RARE DISEASE CHALLENGE
RaDiChal'21

Pre-Project Report

TEAM NAME

Genes Of The Future (GOF) Team

TEAM MEMBERS

Abdurrahim TOPRAK

Beyza Gül ATEŞLİ

Enes Buğra YALÇINKAYA

TARGET DISEASE

Family Mediterranean Fever (FMF)

1. Project Summary

Project Title: Increasing the expression of *MEFV* gene and Pyrin (Marenostrin) Protein by correcting c.2040G>C Transversion and providing c.2080A>G transformations with CRISPR Prime Editing Method.

In our project, we target M680I and M694V mutations in the 10th exon of the MEF gene, which is high worldwide. While doing our targeting, we also target c.2040 C transversion and c.2080 A>G Mutations and plan 17 mutations that are insufficiently targeted within the region containing 40 bp. We aim to achieve our targeting and using the CRISPR Prime Editing method.

In the *MEFV* gene, we intend to correct the c.2040G>C Transversion we have targeted with the CRISPR Prime Editing method and realize the M680I – M694V targets for c.2080A>G edits. At the same time, we target a total of 17 mutations with Prime Editing for those that have been investigated and validated to be pathogenic or less pathogenic with lower incidence than will occur between these approximately 40 bp. It is transmitted to all tissues in myeloid structure, as root in BM (Bone Marrow) and in the tunnel tunnel. Peripheral main white blood maker is those in the blood. The hematopoietic stem cell (HSC) is a multipotent cell that has both the potential for self-renewal and creates all cancer competition. We rely on the expressed myeloid application of the *MEFV* gene. Our research is by hand observation from the production in production in the myeloid production we are targeting. We aim to form hematopoietic root, from teaching to cultivate more difficult than direct myeloid implantation. In our project, we target the M680I and M694V mutations that occur in the 10th exon of the *MEFV* gene, which is common and has a high worldwide prevalence (incidence, %). While doing our targeting, we target c.2040 G>C transversion mutation and c.2080 A>G Mutations and 17 low incidence mutations in the region containing 40 bp between these insertions. The project is CRISPR Prime Editing (PE), a transcriptional and fully functional editing method. PE consists of major guided RNA (pegRNA consisting of primer region (PBS) forming the sequence contained in PAM native RNA (PAM)) and nCas9(H840A)-reverse transcriptase source (RT), which consists of reverse transcriptase pattern. We build RISP, to reach the mutations we target.

2. Problem:

Familial Mediterranean fever (FMF) is a recessively inherited systemic autoinflammatory disease. Familial Mediterranean fever (FMF) is the most common autosomal recessive autoinflammatory disease (AID) in populations predominantly in the Eastern Mediterranean regions. It is a recessively inherited disease characterized by recurrent, self-limited fever, episodes of serositis, and infiltration of affected tissues by multiple neutrophils (Bernot et al. 1998), (Tidow et al. 2000), (Gangemi et al. 2018). It is characterized by an uncontrolled activation of the innate immune system, resulting in short-term fever and serositis, chest, abdominal, joint and muscle pain (Gangemi et al. 2018).

Familial Mediterranean Fever (FMF) is clinically characterized by attacks lasting between 24-72 hours. The *MEFV* gene that causes this disease is a part of 10 exons in the short

