percent chance of developing breast cancer by age 70, as well as having a 40 to 60 percent chance of developing ovarian cancer. Yet, only about 10-20 per cent of all diagnosed breast cancers have a family history in part because many diseases are regulated by other genetic and environmental factors,

**Genetic Testing and Screening**

There are several types of genetic tests available to the developing fetus or newborn baby, which identify genes that affect the health of the child. Pre-implantation Genetic Diagnosis (PGD) is done in pre-implanted embryos, allowing the couple to select an embryo that does not contain the gene causing the specific disease. Dr. Mark Hughes developed PGD in the mid-1980’s, with Robert Winston and Alan Handyside, as a screen to test which embryos will develop cystic fibrosis. PGD is performed on an embryo, created via in vitro fertilization (IVF), which has developed to the 8th stage. Using micro-manipulation techniques, one of the cells is removed and tested for a specific mutation using PCR (polymerase chain reaction). Sometimes, chromosomal aberrations, as seen in Down’s syndrome, are detected in this removed cell using fluorescence *in situ* hybridization technologies. Using PGD, embryos are selected that do not express two defective genes or even an embryo that does not express one defective gene (such an embryo will not develop into a child who will be a carrier for the recessive disorder). One or two of these embryos are implanted into the woman. As of 2014, over 1,000 healthy babies were born using PGD.[[4]](#footnote-4) The list of diseases that now can be screened using PGD is over one hundred and includes cystic fibrosis, Down’s syndrome, Duchenne muscular dystrophy, Huntington’s disease, certain forms of early onset Alzheimer’s disease, sickle-cell disease, and Tay-Sachs disease. PGD can also be used for sex selection. As of 2015, the error rate for misdiagnosis varied between 0.5-1% depending on which diseases were screened (Tiegs et al., 2015). Some of the errors result from a rare phenomenon that the cell removed from the 8 cell embryo may not be representative of the other cells. This phenomenon is called mosaicism. In other words, one cell would appear to lack the genetic defect whereas the remaining cells in the embryo would be abnormal or vice versa.

There appears to be a misnomer in calling this test Pre-implantation Genetic Diagnosis. In reality, this test is a way to screen pre-implanted embryos for specific genetic mutations. Therefore, it should be renamed Pre-implantation Genetic Screening (PGS) or Pre-implantation Genetic Testing (PGT).

Prenatal diagnostic testing is another way to assess reproductive risk. Prenatal diagnostic testing involves testing the fetus before birth to determine whether it has a certain hereditary or spontaneous genetic disorder. The most common tests used to detect abnormalities in a fetus include ultrasonography, chorionic villus sampling (CVS), amniocentesis, and percutaneous umbilical blood sampling. CVS involves removing a small amount of tissue called the chorionic villi, which is located on the outside of the fetal gestational sac and will later become the placenta. The chorion, as fetal tissue, shares its genetic makeup with the fetus, not the mother. The chorion has many small, finger-like projections on its outer surface, and a few of its cells may be carefully removed without disturbing the pregnancy. The chorionic villi cells may be used for chromosome analysis or other genetic testing, but cannot be used to test for open neural tube defects. CVS is available from 10.0 to 13.3 weeks of pregnancy. The CVS may be performed trans-abdominally by guiding a thin needle through the abdominal wall to the chorionic villi and then withdrawing a small amount of this tissue.

There are considerable efforts to test the genetics of a fetus by obtaining fetal DNA from the blood of the pregnant woman. During pregnancy, 5% to 15% of noncellular — so-called "cell-free" — DNA fragments in the maternal blood are of placental origin. While the amounts of fetal DNA is low, genetic analysis of DNA can be done on obtaining only several molecules of fetal DNA. Employing this type of prenatal testing could reduce costs by as much as 90%. In addition, cell-free fetal DNA testing has a very low false-negative rate (0.5%), which means that only women confirmed to be at high risk for fetal abnormality need to subsequently undergo amniocentesis.[[5]](#footnote-5)

Most genetic tests are offered primarily to couples with an increased risk of having a baby with a genetic abnormality (such as Down’s syndrome) or a chromosomal abnormality (particularly when the woman is aged 35 or older). In Sardinia, for instance, where beta thalassemia is a relatively common genetic condition, prenatal genetic screening programs have produced striking results. Following fetal diagnosis of homozygous beta thalassemia, most couples decide to terminate the pregnancy. Overall, since the introduction of widespread genetic education, counseling, and screening programs in Sardinia, "the incidence of beta thalassemia major has been reduced from 1 of every 250 live births in 1975 to 1 of every 4000 in 1996, with 94% of the cases prevented" (Cao and Kan, 2013). Notable reductions in incidence due to targeted prenatal testing are reported for other disabling conditions as well, such as Tay-Sachs disease among Ashkenazi Jews, spina bifida in Britain, and Down’s syndrome in the United States (Harper and SenGupta, 2012).

