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The New Fountain of Youth? The Anti-Aging Potential of Genome Editing

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Today's society heavily emphasizes the importance of appearance, thereby influencing individuals to view aging in a negative light. CRISPR-Cas9 technology has the potential to combat the effects of aging via the upregulation of particular genes to protect telomeres. This article aims to investigate the anti-aging potential of genetic engineering as well as analyze bioethical concerns relating to CRISPR technology. Preliminary research in cell cultures, mice, worms, and flies have demonstrated the possibility of using gene therapy to slow the natural process of aging through the introduction of telomerase and neutralization of free radicals. However, concerns involving the legalization of genetic engineering question whether CRISPR-Cas9 technology is ethically responsible, considering environmental and economic implications.

Introduction

CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is a set of DNA sequences found in bacterial cells.[1,2] When paired with the protein Cas-9, the CRISPR DNA sequence can aid in developing immunity to recognized viruses.[2,3] The defense is based on the recognition and cutting of specific foreign DNA sequences specified in a bacterium's CRISPR library. This system has been adapted into a revolutionary technology that drastically decreases the costs of genome editing, allowing scientists to manipulate DNA in a way they have never been able to before. Using CRISPR technology, scientists can delete, insert, or introduce mutations to the genome and turn 'ON' and 'OFF' specific genes.

The scientific community generally classifies genetic engineering into two major categories: therapeutic and reproductive. One possible application of CRISPR gene therapy is to eliminate genetic diseases (such as Huntington's disease and Duchenne's muscular dystrophy) by either cutting out or turning off the mutated genes in somatic cells, which are any cells other than reproductive cells. Another application of CRISPR-Cas9 is called germline genetic engineering (GGE), which introduces changes to sperm, egg, or progenitor cells that will be heritable for future generations.[4,5] GGE is the more

4 Baltimore D, Berg P, Botchan M, et al. A prudent path forward for genomic engineering and germline gene modification. *Science. 2015;*348(6230):36–39.

5 Gyngell C, Douglas T. The ethics of germline gene editing. *J*

My name is Kirra Borrello and I am currently a junior majoring in Cell and Molecular Biology and minoring in Public Health. Born and raised in Hawai'i, I am passionate about attending medical school and becoming a pediatric specialist to take care of my community. As a part of my honors sophomore seminar class, I decided to explore the potential of genetic engineering, which is an emerging biotechnology with possible applications to the health care field. With the help of my mentor Zoia Stoytcheva, I was able to navigate through the difficulties of relatively limited research about this semi-new topic. I am honored to have the opportunity to have a publication in *Horizons* and am excited to continue to expand my knowledge both at UH Mānoa and beyond.

controversial application of CRISPR-Cas9 because it is irreversible and may change the evolutionary gene pool. Associated with enhancements, genetic engineering to germline cells could hypothetically put a perfectly healthy individual at an advantage over others with "normal" innate abilities for no justified medical reason. This could include editing an embryo's genome to manipulate variables such as height, intellectual ability, and hair or eye color.

Genome editing via CRISPR-Cas9 is becoming a hot topic in the public eye.[6] A survey conducted in 2017 shows that the public largely supports genetic engineering for therapeutic purposes in both adult and prenatal patients, but objects to gene therapy for enhancement purposes in both populations.[7] Where does anti-aging fall on this spectrum of treatment versus enhancement? Is anti-aging genome editing a treatment for the age-related degeneration of the body or a method of enhancing humans to live a longer, possibly more rewarding life?

The Biology of Aging

While many people have a general concept of "aging" (gray hair, wrinkles, difficulty with movement, hearing, memory, and communication), aging encompasses complex physiological degeneration of cardiovascular tissues and skeletal muscles, as well as changes in the brain and other organs. On a biological level, changes to chromosomes involving the length of the telomeres can help to explain genetic changes caused by aging. One effect of these changes is that muscles experience a decrease in functional capability and efficiency, leaving the organism more susceptible to infections and disease. To further explain the concept of aging, it is important to consider the effect of evolution and the length of telomeres.[8,9]

