

Aftermath of the Human Genome Project: an era of struggle and discovery

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Abstract: The collaboration between the Human Genome Project and the Sanger Institute, spurred on by their contemporary- Celera Genomics, in the race to sequence the human genome, opened the door to a generation of research concerned with the study of the nucleic manual. Following this monumental achievement, the genomic era was born, heralding the development of gene therapy, personalized medicine, pharmacogenomics, and CRISPR/Cas9 gene-editing systems. These 'omic' disciplines offer a wide range of applications in the treatment of disease and the betterment of the quality of human life. Their notability, however, manifests in the unique mix of sociological and scientific discourse that ensues. Current genomic-era milestones present a unique opportunity to view scientific advance in a thoughtful light, lacing together discoveries, history, and ethical questions, while encouraging the public to consider the varied facets of this scientific evolution with the same curiosity and enthusiasm as the trailblazers of the Human Genome Project. Consider it an invitation to reflect on the fruits of the genomic era.

Key words: Human Genome Project, gene therapy, personalized medicine, pharmacogenomics, 'omic' disciplines, genomic era, CRISPR/Cas9

1. Introduction

"Science is essentially a cultural activity. It generates pure knowledge about ourselves and about the universe we live in, knowledge that continually reshapes our thinking." – John E. Sulston, Winner of the Nobel Prize in Physiology or Medicine in 2002 (Human Genome Project and the Sanger Institute).

The conception of the Human Genome Project marked a decision unparalleled in the reach of its ambition and scope of its achievement. With its birth, researchers indulged humankind's primordial curiosity—the desire to expose the fundamental substance of development. Sequencing the human genome represented the equivalent of decoding the Rosetta stone of genomics and its closure signified the conclusion to an era illiterate in the language of its inhabitants' microscopic manual. Furthermore, Next Generation Sequencing methods catapulted the depth of genome research to an unprecedented level enabling the rapid sequencing of human genomes and RNA sequencing as well as the analysis of DNA to protein interactions. These accomplishments unleashed a wave of new techniques that revolutionized the face of medicine leading scientists, as well as society, on an intriguing, albeit provocative foray into the worlds of gene therapy and personalized medicine.

No longer did researchers view the human genome through a static and informational lens; rather, they

regarded it as a dynamic force teeming with opportunities for extraordinary applications in the identification of homologous regions between species, the classification of gene function, and the therapeutic introduction of DNA through gene therapy. While met with a multitude of answers long sought after, such genomic studies also encountered the resilient force of many questions and uncertainties. Witnessing its share of failure and death cemented the controversial nature of this pursuit and established the compelling spirit of its humanism.

Nevertheless, progress surged forward at the hands of the researchers guiding this determined venture into previously uncharted territory. Their diligent efforts cleared the way towards an augmented understanding of disease. In tandem with these occurrences, personalized medicine and pharmacogenomics originated, customizing cures to the patient's genome. These approaches promise greater effectiveness in treating diseases such as cancer, as well as combating the rise of antibiotic resistance. Encompassing fields such as proteomics, genomics, and pharmacogenomics, the 'omics' represent the quantifiable manifestation of genetic architecture studies. In fact, the 'omic' fields are an actuation of scientific reductionism in every sense of the word, especially regarding their literal dissection of the objects of their focus. Combined with CRISPR/Cas9, the 'omics' form the sturdy foundation

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to guide medical and scientific study towards an era of definite and systematic genomic modifications. As these fields continue to develop, researchers are on the brink of finalizing an exquisite gene editing technology based on prokaryotic immune defense mechanisms—CRISPR/Cas9.

2. Paving the way with the Human Genome Project

2.1. A tumultuous undertaking

Genome Sequencing—two words that represent a tumultuous combination of unprecedented enlightenment and yet unchecked power in the forms of genetic engineering and gene therapy. The expression of either the former or the latter opinion depends on the individual. However, it is vital to the understanding of the process itself to realize that the unique blend of such judgments is a hallmark of the genetics field. Within the efforts of the scientists seeking to unveil *the very make* of the human being, one recognizes the potential for advancing quality of life through advanced medical care and successful eradication of disease. Certainly, these benefits are the aspirations of gene therapy, a field whose foundation rests on the milestones established by genome sequencing. Despite the well-meaning intentions of these goals, the doubt that such methods may intervene with a particular order or may be the root of lasting harm worries many.

Such conflict is a present reality. At times, it represents drawbacks to furthering a blossoming field budding with untapped knowledge. Other times, it proposes questions whose answers are necessary for understanding the ethical implications of an era born mere moments ago in humankind's long history (e.g., Figure 1). Most importantly, it portrays the long reach of science as an impetus of change in today's society. At such a checkpoint in the discussion, it becomes necessary to question the reasoning at the heart of the proposed action. What drove the desire to sequence the human genome and what are the events preceding its success? Moreover, what are its implications and the results that have stemmed from its discovery? How did sequencing the human genome lay the proper groundwork for further expansion into the fields of gene therapy and personalized medicine?

2.2. Establishing a fertile foundation

Sequencing the human genome marked the zenith of a burgeoning mountain of genetic information obtained during the years preceding its achievement. The program's success is a product of the collaborative and innovative efforts of the Human Genome Project, an international research program initially founded by the Department of Energy and the National Institute of Health in 1990 (Watson and Cook-Deegan, 1991), and the Sanger Institute, the British extension that pioneered pilot sequencing projects providing integral support to the

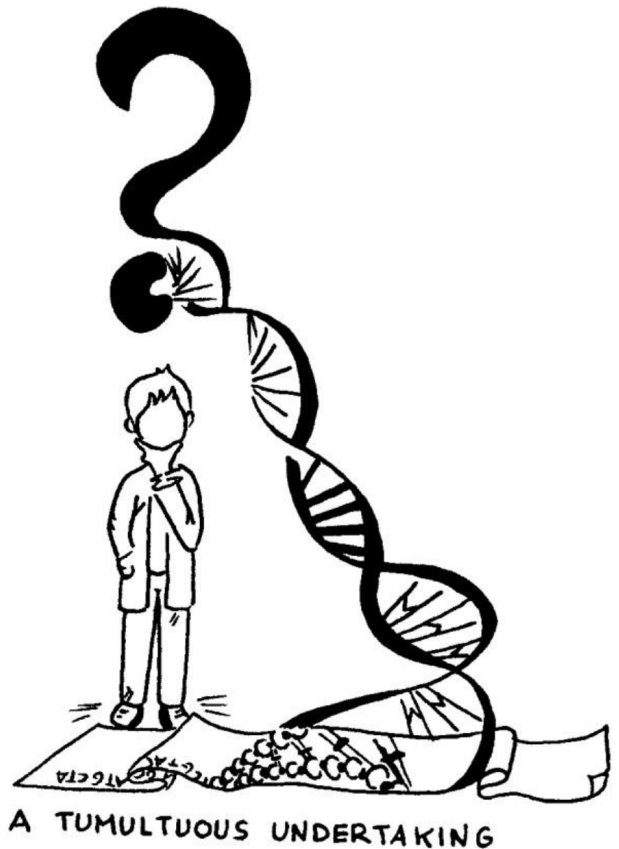


Figure 1. A tumultuous undertaking. The Human Genome Project was met with many hopes, criticisms, and worries. Many questioned the scientific path after its completion. These doubts were not solely confined to the opposition, however, but characterized the researchers involved, as well. In fact, it could be said that although they were directly involved in the Human Genome Project, the scientists, themselves, fell prey to the most questions because they were embarking on a journey no one else had taken before!