 Newborn genetic screening is aimed at identifying infants who have genetic conditions that can be helped by early intervention. In many cases, this early intervention means the elimination or reduction of symptoms that would have left an unscreened individual with a lifetime of disability. Historically, this type of screening was strongly influenced by a genetic disease called Phenylketonuria (PKU). PKU is a genetic metabolic disorder that is easily treated by restricting certain foods from the diet; if left untreated, however, the disorder causes severe mental retardation. PCR is a common method for screening newborn babies for PKU**.**

Carrier screening is usually carried out in adults and involves identifying unaffected individuals who carry one copy of a gene for a recessive disease condition. The most common tests in carrier screening are cystic fibrosis, Tay-Sachs, and sickle cell trait. As a case in point, since carrier screening has begun for Tay-Sachs, the incidence of babies born with this disease has decreased dramatically in New York City alone. It is unusual to see a baby with this condition after 2000. Individuals can also undergo pre-symptomatic testing for predicting adult-onset disorders such as Huntington's disease or for estimating the risk of developing adult-onset diseases which have multifactorial etiologies like cancers, ischemic heart disease, asthma, diabetes, and Alzheimer's disease.

**Epigenetics**

No chapter in the genetics of disease can omit discussing epigenetics. Epigenetics is a hereditable process but differs from Mendelian genetics. In Mendelian genetics, changes in the base pair sequence of a gene can be a critical determinant of its activity. Epigenetics is the study of changes in gene activity that are caused by chemical modifications of specific base pairs or proteins that govern gene expression. It can be viewed as the software of the genome. What scientists have learned over the past several decades is that these changes can be passed down at least one successive generation. Epigenetics regulates gene expression by orchestrating a set of chemical reactions that switch parts of the genome off and on at strategic times and at specific DNA locations. The epigenetic changes include DNA methylation and histone modification, which regulate high-order DNA structure and gene expression. Epigenetic regulation of gene activity involves a structure called an epigenome that sits on top of the genome, just outside it (hence the prefix epi-, which means above). The epigenome consists of chromatin, a protein-based structure, around which the DNA is wrapped, whose activity can be regulated by post-translational modifications and methylation of specific bases such as cytosines. In general, chromatin and DNA methylation results in gene silencing. On the other hand, the addition of acetyl groups unwinds the DNA around the histone spool and makes it easier for the RNA to transcribe a particular gene.[[6]](#footnote-6) It is through epigenetic marks that environmental factors like diet, stress and prenatal nutrition can make an imprint on genes that is passed from one generation to the next. As James Watson said in 2003 “you can inherit something beyond the DNA sequence. That's where the real excitement of genetics is now."

 Drugs exist that can remove methyl groups. Such medications could have novel clinical applications – years of trauma and abuse could potentially be wiped away with a single dosing. Besides the obvious need for further safety investigations (potentially beneficial methyl groups could be erased as well), would such treatments violate an ethical obligation to not alter the human genetic code? As epigenetics have revealed, our evolutionary connection to our ancestors is more complex than simple nucleotides. It is also made up of epigenetic modifiers that have been passed down from generation to generation.[[7]](#footnote-7)6

At first glance, epigenetic trans-generational inheritance of acquired characteristics is reminiscent of a theory of genetics proposed by Jean-Baptiste Lamarck (e.g., a giraffe, through evolutionary processes, has a long neck because he must reach the highest branches to obtain food). In fact, the current underlying mechanisms of epigenetics provide scientific evidence describing how the environment can trigger heritable changes. There is ample evidence in animals and even in human beings that environmental factors shape health and disease via epigenetic mechanisms that mediate gene-environment interactions. According to Dr. Moshe Szyf, a leading geneticist, epigenetics is a physiological mechanism by which the genome senses the world and changes itself (Narain, 2012).