From an evolutionary standpoint, the main goal for any organism is reproductive success–to live long enough to successfully pass on genetic material to future generations. Therefore, organisms are biologically optimized to maximize fitness early on in life, with little focus or regard for what happens once it has successfully reproduced. This concept is directly related to a situation biologists refer to as antagonistic pleiotropy–the phenomenon in which some genes that enhance fitness early on in life are actually detrimental later in life. For example, high levels of testosterone in human males lead to increased fitness in early life, but later in life, high levels put the individual at a higher risk for prostate cancer. Furthermore, because evolution has favored physiological investment in rapid reproduction rather than repair, most organisms are genetically inclined to gradually deteriorate after exiting the "normal" reproductive stage.[8] This is evident as women's fertility begins to decrease after about the age of 30; less of the produced eggs are viable, with higher rates of miscarriages and mutations.[10,11]

On a molecular level, biologists can begin to understand aging by inspecting telomeres, structures that serve as effective biomarkers for the effects of aging.[9,12] Telomeres are repetitive DNA sequences found at the ends of chromosomes that serve as protection for the coding regions of DNA that carry genetic information, like front and back covers of a book. Due to the particulars of how DNA is copied by a cell, telomeres shorten with each division of the cell, resulting in the loss of a bit of the protective margin. Eventually, the telomeres become too short and are no longer able to adequately protect the chromosome's coding region, which leads to loss of information and impaired cellular function.[13,14,15]

Throughout this paper and in anti-aging literature, the effects of aging are commonly evaluated based specifically on leukocyte telomere length. Biologists typically focus specifically on leukocytes, cells of the immune system present in the blood, because they are easy to access through repeated, non-invasive procedures and allow tracking of an individual's telomere length change over time.

Research has identified many catalysts of telomere shortening including obesity, stress, and smoking. Huzen et al. found that having a higher body mass index (BMI) correlates with more rapid telomere attrition, DNA damage, and even a higher cancer incidence, supporting the hypothesis that obesity accelerates the aging process.[16,17] Additionally, metabolic stress increases the production of free radicals, highly reactive molecules that can affect the function of mitochondria.[18,19]

10 Andersen AM, Wohlfahrt J, Christens P, et al. Maternal age and fetal loss: population based register linkage study. *The BMJ*. 2000;320(7251):1708-1712.

11 Somigliana E, Paffoni A, Busnelli A, et al. Age-related infertility and unexplained infertility: an intricate clinical dilemma. *Hum Reprod*. 2016;31(7):1390-1396.

12 Brane AC, Tollefsboi TO. Targeting telomeres and telomerase: studying in aging and disease utilizing CRISPR/Cas9 technology. *Cells*. 2019;8(2):186.

13 Vidaček NS, Nanić L, Ravlić S, et al. Telomeres, Nutrition, and Longevity: Can We Really Navigate Our Aging? *J Gerontol Biol Sci*. 2018;73(1):39-47.

14 Gorgoulis V, Adams PD, Alimonti A, et al. Cellular senescence: defining a path forward. *Cell*. 2019;179(4):813-827.

15 Gil J. Cellular senescence causes ageing. *Nat Rev Mol Cell Biol*. 2019;20:388.

16 Huzen J, Wong LS, van Veldhuisen DJ, et al. Telomere length loss due to smoking and metabolic traits. *J Intern Med*. 2014;275:155-163.

17 Shammas MA. Telomeres, lifestyle, cancer, and aging. *Curr opin clin nutr and metab care*. 2011;14(1):28-34.

18 Starckweather AR, Alhaeeri AA, Montpetit A, et al. An integrative review of factors associated with telomere length and implications for biobehavioral research. *Nurs res*. 2014;63(1):36-50.