sequencing effort (Human Genome Project and the Sanger Institute). Moreover, Celera Genomics' privately funded sequencing endeavor headed by J. Craig Venter imbued the sequencing effort with a competitive edge, spurring its completion (Shampo and Kyle, 2011). In June of 2000, the project announced the sequencing of the majority of the human genome, followed by its official publication in the February 15 issue of *Nature* less than a year later (Collins and McKusick, 2001). Similarly, Celera Genomics published their results in *Science* within the same year, highlighting the spirited vitality that defined the race to sequence the human genome (Shampo and Kyle, 2011). Two years later, in April of 2003, a more accurate version was released providing scientists conducting research on the sequence with greater precision and additional information (Lander et al., 2001).

The primary goal of the program was to identify the sequence of bases that constituted the human genome and to advance sequencing technology and methods. Researchers also sought to understand how the human genome, by virtue of its makeup, interacts with the environment to promote and regulate a cascade of reactions governing bodily functions and development. In a nod to specialists of evolutionary biology, a field that had been steadily gaining recognition, scientists also aspired to identify homologous and conserved genetic regions between humans and other species. The rich influx of information afforded by the Human Genome Project increased understanding of evolutionary relationships, giving credence as well as substantial data to the emerging field of comparative genomics (Collins et al., 1998).

Headed by the likes of intellectuals James Watson, Michael Morgan, and Francis S. Collins, the project achieved unrivaled heights, effectively dissecting the human code into its component alphabet, an amazing feat bearing in mind where, how, and in what it is housed. From this complexity, researchers accomplished the equivalent of unravelling the tangle of chromatin, unwinding the double helix, and cleaving the nucleotide rungs for meticulous examination. However, to do so required the establishment of a fertile foundation ripe with potential for incredible discoveries. The greats of the genetic field extending as far back as the Augustinian monk Gregor Mendel, with his studies of the delicately quaint pea flowers to the groundbreaking discovery of the double helix structure of DNA by Rosalind Franklin, James Watson, and Francis Crick sowed this foundation and planted its seeds (e.g., Figure 2). Moreover, the sequencing of the human genome was one in a long series of other genetic assignments taken under the wide wings of the Human Genome Project such as those involving model organisms *Drosophila melanogaster*, the common fruit fly, and *Caenorhabditis elegans*, a small roundworm (Lander et al., 2001). Once complete, these and other decoded genomes facilitated greater understanding of sequencing processes and established standards of reference to which the human genome may be compared (Lander et al., 2001; Celniker et al., 2009).

2.3. The remarkable nature of the human genome

With the multitude of organisms whose genomes were studied previously, from bacteriophages to complex, multicellular organisms, the extensive study of the human genome proved an interesting, yet challenging task. Nevertheless, the abundant information garnered during this period remained unmatched by the daunting assignment ahead and yielded many unexpected surprises regarding the remarkable features characterizing the human genome.

Before the project's launch, estimates concerning the number of genes within the human genome ranged from

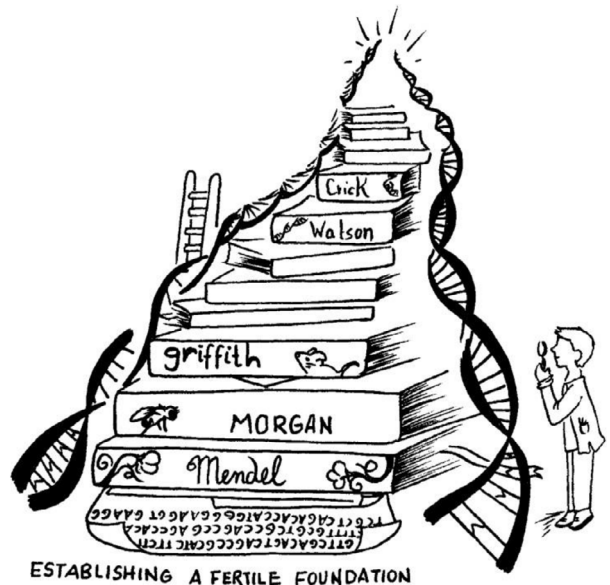


Figure 2. Establishing a fertile foundation. The Human Genome Project represented the culmination of an extensive foundation laid down before its initiation. The likes of Mendel, Morgan, Sanger, Watson, Crick, and Rosalind Franklin present but a few of the inquisitive intellectuals behind the eventual elucidation of the enigma—the human genome.

35,000 genes to over 100,000 with protein-coding genes numbering up to 40,000; however, results revealed that protein-coding genes averaged a simple 21,000 genes, less than initial expectations (Lander, 2011). Conversely, organisms such as lilies and salamanders boast larger genomes, disputing the assumption that greater complexity yields a more extensive genome (Cooper, 2000).

Moreover, results indicated that protein-coding genes composed a meager 1.5% of the typical individual's genetic constitution. Conserved noncoding elements (NCEs), DNA sequences that do not produce proteinaceous products but rather assume regulatory functions comprise the remainder of the genome. In addition, noncoding RNA (ncRNA), RNA transcripts that are not translated into proteins, encompass more than 10% of the genome (Lander, 2011). This revelation challenged the notion that transcription was limited solely to protein-coding regions of the DNA.

Despite these novel accomplishments, the Human Genome Project approached transposons, DNA segments that move between chromosomes, in a manner conflicting with current views. Rather than consider them active, shuffling bearers of genetic information, these genes were treated largely as cumbersome weights without any apparent purpose. Ironically, the Human Genome Project required correction, a responsibility the field of comparative genomics proficiently assumed. By studying

placental mammals and marsupials, researchers proved that up to 15% of CNEs that arose in these species 150 million years ago to 90 million years ago are derived from transposons by virtue of their ability to interact with the host's transcription mechanisms (Lander, 2011). Although constituting at least 45% of the human genome (Pace and Feschotte, 2007), the evolutionary history supporting the proliferation of transposable elements remains furtive. Recent research, however, indicates that casposons, a class of self-synthesizing transposable elements in prokaryotes, encode a homolog of the CRISPR-associated Cas1 endonuclease highly influencing the development of CRISPR/Cas systems (Krupovic and Koonin, 2016).