A 1974 experiment on mice (Bailey et al, 1974) may present evidence for epigenetic influences within the ovum. Two strains of mice, which we will call “A” and “B”, are relevant. The researchers discovered a gaping difference in the violence levels between two groups of male mice that were from distinct combinations of these two strains:

* The offspring of (“A” female mated with “B” male) female mated with (pure bred “B”) male tended to be much more violent than:
* The offspring of (“B” female mated with “A” male) mated with (purebred “B”) male.

There should be no chromosomal difference between the two groups (all should have “B” Y chromosomes and within both groups there should be a relatively equal amount of “A” and “B” X chromosomes). This logic led the researchers to conclude that there must be a cytoplasmic difference in the ova of strain “A” and strain “B” that affects the mouse pup’s inclinations towards violence (the researchers suggested that perhaps it was simply a “mitochondrial protein or enzyme which interacts either with other cytoplasmic or nuclear factors”). It is also possible that epigenetic factors during pregnancy may affect behavior.

 **Genetics and Human Behavior**

Unlike genes that are directly responsible for diseases like Hungtinton’s Disease, Tay Sachs or Alzheimer’s disease there are genes that, in combination with environmental factors, influence human behavior. For example, it has been known for a long time that certain human behavioral characteristics are rooted in our genetic background and mimic behaviors observed in other members of the animal kingdom. However, studying genes that affect behavior creates a unique set of scientific problems. The majority of behavioral genetics studies have focused on genes that influence criminal tendency, cognitive ability, novelty seeking, mental disorders, addiction to drugs or alcohol, and sexual behavior. Most geneticists interpret current scientific data to show that these behavioral traits are complex in their pattern of inheritance and involve a combination of many genes interacting with environmental factors. The following section will briefly summarize genes that affect intelligence, sexuality, violence and other behaviors.

 *Intelligence.* Genes that influence intelligence have been a keen interest in major research centers around the world. A variety of methodologies have been employed to examine the genetic contribution to cognitive ability (intelligence or I.Q.). Yet, there are problems inherent in studying the genetics of intelligence. These studies require the investigators to define intelligence in a measurable and definitive fashion. For example, is IQ a sufficient measure of intelligence? The problems of cognitive assessment may in part be responsible for the scarcity of well-designed studies to characterize specific genes that contribute to the development of intelligence. Furthermore, intelligence is often seen as a highly complex trait, with many possible influential genetic factors. Therefore, the precise genetic and epigenetic polymorphisms underlying normal-range intelligence differences remain mysterious and vastly undefined (Haggarty et al., 2010).

 Studying the genetic role of intelligence also highlights how environmental factors may account for the difficulties in identifying specific genes. Specifically, it is difficult to sort out environmental versus genetic or epigenetic factors that influence behavior, in part because genes that regulate aspects of behavior appear to be highly responsive to environment stimuli. Traditional research strategies, which include studies of twins and adopted children, are often used to distinguish between biological and environmental influences on specific behaviors (nature vs. nurture). Many of these studies have not yielded sufficient results to dramatically expand our understanding of how environment and genetics interact to affect behavioral characteristics.

 The inability to positively identify intelligence genes via genome-wide scans or state-of-the art technologies is leading some scientists to propose that genes do not play a major role in determining intelligence. Rather, environment and maternal effects may be the critical parameters that account for intellectual abilities.

*Sexuality*. There has been a great deal of effort to examine the role of genes in sexual behavior. Such studies have been going on for decades and usually involve trying to identify sexual patterns among monozygotic twins, dizygotic twins, or adoptive siblings. Many studies have focused on homosexual behaviors and several papers utilize two lines of evidence that homosexuality is influenced by polymorphic genes: (i) twin studies indicate that there are both genetic and environmental factors that contribute to the expression of the homosexual phenotype (Ramagopalan et al., 2010), and (ii) male homosexuality appears to be inherited more frequently from the matrilineal lineage. These studies suggest the existence of polymorphic, heritable maternal effects and/or polymorphic X-linked genes influencing male homosexuality. In some studies the researchers found that 52% (29/56) of monozygotic twins, 22% (12/54) of dizygotic twins, and 11% (6/57) of adoptive brothers were homosexual. Thus, heritability of homosexuality was considered to be substantial under a wide range of assumptions about the population base rate of homosexuality and the ascertainment bias. However, the rate of homosexuality among non-twin biologic siblings was significantly lower than would be predicted by a simple genetic hypothesis and by other published reports. From the rates of homosexuality observed in monozygotic and dizygotic twins, ordinary siblings, and adoptive brothers and sisters of homosexual men and women, overall heritabilities of 31 to 74% for males and 27 to 76% for females were estimated. The observation that male homosexuals usually have more gay brothers than gay sisters, whereas lesbians have more gay sisters than gay brothers, suggested that the factors responsible for familial aggregation are at least partially distinct in men compared to women.

