RaDiChal'21
FINAL

2021 Rare Disease Challenge

Study of the Correction of The Single Nucleotide Mutations in MEFV gene in Autologous CD34+ Hematopoietic Stem Cells to Producing Functional Protein for Treating FMF Disease With Prime Editing

FMForce

Affiliation

Bilal BOSTAN[1], Hacer AZAK[1], Merve SOYYİĞİT[1], Ömer Faruk AYDOS[1]
[1]Necmettin Erbakan University , Meram Faculy of Medicine

Abstract

FMF; It is an autosomal recessive genetic disease seen in Mediterranean populations. FMF, which is the most common autoinflammatory disease, is mostly seen in Turkey in the world.

The main cause of Fmf is the non-functional production of pyrin protein due to single point mutations in the MEFV gene. Expression of the pyrin protein occurs at a high rate in peripheral blood leukocytes, especially mature granulocytes and monocytes. (Figure 2). In this study, we proposed a new treatment idea for FMF disease, which has no definite cure. Our project offers the promise of correcting single point M680L, M680V, M680IGC, M680IGA, G687D, Y688C, Y688F, Y688X, M694V, M694L, M694K, M694I, K695R, K695N, V726A with the PE2 system, thus covering 70% of FMF patients.

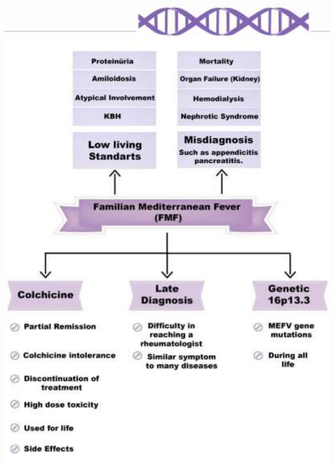


Figure 1:FMF Problem Tree.

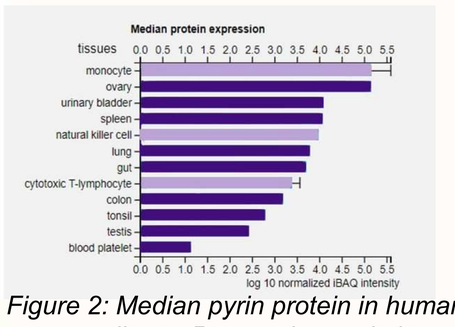


Figure 2: Median pyrin protein in human according to Proteomics website.

Our study was designed as transferring CD34+ HSCs obtained from the patient to the patient after proving that they were regulated with PE2 system elements in vitro and produced functional pyrin. Transfer of PE2 elements will be done by neon transfection method. In addition, several systems with different pegRNA and electroporation parameters will be tried and the most efficient will be found. Animal after cell studies in vitro Human clinical trials will be initiated if the trials result in a positive result.

Projects

Our study consists of 3 main steps; Including in vitro ready-made HSC studies, animal trials and human clinical trials. The following steps will be followed for the study of editing single point mutations causing FMF disease.

1-) The patient who has been found to have the mutations we intend to correct; CD34+ hematopoietic stem cells from peripheral blood will be isolated by stem cell apheresis.

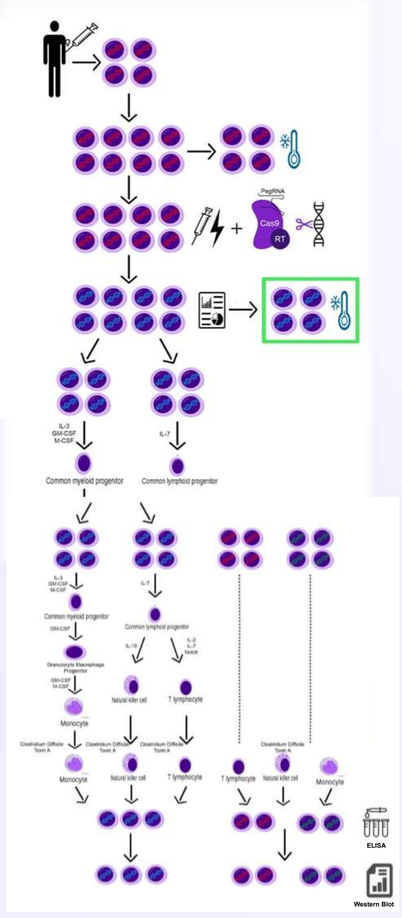
2-)Cultured isolated cells will be replicated. Some of the cells will be frozen and stored.

