Gene Editing Tool – CRISPR

THE USE OF CRISPR ON THE TREATMENT OF COELIAC DISEASE

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Abstract

Literature Review

Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 or simply, CRISPR-Cas9, is an effective gene editing tool allowing researchers to manipulate DNA sequence(s) for desired gene(s) function (L. Selokar, 2018). The key underlying ethical dilemma is one that applies to any application of human genetic modification: it may inadvertently affect the genome forever (Newson & Wrigley, 2016). Attempts to develop wheat with hypoimmunogenic gluten were obstructed by strict regulation of unintended GM risk at the expense of reducing the existing immunogenicity risks of patients (Jouanin A. *et al*, 2018).

Methodology

The method for collecting data on the use of CRISPR-Cas9 for Coeliac Disease will be undertaken through an extensive literature review of peer reviewed and current studies, an analysis of secondary research through databases and a thematic analysis of a survey of professors/PhD students opinions in regard to the topic.

Results

A collection of data through a qualitative and quantitative survey resulted in ten responses from professors with knowledge within the field of the CRISPR-Cas9 technology. The thematic analysis analyses the results from the survey and the extensive review of peer-reviewed journals and articles. Themes were organised into a global theme of ‘CRISPR’ with sub themes ‘Ethics and Legal Issues’, ‘Use on Humans and Plants’ and ‘Improvements’.

Conclusion

This study on the use of CRISPR as treatment for Coeliac Disease had an objective of outlining the state of CRISPR-Cas9 as a possible treatment for Coeliac Disease, taking into account the ethical and legal implications of the technology.

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Introduction

Coeliac disease (CD) is a chronic autoimmune disorder characterised by the inability of the digestive system to digest gluten, found within wheat, barley, oats and rye. The immune system reacts to the gluten, attacking and damaging the intestinal lining, leading to severe symptoms such as diarrhea, abdominal pain, and malnutrition. The current treatment for CD is the elimination of gluten from the diet, which can be difficult and costly to maintain and often leads to non-compliance. The recent modern revolutionary gene editing technology of CRISPR-Cas9 has the potential to provide treatment for CD by modifying the genes responsible for producing gluten. This paper reviews the current literature on CRISPR-Cas9, its possible application to CD, its potential to provide a treatment and the ethical issues that may restrict its use. The methodology used in this study is a comprehensive literature review, analysing studies and reports on the use of CRISPR-Cas9 in the treatment of CD, and a survey of experts in the field.

Literature Review

What is CRISPR-Cas9?

Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 or simply, CRISPR-Cas9, is an effective gene editing tool allowing researchers to manipulate DNA sequence(s) for desired gene(s) function (L. Selokar, 2018). CRISPR-Cas9 was developed from a naturally occurring genome editing mechanism that bacteria use as an immunological response and discovered in 2012 by American scientist Jennifer Doudna and French scientist Emmanuelle Charpentier (Fridovich-Keil, 2023). The process involves two main steps. To begin, components of a custom-designed nuclease (known as an endonuclease) are injected into a recipient cell, where they self-assemble, and the endonuclease selects a DNA sequence and cuts one or both of its strands (Newson & Wrigley, 2016). The recipient's cell's internal DNA-repair system then repairs the cut and introduces the desired alteration as shown in *Figure 1* (Newson & Wrigley, 2016).

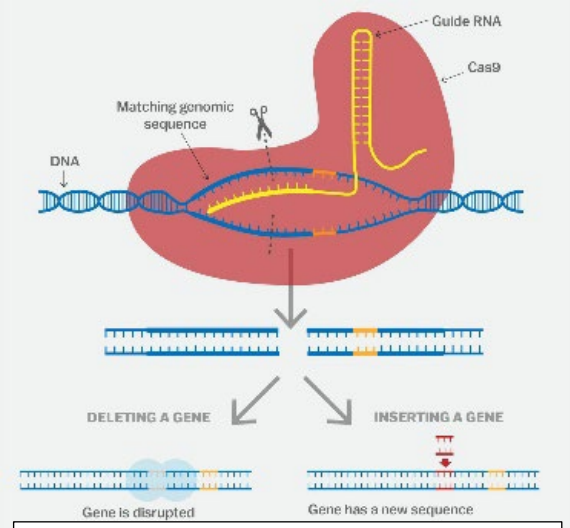

Figure 1 – Steps of CRISPR (*Zarracina*, 2018)

Ethics of CRISPR-Cas9 and Current Use

The idea of genetically engineering the human race has plagued the modern mind for more than a century, bringing both worries and hopes. The key underlying ethical dilemma is one that applies to any application of human genetic modification: it may inadvertently affect the genome forever (Newson & Wrigley, 2016).

