RADICHAL

RARE DISEASE CHALLENGE

PROJECT PRELIMINARY REPORT

PROJECT NAME: REGULATION OF M694V MUTATION WITH PRIME EDITING IN HEMATOPOIETIC STEM CELLS

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1-NECMETTİN ERBAKAN ÜNİVERSİTESİ

Familial Mediterranean Fever (FMF) is an autoinflammatory disease caused by mutations in the MEFV gene (Moradian, et al., 2017). The MEFV gene is localized to the short arm (16p13.3) of chromosome 16 and encodes a 781 amino acid protein (pyrin) (Gonul, Bal, Torun, Uguz, & Yıldırımkaya, 2008).

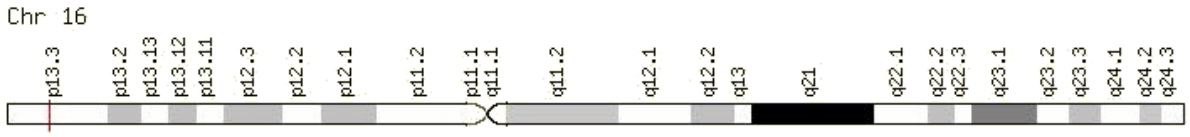

Figure 1. Chromosomal location of the MEFV gene

Although the frequency of carrier varies between societies, it is 1/5 in Turks, 1/6-8 in North African Jews, 1/11 in Israel, 1/6-7 in Armenians, 1/4.3 in Arabs. The incidence of FMF in Turks has been reported as 1/1075 (ER, 2015).

FMF is characterized by apparent fever and pain attacks. Although the duration of the attack usually varies between 2-4 days, there may be longer or shorter seizure types (Ustebay, UlkerUstebay, & Yılmaz, 2015). Attack severity and findings may differ from attack to attack (Cosan,2008).

There are no definitive diagnostic examination finding and no specific laboratory test for FMF disease. Genetic testing can be helpful but difficult because 377 different MEFV variants have been reported so far (Ciaula, Stella, Bonfrate, Wang, & Portincasa, 2020). FMF gets diagnosed clinical findings, family history, biochemical and genetic laboratory data, response to treatment, and exclusion of other familial periodic fever syndromes (Ustebay, UlkerUstebay, & Yılmaz, 2015). The most basic feature that determines the prognosis of FMF disease is the presence of amyloidosis (Ustebay, UlkerUstebay, & Yılmaz, 2015). A highly effective colchicine drug is used in the treatment of FMF. Amyloidosis is almost always observed in patients who do not regularly use colchicine or in patients with delayed diagnosis (Satis, et al., 2020).

The most common mutations are M694V, M680I, V726A, M694I in exon 10 and E148Q, E148V in exon 2 (Arpacı, Dogan, Erdogan, El, & Cura, 2021). Among them, M694V homozygous mutation was found to have the highest risk of developing amyloidosis (Ugan, Ermis, & Sahin, 2011).

Pyrin, the protein mutated in FMF, regulates caspase-1 activation and thus IL-1β production through cognate interaction of the N-terminal pyrin motif with the ASC adapter protein. When there is a mutation in the MEFV gene, there will be uncontrolled IL-1β synthesis and a continuous inflammation state through caspase-1 activation as a result of disruption in pyrin function (Cosan, 2008). The fact that Pyrin expression is directly proportional to IL-1β release has shown that plays a (proinflammatory) role in the formation of inflammation of Pyrin (Taskıran, 2012). Pyrin is produced in certain white blood cells (neutrophils, eosinophils, and monocytes) that play a role in inflammation and fighting infection. Pyrin can direct the migration of white blood cells to sites of inflammation and stop or slow the inflammatory response when they are no longer needed (Ural, 2006).

In our project, in order to reduce the dependence on colchicine, gene regulation was carried out in hematopoietic stem cells, which are the origin of blood cells such as neutrophils, eosinophils and monocytes, where pyrin protein is produced by targeting M694V, the most common mutation, by applying Crispr Prime Editing method.

Genome editing through homologous recombination (HR) (gene targeting) in human hematopoietic stem cells has the power to reveal gene-function relationships and transform potentially curative hematological gene and cell therapies (Bak, Dever, & Porteus, 2018). Gene correction in HSCs should lead to permanent gene correction across different lineages (Morgan, Gray, Lomava, & Kohn, 2017).

Prime editing allows for a variety of genomic modifications to target regions without requiring double-strand breaks or donor templates (Chow, Chen, Shen, & Chen, 2020). In the prime editing method are used Cas9 nickase-fused reverse transcriptase and a prime-editing guide RNA (pegRNA).

