RaDiChal'21
FINAL

2021 Rare Disease Challenge

eEXON sPLİCİNG by KNOCK-IN METHOD USING PHYTSSİLOGİCAL PROMOTER İN ömefv gene

Affiliation

Ayşenur SAYGILI*, Betül ÇAKMAK*, Kübra MAÇ*, Sena KIZILBOĞA*
*İNÖNÜ UNIVERSITY

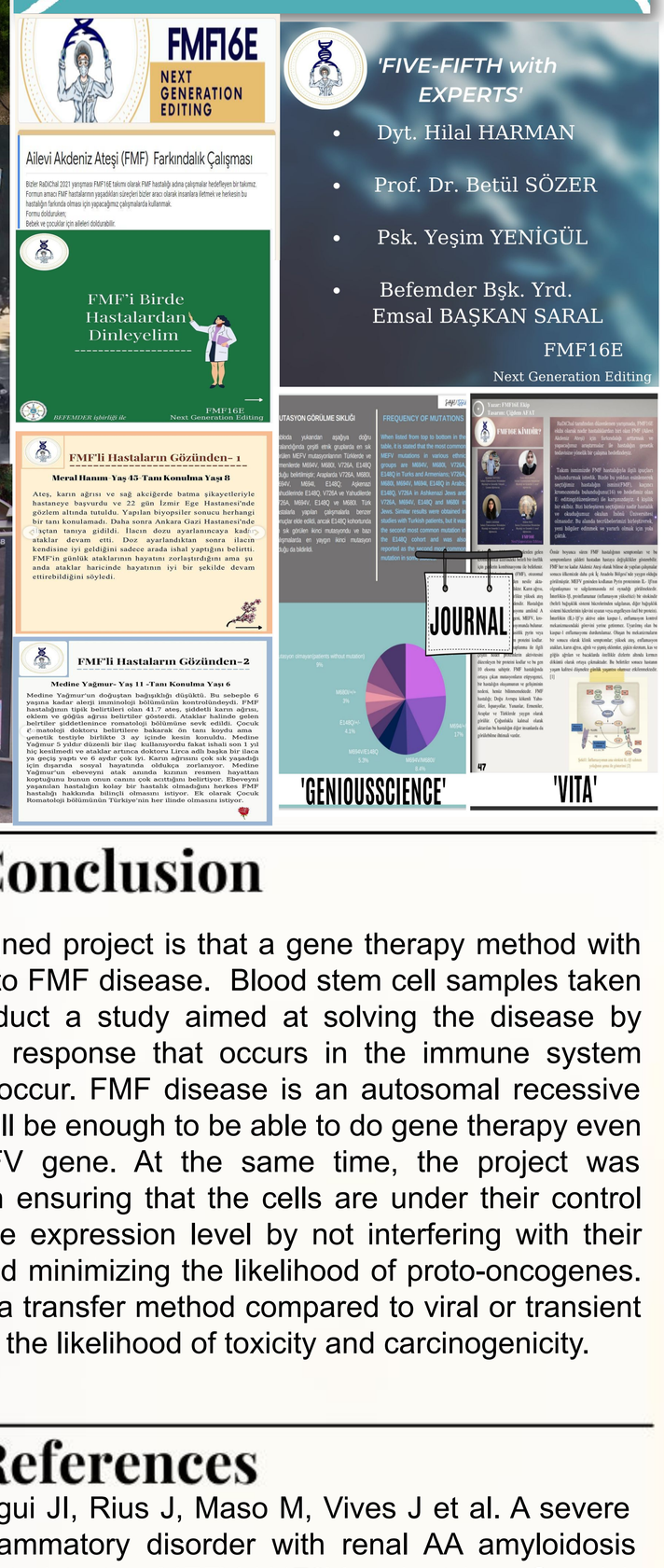
Abstract

FMF (familial Mediterranean Fever) disease is an autosomal recessive disease caused by mutations in the MEFV gene located in the short arm (P13.3) of chromosome 16 of the human genome(1). The MEFV gene consists of 10 exons and 781 amino acids and contains the codes of the Pyrin protein(1). Mutations are mostly caused by the MEFV gene 2., 3., 5. and 10th. it is found in their exons (2). Pyrin protein affects the inflammatory mechanism. As the PKN1/PKN2 proteins are unable to phosphorylate mutant Pyrin and subsequently 14.3.3 proteins cannot bind Pyrin, an inflammatory response results from the binding of ASC and Pro-caspases to Pyrin. (2). Since the main effect organ in FMF disease is blood, treatment with hematopoeitic stem cells is expected to be a solution to the disease. This designed project is designed to be carried out ex vivo and then in vivo. It is planned to keep the gene level interference to a minimum without interfering with the physiological promoter region of the gene and to perform all exons with HiFi-Cas9 protein in the CRISPR Knock-in method. It is known that the off-target mutation rate is reduced by 20 times by using HiFi-Cas9. The gRNAs to be used with HiFi-Cas9 have been designed more than once by considering different off target values and their specificity has been increased with the RNP method. Gene tracking will be provided by placing the GFP sequence at the end of the designed donor DNA. For GFP expression efficiency, P2A sequence was added and extra stop codon was added to prevent possible gene transcription. Donor DNA integration is strengthened with 800 bases homology arms added to the right and left parts. In the electroporation method stage, the transfer method was strengthened by using the 4D Nucleofector. At the proof of concept stage, the cytokine levels of the selected cells will be examined and control tests will be performed, taking into account the stem cell differentiation and MEFV gene activity. For in vivo clinical studies, it is planned to move on to human trials after control experiments are provided by examining certain symptoms using humanized MEFV model mice.