 Hamer and his colleagues (Mustanski et al., 2005) performed one of the most complete and largest studies in an attempt to identify a gene for homosexuality. In 1993, he studied pedigree and linkage analyses of 110 families of homosexual men. Increased rates of same-sex orientation were found in the maternal uncles and maternal male cousins of these subjects, but not in their fathers or paternal relatives, suggesting X-linked transmission. Linkage analysis using DNA markers in a selected group of 40 families, in which there were 2 gay brothers and no indication of non-maternal transmission, demonstrated a correlation between homosexual orientation and the inheritance of polymorphic markers on the X chromosome in approximately 64% of the sibling pairs tested. The linkage to markers on Xq28 (on the tip of the long arm) indicated a statistical confidence level of more than 99% that at least 1 subtype of male sexual orientation is genetically influenced. Hamer (LeVay and Hamer, 1994) emphasized that the findings of his study should not be interpreted as 'medicalizing' homosexuality because sexual preference should be viewed, he insisted, as a behavioral variable. His studies were consistent with the observation that homosexuality seems to run in the female line.

 What motivated Hamer’s research in the genetics of homosexuality? Hamer hoped that scientific research would help dispel some of the myths about homosexuality that have clouded the gay and lesbian community in the past years. Hamer also recognized that educating the public about genetics and behavior would eventually improve our understanding of the individuals’ natural rights and human diversity.

In 2014, a new report probed a genome-wide linkage scan on 409 independent pairs of homosexual brothers and confirmed Hamer’s results that there are genes that influence the sexual orientation of males (Sanders et al., 2014). First, they found a region in chromosome 8 that influences male sexual orientation. Second, they confirmed Hamer’s earlier studies that the Xq28 region on the X chromosome also influences male sexual orientation. However other reports suggest genetic linkage of homosexuality to other chromosomes. Yu et al., (2015) report a linkage to chromosome 22. Clearly much more work is required to elucidate the role of genetics to sexual orientation.

In 2015, a new study reported that epigenetic effects influence sexual orientation (Balter, 2015). Researchers found five genome regions where the methylation pattern appears very closely linked to sexual orientation. A model that predicted sexual orientation based on these patterns was almost 70% accurate within this group. However, analysis of these epigenetic regions did not predict sexual orientation in the general population.

 If there are genes that influence gay behavior, then it will be important to understand how this trait provided an evolutionary advantage since, by its intrinsic nature, gay couples do not procreate. Genes that regulate sexuality may be part of the Darwinian "paradox". Evolutionary models have proposed suggesting that polymorphic genes that influence homosexuality confer a reproductive benefit to heterosexual carriers, thus offsetting the fitness costs associated with persistent homosexuality. Genes that confer gay tendencies may in fact offer evolutionary advantages in heterosexual individuals such as making them more loyal, considerate or empathic. Genes that promote same-sex bonding may also reduce aggression within social communities and encourage resource sharing, which may also have provided an evolutionary benefit.

 Two models have been suggested that describe the evolutionary benefits of male homosexuality: heterozygote advantage and sexually antagonistic selection. The former was discussed in the previous paragraph and proposes that the benefits of gay tendencies in heterozygous, heterosexual men were so great that they overpowered the disadvantages from lack of procreation in homosexual men. This theory is only enhanced under the knowledge that historical anti-gay attitudes may have caused gay adults to procreate regardless of their homosexuality. The heterozygote advantage model can be applied to females as well. Researchers recently demonstrated a correlation between increased masculinity in women and a larger amount of sex partners throughout life. The other model, sexually antagonistic selection, can only be applied to male homosexuality. It proposes that female fitness is increased by the presence of alleles inducing male homosexuality, although male fecundity is negatively harmed if not indifferent (Burri et al, 2015).