CRISPR-Cas9 has rapidly become ubiquitous in molecular biology, with applications beyond gene therapy (Foht, 2016). Advances in CRISPR-Cas9 genome editing have enabled efficient targeted modification in most crops, thus promising to accelerate crop improvement through its ability to refine/eliminate and incorporate/add-in genes to promote effective and efficient growth, reduce costs and/or implement pest control (Chen *et al*, 2019).

Attempts to develop wheat with hypoimmunogenic gluten were obstructed by strict regulation of unintended GM risk at the expense of reducing the existing immunogenicity risks of patients (Jouanin A. *et al*, 2018). Additionally, due to the large number of gluten genes and the complexity of the wheat genome, wheat that is coeliac-safe but retains baking quality is unable to be produced by conventional breeding alone (Jouanin A. *et al*, 2020). CRISPR-Cas9 edited wheat varieties could be helpful in the management of CD by reducing the risk of unintentional consumption and reducing the limited dietary range (Verma *et al*, 2022). However, CRISPR-Cas9 has been used for use in production of wheat ie. the wheat crop's two endogenous genes, *TaWaxy* and *TaMTL*, were edited to become more efficient (80.5%) in production (Liu H. *et al*, 2020). The table below (*Figure 2*) outlines examples of plants and crops that have been successfully manipulated through CRISPR-Cas9.

Plant	**Gene(s) targeted**	**Traits**	**Method**	**References**
Apple	MdDIPM4	disease resistance	gene inactivation	Pompili *et al*. 2020
Maize	*ZmPHYC1* *ZmPHYC2*	flowering time/plant height	gene knockout & overexpression	Li *et al*. 2020
Muskmelon	*CmPDS*	albinism (CRISPR trial)	gene knockout	Hooghvorst *et al*. 2019
Oil palm	*EgIFR* *EgMT*	disease resistance	base editing	Budiani *et al*. 2018
Oilseed rape	*BnALS1*	herbicide resistance	base editing	Wu *et al*. 2020
Rice	*Os8N3* *OsProDH* *OsGS3* *OsNAC45*	disease resistance thermotolerance grain length salt tolerance	gene knockout gene knockout & overexpression site directed mutagenesis gene knockout & overexpression	Kim *et al*. 2019 Guo *et al*. 2020 Usman *et al*. 2021 X. Zhang *et al*. 2020
Soybean	*GmPRR37* *GmFT2a/5a*	flowering time & regional adaptability	site directed mutagenesis	Cai *et al*., 2018; Cai *et al*., 2020; Wang *et al*., 2020

Figure 2 - Examples of successful genome editing of plant species (Gan & Ling, 2022)

Coeliac Disease (CD)

Coeliac (also spelt celiac) disease is a T-cell-mediated, autoimmune, hereditary disease that affects the small intestine and is often controlled by eliminating gluten from the diet (McCabe *et al*, 2012). The gluten protein causes damage to the mucosa (lining) of the small intestine, in an affected individual. The component of gluten that causes problems for people with CD is the prolamin fraction, found within the gliadin in wheat, secalin in rye, hordein in barley and avenin in oats (Better Health, 2021). The Better Health Channel (2021) funded by the state government of Victoria concluded that if CD is left untreated, problems that can develop including: malnutrition, osteoporosis, depression, infertility, and a small increased risk of cancer. These factors are due to the inflammation of the villi, referred to as villous atrophy (Gore & Levine 2010, p. 296-298). The strict gluten-free diet allows for the small intestine to heal, reduction of symptoms and reduced risk of long-term effects.

The symptoms of CD vary considerably depending on the individual. Coeliac Australia (n.d.) list the following as symptoms of CD:

- Persistent gastrointestinal symptoms e.g. diarrhoea, constipation, nausea, vomiting, flatulence, cramping, bloating, abdominal pain, steatorrhea
- Prolonged fatigue, weakness and lethargy
- Iron deficiency anaemia and/or other vitamin and mineral deficiencies
- Failure to thrive or delayed puberty in children
- Unexplained weight loss
- Severe or recurrent mouth ulcers
- Skin rashes such as dermatitis herpetiformis

There is no correlation between symptoms and bowel damage, therefore if a coeliac person is asymptomatic (no obvious symptoms), damage to the small intestine can still occur if gluten is ingested (Coeliac Australia, n.d.). Therefore, a strict gluten-free diet should be adhered to for diagnosed individuals.

