An Inquiry About the Relationship between CFTR Variants of Unknown Significance and Cystic Fibrosis

Deniz Muratlı Salih Kaya A. Bera Efe

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Introduction

Cystic Fibrosis



Cystic Fibrosis

- Occurs in every one of 3,200 live births in the US among white Americans ("How common is cystic fibrosis and who does it affect?," 2019)
- Leads to abnormally thick and sticky mucus that results in ("Cystic fibrosis," 2022):
 - Blockage of airways → Lung damage
 - Trapping of germs → Increased likelihood of infections
 - Prevention of proteins needed for digestion from reaching the intestines
 → Decreased ability to absorb nutrients from food
- Reduces lifespan to 44 years ("How common is cystic fibrosis and who does it affect?," 2019)

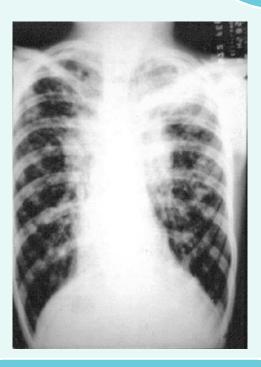
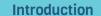


Figure 1: X-ray image of the lungs of a patient with advanced cystic fibrosis ("Cystic fibrosis: Practice essentials, background, pathophysiology," 2021)

Introduction

CFTR Gene & Protein





CFTR Gene

- Provides instructions for making the CF transmembrane conductance regulator protein (MedlinePlus, n.d.)
- Found on Chromosome 7 (7q31.2) (National Center for Biotechnology Information, 2022)
- A 6132 bp sequence that contains 27 exons and 1480 amino acids (National Center for Biotechnology Information, 2019; UniProt, n.d.)

Introduction

CFTR Protein

- Functions as a channel across the membrane of cells that produce mucus, sweat, saliva, tears, and digestive enzymes (MedlinePlus, n.d.)
 - Transports CI- ions and controls the movement of water in tissues (necessary for the production of thin mucus)
- Segmentation (UniProt, n.d.):
 - Binding sites: 401, 434, 458-465, 493, 1219, 1244-1251
 - o Domains: 81-365, 423-646, 859-1115, 1210-1443
 - Regions: 654-831 (disordered),
 1386-1480, 1452-1480 (disordered)
 - o Motifs: 1478-1480

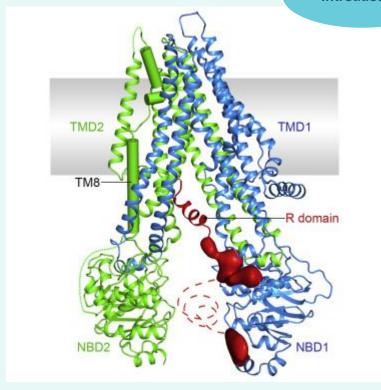


Figure 2: A representation of the CFTR protein (Liu et al., 2017)

Materials and Methods



2.1

Overview of VUS Positions

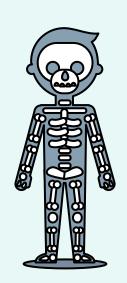
Overview of VUS Positions



Aim

is to form a graph that gives a general idea of VUS positions and possible accumulations.

