Ethical Issues Regarding CRISPR-mediated Genome Editing

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Abstract

CRISPR/Cas9 has emerged as a simple, precise and most rapid genome editing technology. With a number of promising applications ranging from agriculture and environment to clinical therapeutics, it is greatly transforming the field of molecular biology. However, there are certain ethical, moral and safety concerns related to the attractive applications of this technique. The most contentious issues concerning human germline modifications are the challenges to human safety and morality such as risk of unforeseen, undesirable effects in clinical applications particularly to correct or prevent genetic diseases, matter of informed consent and the risk of exploitation for eugenics. Stringent regulations and guidelines as well as worldwide debate and awareness are required to ensure responsible and wise use of CRISPR mediated genome editing technology. There is a need for an extensive dialogue among scientists, ethicists, industrialists and policy makers on its societal implications. The opinion of different elements of the society including the general public as well as religious scholars is also critical. In countries with existing legislative framework, it might be appropriate to allow CRISPR-based research to proceed with proper justification. However, much anticipated future clinical applications must be strictly regulated with newly established regulations.

Introduction

The quest for introducing the site-specific changes in the DNA sequence began when DNA was first discovered. Progress in genome engineering technologies began in 1990s now reaching to a highly advanced, easy, economical and sophisticated method for editing genomes called CRISPR/Cas9. CRISPR (Clustered Regularly Interspaced Palindromic Repeats) technology does not arrive as a breakthrough technology for editing the genomes but other genome editing platforms like TALENS (Transcription Activator-Like Effector Nucleases) and ZFN (Zinc Finger Nucleases) were in use for some time but have lost their popularity because of their complexity, expensiveness and time consumption (Carroll and Charo, 2015; Doudna and Charpentier, 2014; Hsu *et al*., 2014; Jinek *et al*., 2012). The aforementioned techniques require protein engineering while CRISPR needs a guide RNA molecule that recognize the target sequence (Caplan *et al*., 2015). The genome engineering tools offer promising advances in medicine, healthcare, agriculture and food. CRISPR/Cas9 has brought a revolution in the biological innovations. This technology can be used to treat the genetically inherited diseases by correcting the responsible mutation, analysing cancer progression and genetic rearrangements (Doudna and Charpentier, 2014). Despite the wide array of applications, ethicist raises serious

reservations about the CRISPR ability to easily modify the human germline cells, making these changes transmittable to the progeny.

CRISPR was initially discovered in the genome of *E. coli* by Japanese researchers in 1987 and described as short direct repeats interspaced with short sequences. Later on CRISPR was discovered in many bacteria and archaea with a possible role in gene regulation or DNA repair (Guy *et al.*, 2004; Ishino *et al.*, 1987; Makarova *et al.*, 2002; Mojica *et al.*, 2000). In 2005, it was discovered that many spacer sequences that are accompanied with CRISPRs were the derivatives of plasmid and viral sequences (Bolotin *et al.*, 2005). It was proposed by various research groups that CRISPR/Cas9 has a possible role in adaptive immunity and this proposition was confirmed later on in 2007 by researchers working on *Streptococcus thermophilus*. In 2008, Brouns *et al.*, in a study published in *Science*, showed that Cas proteins interfere with the multiplication of a virus inside prokaryotes (*E. coli*), while in the same year the DNA-targeting ability of CRISPR/Cas9 was established (Brouns *et al.*, 2008; Marraffini and Sontheimer, 2008). (For a comprehensive history and mechanism which is behind the scope of this review we recommend 'The new frontier of genome engineering with CRISPR/Cas9' by Doudna *et al.*, 2014.)

Ethical issues regarding CRISPR technology

The rapidly growing technology of genome editing, CRISPR/Cas9, is transforming the field of molecular biology enabling scientists to make desired changes in DNA in a variety of organisms. Soon after it was introduced in 2012, it has been quickly adopted due to ease of its use and simplicity. It is being considered for a variety of applications from agriculture to clinical therapeutics including human germline alterations to correct genetic diseases (Carroll and Charo, 2015). Although the ethical debate on human genetic modifications is not new (as mentioned previously), however, CRISPR/Cas9-mediated genome editing has given it a new edge. Owing to unpredictable and far reaching consequences associated with attractive applications of this technology, a comprehensive dialogue is needed on its ethical and societal implications. The present review discusses the ethical concerns related to this new technology with special emphasis on its possibilities and concerns on human germline modifications. It urges the need for an extensive dialogue among scientists, ethicists, industrialists and policy makers. The opinion of different elements of the society including the general public as well as religious scholars is critical.