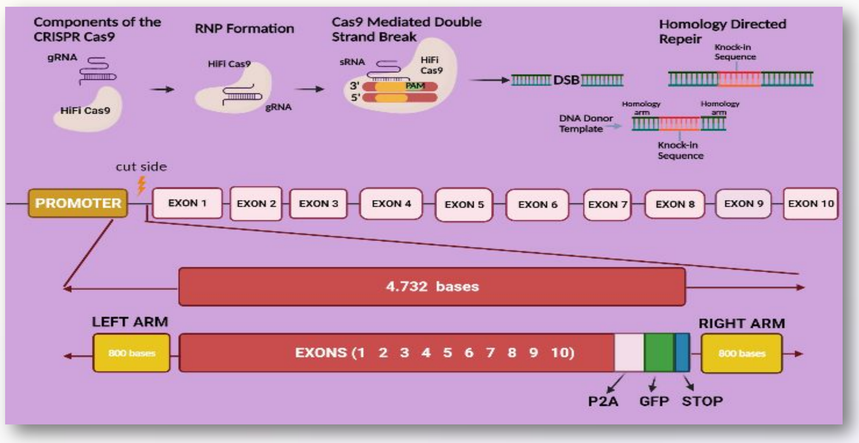
Social Impact

@fmf16e @EFmf16e FMF16E TEAM

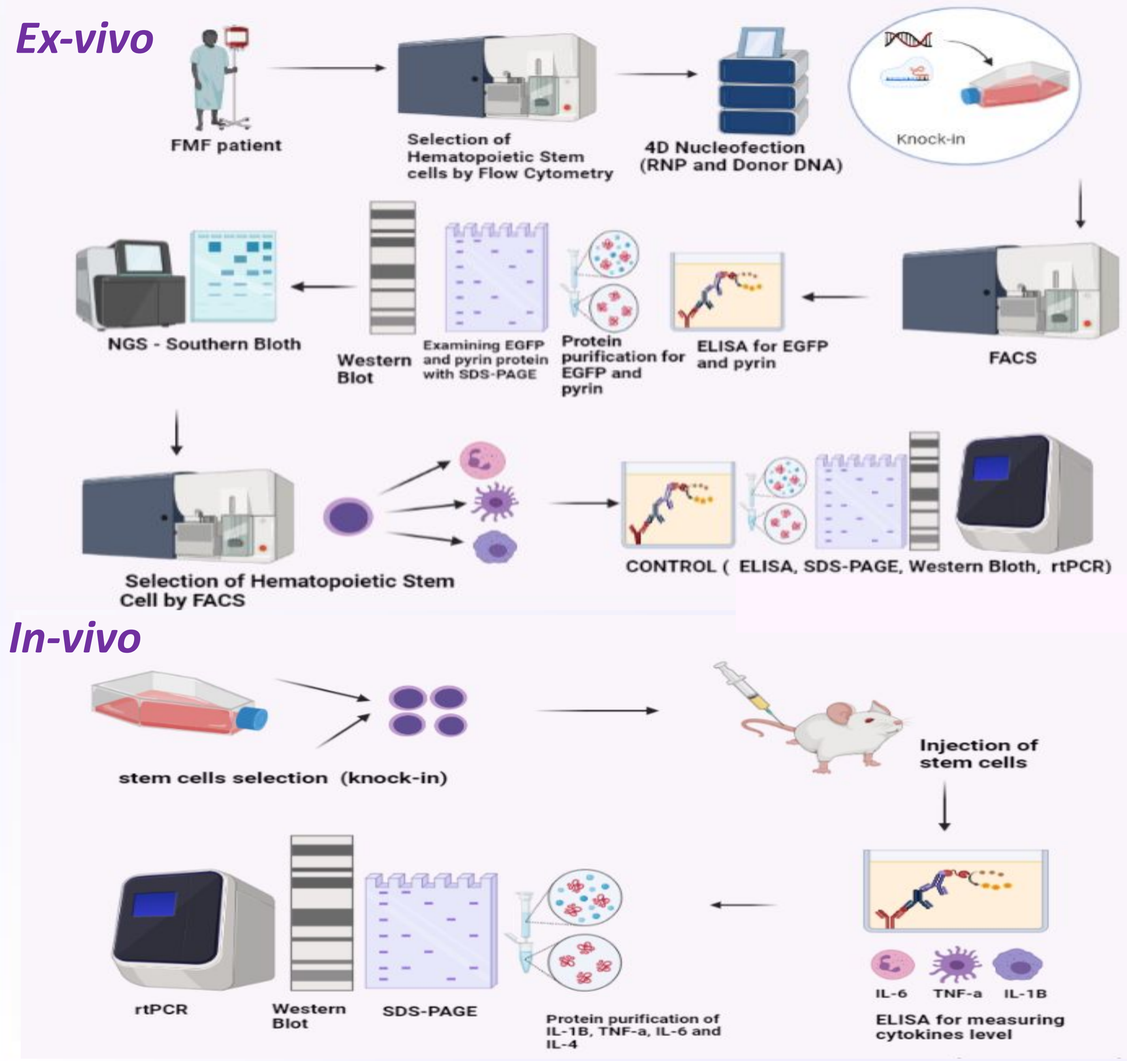
Followers: 325
Likes: 1.438
Sharings: 93
Watchs: 1.377
Reading of Journal: 500



Projects



Ex-vivo



In-vivo

Conclusion

The hypothesis in this designed project is that a gene therapy method with blood cells may be the solution to FMF disease. Blood stem cell samples taken from patients are used to conduct a study aimed at solving the disease by ensuring that the inflammatory response that occurs in the immune system caused by mutations does not occur. FMF disease is an autosomal recessive disease, so it is thought that it will be enough to be able to do gene therapy even for just one copy of the MEFV gene. At the same time, the project was developed with the idea of both ensuring that the cells are under their control without the need to regulate the expression level by not interfering with their promoter region of the gene, and minimizing the likelihood of proto-oncogenes. The use of the RNP method as a transfer method compared to viral or transient methods is intended to minimize the likelihood of toxicity and carcinogenicity.

References

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3. Kang, SH., Lee, Wj., An, JH. et al. Prediction-based highly sensitive CRISPR off-target validation using target-specific DNA enrichment. Nat Commun 11, 3596 (2020).

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