Received: 24 July 2023 | **Accepted:** 04 Sep 2023 **Genel Tıp Derg. Volume** 33/**Issue** 5 (October), 594-598
DOI: 10.54005/geneltip.1332161

ORIGINAL ARTICLE

Different Approach on our Cells and the Use of CRISPR/Cas 9 Technology: Super Gene'ethic

Hücrelerimize Farklı Yaklaşım ve CRISPR/Cas9 Teknolojisinin Kullanımı: Süper Gen Etiği

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How to cite ?

Köksoy H. , Ameen Al Qadri M. S. , Karakaya S. , Bacak P. , Yüksel E. S. , Koçer Ş. , Kav E. , Demir S. , Demirel E. , Akkaya H. , Akmeşe A. N. , Taşkaya A. Different Approach on our Cells and the Use of CRISPR/Cas 9 Technology: Super Gene'ethic. Genel Tıp Dergisi. 2023; 33(5): 594-598.

ABSTRACT

Aims: CRISPR/Cas9, a revolutionary genome editing tool derived from a bacterial system, has transformed molecular biology research, enabling precise and versatile modifications to the genome. This study surveyed 300 participants, including instructors, second-grade medical students, and patient relatives from Selcuk University Faculty of Medicine, to assess their knowledge, attitudes, and ethical perspectives on gene editing.
Methods: A comprehensive survey was conducted, and the participants' demographics were correlated with their responses. Statistical analysis was performed using SPSS 22.0.
Results: The survey revealed significant differences ($p<0.05$) in attitudes based on gender, age, and participation groups. The age group of 18-29 exhibited the highest participation rate (57.3%), while the age group of 60-65 had the lowest (1%). Approximately 50.3% of participants reported no prior knowledge of CRISPR/Cas9 although 64.3% were university graduates. Obtaining approval from ethics committees was considered crucial by 151 respondents for commencing CRISPR/Cas9 research. Interestingly, 63.3% showed eagerness for genetically modified plants and animals, while only 9% expressed interest in studies related to eternal youth. Additionally, 88% found gene editing studies conflicting with their beliefs.
Conclusions: CRISPR/Cas9 holds great promise for biomedicine and healthcare but necessitates enhanced public awareness and informed decision-making. The study highlights the importance of promoting informed discussions on the ethical implications of CRISPR/Cas9 to address the concerns and perceptions of various demographics. These valuable insights contribute to the ongoing discourse on the impact of CRISPR/Cas9 on society.

Keywords: CRISPR/Cas9, Genetic manipulation, Ethics, Genome Editing, Medical Applications.

ÖZ

Amaçlar: Bakteriyel bir sistemden türetilen devrim niteliğinde bir genom düzenleme aracı olan CRISPR/Cas9, moleküler biyoloji araştırmalarını dönüştürerek genomda hassas ve çok yönlü modifikasyonlara olanak tanımaktadır. Bu çalışma, Selçuk Üniversitesi Tıp Fakültesi'nden öğretim elemanları, tıp ikinci sınıf öğrencileri ve hasta yakınları da dahil olmak üzere 300 katılımcıyla; gen düzenleme konusundaki bilgilerini, tutumlarını ve etik bakış açılarını değerlendirmek için anket yapılmıştır.
Yöntemler: Kapsamlı bir anket yapılmış ve katılımcıların demografik özellikleri yanıtlarıyla ilişkilendirilmiştir. İstatistiksel analiz SPSS 22.0 kullanılarak yapılmıştır.
Bulgular: Araştırma cinsiyet, yaş ve katılım gruplarına göre tutumlarda anlamlı farklılıklar ($p<0,05$) ortaya çıkarmıştır. En yüksek katılım oranı yüzde 57,3 ile 18-29 yaş grubunda görülürken, en düşük katılım oranı ise yüzde 1 ile 60-65 yaş grubunda gerçekleşmiştir. Katılımcıların yaklaşık %50,3'ü önceden CRISPR/Cas9 bilgisi olmadığını bildirdi ve %64,3'ü üniversite mezunuydu. 151 katılımcı, CRISPR/Cas9 araştırmasını başlatmak için etik kurullardan onay almanın çok önemli olduğunu belirtmiştir. İlginç bir şekilde, katılımcıların %63,3'ü genetiği değiştirilmiş bitki ve hayvanlara ilgi gösterirken, yalnızca %9'u sonsuz gençlikle ilgili çalışmalara ilgi duyduğunu ifade etmiştir. Ek olarak, %88'i gen düzenleme çalışmalarının inançlarıyla çeliştiğini bulmuştur.
Sonuçlar: CRISPR/Cas9, biyotıp ve sağlık hizmetleri için büyük umut vaat etmektedir. Ancak daha fazla kamu farkındalığını ve bilinçli karar almayı gerektiriyor. Çalışma, çeşitli demografik grupların endişelerini ve algılarını ele almak için CRISPR/Cas9'un etik sonuçları hakkında bilinçli tartışmaları teşvik etmenin önemini vurgulamaktadır. Bu değerli bilgiler, CRISPR/Cas9'un toplum üzerindeki etkisine ilişkin devam eden söylemlere katkıda bulunuyor.