2.4. An undertaking steeped in doubt

Despite great strides in the genomics field, doubt and worry persisted. Revealing the human genome represented the equivalent of divulging a fascinating secret. For some, it embodied the temptation to tamper and alter, and for others the opportunity to explore and develop. Such disagreements in opinion are the product of a healthy society open to discussion and debate. However, they potentially highlighted the absence of fulfilling answers in the face of a new chapter in genomic studies. Such lack bred concern regarding a number of ethical and legal implications which the ELSI, the Ethical, Legal and Social Implications, program addressed. Of the web of apprehension built around the project, the ELSI program addressed four main areas: the privacy and protection of use of obtained genetic information, the ethicality of methods in transferring knowledge extracted in the lab to the clinic, the endeavor to ensure that all those involved in the project understand the benefits and shortcomings before consenting to participate, and the dedication to educate the public and professional sectors on the matter (Collins and McKusick, 2001; Collins et al., 2003; McEwen et al., 2014).

Although contentions surrounding the project's announcement were predominantly ethical, it was, nevertheless, met with staunch opposition questioning its practicality and reliability (Figure 3; Davis, 1992). The opposition contested the funneling of money and support into a project that may not lead to any plausible conclusions (Berg, 2006). By the same token, others challenged the 'favoritism' of such funding, viewing it as a threat to their smaller scale experiments (Davis, 1992; Berg, 2006). Opponents worried that even the brightest of researchers would fall into disillusion and boredom with such tedious work, wasting millions of dollars in backing that could have been distributed elsewhere (Collins et al., 2003; Berg, 2006). Furthermore, they challenged the credibility of research methods employed, arguing that directly attacking the nucleic makeup of the human for answers would prove futile especially in light of the

abundant noncoding, intronic, DNA. Traditional methods of studying each disease and its mutations on their own, they argued, yield more accurate and fruitful information (Berg, 2006).

2.5. Answers continue to be sought

With the closure of the Human Genome Project, additional reservations emerged, addressing, among a number of various topics, the possible effects elucidating one's genome may have on those with intellectual disabilities (Munger et al., 2007). Concerns ranged from worries regarding insurance and employment discrimination to the personal weight that may follow the discovery of a genetic limitation (Collins and McKusick, 2001; Guttmacher and Collins, 2003; Munger et al., 2007).

The answers to these doubts at the time of the project's launch were merely a framework, a skeleton, of the desired answers. With the implementation of the ELSI program, satisfying and complete responses seemed closer at hand; however, with the ELSI program functioning on three distinct, yet interwoven levels, biology, health, and society, all under a broader ethical category (Walther and Stein, 2000), the question of whether definitive and clear answers can be extracted arises (Figure 4).

ELSI research is both diverse in its scope and pointed in the populations it seeks to educate, which include the government, the scientific community, and society. Addressing these three different sectors, united in their humanity yet divergent in their associations, proves difficult in the face of linguistic, moral, and even career orientations. Furthermore, the ethical methods employed vary and include general scientific methods and common moral reasoning as well as bioethics and empirical procedures (Walker and Morrissey, 2014). Applied unanimously or without strict organization, these methods could collectively impede the establishment of a coherent and stable ethical foundation for furthering a field of research highly dependent on the public's understanding and opinion.

3. The birth of gene therapy

3.1. Reaping the benefits of the Human Genome Project

Despite mounting criticism, the Human Genome Project reaped unprecedented benefits and succeeded favorably against the doubts of many. With valuable information gathered from rigorous years of study, researchers developed the human standard to which other organisms may be compared. Establishing these relationships allowed for a more informed analysis of the vital association between disease-causing agents and the human species (Austin, 2004).

While such prospects carry the potent weight of promise, the prize gem nestled securely in the hands of this era manifests in the form of gene therapy. Elucidating

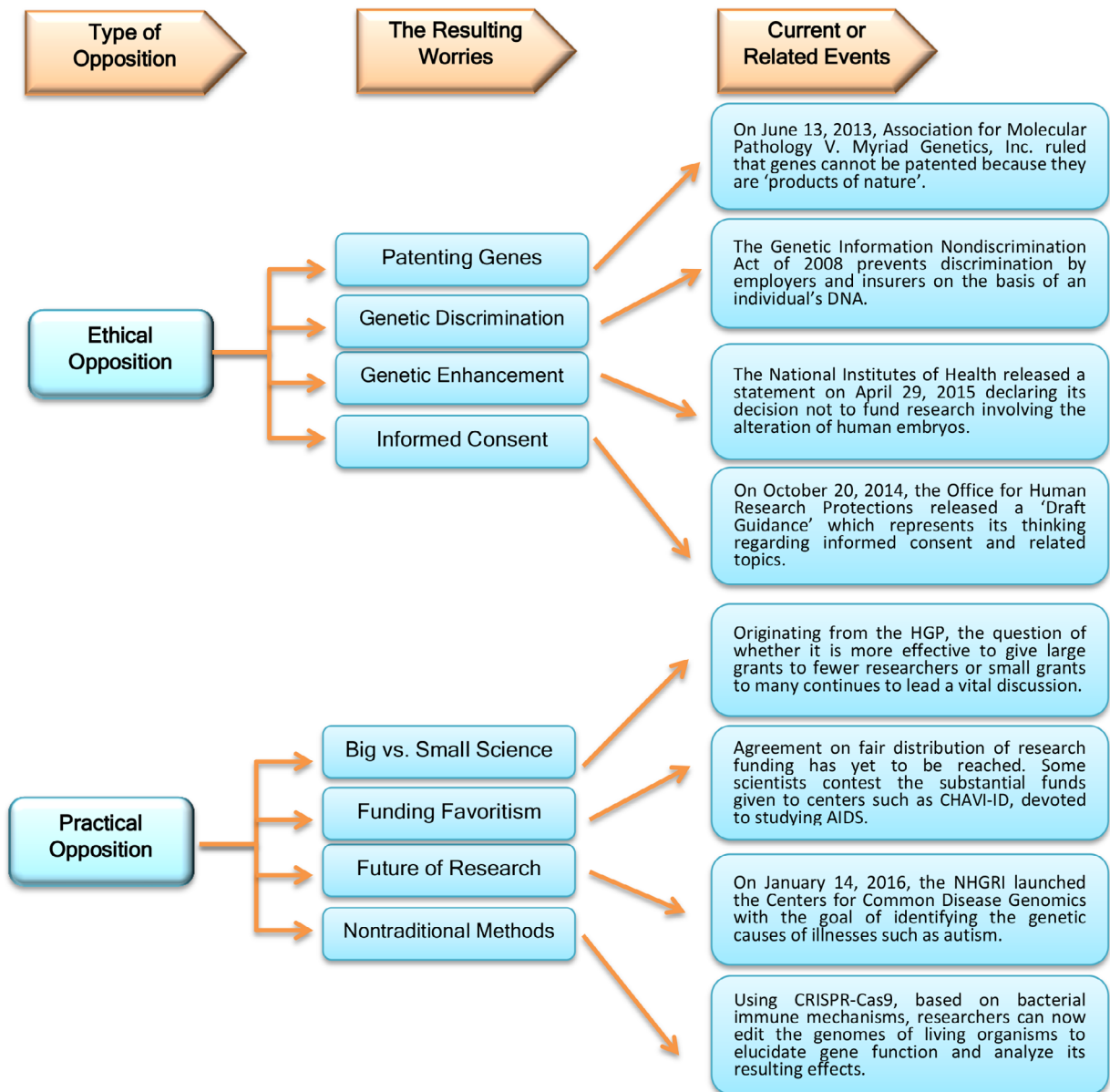


Figure 3. Opposition faced by the Human Genome Project. A predominantly practical and ethical opposition faced the conception of the Human Genome Project. The doubts and questions stemming from the two camps, although different, reflected the thoughts plaguing the public's opinion upon hearing of the weighty endeavor to sequence the human genome. The resulting worries have had repercussions on related tasks in the 'omic' fields today, spanning legal action, heated debate, and criticism.

