

Global Research Assessment of CRISPR: A Scientometric Analysis of Literature Published in Scopus

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ABSTRACT

The study aimed to review the CRISPR research trend by covering major historic milestones in its research development from 2005 to date. The data from Scopus considered for this evidence-based scientometric study. Nine thousand four hundred eighty-nine data were extracted and analyzed for publications, sources, authors, affiliations, countries, and keywords based on different scientometric tools using MS Excel and other scientometric software viz. Biblioshiny (RStudio), VOSviewer. The findings showed the content and research direction of the area and have helped us identify the current situation and find the most productive teams in this field. The co-citation analysis identified the highly cited reference. The cluster analysis and timeline helped to recognize the structure and evolution of this research.

Keywords: CRISPR, Genome editing, Scientometric, Bibliometric, Research assessment.

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INTRODUCTION

In recent years CRISPR-Cas9 gene editing has gained attention and is being widely explored as a solution for treating genetic aberrations. The CRISPR (clustered regularly interspaced short palindromic repeats) is the hallmark of the bacterial adaptive defense mechanism.^[1] Research on gene editing using the CRISPR-Cas9 system is a prevalent and promising aspect of genetic engineering and gene therapy. The CRISPR-Cas9 system consists of two key molecules which introduce a change into the DNA. An enzyme Cas9 acts as a pair of 'molecular scissors' that can cut the two strands of DNA at a specific location in the genome, and a piece of RNA called guide RNA (gRNA).^[2] This consists of a small piece of pre-designed RNA sequence (about 20 bases long that complement target DNA) located within a longer RNA scaffold. The scaffold part binds to DNA, and the pre-designed sequence 'guides' Cas9 to the genome's right part. This makes sure that the Cas9 enzyme cuts at the right point in the genome. This system allows precise edits to the genome, permanently edits genes in organisms, and repairs explicitly mutations. Different delivery methods are used *in vitro*, such as viral, non-viral, and physical methods. Despite all mentioned features, *in vivo* application of CRISPR-Cas9 has to overcome many challenges such as obstacles in delivery, off-target effects, and difficulties in gRNA production,

insertional mutagenesis, and immunogenicity. Successful clinical use of CRISPR-Cas9 has countless applications that will allow easy alterations and modifications of DNA sequences and treatment of genetic diseases.

Review of CRISPR Studies

As mentioned in the introduction, CRISPR is a group of DNA sequences in the prokaryotic genome. A study on *E. coli* genes involved in phosphate metabolism revealed unusual repeating short DNA sequences and similar sequences in reverse,^[3] which later termed CRISPR. Barrangou *et al.* first featured this bacterial CRISPR-Cas9 system as a distinct bacterial adaptive defense mechanism.^[1] Prokaryotic organisms like bacteria and archaea obtain these DNA sequences from bacteriophages and viruses that had infected them.

The system comprises two essential molecules Cas9 (CRISPR associated protein 9) enzyme and gRNA (guide RNA). Cas9 enzyme cuts the two strands of DNA at a specific sequence in the genome. gRNA is lodged in a large RNA framework and is about 20 bases long pre-designed pieces of the RNA sequence complementary to target DNA. First, gRNA is designed to match the mutated gene sequence and then combined with the Cas9 enzyme. The RNA frame binds to DNA, and gRNA guides Cas9 to a specific location in the genome, assuring that Cas9 cuts DNA at the exact desired position. These cuts generate double-strand breaks at target sites, and subsequent DNA repair mechanisms help the desired insertion and deletion. CRISPR-Cas9 delivery to any cell modifies the target sequence.



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Later in the year 2012, there was a breakthrough in CRISPR research, where it was found that CRISPR-Cas9 can design to find and cut specific DNA sequences, and it is *in vitro* use demonstrated that it could be used to edit the human genome.^[4] Further, Feng Zhang and team in early 2013 demonstrated successful adaptation of the CRISPR-Cas9 system in a eukaryotic cell.^[5] A details historial development of the CRISPR has provided in the Figure 1. These studies were milestones to signify the role of CRISPR-Cas9 in targeting, deleting genes, and editing any genome.

Review of scientometric studies

Roy has attempted to evaluate India's scientific research output in *Biological Science* from 1901 to 1947. The study examined the growth rate, collaborative pattern, authorship pattern, most prolific author on biological Science in India. It revealed that the mean relative is 0.615, and the Duplication time is 1.007 from 1901 to 1947. It showed that the degree of collaboration is 0.249 and most papers are single-authored (75%). The study also highlighted that the collaborative authorship trending and single authorship have decreased trends. The most ten productive authors have contributed 15% of *Biological Science* literature from 1901 to 1947.^[6] M. K. Singh and Tripathi examined the top ten

research organizations in *Biotechnology* in India from 2001 to 2016 to find a total of 5423 bibliographic records. The study applied a statistical and mathematical approach to know the pattern of publication and types of documents. The study used different Scientometric methods, including collaboration co-efficient, co-authorship index, and collaborative research in biotechnology. The study also used the activity index tool to know the authorship pattern trends and institute data mapping. The USA is the most preferred country for International collaboration in the study domain.^[7] Manendra reviewed authorship and collaborative research in *Biotechnology* in ISBA Countries from 2007 to 2016. A total of 24888 papers considered from the Scopus database and analyzed with different Scientometric tools such as the collaboration coefficient, the pattern of authorship, and Activity Index. The study revealed that multiple authors had contributed more research papers than a single authorship pattern. South Africa is the leading country in the average Index activity among the IBSA countries over 2007-2016, while India (2nd) and Brazil (3rd) took the position. The United States of America is the most dominant international collaboration country among the IBSA countries.^[8] Chinnaraj and Narzary conducted a Scientometric analysis on *Colorectal Cancer* among *Asian Countries* from 2000-2017. A total of 2726 research papers considered indexed in