Anahtar Kelimeler: CRISPR/Cas9, Genetik manipülasyon, Etik, Genom Düzenleme, Tıbbi Uygulamalar.

Introduction

CRISPR-Cas 9 has recently emerged as a promising system. It helps to edit the genome (epigenome and transgenome) at the multiplex level. Due to its specificity, low cost, and easy use, it allows a wide-ranging use on organisms (1). CRISPR-Cas 9; It is an RNA-mediated immune system that protects bacteria and archaea from viruses or plasmids. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is not a human-developed tool. To regions repeated over and over gene regions; If the bacterium wins the war with the virus that threatens itself, it records the gene of the virus and stores it in memory. Cas-9 is a protein that plays an active role in this cutting-splicing process. When the bacterium is infected with a virus, it checks its memory. If it recognizes it in its memory, it gives orders to its DNA. RNA is synthesized from DNA. Cas 9 binds to this RNA, cuts out the virus from that DNA, and the DNA repairs itself. Thus, it is protected from

Peer-Review: Double anonymized - Two External
Plagiarism Checks: Yes - iThenticate
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Selcuk University School of Medicine
Genel Tıp Dergisi | e-ISSN: 2602-3741
https://dergipark.org.tr/tr/pub/geneltip

threat (2). This feature scientist; has given us a unique in-cell gene editing aid that offers the ability to cut, inactivate, or precisely replace a particular region of the genome of any living organism. Jennifer Doudna, one of the biggest developers of CRISPR technology, said that this system was a "molecular surgeon's knife" for the genome (2,3). CRISPR method; It allows the reorganization of the DNA molecule, which determines how all living things and all bodies should be and function. Moreover, if the change is permanent due to regulation, it can be passed on through generations. But if there is even the slightest mistake, the system can break down and be passed on to the next generation (4).

Studies have been started in many health fields such as HIV, Huntington's Disease, Cancer, Ophthalmology, Down Syndrome, Heart Failure, Diabetes, and Cholesterol. GMO plant products, and animals whose hair color changes are included in the studies. Experiments are currently mostly carried out on bacteria, plants, and animals. It is unethical to conduct experiments on humans in the world. Yet, there have been unsuccessful human embryo experiments in China. Significant scientific, religious, and ethical debates exist in the world (5).

CRISPR-Cas9 technology, derived from prokaryotic organisms, utilizes clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated genes (Cas) to achieve adaptive immunity. The system involves the Cas9 nuclease, which targets and cleaves specific foreign nucleic acid sequences, leading to their destruction. Researchers are increasingly interested in exploring the potential of CRISPR-Cas9 for editing genomes, treating complex diseases, and other biotechnological applications (11).

Researchers are also interested in the mechanism and development of CRISPR/Cas9, its current applications in cancer research, diagnosis, and therapy, and its potential in emerging areas of oncology research. The review also addresses challenges and future directions in this field (12).

CRISPR-Cas9 and nuclease-deactivated Cas9 (dCas9) systems have revolutionized genome engineering and regulation. Inducible CRISPR-dCas9-based transcriptional systems have been developed to precisely control genetic manipulation. Current strategies focus on conditional control of gRNA function and dCas9 protein using external and internal triggers like light, small molecules, and oligonucleotides (13).