the enigma at the heart of an organism's makeup—the *genome*, ushered in an age of specific, deliberate and human-driven modification. Lauded as 21st century medicine, gene therapy seeks to introduce the functional, or curative, form of a gene into a host to treat symptoms and signs of the mutated, or deleterious, form of the gene (Verma and Weitzman, 2005; Naldini, 2015). Unlike the relatively straightforward task of sequencing a viral or bacterial pathogen's genome to elucidate its structure and function, gene therapy must manipulate its knowledge of

the human genome to traverse the complex mechanisms of the human's defenses and ingratiate its message within the DNA machinery (Verma and Weitzman, 2005).

Over the years, the understanding of disease gradually evolved into a multifactorial system. Bacterial and viral pathogens, as well as mutations, no longer represent the sole culprits behind the debilitating circumstances. Rather, complex relationships between the cause of disease and the genome of the patient were established as main contributors (Verma and Weitzman, 2005). Ultimately,

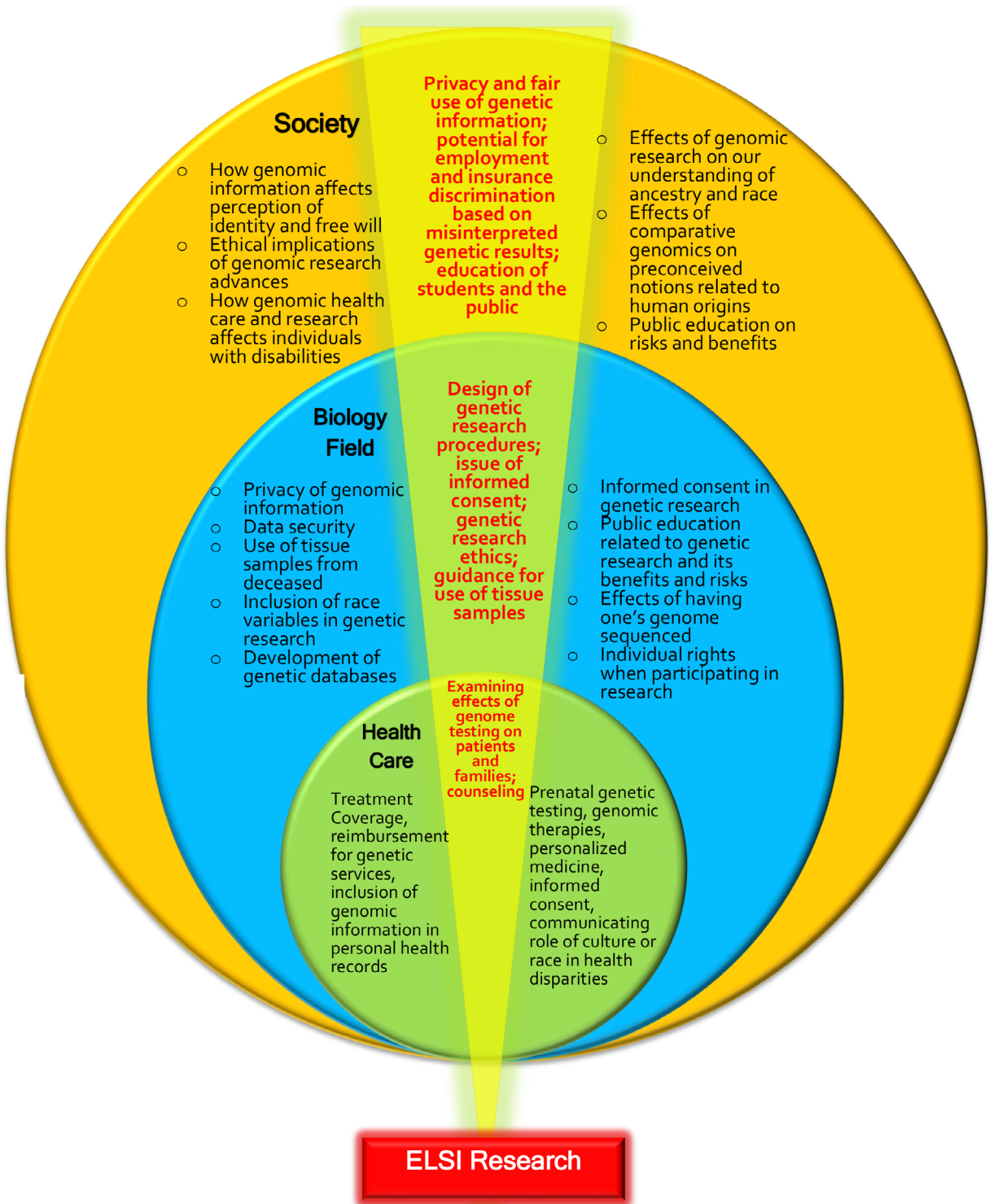


Figure 4. The scope of ELSI research. The scope of ELSI research encompassing the following sectors: society (in orange) as the overarching public and its concerns, the biology field (in blue) as the source of scientific research, and the health care field (in green) as the medium of practical application for derived research. Points of interest for further ethical research are delineated within each section as the ELSI program sheds light on particular topics (in yellow); points listed within the yellow area are those topics that ELSI has made significant strides in researching. Although substantial progress has been made regarding highly controversial topics such as employment discrimination based on the individual's genome, the nature and speed at which 'omic' fields are advancing leaves much to be addressed.

effective management of disease would depend on the meticulous study of the patient's genetics and the resulting interactions with the disease-causing agent, the concept central to gene therapy (Marraffini, 2015).

3.2. The vehicles of gene therapy

For successful completion of its mission, gene therapy must deliver the appropriate amount of the transduced gene within a vector without initiating a toxic effect within the host or potential offspring (Friedmann and Roblin, 1972; Kay et al., 2001). In recent years, vectors have withstood the most of researchers' scrutiny for their critical role in DNA introduction. Common vehicles of nucleic acid introduction include retroviruses and adenoviruses (Wu et al., 2002), the pathogens underlying acquired immunodeficiency syndrome and the common cold, respectively. Viral vectors are attractive candidates for the introduction of corrective DNA for their ability to insert the desired genes and target the chosen organs with an exceptional precision rarely observed in other vectors. As of 2014, about 70% of clinical trials conducted incorporated modified viral vectors for the purpose of nucleic acid introduction (Yin et al., 2014). Despite their commendable qualities, viral carriers are often associated with carcinogenesis, broad tropism, and immunogenicity, a detrimental situation that defined the tragic death of Jesse Gelsinger, a teenager suffering from ornithine transcarbamoylase deficiency (OTC deficiency), which prevents the successful elimination of ammonia, in the year 1999 (Marshall, 1999; Sibbald, 2001; Douglas, 2007; Yin et al., 2014).