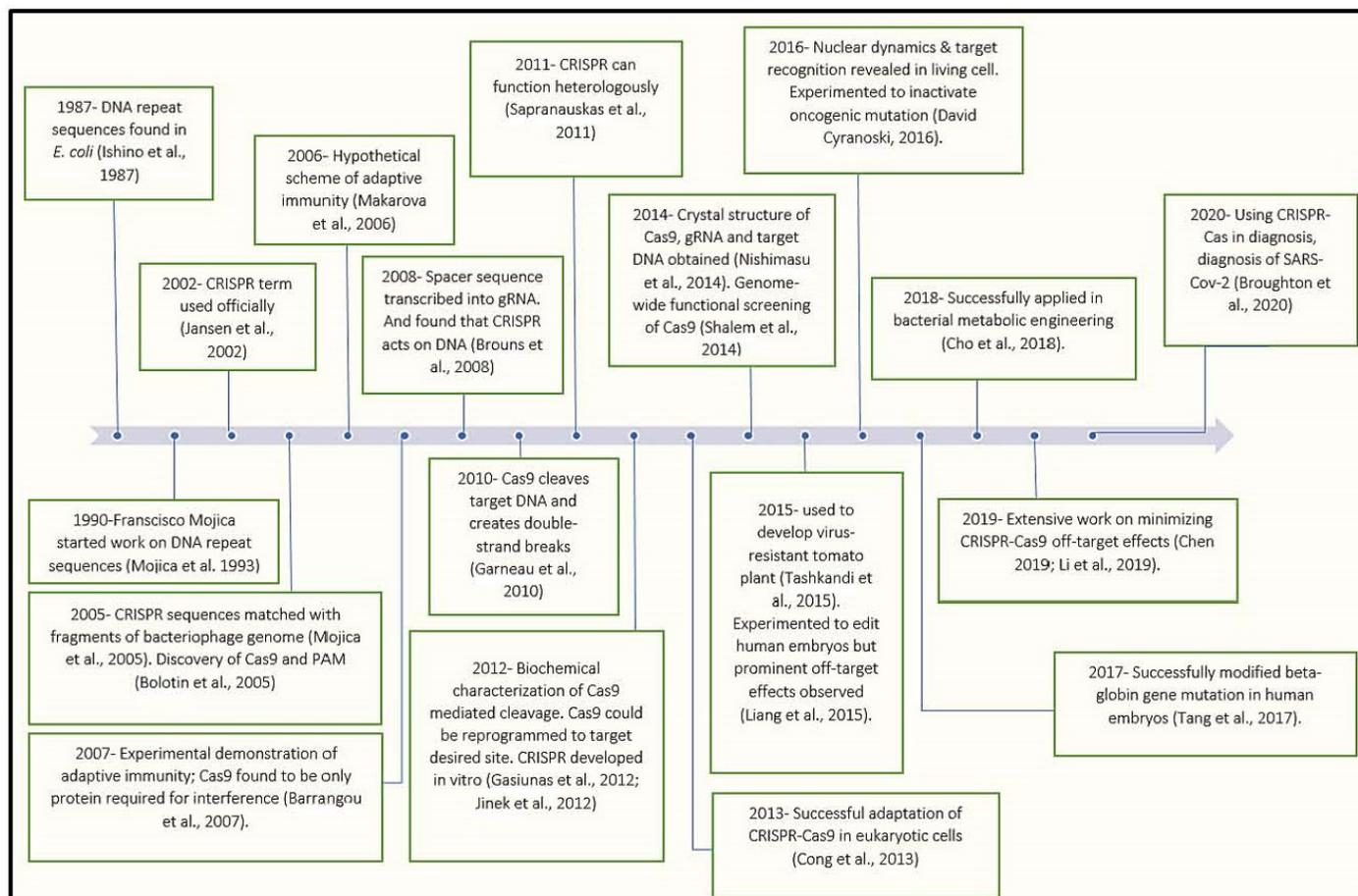


Figure 1: CRISPR timeline.

PubMed. Most of the articles published in the English language, 2661 (97.61%), the maximum number of documents published as journal articles, 2225 (81.62%). The degree of collaboration is 0.84; the relative growth rate decreased while doubling time was increased; the mean relative growth rate is 0.28. Thailand contributed the highest number of publications, 838 (30.74%), Wang J with 31 (1.31%) papers found as a most productive author who collaborated with 82 other scientists of the same field of colorectal cancer during 2000-2017.^[9] Rahaman *et al.* was conducted a study to determine the trends and characteristics of new coronavirus publications. 2661 Scopus publications were downloaded in BibText format and analyzed with Bibliometrix and Vosviewer over 25 years. The findings reveal the highest number of publications in 2020. (779). The data suggests that 68 percent of the 1806 publications are in the form of research papers. "Journal of Virology" (JIF = 4.324) was the most productive source in coronavirus research. "Yuen KY" was discovered to be a prolific author (75), as well as having more collaborative publications (62). Researchers who are currently conducting or planning coronavirus research will benefit from this study.^[10] Sathiyapriya and Palanisamy highlighted the research output of *Immunology* in India from 1993 to 2017. The research productivity of India was noted as 397 papers from 1993 to 2017. The study focused on the language-wise publication, document type, publication, year-wise distribution of documents, authorship pattern, degree of collaboration, keyword analysis, the applicability of Zipf's law method, and measure of *h*-index for highly productive authors.^[10]

Objectives

- To cover major historical milestones in the research development of CRISPR, from 2005 till date.
- To explore current research trends in CRISPR by keyword analysis.
- To elucidate increasing trends towards multi-author collaboration in recent years.
- To identify author groups from a map of authors obtained by network analysis.
- To investigate the developed research network between countries, authors, and affiliations.
- To identify the highly involved funding agencies investing in CRISPR research.
- To identify the most cited documents.

Data collection

A massive amount of published literature was found indexed in the Scopus database, i.e., thirty thousand (30,616) from 1946 to 2021. Analyzing such colossal data is complex, and it might cause inaccuracies in the results. Hence, this issue acknowledged by

incorporating the following search strategy (Title = CRISPR) on 27th January 2021 at Imam Abdul Rahman bin Faisal University. Therefore, data collected under the 'Title' search rather than 'Topic search to get accuracy in the results, and 9489 data collected, spread over the years 2005 to 2021. The data collected in BibTex and CSV format to analyze by using professional bibliometric software such as Biblioshiny^[11] and VOSviewer.^[12] Microsoft Excel is also incorporated in mapping and visualization.

RESULTS

General information

The collected data comprises 1629 different sources, including journals, books, and conference proceedings. The total data collected in the given period included 9489 documents. The average years from publication for each document found as 3.23. Each document received approximately 32.95 citations. The average citation of each document per year is 5.414. Total references of these documents sum up to 288505.

Types of CRISPR publications

The data shows that the research published on CRISPR comprises research articles, book chapters, editorials, conference papers, reviews, etc. The 69.66% of publications (6610) are articles, followed by 1275 reviews, 408 book chapters, 402 notes, 207 letters, 175 errata, 162 short surveys, 133 editorials, and 106 conference papers. In contrast, very low publications are books and data papers, i.e., only 5 and 3, respectively. There are three articles in the press in this period Table 1.

Annual scientific publications

Before 2010, the number of CRISPR publications was very low, with less than 20 articles published every year, indicating that this field was at the preliminary stage. However, publications kept rising gradually. Once CRISPR got discovered as a gene-editing tool in 2012, the number of publications upsurged to more than double the number in 2013 (from 68 in 2012 to 181 in 2013). Until 2016, the number of publications kept doubling from 2017 to 2020 constant rise in the publication observed. The maximum number of publications (2150) recorded in the year 2020. The lowest publication recorded is 1 document in 2006, and this is the document with the highest mean (549.28) of total citation per article with 15 citable years Table 1.

The lowest mean total citation per article was recorded in 2020 (where the number of publications is highest) and only one citable year. The highest mean of citation per year (39.234) recorded in 2007, and the lowest mean of citation per year is 2.37 recorded in 2020. Table 2). Overall, the outputs increased continuously this decade, with the appearance of some reviews and new findings of CRISPR-Cas9 relation and innovation of methods to use CRISPR in genome editing. Nevertheless, the average number of

publications has not reached a high level, suggesting that this field has lots of scope for further detailed research and needs attention.

Most Globally cited publications

The top 20 globally cited papers arranged in Table 2. 'Multiplex Genome Engineering Using CRISPR/Cas Systems' by Le Cong *et al.* received the highest citations (7506),^[5] followed by 'Genome engineering using the CRISPR-Cas9 system' by F Ann Ran (4302 citations),^[13] and paper 'CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes' by Rodolphe Barrangou (2864 citations).^[1] 'CRISPR/Cas, the Immune System of Bacteria and Archaea' by Philippe Horvath with 1256 citations noted as the least cited paper in the top 20 list.^[14]

Publications with highest contributes

By analyzing Figure 2, It is observed that clusters gathered closely, the documents are not extremely apart, suggesting a similarity between the topics discussed in each cluster's documents. One example can be represented by the paper of Soleimani F *et al.* from the green cluster,^[31] the documents of Li J *et al.* from the red cluster,^[32] and Roberts B *et al.* belonging to the blue cluster.^[33] Despite these papers being from three different clusters, one is close to the other in the Figure, and all are focused on using CRISPR-Cas9 gene editing. The difference consists in the way in which the topic is developed. Soleimani F *et al.* and Li J *et al.* used CRISPR-Cas9 on mice embryonic stem cells to modulate pluripotency genes and GADD45A gene, respectively,^[31,34] while Roberts B *et al.* used CRISPR-Cas9 for systematic gene tagging.^[33]

Red Cluster #1 represented by Shetty DK *et al.* (2016), Li C *et al.* (2019), Li J *et al.* (2020), and Feng Y *et al.* (2020) with their work published in *Stem Cell Research*.