 Emanuele et al., (2007) examined whether genes affect human romantic bonding and found a significant association between a certain neurotransmitter gene (the dopamine D2 receptor gene) and a specific style of love characterized as EROS (a loving style characterized by a tendency to develop intense emotional experiences based on the physical attraction to the partner). These associations were present in both sexes. Some studies link gene patterns to the number of sexual partners a person has (Burri et al., 2015). They show that genetic factors responsible for nonheterosexuality are shared with genetic factors responsible for the number of lifetime sexual partners via a latent sex typicality phenotype in human females. Another area that is ripe for exploration is the genetics of transgenderism. As of 2016 there were no significant papers published on this topic. Yet, one can expect that more researchers will examine the genetics of transgenderism in the future.

*Violence.* Behavioral genetic research has analyzed thousands of sibling pairs and has pointed to the “inescapable conclusion” that genetic factors do contribute, to a certain degree, to the etiology and cause of violence (Ferguson, 2010). Some conclude from these studies that approximately 50% of the variance in antisocial phenotypes is the result of genetic factors (Ferguson and Beaver, 2016). Examining genes that regulate violent behavior has been supported by both academic centers as well as governmental agencies that monitor terrorism and the crime rates within a specific society or country. Violent behavior is affected by social and possibly genetic factors (Tuvbald and Baker, 2011). This research points to the importance of a nurturing social environment in childhood, good early education, and success in academic areas. Peer influence is also of critical importance in predicting violent behavior. Many twin and adoption studies indicate that child and adolescent antisocial behavior is influenced by both genetic and environmental factors, suggesting that genetic factors directly influence cognitive and temperamental predispositions to antisocial behavior. These predisposing factors and socializing environments, in turn, influence antisocial behavior in children. Research also suggests that for some youth with early onset behavior problems, genetic factors strongly influence temperamental predisposition, particularly oppositional temperament, which can negatively affect experiences. When antisocial behavior emerges later in adolescence, it is suspected that genetic factors contribute less. Such youths tend to engage in delinquent behavior primarily because of peer influences and/or have experienced abuse in the home.

Based on genetic analysis, several studies have suggested that genes coding for the monoamine oxidase (MAOA) and tryptophan hydroxylase (TPH) enzymes are linked to specific cases of violent behavior. These genes code for certain enzymes that are responsible for the metabolism or synthesis of three neurotransmitters (serotonin, norepinephrine, and dopamine) that have been associated with the onset of aggression or violence. Serotonin is one neurotransmitter that is responsible for moods, appetite, sexual activity, homeostasis, and sleep. Norepinephrine regulates stress and moods in the brain. Dopamine regulates emotion, the "pleasure center" of the brain, and motivation.

One problem with linking MAOA encoding-genes to behavior is that, in the literature, there are scores of behavioral characteristics that have been ascribed to this enzyme. The same polymorphisms of these genes are said to predict variation in other behavioral and physical traits. The idea that one or two genes could be responsible for so many disparate behaviors is biologically implausible. In addition, the genetics underlying violent behavior is complex. A 2014 study uncovered at least 13 genes that changed during evolution as cats morphed from displaying wild aggressive behaviors to friendly behaviors (Montague et al., 2014).

*Other behaviors.*There are now several studies that link genetic markers to the ability of individual to lose weight. Pathway Genomics is a company that will analyze your genes to provide clues to effective weight loss programs. A study in 2016 examined happiness in twin siblings who were separated after birth and brought up by different families with different socio-economical backgrounds. Following years of observation, the team found that twin siblings who have the same genetics report the same levels of happiness no matter how they are affected by environmental factors. Although other factors play an important role in an individual's happiness, the effect of such factors does not last for long. The study revealed that genetics accounts for 48 percent of the influence behind feelings of happiness while 40 percent is tied to other incidents that happen every day and 12 percent is linked to other elements.