CRISPR-Cas9 is an RNA-mediated immune system that safeguards bacteria and archaea from viral and plasmid infections. However, its recent development has transformed it into a crucial technology for genome editing, targeting, and regulation across various organisms and cell types (2).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron death in the spinal cord and brainstem. Researchers have used CRISPR/Cas9 to correct common ALS-associated mutations in animal models and patient-derived induced pluripotent stem cells (iPSCs), allowing them to study the effects of these mutations and observe differences between patient-derived and gene-corrected iPSCs (14).

In the field of laboratory animal research, CRISPR/Cas9 has significantly contributed to understanding gene functions and has become a popular method for modeling human disorders. Moreover, its expanding application to human genes shows great promise for molecular characterization and the treatment of human diseases. This review provides an overview of the fundamental principles of CRISPR/Cas9 technology and highlights recent advances in its use in translational biomedicine (15).

The purpose of this research questionnaire; The aim is to discuss in depth the knowledge of new genetic arrangements to be made in living things with the CRISPR/Cas 9 protein and the ethicality of this subject, the attitudes and behaviors of people about the benefits and harms of the subject, their religious and moral beliefs with the students of the Faculty of Medicine. As a result of the research survey, it is aimed to contribute scientifically to the ethical debates around the world on behalf of our country.

Material And Method

This research questionnaire was applied to a total of 300 volunteers aged between 18-65, who were Selcuk University Faculty of Medicine faculty members, grade 2 students, and outpatients. 100 people from each group participated in the survey. At the end of the survey, the gender ratio of men and women is equal, including within each group. The questionnaire was prepared according to the Likert scale (6). Volunteers participating in the research; 35 survey questions were asked on the level of knowledge, attitude, behavior, ethical problems, and moral value approaches related to the groundbreaking CRISPR/Cas 9 technique in genetic editing. Consent of the individuals was obtained before each survey.

All information about the participants in the study will be kept confidential and those who do not want to participate in the survey will not be forced. Permission was obtained from the ethics committee with the number 2017/357 on 06.12.2017. The questionnaire forms were brought together and the data were entered into the computer together with the Evidence-based Medicine 2B (EBM 2B) students and the statistics were made. The data were calculated using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc. Chicago, IL, USA) statistical package program, and the $P<0.05$ value was considered statistically significant (7). The data we have were evaluated by performing Two-Sample T-Test, ANOVA, Chi-Square Analysis, Correlation, and Frequency analysis.

Some Survey Questions (Answered according to Likert Scale)(6).

- Have you heard of the CRISPR/Cas 9 protein, the

DNA molecular assistant for genetic studies?

• Do you know which genetic fields are being studied with CRISPR/Cas 9?

• My doctor should change my genetic physical characteristics (hair, height, eye color, etc.) chosen by my family before I was born.

• I volunteer in the treatment studies of diseases by changing genes or gene regions.

• I cannot wait to see genetically modified super plants and super animals.

• Regulation of new drugs and treatments according to a genetic structure is a great health improvement.

• If our genes are rearranged, diseases will no longer exist.

• Permission from the ethics committee should be obtained before any work to be done with CRISPR/Cas 9.

• "Ecological Imbalance" occurs in genetic studies.

• Genetic studies on humans should be ethically permitted.

• Do genetically engineered creatures go against my beliefs?

• The entire survey can be accessed at https://hkoksoy76.wixsite.com/sitem.

Results

Participation in the study in the 18-29 age group was at the highest level with 57.3%. The lowest participation rate in the 60-65 age group was 1%. Of the 300 people who participated in the survey, 64.3% were university graduates; 25.3% of them were graduates of postgraduate programs. The questionnaire is divided into four sections (Knowledge Level, Attitudes, Ethics, and Moral Perspective) (Figure 1,2,3 and 6). Participants' demographics were correlated with survey responses to identify significant trends. Gender, age, and participation groups showed statistically significant differences ($p<0.05$). Interestingly, 50.3% of the participants reported having no prior knowledge of CRISPR/Cas9. The survey data were analyzed using the SPSS 22.0 statistical package (Table 1,2).