There are two genes for CD, human leukocyte antigen (HLA) DQ2 and DQ8, most people with CD have at least one of these genes (Brigham, 2021). HLA-DQ2 and HLA-DQ8 genes are located on chromosome 6p21 indicating an individual with CD possibly has HLA-DQ2 or HLA-DQ8 heterodimers, proteins composed of two polypeptide chains differing in composition, transcribed from major histocompatibility complex (MHC) class II genes of chromosome number 6 as shown in *Figure 3* (Szałowska-Woźniak, D. A. *et al*, 2014; Siddiqui, K. *et al*,

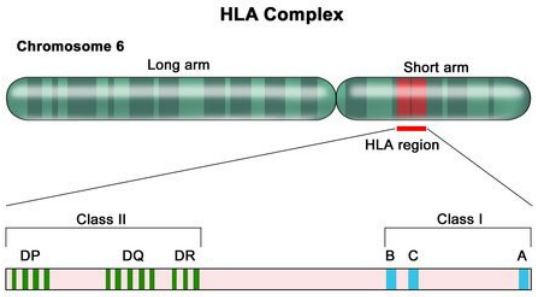

Figure 3 – HLA Complex (Winslow, 2012)

2021; Merriam-Webster, n.d.). The vast majority of CD patients express HLA-DQ2, whilst the remainder usually express HLA-DQ8 (Tonnutti & Bizzaro 2014, p. 463-470). Tonnutti & Bizzaro (2014) continue:

> *HLA-DQ molecules are heterodimers consisting of an α and a β chain. Many different HLA-DQ α and β chains exist, which can combine in different ways to form functional heterodimers. These combinations influence the response to gliadin peptides in different ways, suggesting that the level of risk of developing CD depends on the assembly of the heterodimers.*

Therefore, homozygous HLA-DQ2 individuals are at least five times more likely to develop CD than a HLA-DQ2 heterozygous individual (Tonnutti & Bizzaro 2014, p. 463-470). The role of the genes is defined by Tunnutti & Bizzaro (2014):

> *The role of the HLA-DQ2/DQ8 molecules has become clear in the light of the finding that the tTG enzyme is able to deamidate the glutamine residues of gliadin peptides and convert them to glutamic acid; this modification makes the gliadin molecule negatively charged, allowing it to bind to HLA-DQ2/DQ8 antigens, with consequent exposure of the neopeptides to recognition by the T cells.*

Scientific Research Question
Can CRISPR-Cas9 be effective in the treatment of Coeliac Disease?

Scientific Hypothesis
The technology CRISPR-Cas9 will theoretically be able to treat Coeliac Disease. The research and data will indicate that CRISPR-Cas9 can effectively treat a human with Coeliac Disease through the editing of the genes relating to the genes.

Methodology
The method for collecting data on the use of CRISPR-Cas9 for Coeliac Disease will be undertaken through an extensive literature review of peer reviewed and current studies, an analysis of secondary research through databases and a thematic analysis of a survey of professors/PhD students opinions in regard to the topic.

Peer-reviewed academic journals and articles were assessed and limited to within the range of the past ten years (2012) due to the technologies recent discovery. Each peer-reviewed academic journal and article are from knowledgeable and credited sources.

The selection criteria for participation in the survey was having sufficient knowledge and experience within the field of the CRISPR technology. The professors/PhD students that participated in the survey each were required to meet the criteria. A total of 10 professors participated (although all participated anonymously). The number of professors/PhD Students were limited due to the recent discovery of the technology and lack of knowledge of Coeliac-related diseases in relation to CRISPR-Cas9. All that participated were of knowledge that their responses were to be used for research and data collection purposes. The questions (see Appendix A) were non-biased and allowed each participant to answer freely with relevant

information to the topic. The survey was distributed online to participants via email or organisations and each participant answered anonymously.

The answers of the survey were grouped into relevant categories which were explored within each of the responses. These were created into a global theme, predominant themes and minor themes.

Results

A collection of data through a qualitative and quantitative survey resulted in ten responses from professors with knowledge within the field of the CRISPR-Cas9 technology (see Appendix A). The thematic analysis analyses the results from the survey and the extensive review of peer-reviewed journals and articles. Themes were organised into a global theme of 'CRISPR' shown in *Figure 4*.