Recent ethical debate: from where it started

The rapidly expanding scope of applications of CRISPR/Cas9 is incredible. It has provided a number of possibilities in molecular biology research, e.g. to turn off or on genes for studying their function or to induce mutations in cells to study how and why cells become cancerous. It can be used to alter genes in plants and animals to create drought resistant crops or make police dogs become more muscular. Then comes a rather contentious application to permanently modify the human genome to eliminate the disease-causing mutations or even enhance or introduce desired characteristics in children by adding useful genes. Though the idea of genetically modified humans and designer babies has been popular for a long time in both science and fiction, CRISPR provided a reasonable tool to turn this long cherished dream into a reality (le Page, 2015). Genomic changes made in the non-reproductive cells are not heritable but if alterations are done to the germ cells (that develop into eggs, sperms or embryos), they can be passed to the offspring. The great accomplishment of CRISPR lies in the fact that it can be easily and precisely used in both reproductive and non-reproductive cells (Doudna, 2015).

Until now, altering genes in humans was limited to gene therapy in which DNA modifications were inheritable. With accuracy and efficiency of an editor that could transfer the changes to the next generation, CRISPR has renewed the debate about human germline modifications. With the advent of this technology, there was a sudden surge of genome editing experiments across the kingdom of life, but mainly restricted to organisms other than humans. Recently, there were also speculations that several scientists are secretly using this technology on human embryos. In April 2015, research involving CRISPR-based gene editing in human embryos

appeared in an online journal 'Protein and Cell'. This study, done by a group of Chinese scientists (Liang *et al.*, 2015) fanned the flames of ethical controversy on human germline alterations and triggered a serious debate on issues such as how soon the technology will be able to produce designer babies. Although the experiment was done on non-viable embryos meaning that they could not develop into a live baby, it provided a proof of concept that, with little tinkering of the CRISPR enzymes, the technology might be someday successfully used on live embryos (Krishan *et al.*, 2015; Sharma and Scott, 2015). In general, apart from apprehensions related to human genome, some environmental concerns have also been identified as per CRISPR-mediated modifications in plants such as crop improvement.

Major concerns

Off-target mutations

Off-target mutations is one of the major concerns raised about the CRISPR/Cas9 genome engineering, since they may have deleterious effects on humans and the environment. Research has indicated relatively higher number of off-target mutations in human beings than in zebrafish. Larger genomes can have many identical sites for cleavage and hence CRISPR/Cas9 can cut sequences that are not intended. Such mutations can lead to an abnormal transformation and cell death. A part from that, CRISPR/Cas9 based interventions are difficult to make in cells that are hard to infect. Precise modification through CRISPR/Cas9 will play a major role in acceptance of the technology. The risks and benefits, both should be evaluated (Cong *et al.*, 2013; Hwang *et al.*, 2013; Ma *et al.*, 2014; Rodriguez, 2016; Yang *et al.*, 2013). Furthermore, such off-target mutations can become a threat to the environmental integrity. There is a possibility of transfer of genes among organisms by the ongoing process known as gene drive. In such a pattern, the off-target mutations can also be transferred to the other organisms, hence disrupting the integrity of the environment. Some scientists have warned about the risks of accidental release in the environment of experimental organisms modified using gene drive. The predecessor gene editing technologies like TALENS and ZFN need proteins designed or tailored to bind the DNA which is to be edited. These made-to-order proteins may take years to make. In comparison, CRISPR simply requires a complementary RNA to bind the target DNA taking only a few days to make. This is why CRISPR became popular as an efficient editing technique among the scientists all over the world. It is feared that with a gene editing technology like CRISPR which can make changes in DNA effortlessly, the chances of things going wrong will also be larger. For example, the plants given characteristics like drought resistance or fast growth may become invasive weeds. The most dreaded situation is that the technology may be used to produce a bioweapon such as engineering an infectious pathogen that infect humans or crops (Sarchet and Le Page, 2015). It is very important to underline the regulatory norms for the application of CRISPR. Safety and security measures should be developed to control any organism that can induce environmental damage (Oye *et al.*, 2014).