arm of the 16th chromosome and encodes a protein called pyrin, which consists of 781 amino acids. This disease occurs as a result of mutations in the *MEFV* gene (Kasifoglu et al. 2013). They observed more severe disease expression and increased susceptibility to amyloidosis in patients with a specific *MEFV* mutation that changes amino acid 694 of the pyrin protein from methionine to valine (M694V) (Turkish FMF Study Group, 2005). It is transmitted in an autosomal recessive pattern and mostly affects ethnic groups living around the Mediterranean basin: non-Ashkenazi Jews, Armenians, Turks and Arabs. The gene responsible for FMF (*MEFV*) was mapped to the short arm of chromosome 16 (16p13.3) and the gene range was gradually narrowed to a very small 60 kb range at the time the gene was identified (Bernot et all. 1998). Almost all patients have to use colchicine for life. An ancient anti-inflammatory drug, colchicine is an alkaloid obtained from a plant called Colchicum Autumnale, which is very common in South-Central Europe and North-Central Italy. Goldfinger noticed an improvement in the clinical condition of patients with gout and FMF with the use of colchicine since 1972. It has become the most preferred drug for prophylaxis against FMF and to reduce the risk of amyloidosis (Cerquaglia et al. 2005). Although FMF is a self-limiting disease, it can become complicated as amyloidosis becomes a serious disease. Amyloidosis secondary to FMF is characterized by the polymerization of serum amyloid A (SAA), an acute phase protein, into amyloid fibrils and their accumulation in multiple organs (liver, spleen, heart, and testicles), primarily the kidney, leading to chronic renal failure. This drug slows down the rate of deterioration of kidney function and reduces proteinuria in cases of renal failure due to amyloidosis. By controlling FMF attacks, colchicine indirectly reduces SAA levels and directly inhibits the organization and deposition of amyloid fibers (Cerquaglia et al. 2005).

There are also patients who are resistant to colchicine. The side effects of colchicine are well defined as a result of studies. The most common side effects were stated as gastrointestinal intolerance, diarrhea, nausea and vomiting. Chronic use of colchicine has been proven to cause blood cytopenia, liver dysfunction and myopathy, especially when combined with other drugs such as statins, cyclosporine and clarithromycin. Although the side effects of colchicine are well known, the prevalence and risk factors of its side effects in FMF have not been studied in any systematic study. In addition, studies have shown that colchicine intolerance inhibits FMF management and increases complications of diseases such as amyloidosis as a result of suboptimal dosing. In conclusion, colchicine intolerance covers an important area in the treatment of FMF patients. Suboptimal colchicine dose due to drug intolerance may lead to the development of disease complications due to inadequate control of attacks and chronic inflammation. It is predicted that the use of IL-1 antagonists in such patients may reduce the development of future complications after careful evaluation of the factors that may contribute to the complications (Satis et al. 2020).