Blue Cluster #2 represents work published in *Scientific Reports* by Xie Y (2017) and Xie Y (2018), along with work published in *Stem Cell Research* by Benet N (2013) and Liu CL (2020). Cluster 2 also encompasses work by Roberts B (2017) published in *Molecular Biology Of Cell*.

Green Cluster #3 represents work published in the Journal of Cell Physiology by Soleimani F (2020) and Drost J (2017) published in *Science*. The cluster also includes work by Zhong C (2015), Rattanapornsompong K (2019), and J B (2020) published in Cell Stem Cell-a, Methods in Molecular Biology, and Applied Biochemistry and Biotechnology, respectively.

Sources

Studies regarding CRISPR and its associated researches published in various journals. Table 3 shows the journals' ranking according to the Number of Publications (NP) in the data set, the journal's *h*-index, and Total Citations (TC). As shown in the table, *Science* is the most prolific journal with the highest TC, i.e., 27129, and has started publication in 2007 and *Genome Biology* (TC= 3374). *Science* publishes cutting-edge research. *Science* publishes three crucial papers in the field of CRISPR. In August 2012, *Science* published the most influential paper titled "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity" by Doudna JA, Charpentier E, and their team. This

Table 1: Annual CRISPR publication.

Year	Articles	Mean TC per Art	Mean TC per Year	Citable Years
2005	4	45	33.34375	16
2006	1	549.28	3	15
2007	7	464.55	39.234	14
2008	9	154.89	35.735	13
2009	19	218.36	12.907	12
2010	30	158.51	19.851	11
2011	47	70.029	15.851	10
2012	68	263.26	7.7810	9
2013	181	147.91	32.908	8
2014	344	78.512	21.131	7
2015	654	43.469	13.085	6
2016	1015	29.239	8.6939	5
2017	1311	17.401	7.3098	4
2018	1558	8.5280	5.8003	3
2019	1890	2.3744	4.2640	2
2020	2150	0.24378	2.3744	1
2021	201	533.5	-	0

*TC=Total citation, **NP=Number of publication

paper grabbed a lot of attention across the globe and received the highest number of citations. Another remarkable paper published by *Science* in early 2013 was by Zhang F and the team titled "Multiplex Genome Engineering Using CRISPR/Cas Systems." In the same journal article, one more important paper, i.e., by Church and team titled "RNA-Guided Human Genome Engineering via Cas9," was published.

One more impactful journal is *Scientific Reports*, with the highest *h*-index (61) and highest NP (409). *Scientific Reports* is a journal that publishes articles focused on natural and clinical sciences and encourages to submit papers containing original research work. The following journal in rank with respect to the number of publications is *Methods in Molecular Biology*, with 298 publications. *Methods in Molecular Biology* aim to publish work that covers molecular biology research methods and protocols. The other three journals, *Nature Biotechnology*, *Nature Communication*, and *Nature*, have *h*-index 57, 55, and 54 with 137, 195, and 187 publications.

Along with *Science* and *Genome Biology*, the other two journals *Plos One* and *Cell*, started publication in the CRISPR field at an early stage, i.e., in 2009, *Cell* has 22299 total citations. *Cell* publishes significant findings in experimental biology, cell biology, molecular biology, neurosciences, immunology, etc. *Plos One* has 6145 total citations, and its scope is to publish original research submissions from natural Science and medical research.

Frontiers in Plant Science has published the least number of publications (81) on CRISPR in the selected data set. *PNAS (Proceedings of The National Academy of Sciences of the United States of America)* has 90 publications on CRISPR and has acquired quite a good number of citations. *PNAS* published a significant paper, "Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria," by Virginijus Siksnys and team in 2012, which discussed the work similar to that of Doudna and Charpentier.

Table 2: Most globally cited publications.

Rank	Title	Reference	TC	TC / Year
1	Multiplex Genome Engineering Using CRISPR/Cas Systems ^[5]	DOI: 10.1126/science.1231143	7506	834
2	Genome engineering using the CRISPR-Cas9 system ^[13]	DOI: 10.1038/nprot.2013.143	4302	478
3	CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes ^[1]	DOI: 10.1126/science.1138140	2864	190.93
4	Development and Applications of CRISPR-Cas9 for Genome Engineering ^[15]	DOI: 10.1016/j.cell.2014.05.010	2696	337
5	The new frontier of genome engineering with CRISPR-Cas9 ^[16]	DOI: 10.1126/science.1258096	2431	303.85
6	Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells ^[17]	DOI: 10.1126/Science.1247005	2262	282.75
7	One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering ^[18]	DOI: 10.1016/j.cell.2013.04.025	2172	241.33
8	Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression ^[19]	DOI: 10.1016/j.cell.2013.02.022	2157	239.66
9	ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering ^[20]	DOI: 10.1016/j.tibtech.2013.04.004	1957	217.44
10	Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity ^[21]	DOI: 10.1016/j.cell.2013.08.021	1884	209.33
11	High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells ^[22]	DOI: 10.1038/nbt.2623	1749	194.33
12	Efficient genome editing in zebrafish using a CRISPR-Cas system ^[23]	DOI: 10.1038/nbt.2501	1723	191.44
13	CRISPR-Cas systems for editing, regulating and targeting genomes ^[24]	DOI: 10.1038/nbt.2842	1712	214
14	Improved vectors and genome-wide libraries for CRISPR screening ^[25]	DOI: 10.1038/nmeth.3047	1702	212.75
15	Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System ^[26]	DOI: 10.1016/j.cell.2015.09.038	1539	219.85
16	Genetic Screens in Human Cells Using the CRISPR-Cas9 System ^[27]	DOI: 10.1126/Science.1246981	1483	185.37
17	Small CRISPR RNAs Guide Antiviral Defense in Prokaryotes ^[28]	DOI: 10.1126/Science.1159689	1306	93.28
18	RNA-guided editing of bacterial genomes using CRISPR-Cas systems ^[29]	DOI: 10.1038/nbt.2508	1296	144
19	Evolution and classification of the CRISPR-Cas systems ^[30]	DOI: 10.1038/nrmicro2577	1295	117.72
20	CRISPR/Cas, the Immune System of Bacteria and Archaea ^[14]	DOI: 10.1126/Science.1179555	1256	104.66