When these sections are compared with demographic characteristics; There is a significant difference between gender, age, and the groups that participated in the survey. ($P<0.05$). The number of participants who have no information about Crispr/Cas9 is 50.3%. Gene editing; It was determined that there was a significant relationship in all other demographic domains, except for marital status in the field of medical benefit, disease recovery, and treatment. Examples of some of the interesting questions used in the study and their results are also given graphically (Figure 4,7,8,9 and 10). Before starting the works; 159 people "Strongly Agree" to the question "Permission should be obtained from the ethics committee". There are 190 people (63.3%) looking forward to seeing genetically modified plants and animals. 27 (9%) people say eternal youth work is just for them. 88% of the respondents find these studies contrary to their beliefs.

Table 1: Participants' demographics were correlated with survey responses to identify significant trends. Gender, age, and participation groups showed statistically significant differences ($p<0.05$).

	Knowledge Level	Attitudes	Ethics	Morality
Gender	Significant	Ø	Ø	Ø
Age	Significant	Ø	Ø	Ø
Education Level	Ø	Ø	Ø	Ø
Survey Group	Significant	Ø	Ø	Ø
Marital Status	Ø	Ø	Ø	Ø

(Note: "Ø" indicates that there is no significant correlation between the respective variable and the factors of knowledge level, attitude, ethics, and morality in the survey.)

Table 2: Participants' demographics

Gender	Female	150	%50.0
	Male	150	%50.0
Age	18-29	172	%57.3
	30-39	55	%18.3
	40-49	45	%15.0
	50-59	25	%8.3
	60-65	3	%1.0
Marital Status	Married	124	%41.3
	Single	173	%57.7
	Other	3	%1.0
Education Level	Elementary School	6	%2.0
	Middle School	9	%3.0
	High School	16	%5.3
	University	193	%64.3
	Postgraduate	76	%25.3
Survey Group	Selcuk University Medicine Faculty Members	100	%33.3
	Selcuk University Medicine Second-Grade Students	100	%33.3
	Selcuk University Medicine Faculty Patient Relatives	100	%33.3

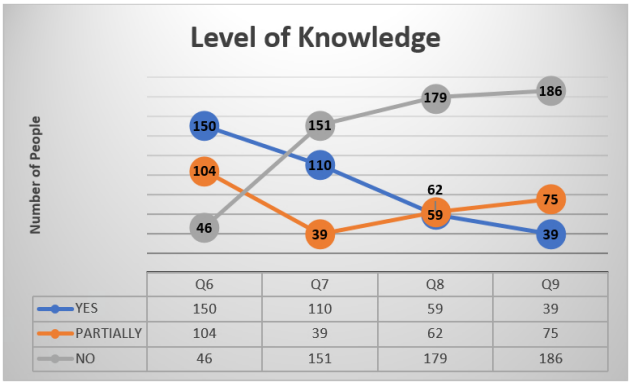


Figure 1: Point of View: Level of Knowledge Questions

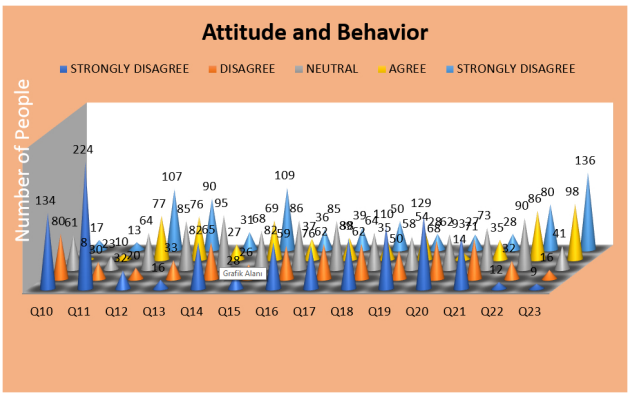
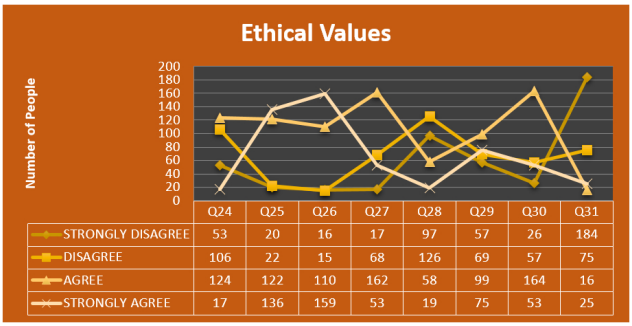