3. Solution

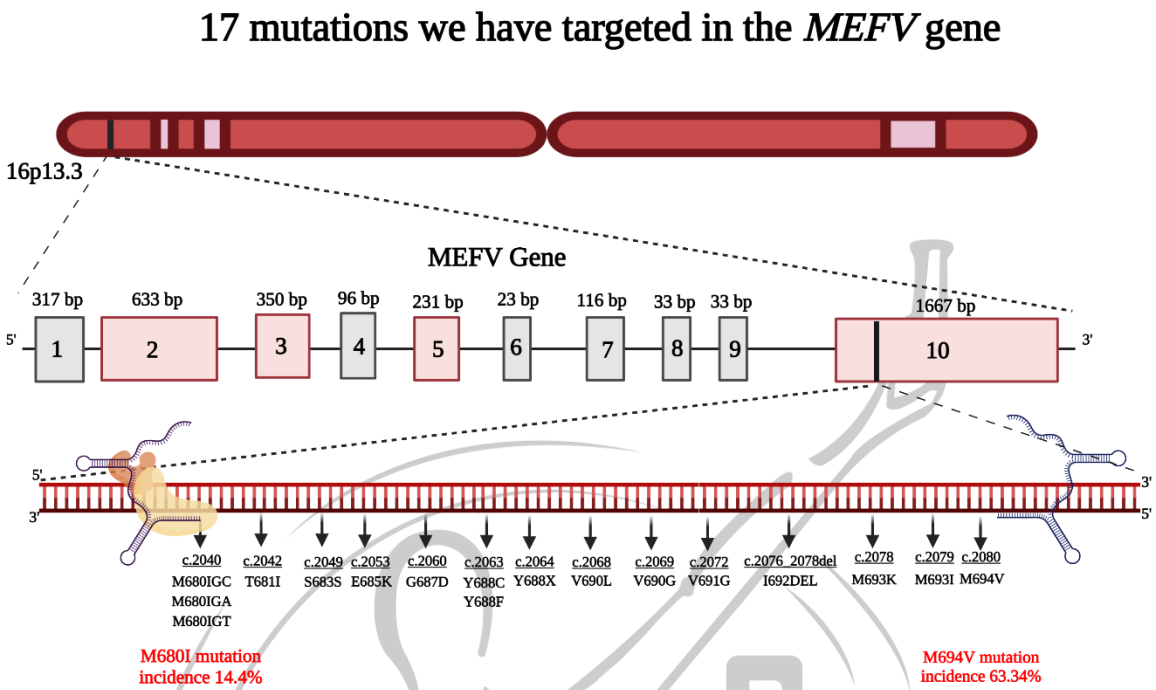

Figure 1. Display of 17 mutations we targeted between c.2040 – c.2080 in the *MEFV* Gene

Considering the Off-target and Base editing scores of Base Edit DNAs, we created an edit site in exon 10 of the *MEFV* gene. This part we will edit includes both the M680I mutation and the M694 mutation. While targeting, we also targeted the c.2040 G>C transversion mutation and the c.2080 A>G mutations and a total of 17 mutations with a low incidence occurring within the 40 bp-containing region between these regions.

Table 1. Mutations that occur frequently on the 10th Exon in the *MEFV* Gene and their incidence (%) (2005 – 2017)

M694V (%)	M694I (%)	M680I (%)	V726A (%)	E148Q (%)	Number of Samples Examined	Resources
63,34	0,83	14,94	9,87	9,59	1835	(Torun et all. 2017).
46,26	-	5,97	13,43	16,41	67	(Erden et all. 2008).
51,7	0.39	17,3	17,57	5,88	197	(Diri & Koşan. 2010).
31,72	1,08	12,9	3,76	9,68	93	(Yeşilada et all. 2005).
18,3	1,9	6,7	8,6	30,8	104	(Evliyaoglu et all. 2009).
36,69	1,83	3,66	13,45	32,71	560	(Dönder, et all. 2012).

There are mutations with high prevalence in Exon 10 and Exon 2 observed in the *MEFV* gene in Table 1. In our country and in the world, the prevalence of mutations is observed as a result of our research. As a result of our research on M694V and M680I mutations on exon 10, which is one of the mutations with a high incidence, we are targeting this mutation range because of the relatively higher incidence of other mutations.

We aim to correct M680I – M694V mutations in the *MEFV* gene, where mutations that cause FMF disease occur, by correcting the c.2040G>C Transversion we have targeted with the CRISPR Prime Editing method and providing c.2080A>G transformations. In addition, since this correction will occur between approximately 40 bp, we actually target a total of 17 mutations with the Prime Editing method for the pathogenic or less pathogenically investigated and validated mutations with a low incidence. We are based on the realization of this correction

and the myeloid cells in which the *MEFV* gene is expressed. In line with our research, we have observed that the myeloid cells we have targeted are derived from hematopoietic stem cells in the production. We aim to edit hematopoietic stem cells, as we anticipate that it may be more difficult to obtain direct myeloid cells.