19 Whaley-Connell A, McCullough PA, and Sowers JR. The Role

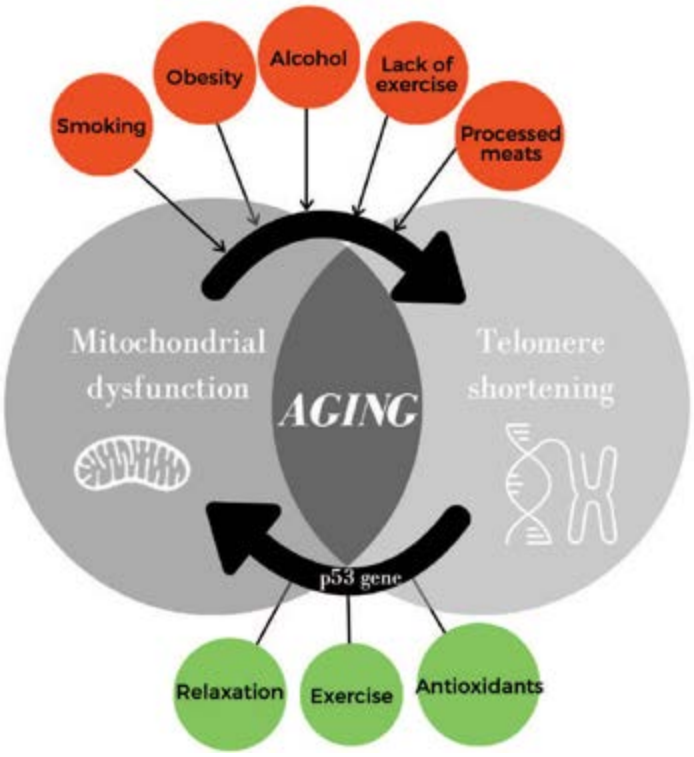

Figure 1 Influence of endogenous and exogenous factors on aging. The two main factors of aging, telomere attrition and mitochondrial deterioration, can be either positively (green bubbles) or negatively (red bubbles) influenced by both endogenous (internal) and exogenous (external) factors.

Because of their high reactivity, free radicals can cause severe damage to critical cell components such as lipids, proteins, DNA, and telomeres. Cigarette smoking also produces free radicals causing damage to telomeres and the coding region of DNA. Valdes et al. found that smoking one pack of cigarettes a day for four years corresponded to an 18% loss of leukocyte telomere length.[16,20]

Anti-Aging Prospects

In our society today, aging generally has a negative connotation. The cosmetic industry makes millions of dollars selling products that claim to make people look younger. Currently, there are few options for those who are interested in reversing the natural cycle of aging.

Geroprotectors are agents that target and combat cellular senescence, therefore prolonging lifespan. For example, antioxidants may donate electrons to neutralize free radicals generated by smoking and oxidative stress. Though there are opposing positions on antioxidant supplementation, high levels of antioxidants have been shown to have a positive effect on leukocyte telomere length as the neutralized free radicals do not damage DNA. Some common dietary antioxidants are vitamins and minerals, polyphenols, and omega fatty acids. A new emphasis on personalized vitamins in the commercial sphere has made vitamin and mineral supplements one of the most popular avenues of acquiring antioxidants. Vitamins C, D, E, folates, beta-carotene, as well as minerals such as zinc and magnesium have been shown to protect telomeres in humans.[13,21,22] Specifically, the Mediterranean diet has been cited for averaging the longest telomere length in humans. Rich in fruits, vegetables, nuts, and legumes, the Mediterranean diet incorporates many antioxidant-rich foods that combat telomere attrition.[13,23,24]

As a different approach, meditation and mindfulness have been positively associated with telomere length. Meditation is a method of stress management and prevention, which helps in depleting the number of free radicals created by oxidative stress. Additionally, preliminary studies associate mindfulness meditation with increased activity of telomerase, an enzyme that adds repetitive sequences back onto the 3' end of telomeres.[25,26,27,28] The combined effects of an improved diet, increased exercise, and mandatory meditation sessions have already proven to correlate with significantly longer telomeres,

of Oxidative Stress in the Metabolic Syndrome. *Rev Cardiovasc Med*. 2011;12(1):21-29.

20 Valdes M, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *The Lancet*. 2005;366(9486):662-664.

21 Paul L. Diet, nutrition and telomere length. *J Nutr Biochem*. 2011;22(10):895-901.

22 Pusceddu I, Herrmann W, Kleber MW, et al. Telomere length, vitamin B12 and mortality in persons undergoing coronary angiography: the Ludwigshafen risk and cardiovascular health study. *Aging*. 2019;11(17):7083-7097.

23 Freitas-Simoes TM, Ros E, and Sala-Vila A. Nutrients, foods, dietary patterns and telomere length: Update of epidemiological studies and randomized trials. *Metabolism*. 2016;65(4):406-415.

24 Davinelli S, Trichopoulou A, Corbi G, et al. The potential nutrigeroprotective role of Mediterranean diet and its functional components on telomere length dynamics. *Ageing Res Rev*. 2019;49:1-10.

25 Scutte NS, Malouff JM. A meta-analytic review of the effects of mindfulness meditation on telomerase activity. *Psychoneuroendocrinology*. 2014;42(1):45-48.

26 Jacobs TL, Epel ES, Lin J, et al. Intensive meditation training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology*. 2011;36(5):664-681.