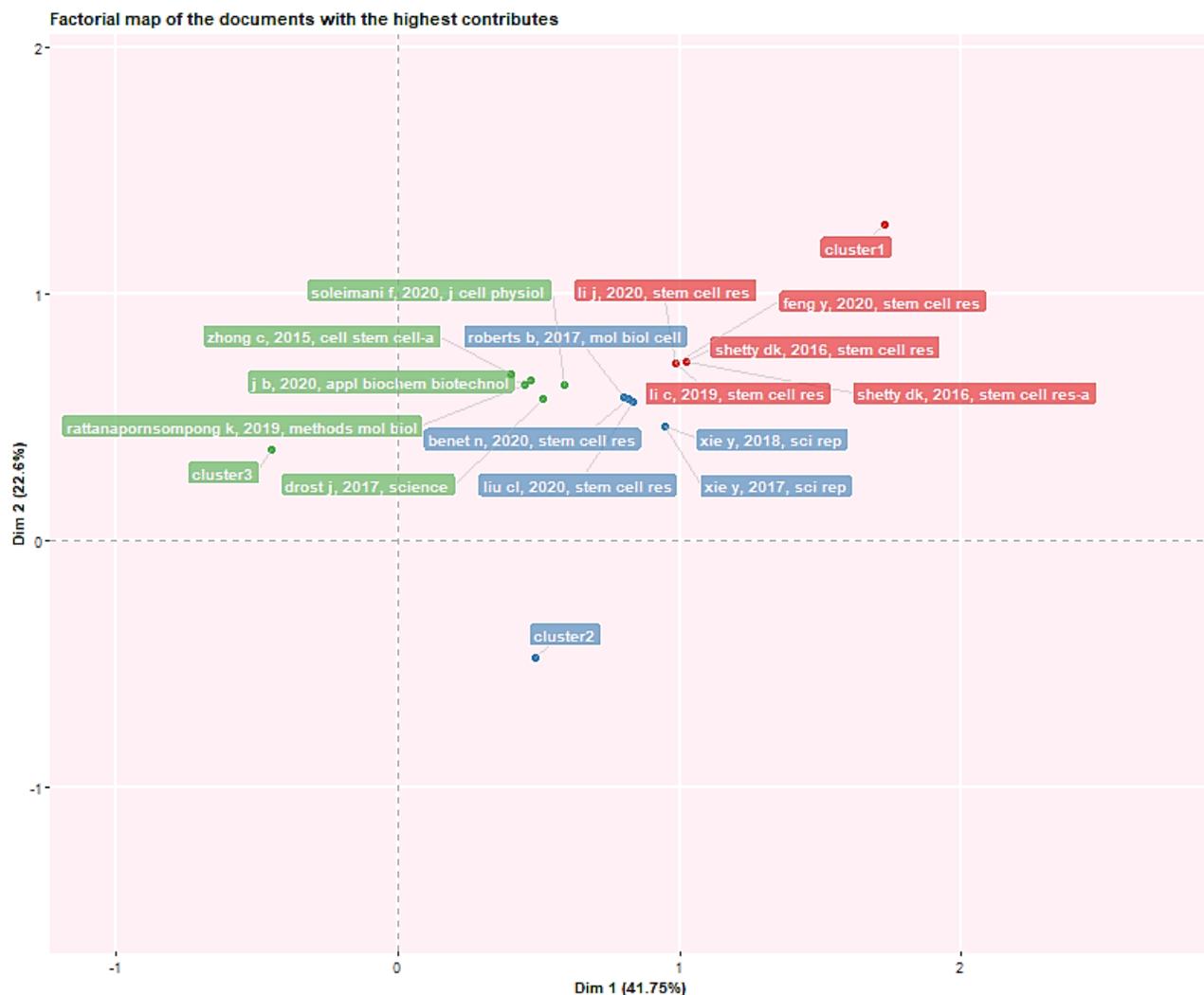


Figure 2: Factorial map of documents with highest contributes.

Authors' general information

The total number of authors are 26857, and they have appeared 57755 times, indicating that each author might have contributed to approximately two publications. 586 authors have single-authored documents to their account, while 26271 are authors of multi-authored papers

Authors' collaboration

Among 9489 documents, 981 are single-authored documents. Analytically there are 2.83 authors per document. Data analysis implies that there are 0.353 documents per author. It also imparts 6.09 co-authors per document, suggesting more collaborative work in this research area, giving a collaboration index of 3.09. Trends in author collaborations show researchers in the field (CRISPR) prefer collaborative research.

Author impact

Zhang F is the fortunate 1st ranked author with regards to total citations (TC), obtaining 38452 TC, and acquiring the highest

h-index (52) (Table 4), even though he has 97 publications (NP) which indicate the significance of his work in the field. Zhang F made the first move towards developing prokaryotic CRISPR-Cas9 immune system as a gene-editing tool and worked on its use in eukaryotic cells. Based on CRISPR, his lab formed a diagnostic nucleic acid detection procedure known as SHERLOCK (Specific High sensitivity Enzymatic Reporter UnLOCKing), which is highly sensitive and can detect very low concentration.^[5] Barrangou R and Li Y started work earlier in 2007. Li Y has 181, and Barrangou R has only 100 publications; however, Barrangou R received higher TC (14025) ranking 2nd with respect to TC. Barrangou had started working on characteristic genetic repeating sequences long before it became popular as a CRISPR.^[1] Barrangou is now making CRISPR to edit the bacterial genome itself.^[1]

Regarding the *h*-index, Wang Y and Zhang Y are at 2nd and 3rd rank with an *h*-index of 44 and 42, respectively. The author "Zhang Y" is a prolific writer in the field of CRISPR in terms of the number of publications (NP) as he has the highest number of publications,

that is 248, and has received 8691 total citations (TC). The author, "Wang Y," ranked 2nd for publications with 222 NP and 7964 TC. Author Liu Y has the least number of publications, i.e., 92.

Most Relevant Authors and their Production over Time

Figure 3 shows the authors' production over time and reports the 20 authors with at least eight years of contribution to CRISPR in the data set. The size of the dot expresses the number of published articles, while the intensity of the color refers to the total citations per year. Though the study considered the data set from 2005 to date, the Figure shows productivity from 2007.

In terms of the number of articles contributed, Zhang Y. and Wang Y. are at the first and second rank, respectively, contributing 265 and 236 articles between the period 2013–2020, followed by Liu Y. and Li Y with 195 and 191 publications. Though Liu Y started work in 2014, the larger dot in 2020 represents that he has published more documents.

Although these authors have published more, it is interesting to see that the author with the highest impact in literature is Zhang F. The darkest dot in 2013 represents the most significant work ever published in the data set. Author Barrangou R is constantly contributing every year from 2007 till 2021. Author Li Y is in 4th position with respect to the number of publications and started

work in the early years, but he has not contributed in the field for four years (2008 to 2012). From 2013, all researchers in the field reported constant contribution, indicating that after the publication of a pivotal paper by Doudna and Charpentier regarding the use of CRISPR-Cas9 as a gene-editing tool,^[16] research in the field of CRISPR has burst. Finally, the literary production over time confirms the growing interest over the years for the research topic.

Co-citation (Cited authors)

Co-citation analysis is one of the distinct methods to study the structure of science. It involves tracing documents that have together been cited in the source articles. Author co-citation involves analyzing the intellectual development of the scientific disciplines. If the same documents co-cited by many authors, a research cluster forms, and each cluster's documents share common research themes. Figure 4 represents the most locally cited authors and co-citations. Each dot represents an author; the size of dots varies in proportion to the number of citations. Lines between dots show the link between authors reflecting that they have been co-cited. The number of lines attached to a dot represents the link strength of that author. Therefore, with the help of link clustering and multidimensional scaling techniques, mapping the structure of specialized research areas can be done with co-citation network analysis.^[35] Co-citation was selected

Table 3: Top 20 source impact.

Source	h-index	TC	NP	PY-start
Scientific Reports	61	11430	409	2013
Methods in Molecular Biology	14	1194	298	2012
Nucleic Acids Research	50	11455	221	2011
Plos One	42	6145	197	2009
Nature Communications	55	9229	195	2012
Nature	54	17625	187	2010
Nature Biotechnology	57	25736	137	2013
Molecular Cell	42	6627	109	2010
Science	45	27129	104	2007
Plant Biotechnology Journal	29	3161	98	2014
Acs Synthetic Biology	25	2566	93	2013
Stem Cell Research	5	152	91	2016
Proceedings of the National Academy of Sciences of the United States of America (Pnas)	40	5495	90	2011
International Journal of Molecular Sciences	16	805	85	2013
Nature Methods	38	9161	84	2013
Frontiers in Plant Science	27	1990	81	2015
Cell	46	22299	75	2009
Genome Biology	29	3374	75	2007
Molecular Therapy	26	2086	71	2012
Molecular Therapy-Nucleic Acids	20	1880	71	2014

Table 4: Author Impact.