 **Synthetic Biology**

The last part of this chapter will focus on synthetic biology. The UK Royal Society has defined synthetic biology as “an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems.”[[8]](#footnote-8)

Scientists have been attempting for years to expand nature's genetic four letter alphabet, consisting of the nucleotide bases cytosine, guanine, adenine and thymine (represented by the letters "C," "G," "A" and "T," respectively). In 2014, Romesberg et al., published a seminal paper culminating 14 years of NIH-funded research and reported the synthesis of two new synthetic nucleotides X (d5SICS) and Y (dNaM). Impressively, they were able to generate bacteria that could replicate DNA containing this new base pair. Romesberg hypothesized that his expanded genetic alphabet is either so foreign that the genetic framework simply doesn't recognize it as an error, or, more likely, has no way to fix or change the X and Y letters. To ensure that such bacteria never leave the laboratory, he modified the E. coli to replicate these nucleotides in the DNA, but did not use a customized clip of genetic code to build proteins, so the new letters were not expressed in any new genes. His X and Y nucleotides are hidden away on a length of DNA that essentially functions as untranslatable code (Chen et al., 2014).

The practical translational application of expanding our base pairs remains to be investigated. However, in the area of computing, the expanded DNA code may offer a significant technological advantage. Biomolecular computing using DNA offers an alternative non-microchip technology for storing information. Using our four base-pair system, a DNA-computer with one liter of fluid would contain six grams of DNA and would have a memory capacity of 3072 Exabytes (one billion gigabytes). The idea of using an expanded DNA code would increase the storage of these computers dramatically and improve the power of biomolecular computing. The development of aptamers is another application of synthetic base pairs. Aptamers are small DNA molecules capable of specifically binding proteins or other cellular targets. One could view aptamers as a chemical equivalent of antibodies and could be used to target tumor cells (Sefeh et al., 2014). Incorporating synthetic bases into aptamers could affect tissue targeting and improve specificity.

Some ethicists fear that incorporating synthetic base pairs into humans in a clinical situation would be very risky. This is because our bodies would be completely defenseless against modified base pairs without any mechanism to identify or break down such artificial material. Thus, unseen complications or mutations caused by the meddling of the natural genetic code occur would be unstoppable.

A research team at Stanford successfully created a biological transistor, cleverly named a transcriptor, within human cells. Like transistors, transcriptors are on-and-off switches, gatekeepers or “gates” of information input, storage, and output. Transcriptors give cells already programmed to store and transmit information a “brain,” a system of logic governing the way they deal with that information. It is “biological internet” that transmits genetic information between cells and a rewritable DNA data storage system. The transcriptor, similar to the way a transistor amplifies electrical signals, can allow small changes in enzyme activity to trigger much larger changes in gene expression. The transcriptor has the ability to report whether the cell has been exposed to a specific stimulus. Such technology has the potential to revolutionize disease detection.[[9]](#footnote-9) The insertion of one or more transcriptors into bacteria transforms them into microscopic calculators.

Clinical samples are complex environments, in which it is difficult to detect signals. Scientists have used the transcriptor's amplification abilities to detect disease markers in the blood and digestive system, even if present in very small amounts. They also succeeded in storing the results of the test in the bacterial DNA for several months. As a proof of concept for creating intelligent bacteria, the authors connected the genetic transistor to a bacterial system that responds to glucose, and detected the abnormal presence of glucose in the urine of diabetic patients. In future, this work might also be applied to engineering the microbial flora in order to treat various diseases, especially intestinal diseases.[[10]](#footnote-10)

**Gene Editing**

Any discussion on genetics must include new technologies in gene editing (Kaufmann et al., 2013). Currently there are at least four different systems by which the base pair of DNA can be targeted to either replace or delete the DNA. These gene editing technologies are:

1. Zinc finger nucleases,
2. TALEN From the French word “claw”

(Transcription Activator-Like Effector Nucleases),

1. BuD nucleases,
2. CRISPR/Cas9 (Clustered Regularly Interspersed Short

Palindromic Repeats) nuclease.

All of these systems rely on proteins or RNA to target specific sites on the DNA, a functional element to initiate double stranded breaks in order to excise the DNA, and an element that allows the DNA to be repaired.[[11]](#footnote-11) In some situations, single base pairs can be changed. In other situations, specific regions of the DNA can be excised. The major difference between these different systems is how they recognize and target specific sites on DNA.

The potential applications to correct human diseases are vast. In 2014, Sangamo Biosciences used zinc finger systems to knock out CCR5 in human T cells from HIV+ patients. The HIV virus requires this receptor to enter T cells. The researchers then safely returned those cells to the patients and raised their T cell counts. (Tebas et al., 2014; Kaminski et al., 2016).