27 Ho RTH, Chan JSM, Wang C, et al. A Randomized Controlled Trial of Qigong Exercise on Fatigue Symptoms, Functioning, and Telomerase Activity in Persons with Chronic Fatigue or Chronic Fatigue Syndrome. *Ann Behav Med*. 2012;44(2):160-170.

28 Hoge EA, Chen MM, Orr E, et al. Loving-Kindness Meditation practice associated with longer telomeres in women. *Brain Behav Immun*. 2013;32:159-163.

lowered lipoprotein (LDL) cholesterol, and an overall reduction of stress.[29]

CRISPR genetic engineering is a promising prospect in delaying telomere attrition to slow, or even reverse, aging. Thus far, research in cell cultures and animals have found that genetic engineering does have the ability to increase the lifespan of an organism.[30,31,32,33] For example, Cynthia Kenyon and her team experimented with *Caenorhabditis elegans* (a species of roundworm) and found that mutations that damaged the *daf-2* gene resulted in a lifespan twice as long as normal.[34] Further research revealed the reason–when the *daf-2* gene was damaged, the cell engaged a gene regulator protein called FOXO that helped to strengthen the immune system and activate genes that code for DNA repair.[34] The *daf-2* gene in roundworms is similar to the insulin or IGF-1 receptors in mammals, which means there is potential for translation to humans.[34]

In Hawai'i, a recent research breakthrough may serve as an interesting reference point for future possible human trials. Cardax, a life science company, developed an astaxanthin compound (CDX-085), which is considered one of the strongest natural antioxidants. Dr. Richard Allsopp, with the University of Hawai'i John A. Burns School of Medicine (JABSOM), found that astaxanthin activates the FOXO3 gene, which is associated with longevity in mammals.[34,35] Allsopp manipulated mice's diets into three groups: no astaxanthin (control group), a low dose of CDX-085, and a high dose of CDX-085. The mice that were given a high dose of CDX-085 had significantly higher (90% more) activity in the FOXO3 gene.[35] Since all mammals, including humans, possess the FOXO3 gene, there is possibility for the successful translation of this research to humans in clinical trials.

In yet another experiment, Chen and colleagues found that transcriptional enhancement of the Klotho gene in both mice and humans leads to increased expression of antioxidants and reduces cognitive deficits found in Alzheimer patients.[36] The Klotho gene is associated with neuronal viability, defense against free radicals produced by oxidative stress, and remyelination of damaged axons.[37] When the Klotho gene is upregulated, the dorsolateral prefrontal cortex (the part of the brain responsible for memory and attention) is larger and functions better.[37] Furthermore, the study found genetic engineering via CRISPR technology could be used to increase expression of the Klotho gene with single-guide RNAs (sgRNAs) to target the promoter region and enhance transcription of the Klotho gene.[37] The discovery of the function of the Klotho gene is promising to advance gene therapy for age-associated neurodegenerative diseases.

The success of these experiments has scientists hopeful for the future prospect of genome editing to be used for anti-aging. The next step includes human clinical trials to determine whether the research is medically applicable.

Bioethical Concerns of Anti-Aging via Genetic Engineering

Because of its immense power, the applications of CRISPR-Cas9 technology are controversial and heavily debated. GGE is among the most disputed as it would irreversibly affect future generations. While there is potential for genetic engineering to eventually be used to reverse or slow aging, there are many bioethical concerns when it comes to the various applications of CRISPR technology.

One debate of genetic engineering is treatment vs. enhancement. The general public supports therapeutic gene therapy to treat genetic disease and disapproves of genetic engineering for enhancement purposes.[7] Where does anti-aging lie on this spectrum? It could be argued that anti-aging is considered a treatment for the effects of aging such as decreased cognitive capacity and muscle strength. However, anti-aging could also be considered an enhancement, as it would extend (the lifespan) beyond human capabilities as they stand today.

Another aspect to consider when thinking about the genetic engineering debate is the environment. Today, our population is the biggest it has ever been in our Earth's history. According to the United Nations, the world population is about

29 Ornish D, Daubenmier J, Weidner G, et al. Increased telomerase activity and comprehensive lifestyle changes: a pilot study. *Lancet Oncol*. 2008;9:1048-1057.

30 Bodnar AG, Ouellette M, Frolkis M, et al. Extension of Life-Span by Introduction of Telomerase into Normal Human Cells. *Science*. 1998;279(5439):349-352.