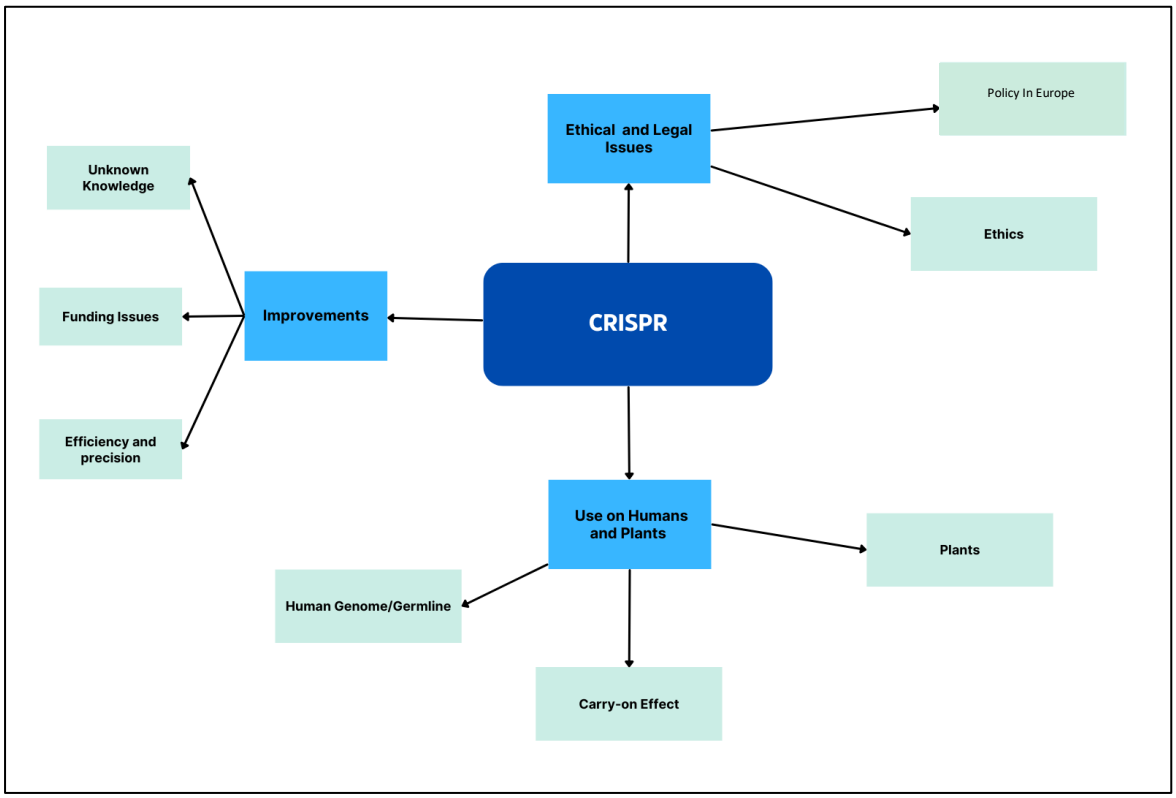

Figure 4 – Thematic network of themes

The use of CRISPR-Cas9 on humans and plants can be broken into three sub themes: plants, carry-on effect and human genome/germline.

Plants

Within the survey, the use of CRISPR-Cas9 on plants was mentioned by three participants (see Appendix B) explaining that, 'For plants, a major regulatory framework change would enable commun [sic] use of the CRISPR technology' and 'One idea would be to use genetically modified wheat that has low or modified gluten that would be safe for patients suffering celiac disease.' However, '… eradicating the disease trough [sic] the modification of the HLA locus, that sounds more difficult to me.' This aligns with the review of literature

stating that ‘develop(ing) wheat with hypoimmunogenic gluten were obstructed by strict regulation’.

Human Genome/Germline

The sub-theme ‘human genome/germline’ attempts to coincide with the applications of CRISPR-Cas9 to humans and its possible implications. Most of the professors mentioned (see Appendix B) the difficulties of using CRISPR-Cas9 on humans due to there being “Too many genes involved in CD, making this approach difficult to use in most people” and “To totally eradicate the "Coeliac Disease gene" in human, one would need to edit human germlines or embryos”. Furthermore, “A person carrying HLA DQ2 or DQ8 does not necessarily mean that that person will develop the disease” meaning that “There is no need to eliminate the gene entirely - DQ2 has isoforms. Isoforms are different combinations of introns combining from a single gene. eg. if a gene X has 5 introns (1,2,3,4,5) the introns could combine as 1,2,3,4,5 or 1,2,3 or 3,4,5 or 1,3,5 etc. They are all gene X, just with various isoforms. Isoform 5 (DQ2.5) is mostly responsible for CD, so using CRISPR to affect DQ2.5 rather than all of DQ2 would be the way to go if you were going to do it.” However, “While most effort is currently focused on somatic cell editing there is potential to modify the germ line and eliminate disease causing genomic variants.” The modification of human genomes is minorly reported due legal implications (see “Theme: Legal and Ethical Issues”) resulting in a lack of research available to obtain.

Carry-on Effects

The use of CRISPR-Cas9 on human genomes and plants both can engage with the risk of carry-on effects due to the lack of understanding of both the human and plant genomes. Six participants acknowledged this with some stating, “Altering DQ2-DQ8 genes could potentially cause other issues within the immune [sic] system response which are not yet foreseen”, “(HLA) DQ2 and DQ8 play an important role in the immune response to many pathogens. Eradicating them could lead to susceptibility to many diseases” and “Eradication of 'disease genes' may improve symptoms, but will also likely induce unintended consequences.” The ‘eradication’ of the genes causing CD in humans may have off target affects upon other genes within the genome possibly altering the normal function of the human body. Reducing or eliminating off-target effects is “advantageous” and may “give rise to neoplastic transformation”. Additionally, “You will have to access bone marrow (painful), effect the change with crispr, eradicate remaining bone marrow (radiation) and provide a bone marrow transplant back in of the original harvest that was modified with crispr (dangerous if it does not take).” The risk of “inadvertently affect(ing) the genome forever” isn’t necessarily ethical on humans or plants. However, the applications to plants has been undertaken through rigorous testing and attempts to reach the modern era of agriculture.