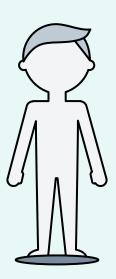
Data Analysis



Formatting Data



Plotting



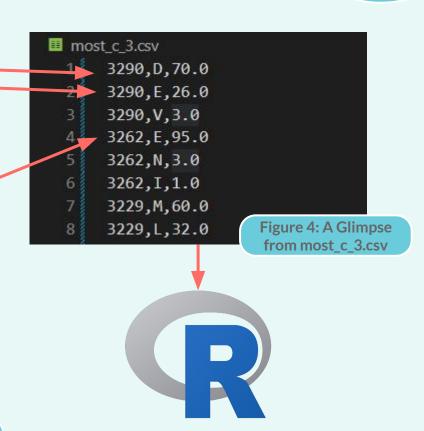
Overview of VUS Positions

from results 800.txt



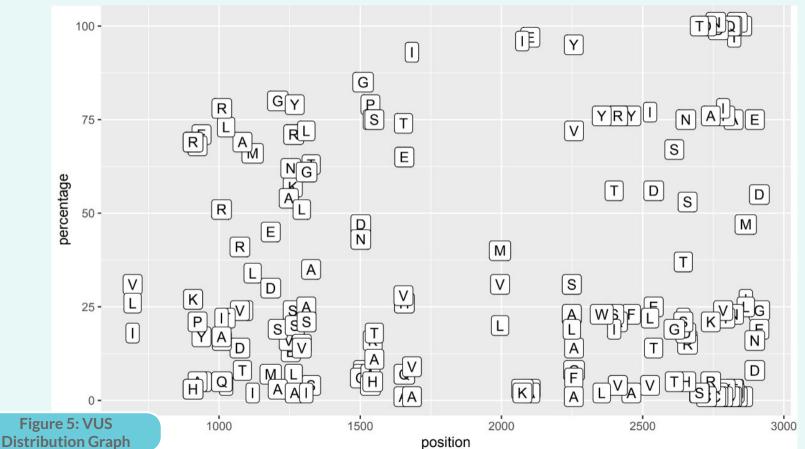
```
The most common characters in the profile:
-D with 70.0% -
-E with 26.0%-
-V with 3.0%
-T with 1.0%
Chemical properties of the most common characters:
-Acidic with 70.0%
-Acidic with 26.0%
-Aliphatic with 3.0%
-Polar uncharged with 1.0%
Substitution: D -> N
Position in the aligned sequence: 3290
Chemical property of the reference character: Acidic
Chemical property of the substitution character: Polar uncharged
Change in entropy: 0.013867385915318664
Substitution score (BLOSUM62): 1.0
In a critical part of the protein? False
Automated prediction: Possibly (<likely) non-pathogenic
The most common characters in the profile:
-E with 95.0%
-N with 3.0%
-I with 1.0%
-A with 0.0%
Chemical properties of the most common characters:
-Acidic with 95.0%
                                                   Figure 3: A Glimpse
-Polar uncharged with 3.0%
```

Aliphatic with 1 0%



Overview of VUS Positions





2.2

Databases

Used Databases

- Leiden Open Variation Database to obtain variants of unknown significance
- NCBI's BLAST and PSIBLAST databases



Materials & Methods

Figure 6: A screenshot of the homepage of LOVD

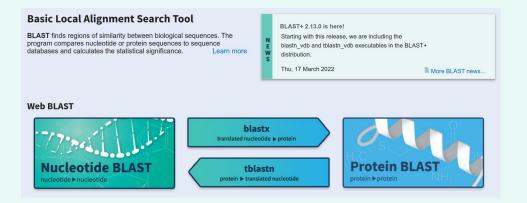


Figure 7: A screenshot of the homepage of BLAST

2.3

Metrics Used

Metrics Used

- BLOSUM62 substitution score
- Shannon's entropy
- Chemical property
- Most common amino acids in the position

```
The most common characters in the profile:
-E with 95.0%
-N with 3.0%
-I with 1.0%
-A with 0.0%
Chemical properties of the most common characters:
-Acidic with 95.0%
-Polar uncharged with 3.0%
-Aliphatic with 1.0%
-Aliphatic with 0.0%
Substitution: K
Position in the aligned sequence: 3262
Chemical property of the substitution character: Basic
Change in entropy: 0.011795103044278221
Substitution score (BLOSUM62): 1.0
Automated prediction: Possibly (<likely) non-pathogenic
```

Figure 8: Sample output

2.