31 Bernardes de Jesus B, Vera E, Schneeberger K, et al. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol Med*. 2012;4:691-704.

32 Jaskelioff M, Muller FL, Paik J, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011;469:102-107.

33 Kenyon C. Experiments that hint of longer lives [Video]. *TED Talks*. https://www.youtube.com/watch?v=V48M5j-6zdE Published November 17, 2011. Accessed February 11, 2020.

34 University of Hawai'i Cancer Center. Astaxanthin compound found to switch on the FOX03 'Longevity Gene' in Mice. Science Daily. https://www.sciencedaily.com/releases/2017/03/170328092428.htm. Published March 28, 2017. Accessed February 28, 2020.

35 Davy PMC, Willcox DC, Shimabukuro M, et al. Minimal Shortening of Leukocyte Telomere Length Across Age Groups in a Cross-Sectional Study for Carriers of a Longevity-Associated FOX03 Allele. *J Gerontol*. 2018;73(11):1448-1452.

36 Chen C, Zeldich E, Li Y, et al. Activation of the Anti-Aging and Cognition-Enhancing Gene Klotho by CRISPR-dCas9 Transcriptional Effector Complex. *J Mol Neurosci*. 2018;64:174-184.

37 Kurtzman L. Brain Region Vulnerable to Aging is Larger in Those with Longevity Gene Variant. University of California San Francisco. https://www.ucsf.edu/news/2015/01/122761/brain-region-vulnerable-aging-larger-those-longevity-gene-variant. Published January 27, 2015. Accessed March 3, 2020 from

7.8 billion people.[38] The urbanization and globalization of our planet is already depleting many of the natural resources our Earth offers, and we must ask–what is the carrying capacity of our Earth? Estimates from experts approximate the carrying capacity at anywhere between 500 million and 10 billion.[39,40] Our growing population is accelerating climate change and causing loss of species and biodiversity.[41] Anti-aging technology would extend the lifespan of our oldest generations, further increasing the population living on Earth. Is this ethical? At the extreme, an enormous population has the potential to cause resource wars and global food and water shortages.

Economically, anti-aging genetic engineering may not have a large effect, but legalization would open the door to other applications of CRISPR-Cas9 technology used for enhancements. As it is expected to be extremely expensive (prices are estimated to be around $1M), only the wealthy upper class would be able to afford the pricey technology.[42] Theoretically, the wealthy could pay to alter themselves or create "designer babies" to be smarter, stronger, and more attractive than "normal" people. This would increase socioeconomic gaps and economic disparity for generations to come.[43]

Figure 2 World map displaying CRISPR policies by country.[44,45] The landscape of national policies regarding CRISPR is varied. Many countries have unenforceable or ambiguous regulations that do not adequately monitor the use of CRISPR-Cas9 technology, while other countries have no laws in place to manage CRISPR research yet.

The climate of the genetic engineering debate is widespread. Geographic acceptance shows that the majority of the Americas and Europe have some sort of regulation in regard to genome editing (Figure 2). Currently in the United States, there are no legal bans on genetic engineering, but federal funds may not be used to alter human embryos.[44] Additionally, research with CRISPR-Cas9 technology must be approved by the Food and Drug Administration (FDA).[46] Although many countries do have some sort of ban or regulation in place, there is a lack of enforcement of these guidelines. For example, germline genetic engineering is banned in Canada under the 2004 Assisted Human Reproduction Act with possible punishments including hefty fines and up to ten years in jail.[44] However, there is no regulatory body to prosecute the laws set forth by the Act. Similarly, in China, scientific research is often approved locally without fully understanding the experiment at hand. This broken system allowed Dr. Jiankui He to infamously create Lulu and Nana, the first "designer babies" modified with CRISPR-Cas9 technology in 2018.[47]

Geographical acceptance may be tied to religious acceptance as well. Israel has ambiguous regulations on genetic engineering (Figure 2). Judaism (the prominent religion in Israel) generally supports scientific research if it is for the benefit of mankind which suggests the Israeli support further research on gene therapy.[45] Similarly, one interpretation of Islamic law believes "in the presence of two evils, the one whose injury is greater is avoided by the commission of the lesser," meaning Muslims support gene therapy but not genetic engineering

38 United Nations. World Population Prospects 2019. Department of Economic and Social Affairs. https://population.un.org/wpp/Download/Standard/Population/. Published August 28, 2019. Accessed June 15, 2020.