Theme: Ethical and Legal Issues

The ethical and legal issues is broken into two sub themes: ethics and legality.

Ethics

The ethical implications associated with the use of CRISPR-Cas9 on humans (or more broadly mammalians) reduces the capabilities for CRISPR to be used in the treatment of

diseases such as CD. The implications were presented as a “major factor holding back the use of CRISPR in mammalians” as “Changing the genome before a baby is born … is considered unethical because the baby does not consent” and “It is generally held as unethical unless we can be absolutely sure it is safe”. For CD, “one would need to edit human germlines or embryos which causes ethical issues” for the eradication of its genes.

Policy in Europe

The ethics definition was strongly entwined to that of legality, with quotes including “For plants, regulatory hurdles are the main factor holding back the CRISPR technology”, “Policy in some parts of the world, such as Europe forbid the use of genetically modified foods” and “With appropriate oversight and government legislation we will potentially be able to modify embryos that would otherwise develop a known genetic disease”. The “legal restrictions on modifying people” and regulatory hurdles within Europe eliminating the use of genetically modifying plants via the technology has severely reduced the ability for CRISPR-Cas9 to be applied within the modern world.

Theme: Improvements

Improvements of CRISPR is comprised of 3 sub themes: Unknown Knowledge, Funding Issues and Efficiency and Precision

Unknown Knowledge

Unknown knowledge was mentioned twice throughout the survey but was extensive within the two responses upon the topic. The lack of knowledge of CRISPR-Cas9 can be related to the lack of applications to humans and plants due to ethical and moral implications (see Theme: Ethical and Legal Issues). “Knowledge of systems biology, that is to say that we don't yet know the complete interactions of molecules, metabolites, toxins, infectious agents etc, therefore we cannot predict outcomes when modifying genes”. The use of CRISPR-Cas9 on CD to eradicate its genes may require one to “modify the recognition pocket, thus the panel of peptides they recognise, but that requires more research”.

Funding Issues

To apply CRISPR-Cas9 the funding required is substantial therefore reducing the possible applications/research of the technology. Although the “Costs will decrease as the technology improves”, currently “Gene therapies are very expensive” which doesn’t allow the technology to advance and becoming more accessible to research, as one participant stated, “Investment in basic research is a major issue. We need more money to study all of these opportunities”. The further advances of CRISPR-Cas9 may allow the technology to be more sophisticated and extensive in its treatments but “the prohibitive cost of these potential treatments” will reduce the applications. “We also need a way to make CRISPR work through oral ingestion … it will happen its just slow”.

Efficiency and Precision

Efficiency and precision are some of the most important factors in relation to the use of CRISPR-Cas9. The importance of being efficient and precise can reduce the cost (see Funding Issues) and the risk of unintentionally causing alternate effects. It is also important to be efficient as it must edit “enough cells to make a difference for the disorder”. “We need

to find ways to optimise both the efficiency and the specificity of CRISPR therapeutic systems". Future advancements are likely to make the technology "become more specific and precise".

Discussion

This is the first study to provide a unique insight into the use of CRISPR-Cas9 on the treatment of CD. The study was from a qualitative perspective and was broken down into three key themes: (1) use on humans and plants, (2) improvement and (3) ethics and legal issues. Treating CD for individuals suffering will offer freedom of diet, reduced risk of problems caused by untreated CD, reduced cost of diet etc. However, the treatment of CD through CRISPR-Cas9 is complicated and is restricted due to ethical and legal issues. Therefore, this research has important future applicable knowledge.

Main Findings

The modification of the wheat plant seems more feasible in comparison to the modification of the entire human genome as stated in the survey, "Why change the human body when you can change the plant?" (Anonymous, personal communication, June 21, 2023) For this to occur 'a major regulatory framework change would enable' the technology to be used 'to develop wheat with hypoimmunogenic gluten' this would create a process that is potentially more cost effective and create a vast variety of dietary options for Coeliac patients. However, the lack of benefit towards a farmer for the crop to replace current efficient wheat plants would minimise the use of the wheat with hypoimmunogenic gluten thus increasing the cost of products that implement the crop due to the lack of supply over demand. Therefore, the plant must provide either a benefit to the farmer (e.g. increase in efficiency and production yield) or non-Coeliac consumers (e.g. health benefits or reduced cost).

The ethical implications and legal restrictions of CRISPR-Cas9 have become one of many technical challenges related to its development. Various governing bodies constrain its use due to the lack of knowledge of the risks it may impose including the regulatory restrictions within Europe on the use of CRISPR-Cas9 on plants. However, the knowledge of these risks may only become known through the use of the technology which is restricted through the 'Principle of Bioethics'. The 'Principle of Bioethics' stated by Garvan Institute of Medical Research (2019) outlines the four bioethical principles as (1) respect for persons, (2) maximise good, (3) minimise harm and (4) justice. These principles reduce the ability for CRISPR to be moral and ethical thus imposing legal restrictions upon the technology further impacting its possible future applications.