Regulatory issues

The simplicity of CRISPR/Cas9 to induce genetic modifications makes it equally difficult to identify the respective modified organism outside the laboratory and therefore raises concerns over their regulations. If CRISPR/Cas9 continues to flourish across the world, we can expect an expanded market of genetically modified organisms which will raise questions on their regulations. In addition, the patenting issues need to be resolved. There exists a friction between the scientific communities about the patenting of genetically modified organisms for therapeutic uses. Many economic interests revolve around the CRISPR/Cas9. It is assumed that patenting of CRISPR/Cas9 techniques can give enormous powers to the relevant companies (Ledford, 2015b; Rodriguez, 2016).

Genetic enhancement

For safety reasons, genetic enhancement is banned in the germlines (except in the UK where the CRISPR/Cas9 modification of human embryos has been allowed since February, 2016), however, CRISPR/Cas9 provides an easy way to manipulate the DNA sequences in the somatic cells and hence can introduce a desired phenotypic trait. For instance, one can easily improve the genetics of a sportsman. It can be used to improve the health

but it may happen in the future that the criminal justice system mandates genome editing of genes related to violence for repeat offenders or violent dangerous criminals (Rodriguez, 2016). Genetic diseases that are caused by mutations in the DNA can theoretically be controlled by editing the germlines, and such modifications will be passed on to generations. Questions are raised because of the introduction of a novel change to the human genome pool (Kohn *et al.*, 2016). It will also raise an ethical debate owing a three W's pattern, like 'Who' is going to decide 'What' kind of modification for 'Whom'? Parental and guardian and extent of their authority, informed consents area some of the other ethical issues. Whether parents will be the only autonomous entity to decide for their child, or would that be tantamount to usurping the interest of future generations who cannot provide their consent at the time of the decision (Lander, 2015).

A part from that, we assume to have a significant increase in the genetically altered organisms through CRISPR/Cas9, which raises questions on the uninform level of regulations. What will happen if such modifications are being used for the non-health purpose. Societal problems will emerge if an individual or a group becomes genetically superior to other. Technology abuse and issues on the Dual Use Research Concerns (DURC) is also raised.

Patient safety

Patient safety is of vital importance among the arguments made for the acceptable application of this technology (Lanphier *et al.*, 2015). Considering the use of germline editing research in a clinical application where inheritance of a certain genetic disease may be prevented, it may relieve the parents' suffering and worries that stem from the risk of that genetic disorder in their child (Ishii, 2015). But even before a much anticipated human therapeutic application turns into a reality, it is important to proceed with extreme caution in order to avoid the undesirable effects. Recently, a synthetic biologist at Massachusetts Institute of Technology and Harvard, Feng Zhang, successfully altered the Cas9 enzyme to reduce the number of off-target mutations (Ledford, 2015a). If combined with other modifications, it is likely that the error rate may be further reduced to a safe range (Ledford, 2015a). With this possibility, the safety concerns of the CRISPR mediated germline editing may be somehow alleviated. Regrettably, even with fast pace of scientific research, the clinical application of this technology seems to be a distant dream.

Another safety concern is related to the clinical trials of CRISPR/Cas-based gene therapies. In case of chemically synthesized or natural substances and even somatic gene therapy using gene editing techniques, health safety of the subjects can be guaranteed by carefully controlling the administered doses. Also, these therapeutics degrade after a certain period of time. However, germline modifications are irreversible and may either require entirely new guidelines for the trials or improvement of existing principles adapted for somatic gene therapies in order to protect patients from adverse side effects (Baumann, 2016).

Overall, the risk potential of CRISPR/Cas technology varies for various applications. Some may be acceptable or anticipated to be applicable in the near future after addressing a few ethical and safety concerns. Others may not even be practical any time soon (Table 9.1 summarizes various potential applications and their estimated risk level). On the bright side, it may provide huge benefits in terms of improving health and environment, but it largely depends on the wise use of this technology (Sarchet and Le Page, 2015).

Ethical standpoint

The CRISPR-based gene editing experiment for human germline modification generated concerns on issues such as challenges to human safety and dignity and the risk of exploitation for eugenics. As a result, a group of stakeholders called for a voluntary moratorium on human genome research until a national or international consensus regarding the acceptance of this technology in society is reached (Ishii, 2015). The moratorium was called in a meeting held in Napa, California, with the primary goal of initiating a public debate on ethical and social impacts of the technology (Sheridan, 2015).