Rank	Author	h-index	g-index	m-index	TC	NP	PY-start
1	Zhang Y	42	90	4.667	8691	248	2013
2	Wang Y	44	85	4.889	7964	222	2013
3	Liu Y	28	49	3.500	2917	181	2014
4	Li Y	37	77	2.467	6402	181	2007
5	Wang X	33	57	3.667	3737	174	2013
6	Li J	35	71	3.889	5310	170	2013
7	Na Na	7	11	0.700	166	161	2012
8	Wang J	30	59	3.750	3713	142	2014
9	Chen Y	36	79	4.000	6446	138	2013
10	Zhang X	31	63	3.444	4296	140	2013
11	Zhang J	31	64	2.583	4312	135	2010
12	Liu X	24	51	2.400	2783	112	2012
13	Wang H	31	83	3.100	6946	118	2012
14	Wang L	25	51	2.778	2932	112	2013
15	Li Z	23	53	2.556	2987	113	2013
16	Barrangou R	41	100	2.733	14025	100	2007
17	Li X	22	40	2.750	1775	97	2014
18	Zhang L	28	52	3.111	2822	96	2013
19	Zhang F	52	97	4.000	38542	97	2009
20	Liu J	30	61	3.333	3793	92	2013

*TC=Total citation, **NP=Number of publication

Top-Authors' Production over the Time

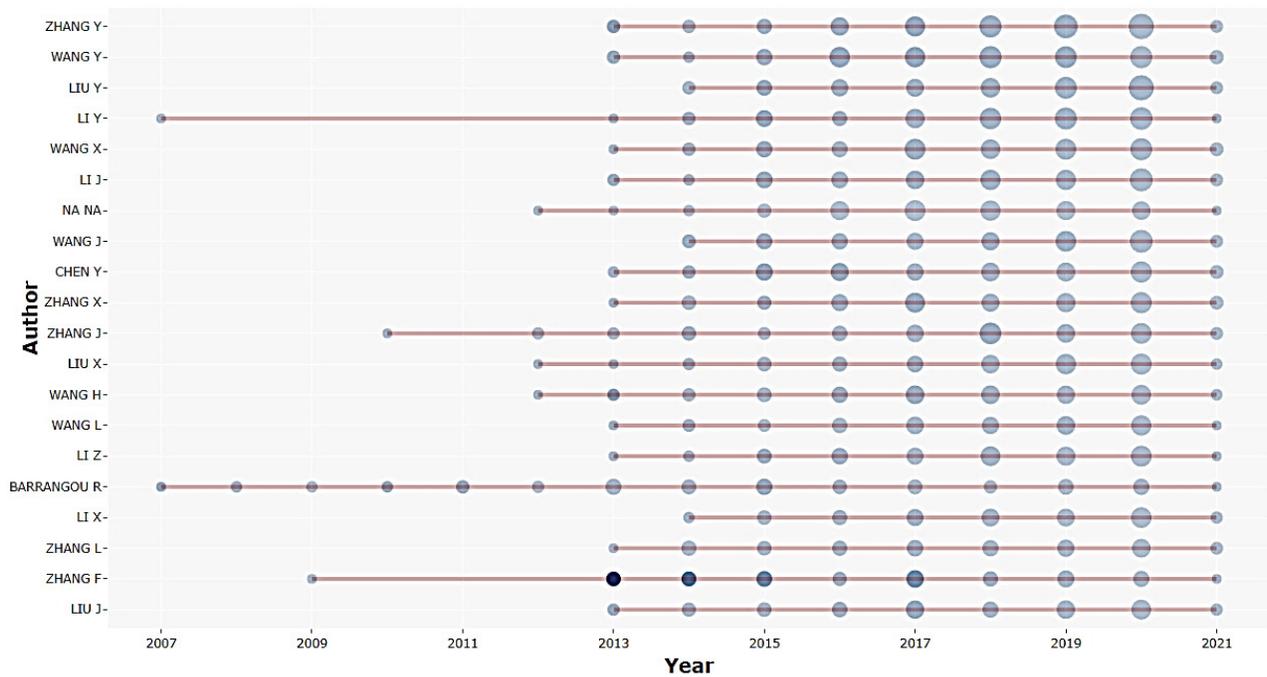


Figure 3: Top-Author's production over the time.

from 'types of analysis,' and cited authors selected from a 'unit of analysis,' a full method used for calculation, minimum 640 citations of an author considered for analysis. Out of the (72258) authors, (70) meet the thresholds. For each of the (70) authors, the total strength of the co-citation links with the other authors calculated and the authors with the greatest total link strength selected. Full item found (70), cluster (2), links (2415), and total link strength (2695083).

Cluster 1 consists of 38 co-citations of cited authors (most prominent are Zhang F, Hsu PD, Jinek M, Mali P, Ran FA, Joung JK).

Cluster 2 represents 31 co-citations of cited authors (Barrangou R, Doudna JA, Charpentier E, Horvath P, Makarova KS, Moineau S).

The majority of dots in the same cluster are closer, which means that the authors with similar research interests are closer to each other in the network. For example, from orange cluster (#1), Sander JD, Joung JK, and Scott DA are very close; similarly, Aach

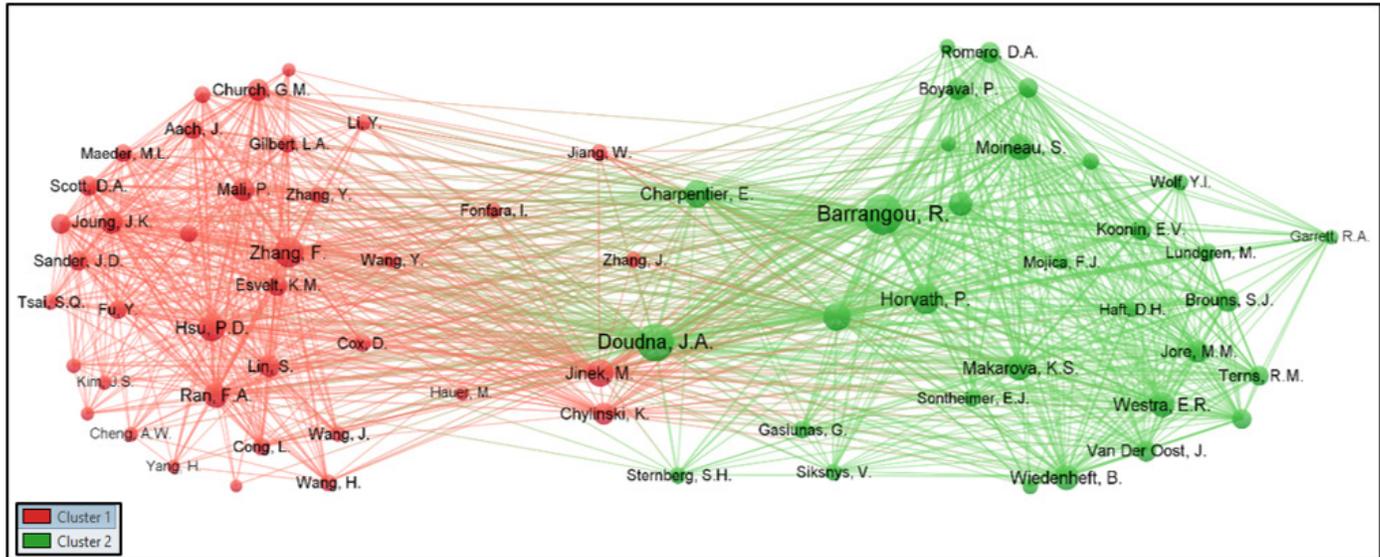


Figure 4: Most locally cited authors and their co-citation.