To remediate the drawbacks associated with viral usage, nonviral vectors, such as plasmids, are used. Packaged within liposomes, plasmids can bear larger sequences and do not provoke an immune response within the host; nevertheless, the inefficiency with which they integrate the desired gene in the host genome challenges these benefits. This discrepancy in efficacy of integration between synthetic and viral vectors is the product of the viral organism's evolutionary and developmental history assembled around a particular penchant for targeting the mammalian genome (Yin et al., 2014).

The retroviral vector, on the other hand, risks activation or mutation of an essential gene if incorporated adjacent to it, provoking undesired tumor proliferation (Walther and Stein, 2000). In the predominantly fruitful effort to cure severe combined immunodeficiency, retroviral vectors inserted in or near the LMO2 gene caused the unchecked proliferation of white blood cells resulting in the development of leukemia (Hacein-Bey-Abina et al., 2003).

To compensate for these disadvantages, virosomes, a synthetic combination of liposomes and viral surface proteins, represent a compromise between the highlights

of the two vectors. Using virosomes, researchers may take advantage of the precision and efficiency with which viruses insert genes and the decreased risk of producing a harmful immune response proffered by the incorporation of plasmids (Kaneda, 2000).

3.3. Gene therapy makes its official breakthrough

Despite these setbacks, gene therapy managed its official breakthrough with the successful treatment of Ashanti De Silva, a patient with severe combined immunodeficiency or SCID due to adenosine deaminase deficiency (Cavazanna-Calvo and Fischer, 2007). Lacking a functional immune system, those suffering from this illness may die from otherwise mild infections. The culprit behind this disease in approximately 15%–20 % of afflicted children is a deletion mutation in the gene encoding the enzyme adenosine deaminase (Markert et al., 1988), which degrades deoxyadenosine to deoxyinosine (Fischer, 2000). In the absence of this crucial enzyme, deoxyadenosine accumulates to dangerous levels within T lymphocytes, interfering with their proper maturation and viability, which contributes to the development of an impaired immune system within the patient (Fischer, 2000). For Ashanti De Silva, treatment began in the year 1990 with the isolation of some of her white blood cells and their subsequent mixing with a retroviral vector bearing the normal copy of the ADA gene. Ensuing injection of altered T cells into her bloodstream resulted in the continued expression of the ADA protein (Greenberg et al., 2011).

3.4. Doubts resurface in the midst of triumph and failure

De Silva's success heightened hope for gene therapy methods and served as a beacon of light for those pioneering this fragile technology (Anderson, 2000). Unfortunately, the nine years that followed observed the death of Gelsinger (refer to Figure 5 for a timeline of significant gene therapy events), during which confidence in the emerging field plummeted to an unprecedented low (Mullard, 2011). Questions arose concerning the safety and reliability of gene therapy. With Gelsinger's death, a consistent connection was drawn between his fatality and the adenovirus vector applied (Sibbald, 2001). Despite this revelation, his parents allege that they were not informed of the severe side effects experienced (Wilson, 2010) by previous patients or that three monkeys had died from clotting disorders and liver inflammation (Sibbald, 2001), propelling the subject of informed consent into the spotlight. Researchers involved in the previous trials attributed these incidents to the dosage applied and thus the vector fell under no suspicion as a vessel of harm (Sibbald, 2001; Wilson, 2010).

No longer were questions solely honed to address the methods employed as a whole; rather, researchers and participants in gene therapy trials alike found themselves questioning the dependability of the delivery

1988	With congressional funding, the Department of Energy (DOE) and the National Institutes of Health (NIH) begin their collaboration on sequencing the human genome.
1998	In September, the main advisory board of the Human Genome Project announced the completion of the full human genome sequence by 2003- two years ahead of schedule.
1999	Jesse Gelsinger dies from a massive inflammatory response due to injection of adenoviral vectors administered to cure OTC deficiency. He becomes the first to die of gene therapy.
2001	In this year, 90% of the human genome's approximately 3 billion base pairs are published in the February issue of <i>Nature</i> by the HGP. Celera Genomics also publishes their findings in <i>Science</i> .
2002	Four of ten French children and one child in London undergoing gene therapy to cure X-linked SCID are diagnosed with leukemia due to treatment over the course of a year from 2002 to 2003.
2003	April 14, 2003 marks the culmination of the Human Genome Project's mission as the International Human Genome Sequencing Consortium announces the completion of the human genome sequence.
2004	The Chinese Food and Drug Administration approves Gendicine® for the treatment of head and neck squamous cell carcinoma, making China the first country to approve a gene therapy drug.
2007	A patient with LCA shows improved eyesight due to gene therapy treatment at the Institute of Ophthalmology and Moorfields Eye Hospital. Jolee Mohr, a 36-year-old woman, dies after participating in a gene therapy trial for rheumatoid arthritis.
2009	At the annual meeting of the American Society of Gene and Cell Therapy, presented gene therapy results show that six patients with Parkinson's disease had improved motor function up to six months after treatment.
2012	Europe approves the first gene-based medicine - Glybera® for the treatment of familial lipoprotein lipase deficiency (LPLD).
2014	Researchers demonstrate success in improving the severity of bleeding episodes in patients with Hemophilia B through adeno-associated viral vector infusion.
2016	By altering genomes within weeks, the precision and efficiency afforded by the use of CRISPR is being applied to a variety of diseases.

Figure 5. Defining milestones of gene therapy's history. The DOE and NIH's decision to collaborate on the HGP with the Sanger Institute marked a significant step forward in the realm of gene-editing. For gene therapy, this meant a spiraling history marked by successes and shortcomings. The timeline provided, although simplified to give a comprehensive overview of the subject, represents gene therapy's provocative history, beginning with its HGP origins and culminating at the threshold of a new and innovative technology: CRISPR/Cas9.

vehicles and, paradoxically, the authority. Nevertheless, an unanticipated triumph in the year 2000, when French researchers reported the effective treatment of nine out of ten patients with SCID, overshadowed this dark time. An improved outlook invariably foreshadowed brighter prospects, that is, until three of the former ten patients developed leukemia-like disorders due to the vector's insertion into the LMO2 gene (refer to Figure 5; Hacein-Bey-Abina et al., 2003). As a result, worry expanded in the span of a few years to gene therapy's major components (the method, the materials, and the specialists), posing legitimate questions worthy of ample consideration.