PD-1 is a receptor present on activated T cells and regulatory T (T-reg) cells, and its ligand PD-L1 is expressed by most cell types including tumor cells and dendritic cells. Anti-PD-1 antibody produced objective responses in approximately one in four to one in five patients with non-small-cell lung cancer, melanoma, or renal - cell cancer. Su et al., (2016) successfully used CRISPR to shut down PD-1, allowing T cells to attack tumor cells more efficiently.

Gene editing is being tested as a means to cure individuals who have genetic mutations causing diseases such as cystic fibrosis, muscular dystrophy, and various forms of clotting disorders. One example is to obtain adult cells from an individual who has Hemophilia A, one of the most common genetic bleeding disorders, caused by various mutations in the blood coagulation factor VIII (F8) gene. Using TALEN technology, scientists could revert the mutated DNA segment back to its normal orientation in these stem cell to obtain a cell line with the normal gene. Then these stem cells would be used in a bone marrow transplantation procedure to enable the patient to produce normal clotting factors.

A third type of application would be to use viral technology to deliver gene-editing proteins to the liver to cure individuals with type I tyrosinemia (Yin et al., 2014). Patients (about 1 in 100,000) cannot break down the amino acid tyrosine, which accumulates and leads to liver failure. In mice, scientists were able to insert the correct gene in about one of every 250 hepatocytes — the cells that make up most of the liver. Over the next 30 days, those healthy cells began to proliferate and replace diseased liver cells, eventually accounting for about one-third of all hepatocytes and curing the mice.

Finally, gene editing can be applied to embryos generated in vitro to replace a single base pair mutation. This technology has been tested in mice that have genetic-based cataracts (Wu et al., 2013). In this study, about 33 percent of the mutant zygotes that were injected with CRISPR/Cas9 grew up to be cataract-free mice. Clearly, the efficiency of success must be greatly improved before applying this technology to human beings.

The most exciting gene editing system in 2015 was CRISPR. The CRISPR system offers certain benefits over the competing technologies. First the Cas9 is a highly programmable enzyme. The use of a guide RNA has the potential to make target location very specific. Second, this system can be used to multiplex or target multiple sites simultaneously. The potential of CRISPR technology is seen in the rapid development of many companies that plan to begin clinical trials using this gene editing technology. In 2015 Bayer Corp. invested $400 million in a small company called CRISPR Therapeutics and Fidelity Investments and a fund backed by Microsoft Corp. founder Bill Gates invested $120 million in Editas Medicine.

CRISPR has been used to create mice that inevitably get liver cancer. These mice can then be used in drug trials. Researchers are looking to utilize these gene-editing tools beyond medicine, as well. These new technologies are viewed as biological “superpowers”. Their envisioned uses are incredibly widespread, including being used as a solution for hunger (through genetically editing produce) and as an end to reliance on petrochemicals (researchers are working on yeast that consumes plant matter and excretes ethanol). Other companies use CRISPR to create industrial and research materials, such as enzymes in laundry detergent. Other scientists hope to bring back the woolly mammoth by using CRISPR to insert its genes into elephant embryos.[[12]](#footnote-12)

Finally, CRISPR is being used to genetically modify plants and animals. CRISPR is being used to create plants that are resistant to certain viruses (Ali et al., 2015). It has been used to delete the muscle-inhibiting gene myostatin from two beagles[[13]](#footnote-13) and pigs (Wang et al., 2015), in order to produce more athletic animals with double the amount of muscle mass. These genetically modified dogs are expected to have stronger running ability, which is good for hunting and police (or military) applications. CRISPR has also been used to create pigs that can serve as human organ donors. Doctors have been slow to use pigs as organ-donor alternatives for at least two reasons: first, the pig genome has a number of endogenous retroviruses that are harmless to pigs, but that could infect humans; second, the human immune system will target pig-specific proteins in the cell membranes, trying to reject the foreign bodies. The CRISPR system can inactivate 62 of the pig’s endogenous retroviruses in embryos as well as modify genes to make their tissues immune-compatible for human transplants.