The M694V mutation is a single point mutation based on the formation of valine amino acid instead of methionine amino acid by replacing the Adenine base with the Guanine base at the nucleotide 2080 in the exon 10. Conversion of guanine to Adenine will be achieved by prime editing method.

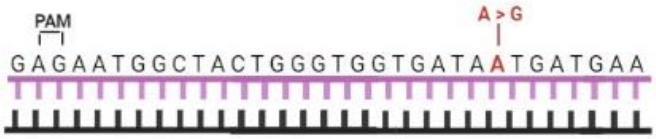

Figure 2. M694V Mutation Display

The plasmid vector designed for the prime editing method will be transferred to the stem cells by electroporation. Electroporation was chosen because of its high efficiency delivery, low cost, reproducibility and ex vivo applicability. Quality controls and possible side-effects will be observed with ex vivo study. The hematopoietic stem cells taken from the patients will be arranged in the laboratory and given to the patients again.

REFERENCES

Arpacı A.,Doğan S.,Erdoğan H.F.,El Ç.,Elmacıoğlu Cura S.(2021). Presentation of a new mutation in FMF and evaluating the frequency of distribution of the MEFV gene mutation in our region with clinical findings.Molecular Biology Reports 48:2025-2033.

Bak R.O., Dever D.P., Porteus M.H.(2018). CRISPR/Cas9 genome editing in human hematopoietic stem cells.Nature Protocols.1(2):358-376.

Chow, R. D., Chen, J. S., Shen, J., & Chen, S. (2020). A web tool for the design of primeediting guide RNAs. Nature Biomedical Engineering. doi:10.1038/s41551-020-00622-8.

Ciaula A.D.,Stella A.,Bonfrate L.,Wang D.Q.H.,Portincasa P.,(2020).Gut Microbiota between Environment and Genetic Background in Familial Mediterranean Fever (FMF), Genes (Basel).11(9): 1041.

Coşan F.(2008). Spondiloartropatilerin Patogenezinde MEFV gen Mutasyonlarının Rolü.Uzmanlık Tezi,İSTANBUL ÜNİVERSİTESİ,Tıp Fakültesi,İstanbul.

Er E.(2015). Ailevi Akdeniz Ateşi Tanısı İle Takip Edilen Hastaların Genotip,Fenotip Ve Laboratuvar Bulguları Açısından Karşılaştırılması.Uzmanlık Tezi.BAŞKENT ÜNİVERSİTESİ,Tıp Fakültesi.Ankara.

Gönül E., Bal C., Güngör TORUN O., Uğuz N., Yıldırımkaya M.M. (2008). Ailesel Akdeniz Ateşi (FMF) Düşünülen Olgularda MEFV gen Mutasyonları Sıklığının İncelenmesi. Türk Hijyen ve Deneysel Biyoloji Dergisi. 65 (1): 1-5.

Moradian, M. M., Babikyan, D., Banoian, D., Hayrapetyan, H., Manvelyan, H., Avanesian, N., & Sarkisian, T. (2017). Comprehensive analysis of mutations in the MEFV gene reveal that the location and not the substitution type determines symptom severity in FMF. Molecular Genetics & Genomic Medicine, 5(6), 742–750.

Morgan R.A.,Gray D., Lomava A., Kohn D.B.(2017). Hematopoietic Stem Cell Gene Therapy– Progress and Lessons Learned.Cell Stem Cell.21(5).574-590

Satış H., Armağan B.,Bodakçı B., Ataş N.,Sarı A.,Yaşar Bilge N.Z.,Yapar D., Bilici Salman R., Yardımcı G.K., Babaoğlu H.,Kılıç L.,Göker B., Haznedaroğlu Ş., Kaşifoğlu T., Kalyoncu U., Tufan A. (2020).Colchicine intolerance in FMF patients and primary obstacles for optimal dosing, Turk J Med Sci. 2020; 50(5): 1337–1343.

Taşkıran Z.E.(2012).Kolşisinin pyrin ve pyrin ile ilişkili proteinler üzerine etkisi.HACETTEPE ÜNİVERSİTESİ,Sağlık Bilimleri Enstitüsü,Doktora tezi.Ankara.

Ugan Y.,Ermiş F.,Şahin M.(2011).Ailevi Akdeniz Ateşi.Süleyman Demirel Üniversitesi Tıp Fakültesi Dergisi.18(4):139-143.

Ural A.U.(2006).Kök Hücreler. TOTBİD (Türk Ortopedi ve Travmatoloji Birliği Derneği) Dergisi. 5:3-4

Üstebay S.,Üstebay D.Ü.,Yılmaz Y.(2015).Ailevi Akdeniz Ateşi,Journal of Academic Research in Medicine.5(3):89-93.