39 Wolchover N. How Many People Can Earth Support? LiveScience. https://www.livescience.com/16493-people-planet-earth-support.html. Published October 11, 2011. Retrieved February 20, 2020.

40 Starkey M. What is the Carrying Capacity of Earth? Population Connection. https://www.populationconnection.org/carrying-capacity-earth/. Published April 13, 2017. Accessed February 20, 2020.

41 Götmark F, Cafaro P, and O'Sullivan J. Aging Human Populations: Good for Us, Good for the Earth. *Trends Ecol Evol*. 2018;*33*(11):851-862.

42 Wilson R, Carroll D. The Daunting Economic of Therapeutic Genome Editing. *The CRISPR Journal*. 2019;*2*(5):280-284.

43 Sherman E. Genetic Engineering Will Make Income Inequality Much Worse. Forbes.com. https://www.forbes.com/sites/eriksherman/2017/08/20/genetic-engineering-will-make-income-inequality-much-worse/#58ea77263d75. Published August 20, 2017. Accessed February 20, 2020.

44 Ledford H. Where in the world could the first CRISPR baby be born? *Nature*. 2015;526(7573:310-311.

45 Vogel KM. Crispr goes global: A snapshot of rules, policies, and attitudes. *Bulletin of the Atomic Scientists*. https://thebulletin.org/2018/06/crispr-goes-global-a-snapshot-of-rules-policies-and-attitudes/. Published June 5, 2018. Accessed February 19, 2020.

for enhancements.[48] Catholics are more conservative in their views. A statement from the Catholic Church by Pope John Paul II, said that Catholics believe CRISPR-Cas9 technology is a gift from God, but realistically stated the possible consequences. The main concern of the Catholic Church is the likely increase in socioeconomic inequality because of their commitment to the poor.[49]

Conclusion

Genetic engineering is a promising technology that has the potential to eliminate genetic diseases, create designer babies, and even reverse aging. In 2015, the first major genetic engineering anti-aging experiment on humans was publicized. Elizabeth Parrish, CEO of BioViva, bypassed FDA regulations by flying to Colombia to receive intravenous infusions of modified adeno-associated viruses (AAD) carrying telomerase.[50,51,52] Unpublished data claims that her telomeres were extended by 10%, or 0.5 kilobases, which the researchers allege corresponds to twenty years of life.[50] However, issues with the experiment such as small sample size (only one person) and the results being within the standard error demonstrate the need for further research to determine whether anti-aging genetic engineering is even possible in high-functioning mammals, and eventually humans.[51]

Though the idea of genetic engineering is extremely exciting, a temporary moratorium should be implemented on CRISPR-Cas9 genetic engineering in humans until more controlled research using other CRISPR derivatives is done on the implications of the technology. In 2017, researchers discovered the C2c2 CRISPR system. C2c2 CRISPR system uses the same CRISPR DNA sequence as CRISPR-Cas9, but utilizes a different enzyme. The C2c2 enzyme targets RNA instead of DNA and can both guide and cleave the RNA.[53] C2c2 CRISPR is less risky than CRISPR-Cas9 because it causes only temporary changes to the genome.

In 2018, researchers found an even more developed approach. CRISPR-Cas13 joins CRISPR c2c2 (now renamed as CRISPR-Cas13a) with three other subtypes (CRISPR-Cas13b, c, and d). The CRISPR-Cas13 system has a much higher specificity and efficiency than C2c2 CRISPR alone. With CRISPR-Cas13, researchers can modify the crispr RNA (crRNA) spacer sequence to target virtually any RNA, to suppress or enhance specific genes temporarily.[53] Preliminary studies show CRISPR-Cas13 suppresses genes 60-90%.[54] CRISPR-Cas13 is also already being used as an efficient diagnostic tool that identifies infectious diseases called SHERLOCK.[55] The CRISPR-Cas13 system has the potential to transform genome editing through temporary modifications to genes.

No further steps should be taken until an international agreement is made and regulatory agencies are appointed. Research via newer, temporary CRISPR derivatives should be used as guidance in decisions on the legalization and regulation of future human genome editing trials.

48 Dayan F. CRISPR Cas-9 genome editing and Islam: A religious perspective. *Bangladesh J Med Sci*. 2019;18(1):7-13.

49 Moraczewski AS. The human genome project and the Catholic Church. *J Int de Bioethique*. 1991;2(4):229-234.