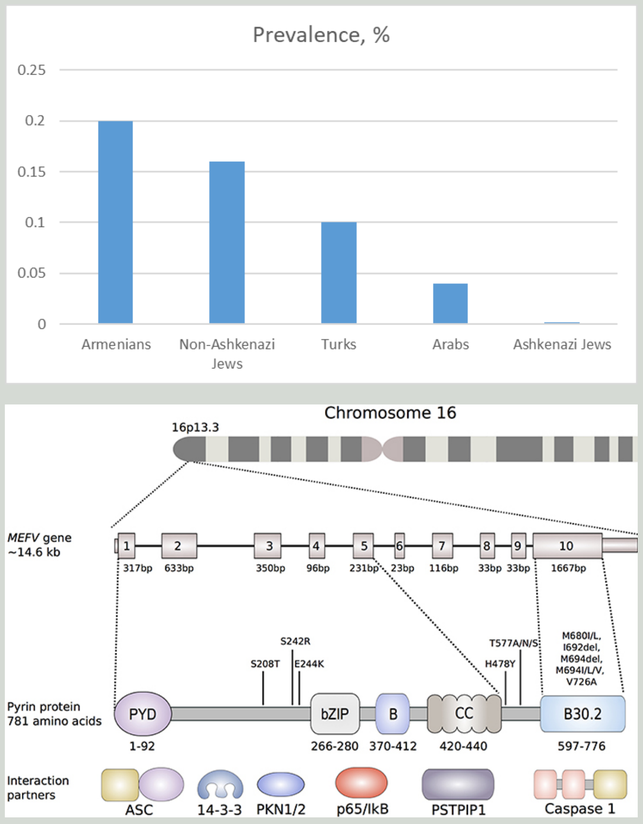
Nanopore sequencing of *MEFV* gene

Introduction

Familial Mediterranean Fever(FMF), is a genetic disorder, which primarily affects populations originating in the Mediterranean region, particularly people of Armenian, Arab, Turkish, or Jewish ancestry.



Adapted from: Front. Immunol. 10:1745. (2019)

The *MEFV* gene encodes pyrin, which functions as an innate immune sensor that can trigger the formation of an inflammasome, allowing the production of inflammatory mediators during infection. Around 50 mutations in the *MEFV* gene are associated with FMF.

Aim:

Our main aim was to investigate if MEFV mutations can be identified with nanopore sequencing.

Materials and Methods

The study was approved by the IRB of the Institute of Molecular Biology NAS RA.

Methods:

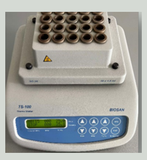
- Oxford Nanopore sequencing
- Electrophoresis
- PCR/ RT-PCR

Materials:

- Oxford Nanopore MinION sequencer
- Electrophoresis machine
- PCR amplificators
- Qubit fluorometer
- Centrifuge/Microfuge
- Vortex mixer
- Thermocyclers
- Magnetic beads and racks
- Pipettes

Bioinformatics programs:

- Open CRAVAT
- Infevers
- IGV, USCS
- Command line
- VCF and BAM files



Termostat

Qubit fluorometer

Vortex Mixer

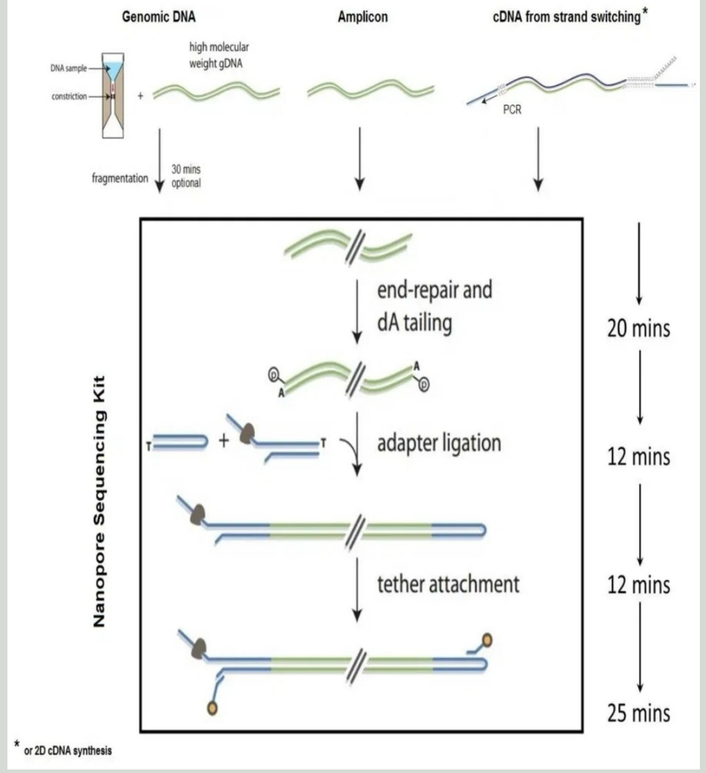
Electrophoresis machine

MinION

Cetrifuge

Magnetic beads and separation rack

PCR Amplificator



Cummings, P. J., Olszewicz, J., Obom, K. M. Nanopore DNA Sequencing for Metagenomic Soil Analysis. (2017).