Strengths and Limitations

The lack of responses in the survey including the use of CRISPR-Cas9 to edit the genome of the wheat plant/crop identifies the deficiency of knowledge of CD and CRISPR-Cas9's applications to plants of the professors surveyed. However, the direct response from knowledgeable sources increases the reliability of the study as these direct responses were selected from criteria that included an extensive knowledge within the CRISPR field.

The study focuses on the use for the technology on humans changing the genome to 'eradicate' the gene thus eradicating CD. However, to truly change the genome of a human

the germline or embryo would need to be accessed and altered which causes a multitude of ethical issues. Hence, using CRISPR to access the genome of wheat plants to become hypoimmunogenic to gluten through removing the gene that codes for the gliadin protein would be more effective.

This study required a deep understanding into both the CRISPR-Cas9 technology and Coeliac Disease itself which was developed through contact with professors and PhD students. Notable contacts included Emmanuelle Charpentier, Aurélie Jouanin, Chao-ting Wu, Anders Sjöstedt, Aydar Mindiarovich Ziganshin, Jan G Shaart and many more. However, CRISPR's use on Coeliac Disease was only within the knowledge of a limited number of the professors and PhD students.

Conclusion

This study on the use of CRISPR as treatment for Coeliac Disease had an objective of outlining the state of CRISPR-Cas9 as a possible treatment for Coeliac Disease, taking into account the ethical and legal implications of the technology. The findings of this study provide key insights into the relationship between CRISPR-Cas9 and its use on CD which can be useful in further research/applications of this topic. Through using a thematic analysis approach to consider CRISPR's use on CD, this study has made a new contribution by suggesting that the application of CRISPR in wheat plants to create wheat with hypoimmunogenic gluten would be more cost-reductive and efficient in treating CD. Working with the understanding that the technology has significant ethical implications due to the lack of knowledge of the effects caused by altering the human genome and considering the complicated nature of CD, the study was effective in creating a guideline for future research and/or applications.

Abbreviations

CD: Coeliac Disease

CRISPR: CRISPR-Cas9

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I would like to acknowledge the traditional custodians of the land of which this study was partaken upon, and I would like to pay my respects to Elders past, present and emerging.

I would also like to express my deepest appreciation to both Ms Aitken, for proof reading my research paper, and Mrs Mantellato, for her expertise in Biology and help in completing my study.

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Appendices

Appendix A

Survey Questions

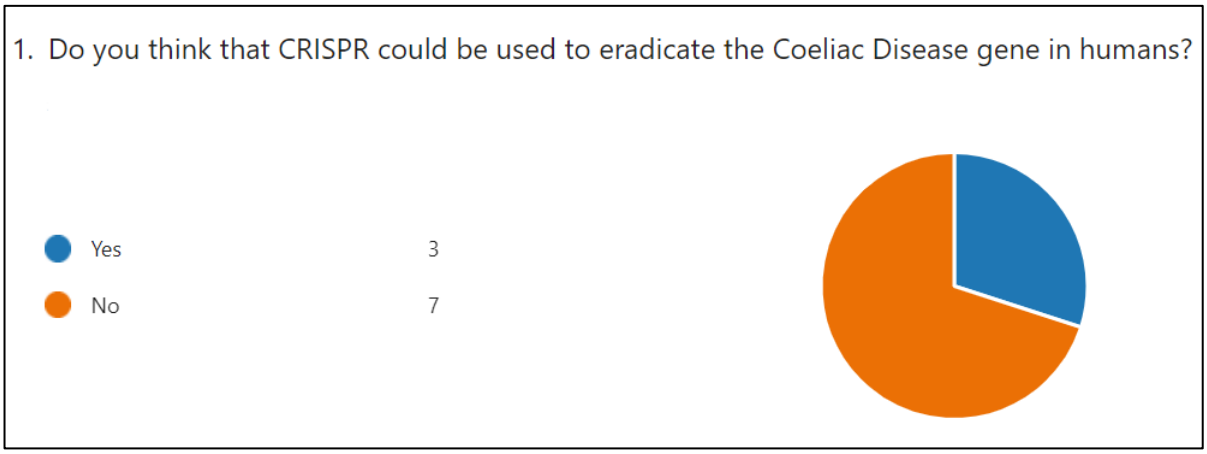

Figure A1 – First Question of Survey "Do you think that CRISPR could be used to eradicate the Coeliac Disease gene in humans?"