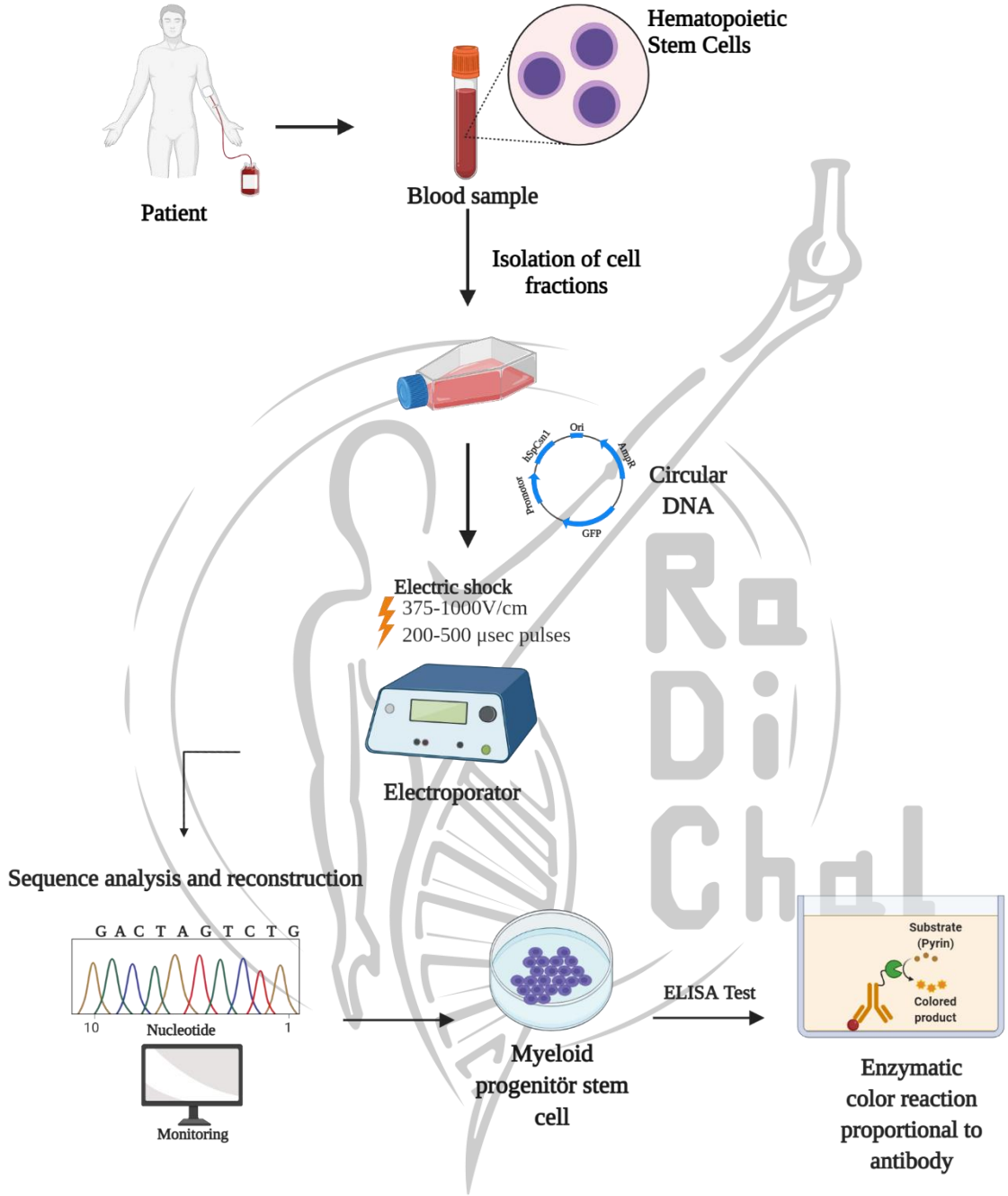

Figure 2. Schematic representation of our project work plan

HSCs obtained from peripheral blood taken from the patient are cultured in the laboratory environment. The cultured stem cells are repaired in the gene region where the M680I (c.2040) and M694V (c.2080) mutations that we have targeted with our pegRNAs occur, by treating them with the pX461 plasmid vector that we have designed. Hematopoietic stem cells, which are repaired, are given to the patient again. The circulating HSCs differentiate into myeloid cells and the disease state is eliminated.

4. Kaynaklar

[1] Bernot, A., Da Silva, C., Petit, J. L., Cruaud, C., Caloustian, C., Castet, V., ... & Touitou, I. (1998). Non-founder mutations in the MEFV gene establish this gene as the cause of familial Mediterranean fever (FMF). *Human molecular genetics*, *7*(8), 1317-1325.