3.5. Adeno-associated viral vectors and alpharetroviral vectors reflect improvements

The shortcomings associated with a variety of vectors, although undesirable, nonetheless, spur a journey forward in search of the optimal gene therapy vector. Adeno-associated vectors, alpharetroviral vectors, as well as various nonviral vectors color the palette of gene therapy. Adeno-associated viruses (AAV) have emerged as highly suitable vectors in the transduction and integration of gene material for their lack of pathogenicity and their ability to infect both dividing and nondividing cells (Daya and Berns, 2008; Zhang et al., 2015). Studying adeno-associated viruses has revealed valuable insight into the nature of the capsid, delineating exposure sites vital to the maintenance of the vector and its infectivity, as well as its production. Using this acquired molecular awareness, researchers may broaden the range of AAV tropism through genetic code expansion, increasing its adaptability to a variety of functions (Zhang et al., 2015). Current research indicates the successful application of recombinant adeno-associated viruses (rAAV) in the transduction of prostate tissue *in vivo* and prostate cancer cells *in vitro*, thus providing a nurturing foundation for abounding study (Ai et al., 2016). Moreover, employing adeno-associated viral vectors, CRISPR/Cas9 mediated removal of exon 23 from the dystrophin gene in mice with Duchenne muscular dystrophy resulted in partial restoration of dystrophin protein expression (Nelson, 2016).

In contrast to the lack of pathogenicity characterizing adeno-associated viruses, alpharetroviruses, originally identified as cancer-causing agents, exhibit a softer side when employed in gene therapy. Researchers observe a more neutral manner of integration with decreased mutagenesis due to off-target attachment in exonic areas of the gene, as retroviruses have demonstrated in the past (Suerth et al., 2014; Kaufmann et al., 2016). Incorporating self-inactivating (SIN) alpharetroviruses into the gene therapy treatment of X-linked chronic granulomatous disease (X-CGD), a rare immunodeficiency disorder, revealed that, administered in low numbers, these vectors can functionally relegate the severity of the disease to

clinically acceptable levels, thereby increasing the survival rates of patients (Kaufmann et al., 2016). Furthermore, in a murine bone marrow transplantation model in which lentiviruses, gammaretroviruses, and SIN alpharetrovirus were administered for assessment of activity, integration of alpharetrovirus vectors decreased near the start of transcription sites and cancer genes compared to lentiviral vectors. Overall, a stronger trend in favoring integration within genes and transcription sites was observed for lentiviral and gammaretroviral vectors than for alpharetroviral vectors. Nevertheless, the resulting 'extragenic' integration defining alpharetroviruses still maintained persistent transgene expression within the model, recommending them as a vessel of low genotoxicity and neutral integration patterns (Suerth et al., 2012).

3.6. Recent successes reignite hope

Although faced with numerous obstacles, gene therapy has been especially beneficial in treating rare diseases. While questions regarding the efficacy of developing an expensive gene therapy treatment for a rare, or orphan, disease that will generate little profit remain, many in the field insist that studies in this area further the general application of gene therapy and increase understanding of its supporting processes (Mullard, 2011).

Hereditary blindness has long been the target of these persistent efforts. Researchers recognize the advantages of treating the eye, a small region both easy to access and moderately sheltered from the often-hindering effects of the immune system (Mullard, 2011). Gene therapy proved modestly successful in improving eyesight in patients with Leber's congenital amaurosis (LCA), a hereditary disorder that is the cause of visual impairment from an early age. Improvements in eyesight were temporary and most noticeable at 6 to 12 months after treatment (Bainbridge et al., 2015).

In addition, the treatment of ten patients with severe hemophilia B induced long-term expression of factor IX, the necessary clotting protein. After treatment, they exhibited enough expression of the protein to significantly reduce bleeding episodes; furthermore, no toxic side effects to initial treatment were observed 3 years after the trials (Nathwani, 2014).

These advances represent a simple portion of the varied achievements accomplished in the field to date. Not every single trial ends on a perfectly positive note. Others, such as the trial involving LCA patients, show moderate progress and leave room for both the acknowledgment of success and renewed resolution. This dynamic between feat and failure inspires scientists to identify the mistake, study its configuration, and develop ways to surpass or stunt its emergence. Although usually unwelcome, mistakes happen to be the avenue through which further learning is achieved and scientific techniques perfected, thus

contributing to the charming and accurately descriptive title “Science as a History of Corrected Mistakes” (Wood and Nezworski, 2005).

3.7. Wavering between support and opposition

Supporters of gene therapy contend that this era is equipped to shoulder the benefits associated with improved and targeted cures for an arsenal of illnesses heavy in the pain they inflict on their victims. In contrast, those opposed to the advance of this face of the genomic revolution claim that supporters rushed into largely accepting a gift, striking in its nascent potential, yes, but burdensome in the magnitude of its ethical consequences.

Such public perceptions of gene therapy depend highly on the way risk in the field is communicated by the media. Risk holds a ubiquitous position in a variety of fields and is associated with uncertainty and the notion that harm may befall something of human value. Although generally accepted as a part of life, the fear of risk increases when faced with altering nature, ethical consequences and trials involving children (Deakin et al., 2009). Furthermore, a multitude of high-profile serious adverse events (SAEs), such as the death of a research patient, has contributed to the disproportionate coverage that fosters this exacerbated fear. Questioning the definition of risk, acceptable range of risk and in whose hands falls the decision to delineate the responses to these questions has become central to the discussion of this topic. On the other hand, there are those that ask the opposite, questioning whether gene therapy is becoming too risk-adverse (Deakin et al., 2009; Mavilio, 2010). Their worries communicate the fear that such a mindset might inhibit progress in the field.

To answer these questions, an awareness of the nature of this era deserves attention. The tireless efforts of researchers throughout the world has allowed the scientific domain to expand into a previously inaccessible realm of medicine, one in which the cure is individualized and unique to the patient. Would one possibly imagine that the distinctiveness and inimitability of each individual's genome imbues the experimental process with greater efficiency (Donnelly, 2011; Zakim and Schwab, 2015)?

4. A personalized approach to medicine

4.1. Enter personalized medicine

The flourishing vigor that exemplifies the genomic era relies on its potential to present a customized approach to medicine (Dias-Santagata, 2010; Chan and Ginsburg, 2011). Rather than depend on the characterization of a cure based solely on the qualities of the disease, the focus of remediation has been directed at defining the genome of the patient and how that influences responses to the disease-causing agent as well as the applied medicine. While not a procedure entirely in itself, personalized medicine presents a methodology whereby family health

history, genomic analysis, clinical decision support, risk prediction, and microbiome interactions are combined and designed to complement the specific needs of patients (Dias-Santagata, 2010; Chan and Ginsburg, 2011; Offit, 2011). Essentially, the administration of drugs devoid of adverse effects and the shift to preventive medicine when the patient is still well epitomizes the vision for the remote future where personalized medicine leads (Dias-Santagata, 2010; Chan and Ginsburg, 2011; Marraffini, 2015). As Feero and Guttman elegantly stated: “Given the diversity of the human species, there is no “normal” human genome sequence. We are all mutants” (Feero and Guttman, 2010).

4.2. A Response to antibiotic resistance and disease mutation

With antibiotic resistance on the rise and cures to various diseases increasingly difficult to formulate, scientists are turning to a personalized medical approach to fill the widening void in available treatments. The escalation of antibiotic resistance in a variety of diseases reflects the agility with which natural selection works to mold the constituents of a range of populations to models of fitness and formidable adaptation. Personalized medicine embodies the reaction to this evolution as researchers hasten to match the speed with which bacterial and viral populations mutate.