Figure 2: Point of View: Attitude and Behavior Questions



	Q24	Q25	Q26	Q27	Q28	Q29	Q30	Q31
STRONGLY DISAGREE	53	20	16	17	97	57	26	184
DISAGREE	106	22	15	68	126	69	57	75
AGREE	124	122	110	162	58	99	164	16
STRONGLY AGREE	17	136	159	53	19	75	53	25

Figure 3: Point of View: Ethical Values Questions

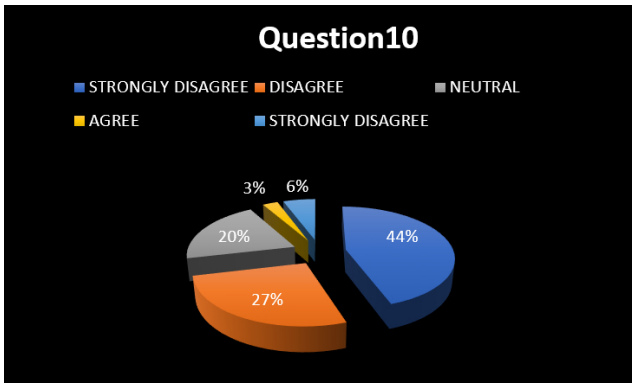
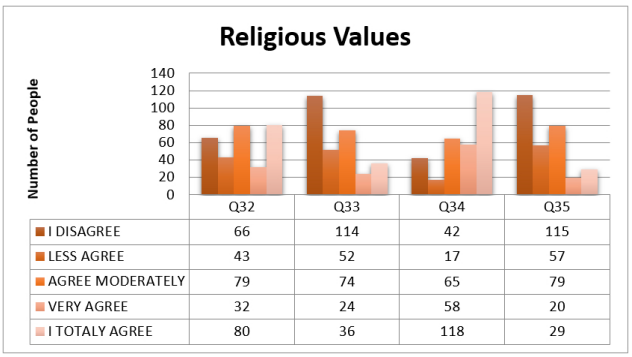


Figure 4: Q10: "Before birth, my family would give consent to any genetic intervention that would be performed on me."



	Q32	Q33	Q34	Q35
I DISAGREE	66	114	42	115
LESS AGREE	43	52	17	57
AGREE MODERATELY	79	74	65	79
VERY AGREE	32	24	58	20
I TOTALY AGREE	80	36	118	29

Figure 6: Point of View: Religious Values Questions

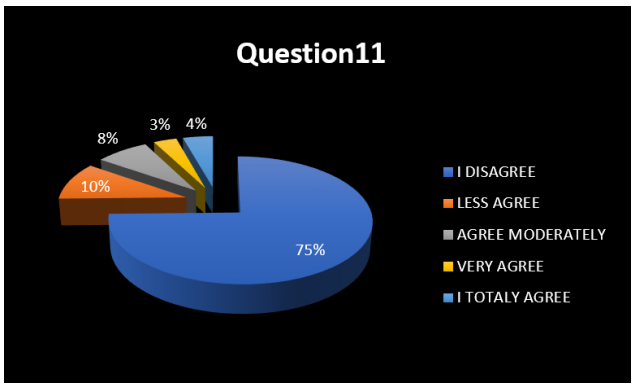


Figure 7: Q11: "Before birth, my doctor should alter the genetic physical traits (hair, height, eye color, etc.) selected by my family."

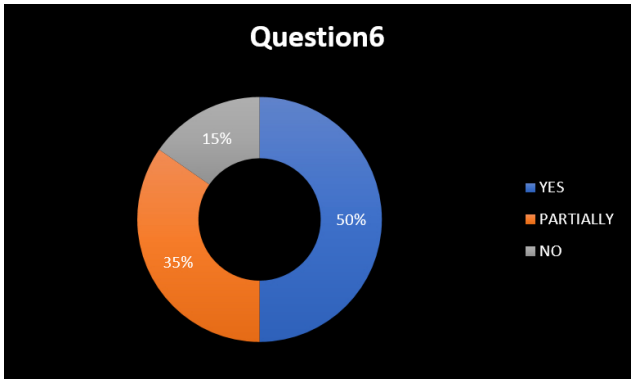


Figure 8: Q6: Are you interested in genetic topics or research?