A variety of viewpoints about the technology in terms of its cost–benefit analysis came from a number of scientists that have been highlighted in this section. An important 'Not So Soon' opinion about the speculated use of this technology for human enhancement came from Janssens and Cecile (Janssens, 2016). They questioned the

Table 9.1 Possible risk level of CRISPR/Cas-based specific applications

Organism	Specific application	Risk level
Human	Cure diseases by replacing endogenous disease-causing genes, correcting disease causing mutations or inserting new genes with protective functions (somatic gene therapies) (Rodriguez, 2016)	Moderate (off-target mutations)
	Germline modification for introducing desired traits such as intelligence etc. or treating complex genetic disorders such as diabetes	Very high (unpredictable effects on future generations, informed consent, need to re-regularize the clinical trial procedures
Animals	Animal models for research to study diseases or development process by mutating or silencing genes. For example, a mouse model to study effect of mutations on cancer (Rodriguez, 2016)	Fairly low or no risk (less off-target effects such as in zebrafish)
Plants	Improving crops, introducing disease resistance, controlling harmful invasive species, reverse pesticide and herbicide resistance in insects and weeds through gene drive (Rodriguez, 2016)	Fairly high (off-target mutations, transfer of traits to unrelated organisms in environment, ecological imbalance due to loss of a population targeted through gene drive)

technical feasibility of CRISPR for enhancement of certain desirable traits. Practically it might be possible in case of a single variant or a limited number of variants, however, what happens when we are dealing with tens or hundreds of variants. Moreover, it is not just a matter of fine-tuning a particular gene, because complex traits such as intelligence happen due to a combination of several genes as well as their interaction with the environment, thus making the editing dangerously unpredictable in the future. This is why initial experiment on human embryos attempted to correct gene mutations in disease such as β-thalassaemia, which is a recessive genetic disorder caused by a single mutation, and not the complex diseases such as diabetes or cancer. Gene editing technology holds great promise for these type of other diseases and they estimated that tinkering with more complex conditions intended for improvement of polygenic traits or diseases will not be feasible.

According to Li and Qian (2015), CRISPR technology is not yet mature enough to fine tune human inheritance. Firstly, because of the chances of off-target mutations in the genome; secondly, it is difficult to envisage the consequences of gene editing in the next generation because all the functions of the gene may not be fully understood. However, it is said that the issues of lower efficiency may be linked to the use of non-viable embryos. And also with a little optimization of the procedure, these technical issues may be addressed (Ishii, 2015).

In all fairness, several important stakeholders recommend not to prohibit the *in vitro* germline research just on the alleged reason that the technology may be used for unethical human experimentation. Sharma and Scott (2015) support the appropriate and justified use of germline editing technology in human embryos. According to their view, as per ethical guidelines, this type of research can only be carried out on embryos before day 14 of culture. Thus, if used in accordance with the ethical procedures, there are no serious ethical concerns with using genome editing technology.

A viewpoint from Lander (2015) on the publication of genome editing techniques applied to human abnormal zygotes, strongly favoured a ban on the technology with the only exception of severe genetic diseases that have no other alternatives. He further commented that until we become scientifically more knowledgeable about the consequences of this technique, it should not be pursued on the human germline. He also pointed out the moral concerns associated with human genome editing, questioning how we would see the children as manufactured products or what would be the consequences of creating a genetic class difference by 'best genomes for the most privileged'.

Global impact of CRISPR/Cas9

CRISPR is no more a word of the scientific community. It has crossed the barriers of the laboratory into the global media and industry with largest

impact on the pharmaceutical and biotech business segments. A lot of investment is pouring into multiple applications with translational medicine and specifically the gene therapy catching the most attention. A number of collaborative agreements for commercial exploitation of CRISPR have been reached between various industries, e.g. Novartis (Cambridge, MA, USA) collaborated with Intellia Therapeutics, and also signed a pact with Caribou Biosciences based in Berkeley, CA, USA (founded by Doudna) (Ricks, 2015; Sontheimer and Barrangou, 2015). With such hype and frenzy, it might not be easy to confine this technology within the boundaries of non-medical use. Thus, stringent regulations and guidelines as well as worldwide debate and awareness will pave the way for this scientific breakthrough of the new genetics era.

Way to go forward … caution!