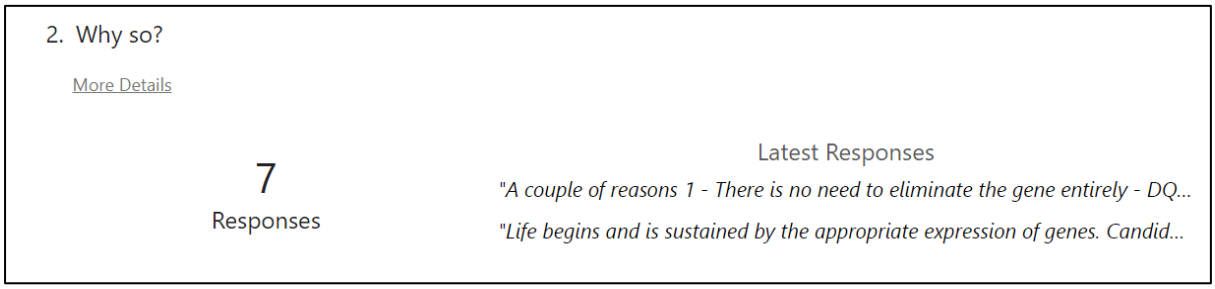

Figure A2 – Second Question of Survey Linked to answering 'No' the first question "Why so?"

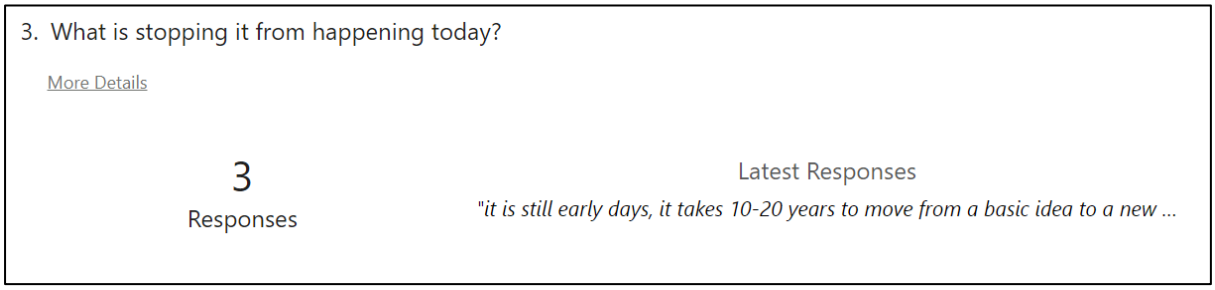

Figure A3 – Third Question of Survey Linked to answering 'Yes' the first question "What is stopping it from happening today?"

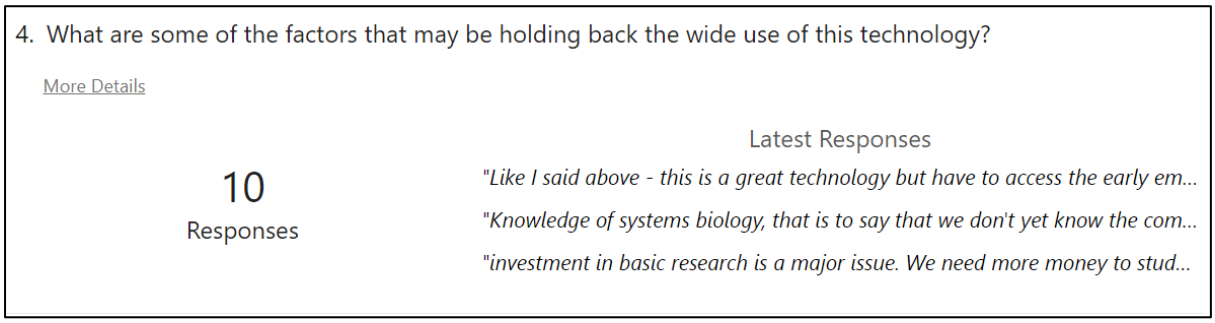

Figure A4 – Fourth Question of Survey "What are some of the factors that may be holding back the wide use of this technology?"

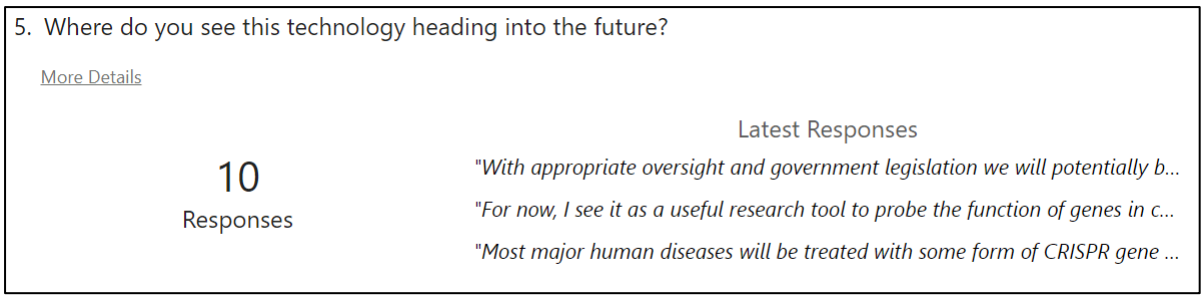

Figure A5 – Fifth Question of Survey "Where do you see this technology heading into the future?"