Although personalized medicine often indicates the extraction of genomic information to assess, for example, the nature of drug metabolism within the patient's body, researchers hope to benefit from it in the rapid identification of infectious diseases and the characterization of the pathogen's antimicrobial resistance profile. If successful, this approach would greatly reduce the unnecessary suffering and deaths often encountered due to the laborious diagnostic cycle and would precisely determine the drug for patient administration (Bissonnette and Bergeron, 2012). Furthermore, in its application to cancer treatment, the personalized medical approach seeks to identify key mutational and genetic anomalies unique to each cancer (Chan and Ginsburg, 2011). Referred to as ‘genetic drivers’, these aberrations present the perfect handicap for exploitation as tumor progression grows increasingly dependent upon them (Chan and Ginsburg, 2011; Baird and Caldas, 2013; De Mattos-Arruda and Rodon, 2013).

4.3. Personalized medicine for the treatment of cancer

The human genome functions as a toolbox, providing a rich trove of information for the analysis of disease mechanisms, implementation of necessary diagnostics, and forecast of disease prognosis through the application of genomic, transcriptomic, proteomic, and metabolomic studies (Chan and Ginsburg, 2011; Chin et al., 2011). These assessment methods focus on whole genome sequencing

with an emphasis on single nucleotide polymorphisms (SNPs), RNA sequencing and gene expression profiles, profiles of the protein products of interest, and metabolic profiles, respectively (Chan and Ginsburg, 2011). While the objective of foremost importance in cancer medicine centers on prevention, detection, and treatment, bridging the gap between discovery science and medicine has proven a slow and difficult process as researchers continue to scrutinize the complex relationships underlying cancer initiation and proliferation (Hamburg and Collins, 2010; Chan and Ginsburg, 2011).

Instigating this search in 1982, researchers discovered the first cancer-related gene mutation, the substitution of glycine with valine in the HRAS gene at codon 12, triggering the activation of the oncogene in T24 human bladder carcinoma cells (Reddy et al., 1982; Chin et al., 2011). The surprising potency with which one point mutation works to confer cells with cancerous properties led to an era of research fixated on identifying the genomic causes of cancer.

To treat a subset of patients with lung cancer, tyrosine kinase inhibitors were administered to block the gene encoding epidermal growth factor receptor (EGFR), a transmembrane protein spurring pathogenesis and progression of tumor growth (Normanno, 2006; Gazdar, 2009). Furthermore, detection of select mutations in the BRCA1 and BRCA2 genes has aided in preventing and treating breast cancer. The prevalence of these mutations depends on ethnicity with a particularly high rate of occurrence in Ashkenazi Jewish and Icelandic populations; additionally, a family history of breast cancer and onset at a young age serve as strong predictors of cancer development, placing these individuals at 40% to 80% risk of contracting the disease (Olopade, 2008). Treating the patient successfully depends on numerous factors, among them the analysis of tumor stage, drug metabolism pathways, dose tolerance in different ethnic populations, type of microRNAs involved (Feero and Gutmacher, 2010), and presence of estrogen receptors in tumors (Olopade, 2008; Baird and Caldas, 2013), all of which are informed by the patient's unique genetic profile. Assessing these qualities guides design of the cure based on the underlying genomic causes.

4.4. The rise of pharmacogenomics

Variations in the genomic sequence between individuals influence differences in the optimal drug dosage required for successful treatment of each patient as well as the side effects experienced (Roden et al., 2006). The field of pharmacogenomics, concerned with the study of how the individual's unique chemical makeup influences their reactions and degree of responsiveness to medicine, has emerged as a critical keystone in the implementation of personalized medicine (Collins and McKusick, 2001; Roden et al., 2006; Chan and Ginsburg, 2011).

Genetic variations affect drug metabolism, targets, and transport, influencing a range of outcomes, among them successful treatment, ineffectiveness, and life-threatening effects (Collins and McKusick, 2001; Roden et al., 2006). By identifying polymorphisms, specific markers of genetic variation within the genome, the physician may administer the suitable amount, thereby preserving the safety of the patient (Roden et al., 2006).

Pharmacogenomic studies have already made great strides in identifying the appropriate dosage of medicine for children with leukemia based on their genes using the TPMT test, which identifies patients at risk of developing severe side effects to thiopurine drugs (Roden et al., 2006; Wang et al., 2011). Currently, genomic measurements made available from the study of tumor-derived cell lines provide a novel way of characterizing the patient's cancer, its behavior, and the therapeutic response to treatment (Goodspeed et al., 2016).

5. Moving forward with CRISPR/Cas9

5.1. CRISPR/Cas9: a paradox of sophistication and simplicity

As gene therapy and personalized medicine continue to take form, the scientific field moves forward with a revolutionary technology, CRISPR/Cas9, drawing on the paradoxical simplicity yet sophistication of prokaryotic immune defense mechanisms. Employing clustered regularly interspaced short palindromic repeat systems (CRISPR), genetic loci defined by repeat-spacer arrays, and a double-stranded RNA-guided endonuclease—Cas9, scientists are currently adapting the molecular structures by which prokaryotic microbes acquire immunity to viral invaders (Brouns et al., 2008; Horvath and Barrangou, 2010; Jiang et al., 2013; Barrangou, 2014; Doudna and Charpentier, 2014; Marraffini, 2015). Within the microorganism operating on a CRISPR/Cas system, viral genome injection initiates the immune process, at which time a small sequence of the viral genome, also known as a spacer, is incorporated into the CRISPR locus, endowing the cell with resistance to further efforts at viral infection (Brouns et al., 2008; Horvath and Barrangou, 2010; Marraffini, 2015). This immunization process not only allows for the spread of viral resistance efficiently and rapidly through a prokaryotic population, but it is also inherited vertically in the offspring of these organisms (Horvath and Barrangou, 2010; Marraffini, 2015). In the lab, manipulation and design of the crRNA guide changes the target site for cleavage, enabling successful genomic editing (Jiang et al., 2013).

However, ensuring that these modifications survive selective forces in subsequent generations requires the employment of a gene drive, which allows the changes to

proliferate even at a fitness cost to the organism. These “selfish genes” induce self-transmission through wild populations by cutting at the homologous chromosomal site, which triggers cell repair mechanisms to copy their sequence into the broken site. In this way, they are inherited more than the Mendelian fifty percent of the time, endowing the gene of interest with greater differential success in comparison to other genes (Sinkins and Gould, 2006; Esvelt et al., 2014).

Innovative applications of this technology include analysis of gene function in mammalian cells, correction of genetic mutations in a variety of disorders, and the study of the development of cancers and other diseases (Doudna and Charpentier, 2014; Wang et al., 2014). Moreover, it promises possible solutions to insect-borne diseases such as malaria and the reversal of pest and herbicide resistance in many invasive species (Esvelt et al., 2014).

5.2. At the cutting edge of CRISPR/Cas9

CRISPR's promise is, nevertheless, diluted with a sobering view of reality. In May of 2015, researchers in China reported substantial off-target cleavage in early human embryos leading to mutations (Liang, 2015). An intellectual divide within the scientific community followed this startling discovery, eventually culminating in a statement by NIH director, Francis S. Collins, declaring the institute's decision not to fund gene-editing procedures in human embryos (see: Statement on NIH funding of research using gene-editing technologies in human embryos).