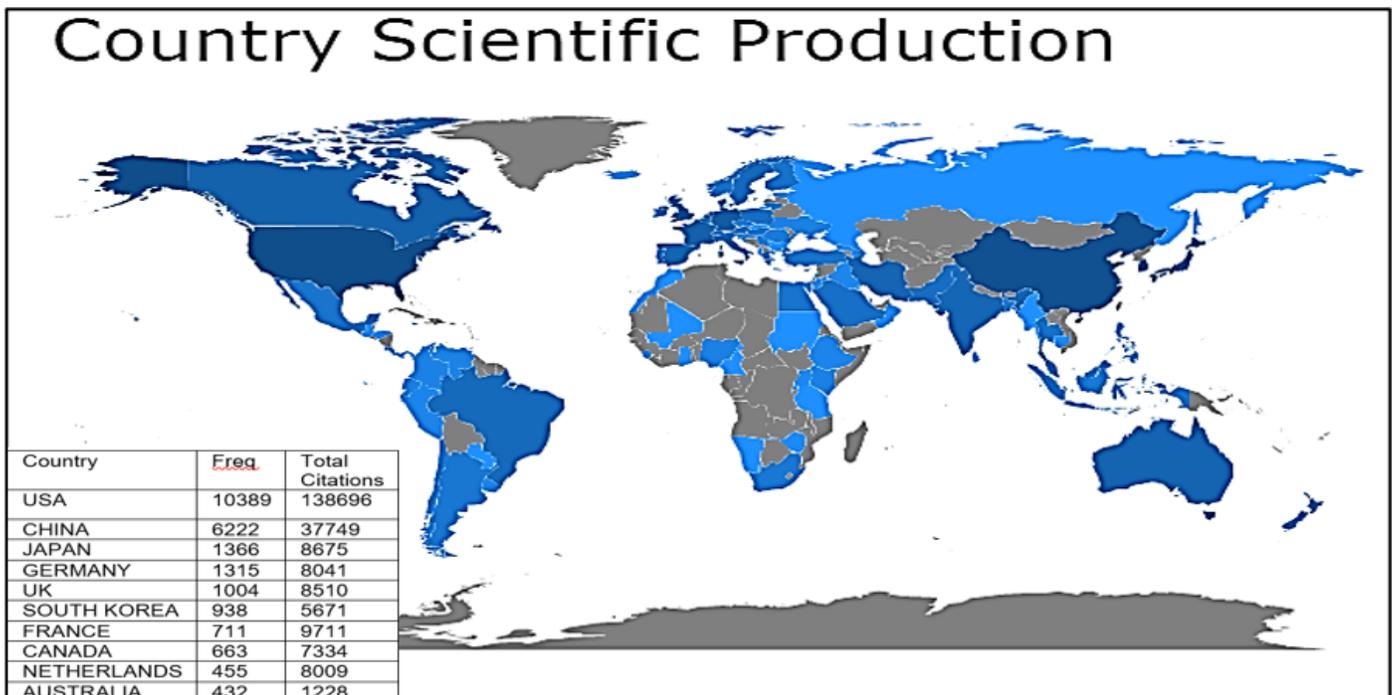


Figure 5: Country scientific production.

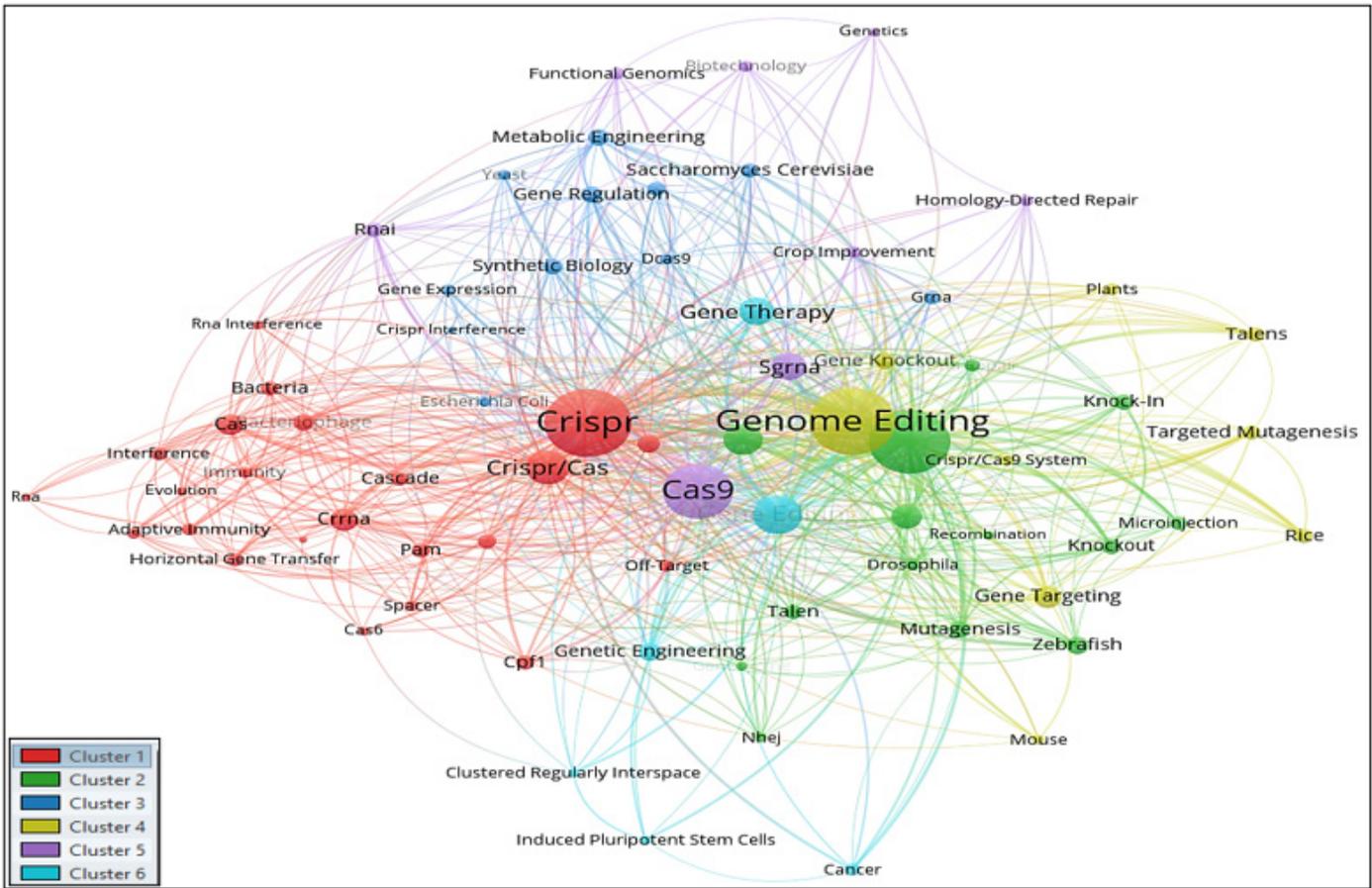


Figure 8: Co-occurrence (Author Keywords).

