RARE DISEASE CHALLENGE
RaDiChal'21

PRELIMINARY REPORT

TEAM NAME

Genius

TEAM MEMBERS

Beril Ay - Efe Sarı - Seray Başak Bozkurt - Yaren Nur Demir

(Acıbadem University)

TARGET DISEASE

FMF (Familial Mediterranean Fever)

1. Abstract

Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by fever episodes and recurrent abdominal, chest or joint pains. The gene that plays a role in the pathogenesis of the disease is the MEFV gene, which encodes the pyrin protein. As a result of MEFV gene mutations, the pyrin protein causes more intense inflammation than normal. Our main point within the scope of our project is to create a disciplined treatment method where we can heal the most patients with the lowest burden. For this reason, we focused on the "Exon 10 M694V" mutation, which has been shown to cause amyloidosis due to the severe course of the clinical picture of Familial Mediterranean Fever and has the highest frequency in our population. We set the repair of the damaged gene in the cell as our main goal. In our project, we will target hematopoietic stem cells (HSC), and our gene design will be transferred to HSCs taken from patients ex vivo. As a gene transfer method, we aim to use the lentiviral transmission route, as it provides a more permanent gene expression and has a higher packaging capacity than AAVs. In order to prevent tumor formation that may occur as a side effect of lentiviral vectors, it is planned to produce viruses as self-inactivating. We plan to target the M694V mutation with the prime editing method, which has a lower off-target effect and higher efficiency than CRISPR-Cas9. Cas9(H840A) nickase will be used as the enzyme.

2. Problem:

2.1.FMF

Familial Mediterranean Fever (FMF); It is a disease characterized by recurrent abdominal, chest or joint pains as well as accompanying fever attacks and the development of amyloidosis over time. The disease is inherited autosomal recessively and is common in certain populations.

Significant fever and pain attacks are seen in FMF. The clinical picture of the disease creates recurrent attacks of fever and serositis. In 90% of the patients, the first attack occurs before the age of 20, which explains the fact that the disease is seen as a pediatric disease. Symptoms of the disease can be seen in 50% of patients within the first ten years of life, and in 5% after the age of thirty. Attacks usually occur suddenly without any symptoms and then disappear spontaneously. Although the clinical findings in attacks can be in various forms, the most common seizure type is a form of seizure in which fever, abdominal pain and joint symptoms are combined.[1,2]

2.2.Etiology

Understanding the symptoms that cause FMF and identifying the gene that causes FMF is a major step forward in recognizing this disease. Mutations in the "Mediterranean Fever (MEFV)" gene cause FMF and prove that this disease is inherited as autosomal recessive.

The cause of FMF is mutations in the MEFV gene located on chromosome 16. Most of these mutations are in exon 10. The M694V mutation in this exon is directly associated with amyloidosis, the severe phenotype of the disease. The M694V mutation is most common among Turkish and Sephardic Jews. [1,2]

2.3.Pathogenesis

The MEFV gene located on chromosome 16 encodes the pyrin protein. The MEFV gene is specifically expressed in monocytes and neutrophils and therefore plays a role in triggering inflammation of these cell lines. Mutated pyrin, which occurs as a result of MEFV gene mutations seen in FMF, causes more intense inflammatory attacks than normal. A 92 amino acid N-terminal fragment of pyrin (pyrin domain) plays an important role in normal pyrin function and FMF attacks. [1,2]

Pyrin proteins have an important role in inflammation and apoptosis. Both pyrin and co-pyrin associate with a common adapter protein known as ASC (apoptosis associated speck-like protein which contains a caspase recruitment domain), which activates caspase-1. Then, IL-1β and NFκB induced in this way trigger the proinflammatory response. While FMF patients are normally in a proinflammatory state, inflammation becomes evident during attacks. [3]

2.4.Clinical Findings

One of the most important symptoms characterized by FMF is fever. The fever can stay high for a few hours to four days, but usually subsides within 24 hours. Fever is often seen together with other clinical findings. Another common symptom of FMF is abdominal attacks. Abdominal pain usually begins a few hours before the fever starts and continues for 1-2 days after the fever subsides.

Joint attacks are the third most common symptom after fever and abdominal attacks. Arthritis/arthralgia occurs in at least half of the patients. Chest pain was reported in 30% of patients. Typical chest pains are usually severe and unilateral. The typical skin lesion seen in FMF is erysipelas-like erythema. [1,2]

Finally, the most important complication of the disease is secondary amyloidosis. The presence of amylodiosis determines the prognosis of FMF. [1,2]

2.5.Diagnosis

FMF is diagnosed with common clinical findings. It makes it possible to consider FMF in a patient with any type of serositis with recurrent and self-limiting episodes of fever for one or more days. [2] If the clinical diagnosis cannot be made definitively, genetic analysis is

performed for the MEFV gene in these patients. If genetic analysis cannot be performed, it is recommended to try colchicine, since FMF is the only periodic fever syndrome responding to colchicine. [4]

2.6.Treatment

Initial treatment of FMF is with colchicine.

Colchicine is primarily effective as a prophylactic treatment for FMF attacks. It is recommended for all patients regardless of the frequency and intensity of attacks. In addition, colchicine treatment is not effective in some individuals, particularly patients with the FMF subtype called colchicine-resistant FMF. Therefore, in the rare cases of heterozygous FMF patients who have been asymptomatic for several (more than five) years and do not show elevated acute phase reactants, it may be possible to discontinue colchicine. [5] Interleukin 1 receptor antagonist drugs are also used as an additional treatment in patients with amyloidosis who have been shown to be resistant to colchicine.

3. Solution

We plan to target the M694V mutation, which causes amyloidosis, one of the most severe complications among the mutations that cause FMF disease. Our gene therapy for the M694V mutation will also eliminate the declining prognosis rate due to amyloidosis, ensuring that FMF patients have the same average life expectancy as healthy individuals.

We target hematopoietic stem cells in all our gene therapy recommendations. Since the pathophysiological process that causes attacks in individuals with familial Mediterranean fever disease is mainly the damaged pyrin protein encoded in the MEFV gene produced in leukocytes, we believe that a gene therapy for hematopoietic stem cells will offer a solution with a permanent and high success rate.

In our project, we aim to correct the M694V mutation with Prime Editing (PE3).

It is aimed to use lentiviral vectors as the transmission method. We think that lentiviral vectors will be more effective in our target cell, hematopoietic stem cells, since they have a larger packaging capacity and provide a longer and more permanent gene expression by integrating into the genome. We will use a self-inactivating vector as a safety precaution, as lentiviruses have a risk of tumorigenesis. At the same time, since we will work ex vivo, we will have the chance to control the cells.

4. References

1. *Üstebay, S., Üstebay, D. Ü., & Yılmaz, Y. (2015). Ailevi Akdeniz Ateşi. Journal of Academic research in medicine, 5(3).*
2. *ÖZALTIN, F., ÖZEN, S., & Fever, F. M. Ailevi Akdeniz Ateşi.*
3. *Turkiye Klinikleri J Int Med Sci. 2006;2(8):9-11*
4. *Livneh, A., Langevitz, P., Zemer, D., Zaks, N., Kees, S., Lidar, T., ... & Pras, M. (1997). Criteria for the diagnosis of familial Mediterranean fever. Arthritis & Rheumatism, 40(10), 1879-1885.*
5. *Goldfinger S. E. (1972). Colchicine for familial Mediterranean fever. The New England journal of medicine, 287(25), 1302. https://doi.org/10.1056/NEJM197212212872514*