Appendix B

Quotes from survey

Human Genome/Germline – "To totally eradicate the "Coeliac Disease gene" in human, one would need to edit human germlines or embryos which causes ethical issues."

"A person carrying HLA DQ2 or DQ8 does not necessarily mean that that person will develop the disease."

"Too many genes involved in Coeliac disease, making this approach difficult to use in most people."

"While most effort is currently focused on somatic cell editing there is potential to modify the germ line and eliminate disease causing genomic variants."

"There is no need to eliminate the gene entirely - DQ2 has isoforms. Isoforms are different combinations of introns combining from a single gene. eg. if a gene X has 5 introns (1,2,3,4,5) the introns could combine as 1,2,3,4,5 or 1,2,3 or 3,4,5 or 1,3,5 etc. They are all gene X, just with various isoforms. Isoform 5 (DQ2.5) is mostly responsible for CD, so using

CRISPR to affect DQ2.5 rather than all of DQ2 would be the way to go if you were going to do it."

Ethics – "Ethical issue is a major factor holding back the use of CRISPR in mammalians."

"Changing the genome before a baby is born - but that is considered unethical because the baby does not consent"

"To totally eradicate the "Coeliac Disease gene" in human, one would need to edit human germlines or embryos which causes ethical issues."

"It is generally held as unethical unless we can be absolutely sure it is safe"

Carry-on Effect – "Altering DQ2-DQ8 genes could potentially cause other issues within the immun(e) system response which are not yet foreseen"

"You would not want to completely delete the HLA gene in an individual as that would perturb their immune system and would lead to problems much more challenging than Coeliac Disease!"

"Off-target effects (editing parts of the DNA, or cell types, by accident) also have to be mitigated and therefore having a more restricted target cell/tissue is advantageous."

"Off target effects that might give rise to neoplastic transformation"

"Eradication of 'disease genes' may improve symptoms, but will also likely induce unintended consequences."

“You will have to access bone marrow (painful), effect the change with crispr, eradicate remaining bone marrow (radiation) and provide a bone marrow transplant back in of the original harvest that was modified with crispr (dangerous if it does not take).”

“(HLA) DQ2 and DQ8 play an important role in the immune response to many pathogens. Eradicating them could lead to susceptibility to many diseases.”

Plants - “For plants, a major regulatory framework change would enable commun [sic] use of the CRISPR technology to improve or solve health issues (allergies, nutrient content, etc.) and agronomic related issues (disease and drought resistant plants, etc.).”

“One idea would be to use genetically modified wheat that has low or modified gluten that would be safe for patients suffering celiac disease … About eradicating the disease trough [sic] the modification of the HLA locus, that sounds more difficult to me.”

Unknown knowledge - “Knowledge of systems biology, that is to say that we don't yet know the complete interactions of molecules, metabolites, toxins, infectious agents etc, therefore we cannot predict outcomes when modifying genes.”

“Rather than eradicating one could try to modify the recognition pocket, thus the panel of peptides they recognise, but that requires more research.”

Legality - "For plants, regulatory hurdles are the main factor holding back the CRISPR technology."

"Technical challenges related to efficiency and efficacy, and societal distrust of the technology, and legal restrictions on modifying people."

"Policy in some parts of the world, such as Europe forbid the use of genetically modified foods."

"With appropriate oversight and government legislation we will potentially be able to modify embryos that would otherwise develop a known genetic disease."

Efficiency, specificity, and precision - "Technical challenges related to efficiency and efficacy, and societal distrust of the technology, and legal restrictions on modifying people."

"We need to find ways to optimise both the efficiency and the specificity of CRISPR therapeutic systems, and we need to somehow address the prohibitive cost of these potential treatments."

"It is also likely to become more specific and precise."

"Efficiency of editing enough cells to make a difference for the disorder"

Funding Issues - "We need to somehow address the prohibitive cost of these potential treatments."

"Gene therapies are very expensive."

“Costs will decrease as the technology improves.”

“We also need a way to make CRISPR work through oral ingestion if we want to target the intestine, it will happen its just slow.”

“Investment in basic research is a major issue. We need more money to study all of these opportunities.”

Other – “Other genes may be involved in establishing or maintaining the inflammation, they may be better targets.”

“Why change the human body when you can change the plant?”

“This means that maybe modifying the HLA locus in a patient might not be enough to reverse the condition, specially if memory cells that recognize gluten antigens are still present after the modification.”

“Also the disease can be managed very well with diet so the ratio of the risk/benefit might not be small enough to justify modifying a person's genome.”