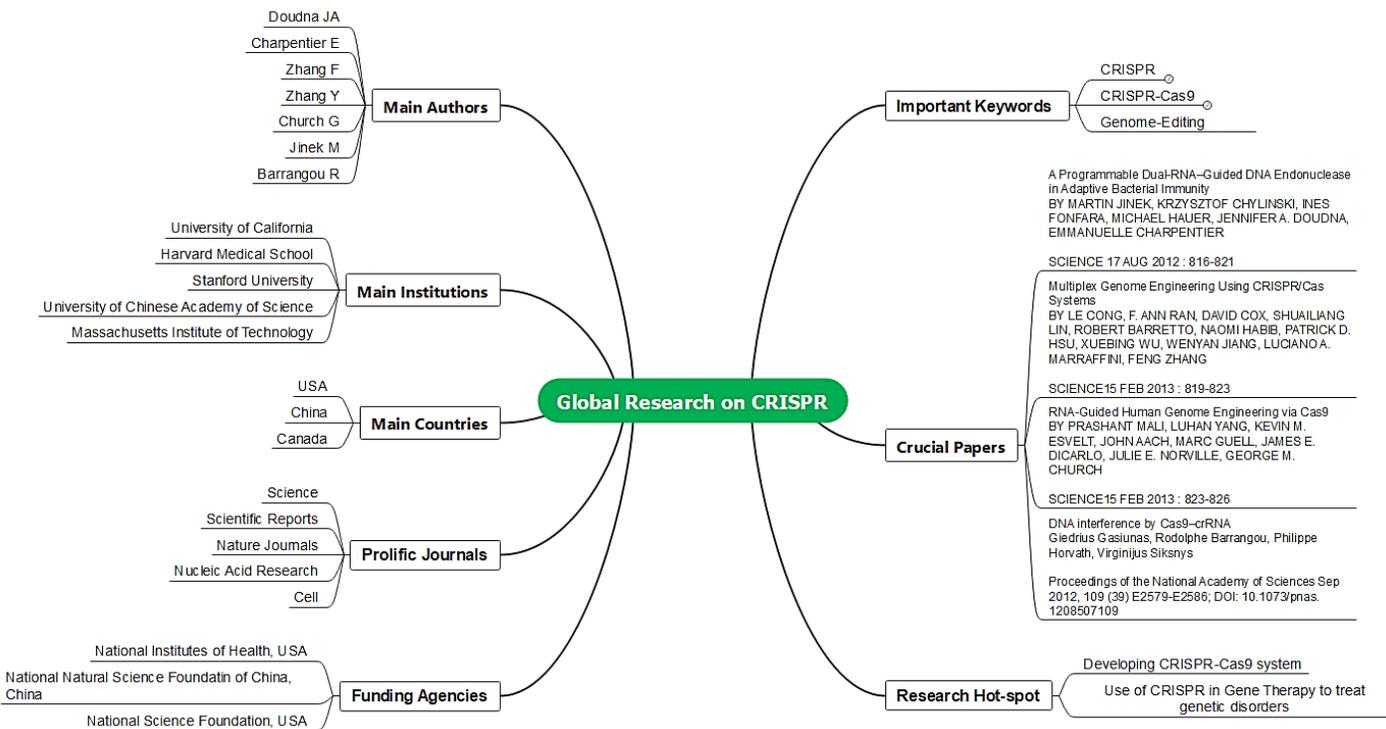


Figure 9: Findings on Global CRISPR Research.

Table 5: Most relevant affiliations.

Affiliations	Number of papers
University of California	1056
Harvard Medical School	379
Stanford University	277
University of Chinese Academy of Sciences	237
Massachusetts Institute of Technology	200
Huazhong Agricultural University	172
Harvard University	168
University of Toronto	158
Broad Institute Of Mit And Harvard	148
University of Georgia	137
University of Washington	137
Baylor College of Medicine	136
Sun Yat-Sen University	130
University of Massachusetts Medical School	123
North Carolina State University	122
Osaka University	122
University of Copenhagen	120
University of Minnesota	117
University of Pennsylvania	114
Seoul National University	110

key component of the CRISPR system, an RNA molecule that recognizes phage sequences, in the bacterium and reported this in 2011. And in the same year, Charpentier teamed up with Doudna. They isolated the CRISPR–Cas9 system components and demonstrated that the system could be programmed to cut specific DNA sequences. Other researchers are recognized as key contributors in the development of CRISPR. They include Feng Zhang, George M Church, and Jinek M. Zhang F received 2402 citations and are at 3rd position in the list. In 2013 Zhang and his team modified the CRISPR–Cas9 system to make precise genome cuts in human and mouse cells. Another profound work is by George Church, who received 1326 citations; Church and his team described RNA-guided human genome engineering via Cas-9 around the same time (early 2013). Jinek M is also among the highly cited authors with 2002 citations and 5th position; he is the first co-author on the landmark *Science* paper.^[16] However, biochemist Virginijus Siksnys at Vilnius University in Lithuania has received only 645 citations, but his work with the team is crucial. Work by Siksnys is similar to Doudna and Charpentier's, and he shared the 2018 Kavli Award in Nanoscience with Doudna and Charpentier. Other authors who appear prominently in the orange cluster are Hsu PD, Lin S, Ran FA, Chylinski K, Mali P, and Esvelt KM. In the green cluster, prominent authors other

than the abovementioned are Horvath P, Moineau S, Jore MM, Brouns SJ, Koonin EV, Boyaval P, Romero DA, and Terns RM.

Top 20 institutions

The top 20 institutions affiliating work on CRISPR are listed in Table 5. The University of California contributed (1056 papers), Harvard Medical School (379 papers), Stanford University (277 papers), University of Chinese Academy of Sciences (237 papers), and Massachusetts Institute of Technology (200 papers). All of them made significant contributions to the research on CRISPR. It is worth noting that 13 of the top 20 institutions are from the United States, demonstrating outstanding contributions from United States institutes in the field of CRISPR. Institutes from three East Asian countries China (3 institutes), Japan (1 institute), and South Korea (1 institute), are also in the top 20 list. There is one Canadian and one Danish institute as well in the list of top 20 institutions.

Country-wise Scientific Productions

Figure 5 represent country wise scientific production of CRISPR during 2005-2021. Based on this analysis, publications on CRISPR come mainly from the USA, with 10389 total publications, similar to the results reported by Rahaman MS, *et al.*^[36] China identified as the second leading contributor (6222 papers), followed by Japan (1366 papers), Germany (1315 papers), UK (1004 papers), and South Korea (938 papers). The USA also seems to be producing the most citable work (received total citations 138696), followed by China (37749 citations), France (9711 citations), and Japan (8675 citations). The analysis reveals the USA is the most productive country in terms of frequency of publications and citations.

Author-Affiliation-Country (Three field plot)

Figure 6 shows a three fields plot is created with the countries in the middle, authors in the left, and affiliations in the right with the number of items limited to 10 for all three fields. The strong relation between the authors, affiliations and the contributing countries is observed in the Figure. Lines represent cooperation between author-country-affiliation, and there is a direct proportion of the thickness of lines to the strength of collaboration. The size of the nodes corresponds to the significance of the country's contribution to the Study in CRISPR.

It can be seen in the Figure that the United States and has made a great contribution; this result aligns with analysis obtained in country-wise scientific production. The USA collaborated with all universities and authors in the plot. The total collaboration of the USA is 14905. The widest band of 7660 collaborations is seen between the USA and the University of California. Wide bands next inline are between the USA and Harvard Medical School, Stanford University, Massachusetts Institute of Technology, Harvard University, Broad Institute of MIT and Harvard, University of Georgia, and the University of Toronto

Table 6: Funding agencies.

Rank	Funding agencies	Country	Papers
1	National Institutes of Health	USA	1364
2	National Natural Science Foundation of China	China	1220
3	National Science Foundation	USA	370
4	National Institute of General Medical Sciences	USA	340
5	Japan Society for the Promotion of Science	Japan	268
6	National Basic Research Program of China (973 Program)	China	224
7	National Cancer Institute	USA	223
8	National Institute of Allergy and Infectious Diseases	USA	176
9	Chinese Academy of Sciences	China	174
10	European Research Council	Belgium	171

with 2238, 1540, 1313, 1040, 906, 520, and 225 collaborations respectively. Huazhong Agricultural University has the least collaboration with the USA, i.e., only 48 collaborations. The USA has collaborated with all the authors in the plot, but China's collaboration with authors is intense. Zhang Y, Li Y, Wang Y, Liu Y, Wang X, Wang J, Chen Y, and Li J has 584, 488, 674, 559, 467, 440, 449, and 510 collaborations with China, respectively. China's least collaboration with the author is 429 (China-Zhang X); however, this number is much more significant than the highest USA-author, i.e., USA-Zhang Y with 288 collaborations. China's institutional collaboration is weaker compare to the USA's. China's highest institutional collaboration is 964 with the University of Chinese Academy of Science. Next is Huazhong Agricultural University and the University of California, with 467 and 254 collaborations, respectively. The USA collaborates more with institutes instead of authors, whereas China prefers collaboration with individual authors compared to institutes.

