2020 Rare Disease Challenge

A Genetic Therapy Trial for FRDA: Excision Of Expanded GAA Repeats

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ABSTRACT

Friedreich's ataxia is a progressive, neurodegenerative, autosomal recessive genetic disease.FRDA, the most common ataxia in the world, occurs at a frequency of 1 / 50,000. Also, 1 in 100 people carry the mutant FXN gene. But these people can live normally. The cause of the disease is the enlarged GAA repeats that can reach up to 1300 in the first intron of the FXN gene (9th chromosome 69037283). These repeats disrupt the chromatin structure of the gene and prevent its transcription.
Thus, the concentration of frataxin, a mitochondrial protein, decreases and affects the iron metabolism in a bad way. In the clinic of the disease, neurological, cardiac and metabolism symptoms are observed. Our project aims to develop genetic therapies for this inherited rare disease. The CRISPR / CAS9 system, which has revolutionized gene therapy in recent years, is the backbone of our project. The aim of our project is to excise the expanded GAA repeats located in the 1st intron of the FXN gene by targeting them upstream and downstream with gRNA / SpCAS9 and finally the correction of the disrupted chromatin structure.
While personalized treatments strengthen its place in the medicine of the future at full speed, we are working to take our place in this trend, thinking that personalized genetic therapies in the treatment of rare diseases will be groundbreaking..

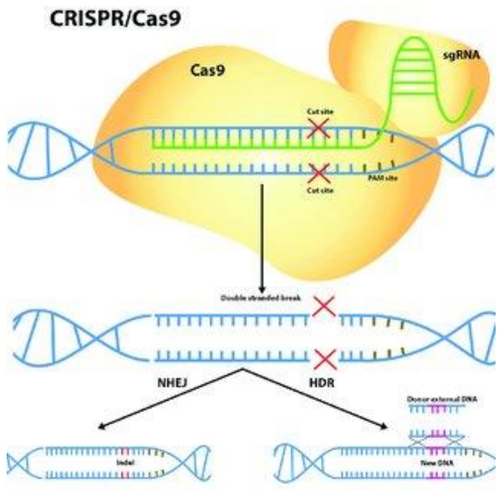
RESULTS

FXN transcription

Transcription of the FXN gene is largely achieved by the SRF and TFAP2 transcription factors. These factors bind directly to the FXN promoter, speeding up transcription. Thus, excision in intron 1 does not prevent our gene from being transcribed.

CRISPR/CAS9

CRISPR is a tool that allows us to make changes in DNA. Basically, it functions with two molecules: CAS9 and gRNA. CAS9 is an endonuclease enzyme obtained from various sources. There are different types of CAS enzymes such as SpCAS9, SaCAS9. This enzyme gains endonuclease activity by targeting PAM sequences on the target DNA. But it also needs a guide RNA for this job. Guide RNAs are determined according to the PAM sequence of the selected CAS enzyme. The Cas9 + gRNA complex binds to the target DNA sequence, forming a double DNA break that we call DSB.



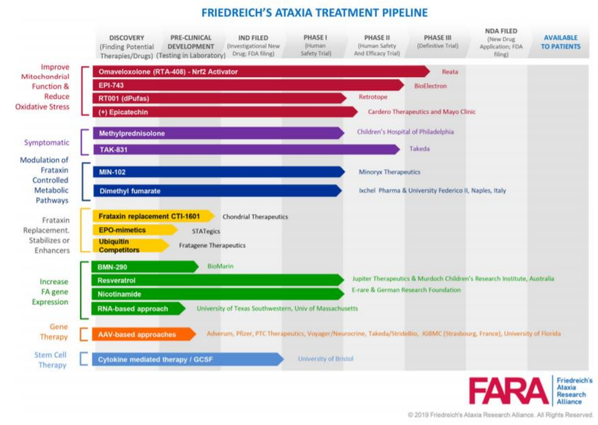
Our Genetic Therapy Design

The aim of our project is to cut the increased GAA repeats in intron 1 of the FXN gene and to reunite the gene with the NHEJ repair mechanism to ensure its non-mutant state. Our design for this is based on CRISPR. We simply aim to transfer 2 gRNAs targeting downstream and upstream of GAA repeats designed in BENCHLING to PX333 plasmid with Golden Gate Assembly method and transferring this system to target fibroblast cells with AAV vector.