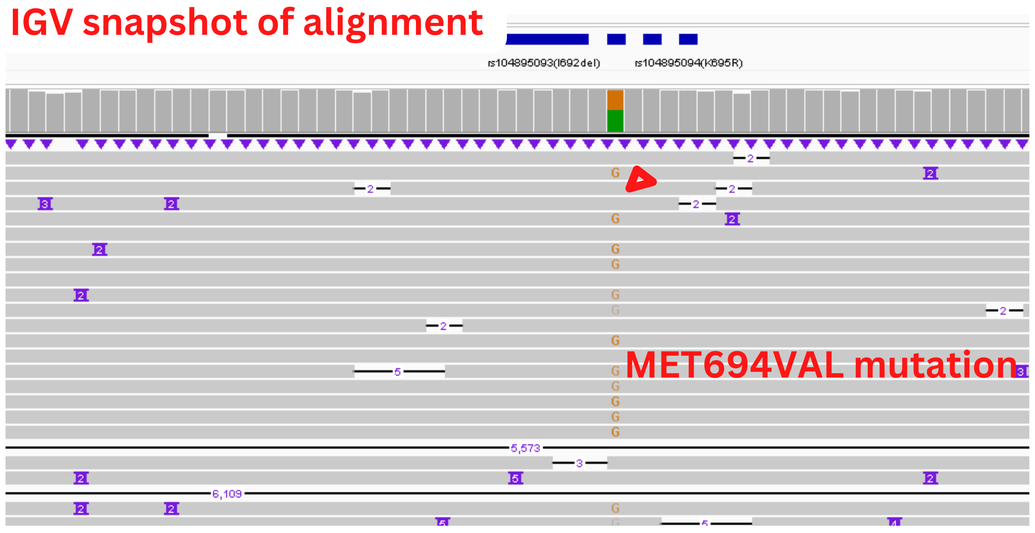
Results and Discussion

DNA samples from two FMF patients (F5 and F11) were used.

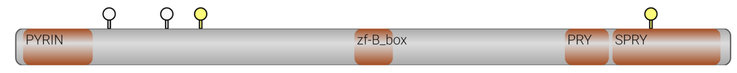
PATIENT	INITIAL CONC. (ng/ul)	LIBRARY CONC. (ng/ul)	SEQUENCING SUCCESS
F5	30.8	22.8	YES
F11	4.14	0.430	NO

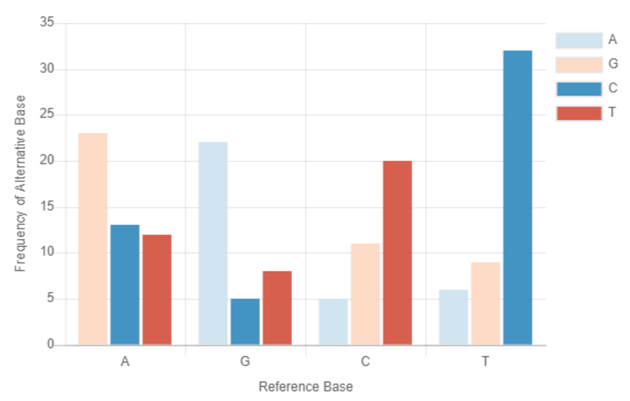
Patient F5 was found to have 4 mutations in the *MEFV* gene, 3 of which are not known to cause FMF, and the mutation at (MET694VAL) had been proven to cause FMF.

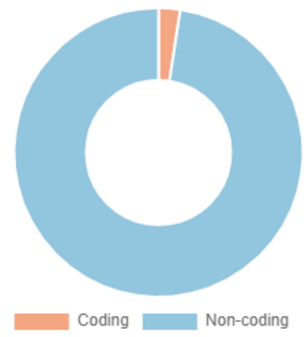
IGV snapshot of alignment



Change	Seq Ont	# Samples
p.Asp102=	synonymous_variant	1
p.Ala165=	synonymous_variant	1
p.Arg202Gln	missense_variant	1
p.Met694Val	missense_variant	1



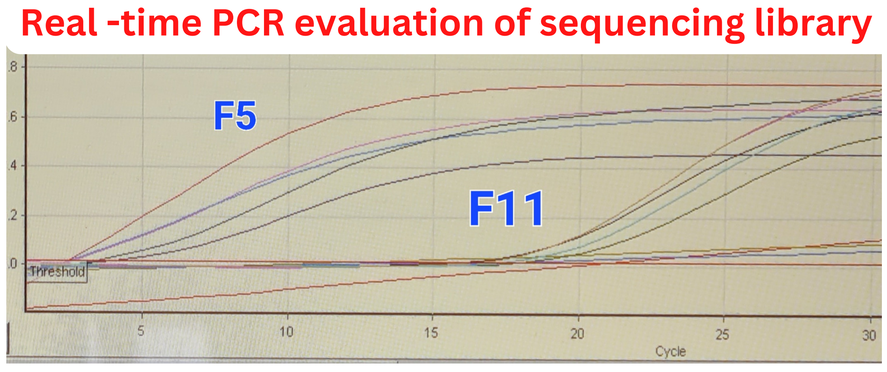




Patient F11 was also found to have the symptoms of FMF, thus the whole process of sequencing was also performed on this sample, however because of low concentration of DNA library, no precise results of mutations were found.

IGV snapshot of alignment

Real -time PCR evaluation of sequencing library



Conclusions

- According to our research, **patient 1(F5)** had 4 mutations, 1 out of which was the causative for FMF.
- The second **patient 2(F11**) also had the FMF, however, as the library concentration was too low, no relevant results about mutations were obtained.
- Nanopore Sequencing is a great and cutting-edge technology for sequencing long DNA fragments, in the presence of a NORMAL DNA library concentration.