Such setbacks have encouraged the development of successful research regarding off-target effects with Farasat and Salis identifying DNA supercoiling, the type of the PAM site, cell division rate, and genome size as valuable factors influencing the nature of Cas9 binding (Farasat and Salis, 2016). According to their results, the binding of one (d)Cas9:crRNA complex to a target DNA site lowered the affinity of subsequent binding in adjacent sites due to resultant negative supercoiling (Farasat and Salis, 2016). In short, the situation poses a limitation in the case that multiple genomic modifications are required in consecutive sites and encourages off-target binding should Cas9 mismatches or noncanonical PAM sites exist (Farasat and Salis, 2016). Furthermore, rates of cleavage drop with increasing cell division as bound DNA sites are replaced through replication and Cas9 and sgRNA concentrations are diluted with increasing cell size (Farasat and Salis, 2016). With this knowledge, researchers can tailor CRISPR application by monitoring the cell cycle, identifying the nature of available PAM sites, computing possible cas9 mismatches, and possibly increasing sgRNA and cas9 concentration.

As with any technology in its early stages, CRISPR achieves a variety of milestones including applications in sarcoma interrogation (Liu, 2016) and inhibited tumor

growth in mice with cervical cancer (Zhen, 2014), as well as cleavage of Hepatitis B viral DNA in cell culture (Kennedy et al., 2015). In fact, for the first time, researchers at China's Sichuan University injected CRISPR-modified genes into a patient with a severe form of lung cancer this year (Cyranoski, 2016). Taking such a step forward indicates achieving an exceptional degree of confidence in the field, which is a sure sign of progress. Interestingly, CRISPR/Cas9 also offers a distinctly rich avenue for scientific debate within the context of ethics (e.g., for interesting discourse, refer to Savulescu et al., 2015; Lanphier et al., 2015; Baltimore et al., 2015). Such discussion may be viewed as a revealing window into the subjective opinions of the individuals behind the greatest objective factual advances in society.

5.3. A discipline of gossamer

With the fusion of various fields with an explosive amount of potential, gene therapy, personalized medicine, pharmacogenomics, and CRISPR/Cas systems collectively represent the culmination of what it means to be in an age where scientific disciplines can be interrelated to one another seamlessly with vast benefit. While the resulting disciplines have become fodder for intense debate, the ability to claim an era where treatment is personalized remains a luxury. Aspirations dwell nearer and human reach has extended higher in the effort to transcend genomic limitations, vanquish disease, and further the understanding of what it means to be human.

Despite the bold nature of this description, science continues to prove itself a discipline of finely spun gossamer, stunning to view in the light but delicate in the face of disturbance. For this reason, care must be taken to ensure that educative awareness of the nature of medical and scientific evolution propagates and matures. For example, the phrase “personalized medicine” evokes in the scientist's mind a developing medical field dependent on genomic analysis, but an image of patients comfortable in the hands of professionals who respect their rights and values for those unaware of these developments (Browman, 2011). This misconception hinders complete understanding of medical evolution today and ignorance, in turn, is the spawn of misunderstanding. Opening a stream of dialogue between the scientific field and the public represents the first crucial step in the enlightening journey ahead.

From treating patients with severe immune deficiencies to identifying optimal drug dosages, the determination of academics investigating the minute coiling helices within human cells is worthy of admiration. While engineers work with a variety of complex but overtly visible machines, geneticists strive persistently in search of understanding a miniscule instructional manual unique in the simplicity of its dimension yet potent in its expression.

For those fascinated by the multifaceted harmony and sophisticated function of the world around them, nothing satiates their curiosity as fully as such a pursuit. Similarly, no venture is as resonant in its effects on the health, happiness, and quality of life of those it successfully treats. While such ambitions may seem too preliminary, especially when controversy concerning gene therapy has yet to be resolved, examples of this kind of determination and forward thinking must be commended. After all, science is the torch wielded by the curious and what would curiosity be without the fuel of imagination?

6. Conclusions

1. The human genome itself is still in the process of being fully analyzed and understood. As the impetus of change in scientific thinking and the basis of much of the medical advances pioneered today, the lack of complete knowledge concerning the human genome is worth noting where gene-editing techniques fail.

2. The ELSI program was established to provide an ethical foundation to the research following the Human Genome Project. However, its methods are too varied, covering a vast field of research intended for three diverse audiences: society, the biology field, and the healthcare field. Spreading ELSI's resources too thin over a broad range of issues may impede the formation of a coherent and firm ethical, legal, and social foundation for current scientific advances.

3. The understanding of disease has evolved into a complex multidimensional web of informants with the patient and disease-causing agent's genomes as key players. Gene therapy takes advantage of this relationship and effectively shifts the focus of treatment towards favoring a human-centered, rather than a disease-centered approach, which had primarily characterized treatment prior to the elucidation of the human genome.

4. Finding the ideal vector has been a difficult task as a unique set of advantages and disadvantages defines each one. While retroviruses and adenoviruses are invariably the most used, their shortcomings—off-target integration and incitement of immune response—spur a search for improvement or replacement. As a result, adeno-associated viruses and alpharetroviruses have emerged as safe, efficient alternatives to their use.

5. While Ashanti De Silva experienced a life-changing alteration in her SCID diagnosis due to gene therapy treatment, others have not been as fortunate. Nine years after her success as the first to be successfully treated with gene therapy, Jesse Gelsinger became the first to

die. His death sparked heated debate concerning a range of issues, including informed consent and the ethicality of gene therapy itself. However, while the successes achieved by gene therapy to date pale in comparison to the shortcomings encountered, they bear acknowledging as a sign that this field has potential, albeit with continued research. Recent accomplishments attest to the viability of the gene therapy field as well as its worth with notable successes in treating hemophilia B and Leber's congenital amaurosis.

6. Understanding the general perception of risk and its effect on public opinion would help alleviate the misunderstandings and preconceptions regarding gene therapy procedures. Research into the nature of risk perception as it relates to experimental trials, children, and death in gene therapy would help guide educative dialogue between societal and scientific sectors.

7. Personalized medicine is slowly changing the diagnostic look at patient care. Rather than assume a one-size-fits-all approach, doctors now look to unique genomic causes to assess the treatment suitable to the patient. Family histories, genetic profile, and ethnic background all converge to inform the causes underlying cancer initiation and proliferation. Personalized medicine and pharmacogenomics both present a methodology whereby these factors can be assessed and quantified into trends useful for the early identification and treatment of disease.

8. As the current emerging technology, CRISPR/Cas9 represents the fine string that ties gene therapy, personalized medicine, and other 'omic' disciplines together. With such a diverse set of applications, however, manifests the preemptive caution to move carefully with furthering this technology for its provocative potential for human application as well as its off-target effects.

9. While various opinions characterize the scientific field and its endeavors, approaching its progress with an appreciation for the advances made, and most of all, the curiosity that only such an elaborate study can bring, would help further the borders of understanding and extend a rich and stimulating dialogue.

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