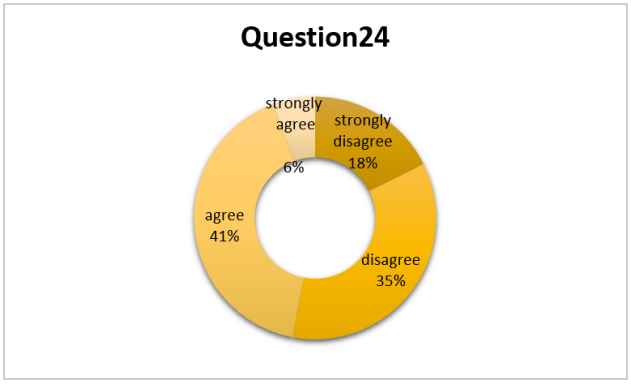


Figure 9: Q24: Genetic research on humans should be ethically allowed.

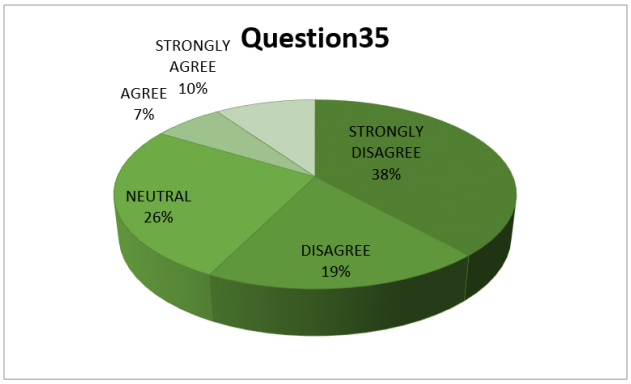


Figure 10: Q35: The intervention in the genetics of living organisms is natural?

Discussion

The CRISPR/Cas 9 Protein studies are poised to break new ground. However, in the surveys conducted around the world, there is 70% of the people are uneasy about the studies to be done (8). Especially in China, there are unsuccessful studies on embryo samples without even getting permission from the ethics committee (9). When you say it will be good, will it be bad? Many people want to volunteer in the field of treatment and sincerely wish for a cure for diseases (10). Just like in our survey, these studies contradict the beliefs of more than 80% of people. As can be seen in the survey study we have conducted, the public does

not take kindly to these studies due to both ethical and religious and moral aspects. However, as long as it is used for the treatment of hereditary diseases, some people approach these studies moderately. A few of the opinions presented in our survey study confirm this. Opinions and suggestions from the participants:

1. It is not ethical and moral for humanity to play in genes for purposes other than health, except for my belief.

2. DNA scissors should only be used in disease treatment methods.

3. We think it would be right to get permission from a higher institution rather than the ethics committee for gene studies.

We think we should draw attention to this point. Genetically modified plants, animals, humans, and bacteria are special and valuable regardless of the organism. These studies require precision. Ethical committees must be established. No one knows what the effects of the study will be years later. Should it work, yes it should. I hope it has shed some light on the most current topic of recent days.

Our people need to be more conscious about the subject of the survey. This research questionnaire shows the level of knowledge and attitude; It is thought that it will contribute positively to other researches.

Acknowledgments

The statistics of our study were made with the contributions of Selcuk University, Faculty of Science, Department of Statistics. We would like to thank Prof. Dr. Coskun KUS and his assistant for their efforts. We had the opportunity to make an oral presentation at the ICONPB-2018 international congress on 10.05.2018. Dr. We would like to thank Osman TUGAY and the congress team. We would like to thank all the participants who participated in our survey for their valuable time.

Author Contributions

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M. S. Qadrı and S Karakaya: Formal analysis, Resources, Data Curation, Statistics, Pollster

P Bacak and E. S. Yuksel: Methodology, Resources Writing - Original Draft Preparation Resources, Data Curation, Project Administration Management

S Kocer and E Kav: Data analysis, Resources, Statistics

S Demir (Yıldız) and E Demirel: Methodology, Resources Writing - Original Draft Curation, Pollster

H Akkaya: Resources, Data Curation, Statistics

A. N. Akmese and Taskaya: Validation, Verification, Resources

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