Canada collaborated with five institutes obtaining its highest collaboration 512 with the University of Toronto. Further accompanied by the University of California, Stanford University, Massachusetts Institute of Technology, and University of Chinese Academy of Science with 87, 17, 7, and 2 collaborations, respectively. Though the number of collaborations are few, all authors have collaborated with Canada. Zhang Y, Wang X, and Zhang X collaborated 15, 11, and 10 times respectively, with Canada.

Eventually, it appears that the USA is the most remarkable country in terms of collaborations. Zhang Y appears to be the most prolific author in terms of his collaboration with countries. The University of California has significant collaborations with countries compared to other institutes.

Research Trends (By keyword analysis)

The research trend identified by analyzing keywords from the evidence of Scopus-based procured documents (on CRISPR research). It has been found that there are 24615 indexed

keywords (Keyword Plus) and 11393 keywords assigned by the authors. However, the analysis was conducted to identify the research trend by considering author keywords. Keyword Plus gives random keywords, whereas author keywords are more relevant to the topic.

The extracted keywords have problems, 1) Case difference such as "CRISPR-Cas9" and "crispr-cas9", which was solved by case conversion. 2) Inconsistent connectors such as "crispr-cas systems" and "crispr cas system" similarly "CRISPR-Cas9" and "CRISPR/Cas9", which is resolved in further analysis. 3) Few words have the same meanings, such as "genome editing," "genome engineering," and "gene editing"; similarly, "human" and "humans." 4) Many keywords are special terms in CRISPR, and the general dictionary is not applicable for the study. Since all analyses use keywords, the limitations have remained in this article without any other forms, which may be improved in future studies.

Word Cloud by author keywords and keywords plus

Figure 7 examined author keywords and keyword plus in the filed of CRISPR. Word cloud displays that the author's keyword "crispr/cas9" has the highest occurrence, while the word "crispr" and "genome editing" are next in line. Next author keywords are "crispr-cas9", "cas9", and "gene-editing". Technically these terms (*crispr/cas9*, *crispr-cas9*, *crispr*, *cas9*) (*gene-editing* and *genome editing*) are similar. From this, we can derive that "crispr-cas9" and "gene-editing" are high occurrence keywords. Closer inspection of word cloud displays similar words such as "crispr-cas," "genome engineering," "gene therapy," suggesting that the use of CRISPR-Cas9 as a gene-editing tool is the hot spot in this research field.

Among the word cloud by keyword plus "gene-editing," "crispr cas system" are the most prominent keywords. Other keywords appearing densely are "genetics," "human," "humans," "human cell," "male," and "female," indicating that majority of the published CRISPR studies are done on humans. The appearance of

"nonhuman," "animal," "mice," "mouse," and "animal experiment" reflect the importance of animal models in the Study of CRISPR. Few keywords appeared as insignificant to the topic and are random such as "article" and "priority journal," still we can assume that the appearance of these words in the cloud represents that most of the published work is in journals in the form of article. "procedure" indicates procedural work, and "guide" in this area mainly refers to guide RNA.

Co-occurrences of author keywords

The interconnection of keywords is determined based on the number of documents they occur together. Co-occurrence selected from "types of analysis" and author keywords chosen from a "unit of analysis"; Counting method: full counting/ Fractional counting, and minimum (6) occurrence of a keyword considered for analysis. Out of the (4255) keywords, (151) meet the thresholds. For each of (151) keywords, the total strength of the co-occurrence links with the other keywords calculated, and the keywords with the greatest total link strength was selected. Full item found (151), cluster (10), links (1493), and total link strength (3754). Some of the keywords are repeated due to different approaches in entering the keywords in the system (e.g., use of the hyphen, dot, comma, dash, slash, and nous). Therefore, this issue has been addressed by merging the same keywords and updating the software clusters. Which resulted in (71) item, clusters (6), links (691), and total link strength (2640) as shown in the Figure 8.

The size of the circle is directly proportional to the occurrence of that keyword. Keyword co-occurrence relationship can reflect the internal connection between keywords.

Cluster 1 (*red*) represents 23 author keywords (Adaptive Immunity, Archaea, Bacteria, Bacteriophage, Cas, Cas6, Cascade, Clustered Regularly Interspaced Short Palindromic, Repeats (Crispr), Cpf1, Crispr, Crispr-Cas Systems, Crispr/Cas, Crna, Evolution, Guide Rna

Horizontal Gene Transfer, Immunity, Interference, Off-Target, Pam, Rna, Rna Interference and Spacer).

Cluster 2 (*green*) consists of 14 author keywords (Crispr/Cas9, Dna Repair, Drosophila, Genome Engineering, Genotyping, Homologous Recombination, Knock-In, Knockout, Microinjection, Mutagenesis, Nhej, Recombination, Talen, and Zebrafish).

Cluster 3 (*blue*) corresponds to 11 author keywords Crispr Interference, Crispr, Dcas9, Escherichia Coli, Gene Expression, Gene Regulation, Grna, Metabolic Engineering, Saccharomyces Cerevisiae, Synthetic Biology, and yeast.

Cluster 4 (*Yellow*) consists of 9 author keywords (Crispr/Cas9 System, Gene Knockout, Gene, Targeting., Genome Editing, Mouse, Plants, Rice, Talens, and Targeted Mutagenesis).

Cluster 5 (*purple*) represents eight author keywords (Biotechnology, Cas9, Crop Improvement, Functional Genomics, Genetics, Homology-Directed Repair, Rnai, and Sgrna).

Cluster 6 (*light blue*) includes six author keywords (Cancer, Clustered Regularly Interspaced Short Palindromic Repeats, Gene Editing, Gene Therapy, Genetic Engineering Induced Pluripotent Stem Cells).

Author keyword co-occurrence is helpful to analyze the research hotspots of the CRISPR-related studies by using the network analysis method. In the network, "Crispr," "Genome Editing," "Crispr/Cas," "Cas9", "Crispr/Cas9" are located in the center of the network, and the size of the circle representing them is bigger, indicating that these keywords are widespread and there is high co-occurrence. Thus, they are the core research contents. CRISPR/Cas9 and gene editing have large cross-study. "Zebrafish", "sgRNA", "crRNA", "Talen", "Gene Therapy", "Guide RNA", "Pam" "Homologous Recombination" are important research areas.

Funding Agencies

Many financial institutions and agencies identified for the production of CRISPR research, out of which the top 10 highly productive funding agencies are listed in Table 6 with respect to the number of research financed. 'National Institutes of Health' from the USA funded 1364 research and bagged the first potions in the top 10 list, followed by 'National Natural Science Foundation of China from China, produced 1220 research, 'National Science Foundation' (370 papers), 'National Institute of General Medical Sciences (340 papers), and 'Japan Society for the Promotion of Science' financed 268 research. The 'European Research Council' from Belgium funded 171 research and ranked 10th in the list.

CONCLUSION

The finding of analysis of CRISPR research revealed that most productive institutes and authors come from America and China, and the high frequency of keywords "*crispr/cas9*" and "*genome-editing*" shows that these are hot spots as shown in the Figure 9. CRISPR's research trends include discovering new CRISPR/Cas9 systems, methods for specific gene editing, and the application of CRISPR/Cas9 in gene therapy and cancer therapy. Furthermore, according to the visual analysis results and critical reading of highly cited references, we predict the future research direction. The study on the use of CRISPR for treating genetic disorders will increase exponentially in the near future. Besides, more genetically modified food will turn up, and new quality measures and verifications will step forward. Discussion trends will shift from basic research to commercialization of the system. There are tremendous opportunities for a molecular biologist to work in this field.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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