3-) Plasmids containing target mutation-specific elements of the Prime Editing 2 system (pegRNA and Cas9) will be transferred to cells through the Neon Transfection s ystem.

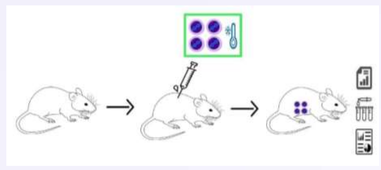
4-)Sanger-TIDE sequence analysis of the regulated cells will be performed.

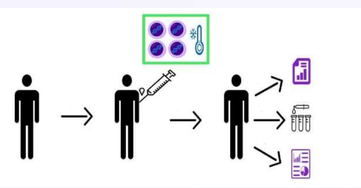
5-) CD34+ hematopoietic stem cells will be differentiated into monocyte, T lymphocyte and natural killer cells that can synthesize Pirin protein by giving the necessary cytokines. Differentiating cells will be stimulated with toxin A.

6-)After stimulation, pirin protein will be isolated from the cellsThen,its functionality and quantity will be analyzed with ELI SA and Western Blot methods



Differentiation will also be achieved in healthy and mutant hematopoietic stem cells. Pirin and IL analyzes; Physiological and pathological conditions will be evaluated after performing Western blot and ELISA. The results of the study will be evaluated by providing positive control of our genetic regulation study.
In case of a positive result of the study, Clinical Animal Trials and Clinical Human Trials will be staged.