A solid precedent for allowing research on human germline editing has been set by the recent approval given to a team of scientists in London, UK, by the country's Human Fertilisation and Embryology Authority (1 February, 2016). The team, led by Kathy Niakan (Francis Crick Institute), plans to alter genes in healthy human embryos using CRISPR/Cas9 technology. In order to proceed the research in an ethically sound way, they will perform the experiment after fertilization and stop at the seventh day after which embryos will be destroyed (Callaway, 2016). Now that an initiative for such type of research has been taken, it calls for a world open dialogue, drafting of reports, establishment of sound policies and guidelines involving the scientific and regulatory authorities as well as the society. An important effort in this regard was a three day International Summit on human genome editing. The national academies of the USA, UK and China organized this international meeting in December 2015, which was hosted in Washington DC. The summit declared that the technology should not be used on the human germline for the purpose of establishing a pregnancy. The statement issued at the end of the summit also cautioned that ethical issues must be addressed before attempting any germline modification for clinical use. It is expected that representatives from the three countries will come up with a consensus report based on the issues discussed in the meeting by the end of 2016 (Reardon, 2015).

The risk of a powerful technology such as that of CRISPR/Cas9 lies in its users rather than the technology itself. In the past, scientists as well as the regulatory bodies have often successfully assessed both the real and seemingly apparent concerns of various scientific developments and established regulatory guidelines to keep those technologies in check. It is widely anticipated that CRISPR will be implemented only after all its risks and benefits have been carefully considered (Sontheimer and Barrangou, 2015). It is suggested that in countries where extensive legislation for human germline research exists, it might be appropriate to allow the Cas9-mediated gene editing research to proceed with proper justification. Its use may be strictly prohibited for any unethical non-medical use. However, they must discuss the social and ethical implications of this technology with the public to gain their confidence. As for the future applications of this research such as to correct genetic diseases in unborn babies, it must be strictly regulated by newly established legislation (Ishii, 2015).

In order to ensure the responsible use of this technology, germline modifications for therapeutic applications in humans should be strongly discouraged until the discussion on environmental, social and ethical concerns is going on among the stakeholders. With regard to its applications in gene therapy, a transparent research must be encouraged to assess its safety and efficacy (Baltimore *et al.*, 2015). In the long run, safety and ethical concerns associated with CRISPR technology should not halt the scientific development aimed at curing human ailments (Lanphier *et al.*, 2015).

Conclusion

Despite the fact that CRISPR genome engineering faces numerous regulatory and societal hurdles, the potential applications cannot be ignored (Fig. 9.1). CRISPR can significantly advance our understanding of diseases at genetic level while it also provides new horizons for treatment and other research applications. There is a great need to uptake CRISPR technology on various platforms that includes participation from experts from ethical, social, religious, legislative and technological

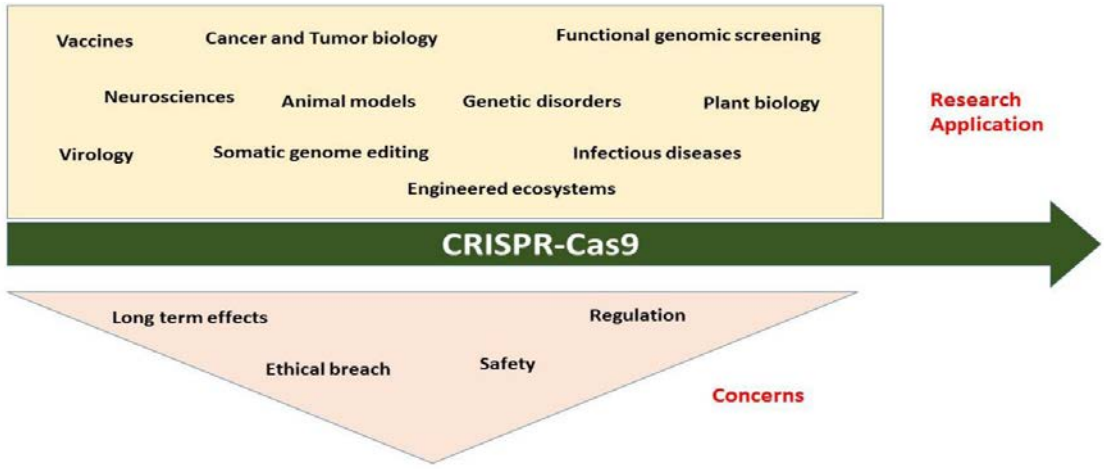

Figure 9.1 Potential applications and concerns about CRISPR.

grounds to develop a long lasting policy regarding benefits and concerns about CRISPR technology. CRISPR/Cas9 initiates many ethical and social issues not only from human perspectives but also for the environment. Risk assessments for ecological and environmental concerns should also be performed.

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