One problem with CRISPR technology is that its components, an enzyme called Cas9 and a strand of RNA to direct the enzyme to the desired sequence, are too large to stuff into the genome of the virus most commonly used in gene therapy to shuttle foreign genetic material into human cells. Recently, a mini-Cas9 was isolated from the bacterium Staphylococcus aureus (Ledford, 2016). This protein is small enough to squeeze into the virus used in one of the gene therapies currently on the market. In December of 2015, two groups used the mini-me Cas9 in mice to correct the gene responsible for Duchenne muscular dystrophy.

CRISPR is also being applied for commercial ventures such as improving the yield to generate cashmere. Most hair on a goat is coarse and thick, unsuitable for fine clothing. Cashmere comes from a second undercoat that goats grow only in the winter, where the hairs are fine and soft and downy. Cashmere is expensive because even goats that are specially bred to produce cashmere produce only about half a pound per goat. Chinese scientist have used CRISPR to disrupt a single gene in cashmere goats to improve the nature of the hair produced and yield. As of 2016, CRISPR modified goats make hair in their undercoats longer and more numerous and boosts the yield by almost 50%.[[14]](#footnote-14)

A novel application of the CRISPR system is called “gene drive”. Gene drive is a technology to accelerate inheritance of particular genes and alter entire populations. By incorporating a CRISPR into the desired gene, scientists can cause a gene to be inherited at a rate faster than Mendelian principles would dictate. Gene drive technologies are being applied to change wild populations of harmful organisms, such as malaria carrying mosquitos, to be less dangerous.[[15]](#footnote-15) By inserting the CRISPR system within a mosquito, it is theoretically possible to create large populations of mosquitoes that will not transmit malaria, Zika, or yellow fever to humans. Gene drives supercharge genetically modified genes so that they defy the normal rules of inheritance. Normally, genetically modified traits are quite difficult to spread within a population of wild insects unless they impart a great evolutionary advantage. But when attached to a CRISPR gene-drive DNA “cassette”, practically every individual in a breeding population will eventually end up being a genetically modified organism. Using gene drive technologies, genes can copy themselves onto a corresponding location in a paired chromosome, thereby overriding typical allele inheritance patterns. Gene drives can also be applied to environmental conservation, notably in fighting invasive species, such as rats on remote islands inhabited by ground-nesting birds, which are wrecking the indigenous ecosystem.

However, the power of gene-drive technology to accelerate the spread of genetic traits also introduces immense potential hazards. It would be possible that either a rogue state or a terrorist cell might decide to generate a gene-drive organism that could pose a threat to human health or to economically important livestock. For example, this could be done by introducing a foot and mouth virus that has the potential to seriously damage the dairy and beef industries or by genetically modifying mosquitoes so that they can deliver lethal bacterial toxins to humans.

In October of 2016 a paper appeared in Nature (Bahal et al., 2016) that reported using nanoparticles instead of CRISPR to alter DNA. FDA-approved nanoparticles were used to deliver peptide nucleic acids (PNA) into the stem cells of mice to remove the beta-thalassemia mutation. Beta-thalassemia is a blood disorder that reduces the production of hemoglobin and leads to a lack of oxygen throughout body, causing weakness, fatigue and serious complications. PNAs containing a strand of healthy donor DNA encoding the hemoglobin gene were injected into the bone-marrow stem cells of live mice. These nanoparticles targeted the mutant DNA region and corrected the mutation to correct the malfunctioning gene. Successful genome editing was achieved in seven percent of cases, with elevated levels of hemoglobin evident for 140 days after treatment. Thus, PNA molecule genome editing provides a complex but efficient alternative to CRISPR.

 **Conclusions**

The genetic composition of an individual can have profound effects on health, behavior, and disease. In some situations, such as Huntington’s disease, the nature of the defect can predict age of onset and severity of the disease. In other situations, environment, diet, and life experiences may alter disease onset and progression. Studying the role of genetics in behavior is compounded by a variety of factors including the complex interaction between genetics and environmental factors. Moreover, it can be difficult to precisely measure human behavior because there are so many variations. This chapter outlined various methods for diagnosing genetic-based diseases and behavioral characteristics including searching for specific genes that influences these diseases and behavior. In part, the overall goals of these studies are: a) to reduce the probability of a child being born with a genetic-based disease or abnormal behavior characteristics and b) to understand how genetic factors contribute to disease and behavior in order to help design new therapeutic interventions.

**Thought Question:** What lesson can you learn from the Supreme Court Decision about patenting genes to the current patent dispute regarding CRISPR?

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