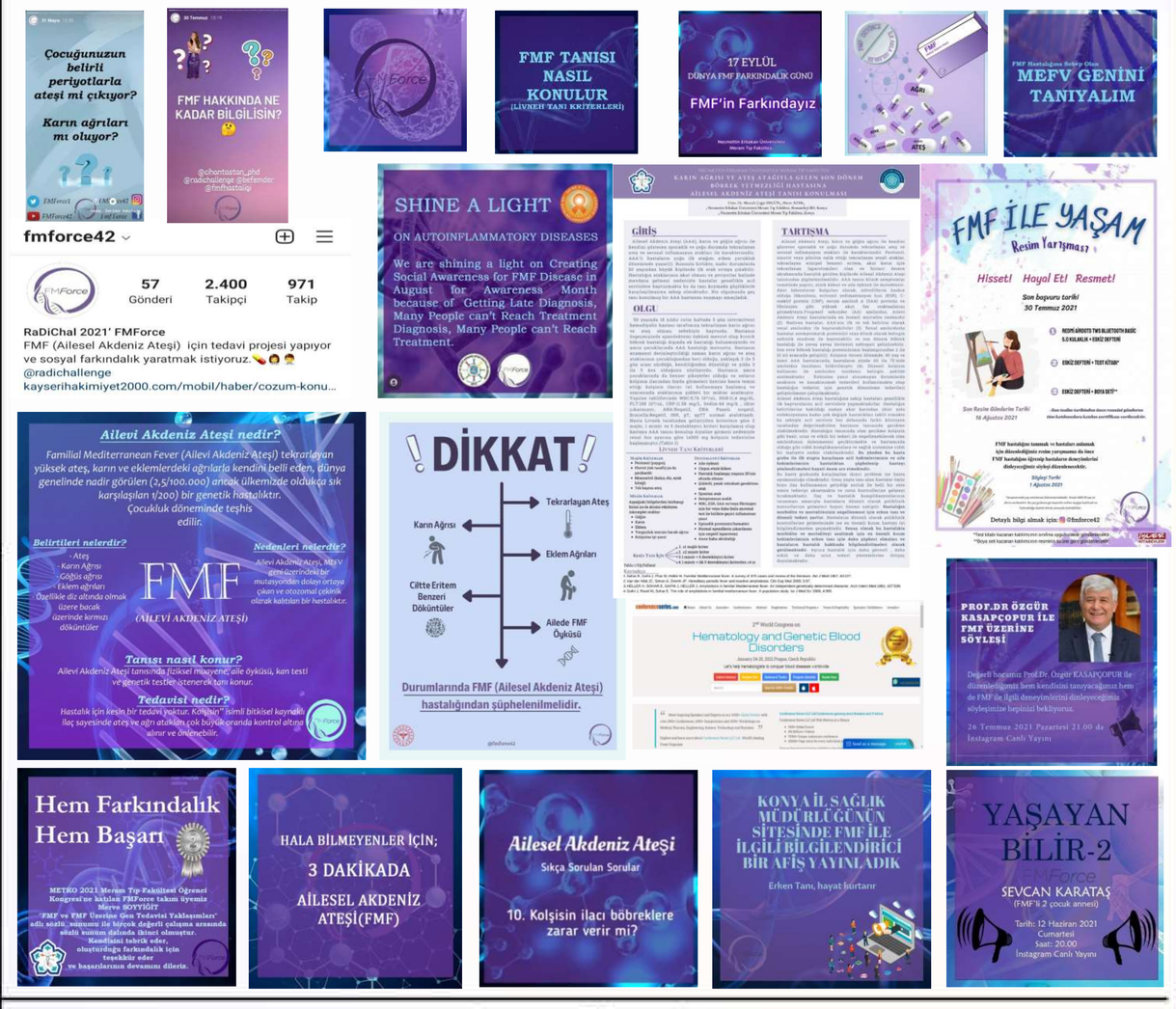


Social Impact

@fmforce42 @fmforce1 FmfForce

Followers: 2400 Likes: 5038 Sharings: 369 Watchs: 109.000

We have organized numerous activities in order to make more people aware of FMF disease and to help them understand FMF patients on this path we set out to create social awareness. Many national and international events such as publications, posts, informative reels on Instagram, publishing posters on the website of the Konya Provincial Health Directorate, congress presentations, preparing scientific posters, publishing news in the newspaper, cooperation with associations for the World FMF Day, acceptance from the international genetics congress, painting competitions. We organized.



Conclusion

Although FMF is considered a rare disease in the world, it is a disease that is too common to be considered rare in Turkey. Although it is a disease that does not have a very high risk of death and has a cure, although it is not certain, these treatments do not work in some patients. At this point; A definitive, genetic treatment is needed.

As a result of the studies, it has been shown that FMF symptoms can be relieved after Allo-SCT.We developed an ex vivo treatment for efficient gene targeting in human HSCs using prime editing 2 and neon transfection system.

An efficient study has been planned using the Neon Transfection System, which has proven itself in genetic editing on HSCs and using pegRNAs targeting 15 different, most common and most severe mutations. Although CRISPR technology is still a new technology, the fact that it allows easy and unique gene editing, the fact that the study is carried out ex vivo and the proof of concept steps are designed considering physiological and pathological conditions and possible risks gives hope for this treatment.

References

1. Promise of gene therapy to treat sickle cell disease. Zulema Romero 1, Mark DeWitt 2, Mark C Walters 3. 11, s.l. : Expert Opinion on Biological Therapy, 2018-11, Cilt 18, s. 1123-1136.
2. Highly Efficient Genome Editing of Murine and Human Hematopoietic Progenitor Cells by CRISPR/Cas9. Michael C. Gundry, Lorenzo Brunetti, Angelique Lin, Allison E. Mayle, Ayumi. 5, s.l. : Cell Reports, Cilt 17, s. 1453-1461.
3.ProteomicsDB.[Çevrimiçi]
4. Allogeneic bone marrow transplantation: cure for familial Mediterranean fever. John Milledge, Peter J. Shaw,. 3, 2002-08-01, Blood, Cilt 100, s. 774-777.
5. R&D SYSTEMS. Hematopoietic Stem Cells & Lineage-specific Markers: R&D Systems. [Çevrimiçi]
6. Infevers - Tabular list. Infevers. [Çevrimiçi

#RaDiChal 2021