Location	Strand	gRNA sequence	PAM	On Target Effect	Off Target Effect
69036771	+	TATCTGACCCAGTTACGCCA	CGG	70.8	90.8
69037619	-	AGGGGTGGAAGCCCAATACG	TGG	67.8	86.4

CONCLUSION

Many therapeutic targets against FRDA disease are being studied by the scientific world. Some of these are shown in the table on the right. Gene therapies are a quantitatively minority among available studies. From this situation, it can be concluded that genetic treatments have some disadvantages that are still difficult to overcome.



As a matter of fact, these problems are the problems we will encounter in the operation of our project. Although the immune response and side effects are relatively less than other vectors make AAVs the first choice for genetic treatments, low cargo capacity prevents the use of AAV in extensive studies. The SaCas9 and double gRNA added to the plasmid, which we wanted in our study, are not available in the literature. This led us to use the px333 plasmid, which combines SpCas9 with double guideRNA. It is important to produce the SaCas9-containing derivative of this plasmid in the laboratory environment as this project is a contribution to the literature. Again, the attempt to increase FXN mRNA by partial intron excision using CRISPR will be a unique contribution to the literature by us.

Some other potential problems ahead are: delivery of the vector to all tissues, toxicity and off-target effects and that may occur inside the cell

REFERENCES

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Kuanyu Li, Anamika Singh, Daniel R. Crooks, , Xiaoman Dai, Zhuangzhuang Cong, Liang Pan, Dung Ha, and Tracey A. Rouault

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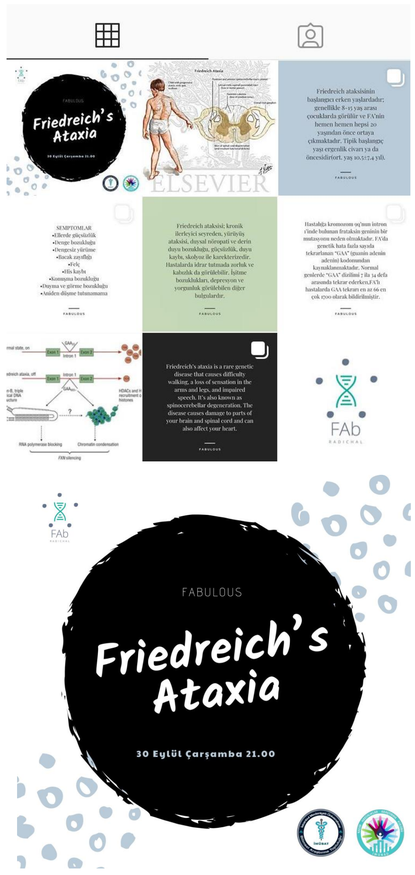
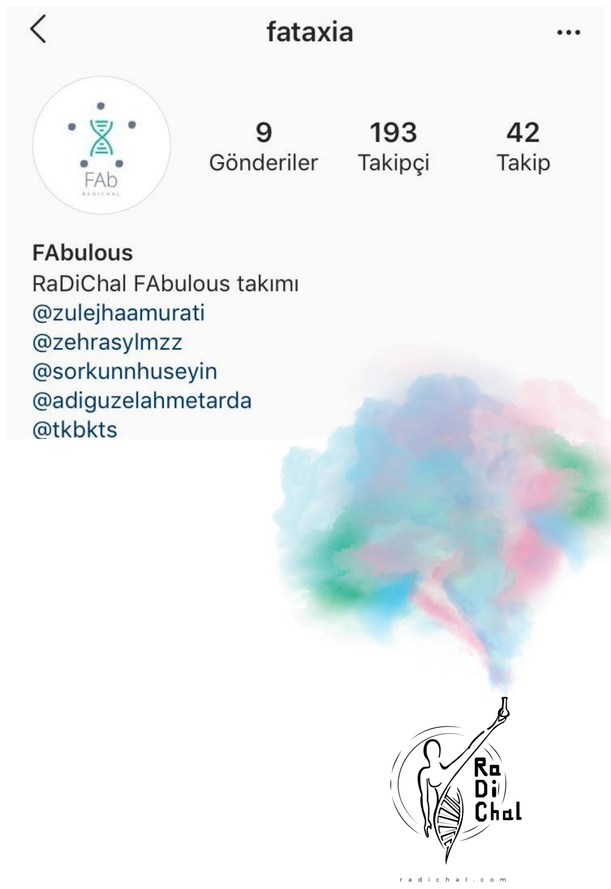
SOCIAL IMPACT

We conducted our social awareness campaign only online within the scope of COVID-19 measures. Our target audience has been medical students, geneticists, pharmacists and biologists. In order to do this nationally and internationally, we shared posts in various languages on @fataxia instagram account. We made informative presentations about FRDA and our project from a ZOOM meeting.





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