[2] Cerquaglia, C., Diaco, M., Nucera, G., La Regina, M., Montalto, M., Manna, R. (2005). *Pharmacological and Clinical Basis of Treatment of Familial Mediterranean Fever (FMF) with Colchicine or Analogues: An Update. Current Drug Targets - Inflammation & Allergy, 4, 117-124.*

[3] Diri, N., & Koşan, C. (2010). Bölgemizdeki Ailevi Akdeniz Ateşi Tanısı ile Takip Edilen Çocuk Hastaların Sonuçlarının Değerlendirilmesi. *Uzmanlık Tezi, Atatürk Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Ana Bilim Dalı*. Erzurum (75).

[4] Dönder, A., Balahoroğlu, R., Çokluk, E., Şekeroğlu, M. R., & Dülger, H. (2012). Retrospektif moleküler bir çalışma: FMF ön tanısı alan hastalarda MEFV gen mutasyonları. Tıp Araştırmaları Dergisi, 10(3), 94-98.

[5] Erden, G., Bal, C., Güngör Torun, O., Uğuz, N., & Yıldırımkaya, M. M. (2008). Ailesel Akdeniz ateşi (Fmf) düşünülen olgularda MEFV gen mutasyonları sıklığının incelenmesi. Türk Hijyen ve Deneysel Biyoloji Dergisi, 65(1), 1-5.

[6] Evliyaoglu, O., Bilici, S., Yolbas, I., Kelekçi, S., & Velat, S. E. N. (2009). Diyarbakır yöresi Ailevi Akdeniz Atesli çocuklarda MEFV gen mutasyon sıklıkları. Dicle Tıp Dergisi, 36(2).

[7] Gangemi, S., Manti, S., Procopio, V., Casciaro, M., Di Salvo, E., Cutrupi, M., … Cuppari, C. (2018). *Lack of clear and univocal genotype-phenotype correlation in familial Mediterranean fever patients: A systematic review. Clinical Genetics, 94(1), 81–94.* doi:10.1111/cge.13223

[8] Kasifoglu, T., Bilge, S. Y., Sari, I., Solmaz, D., Senel, S., Emmungil, H., … Korkmaz, C. (2013). *Amyloidosis and its related factors in Turkish patients with familial Mediterranean fever: a multicentre study. Rheumatology, 53(4), 741–745.* doi:10.1093/rheumatology/ket400

[9] Satiş, H., Armağan, B., Bodakci, E., Ataş, N., Sari, A., Bilge, N. Ş. Y., ... & Tufan, A. (2020). Colchicine intolerance in FMF patients and primary obstacles for optimal dosing. *Turkish Journal of Medical Sciences*, *50*(5), 1337-1343.

[10] Tidow, N., Chen, X., Müller, C., Kawano, S., Gombart, A. F., Fischel-Ghodsian, N., & Koeffler, H. P. (2000). Hematopoietic-specific expression of MEFV, the gene mutated in familial Mediterranean fever, and subcellular localization of its corresponding protein, pyrin. *Blood, The Journal of the American Society of Hematology*, *95*(4), 1451-1455.

[11] Torun, D., Tekgöz, E., Kavuş, H., Öztuna, A., Akar, H., Çınar, M., ... & Tunca, Y. (2017). FMF hastalarındaki MEFV gen mutasyon sıklığı ve mutasyonların dağılımı: Tek bir merkezden geniş bir hasta grubunun analizi. Gulhane Medical Journal, 59(1), 24-27.

[12] Turkish, F. M. F. (2005). Familial Mediterranean Fever (FMF) in Turkey. *Medicine, 84*(1), 1-11.

[13] Yeşilada, E., Savacı, S., Yüksel, Ş., Gülbay, G., Otlu, G., & Kaygusuzoğlu, E. (2005). Ailesel akdeniz ateşi (fmf) düşünülen olgularda MEFV gen mutasyonları. Journal of Turgut Ozal Medical Center, 12(4), 235-238.