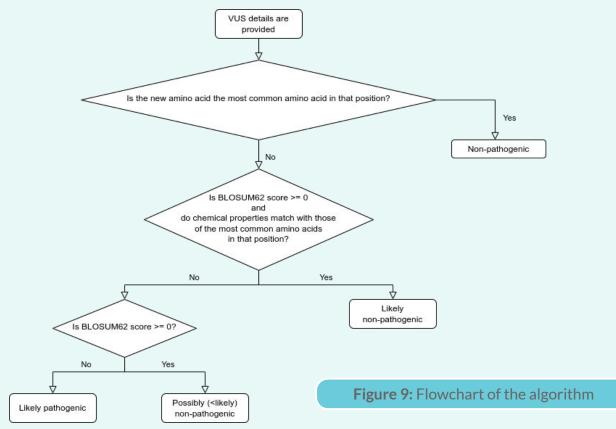
Decision Making Algorithm

Decision-Making Algorithm

- An intuitive and oversimplified decision-making process
- Meant to give an idea; absolutely not conclusive



Decision-Making Algorithm

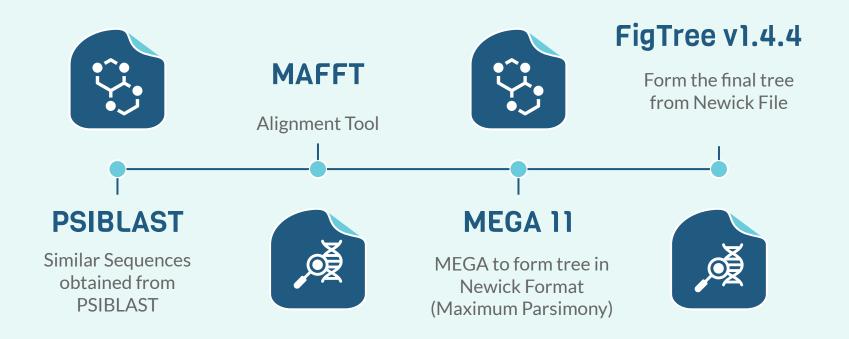


2.5

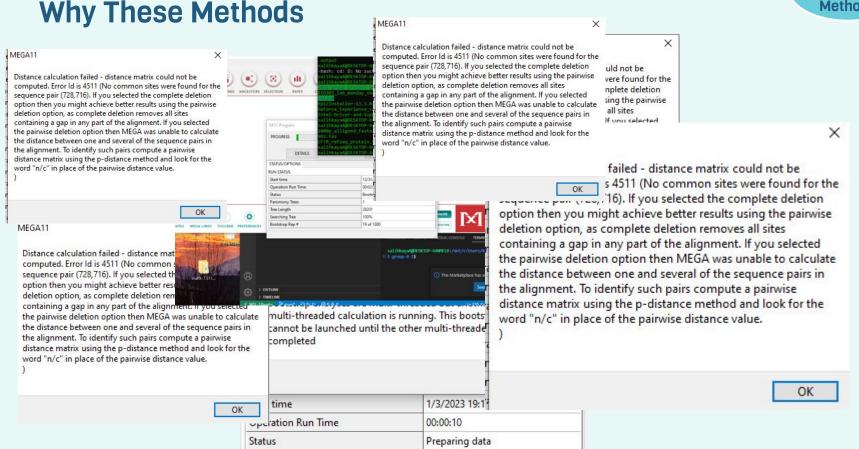
Tree Building



Tree Formation Steps

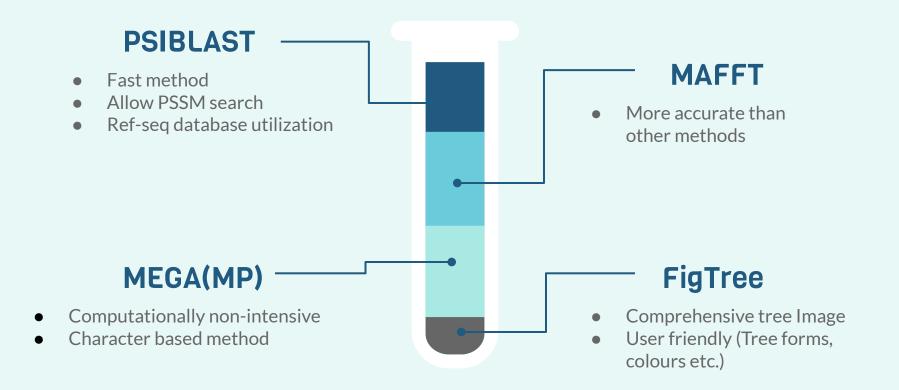


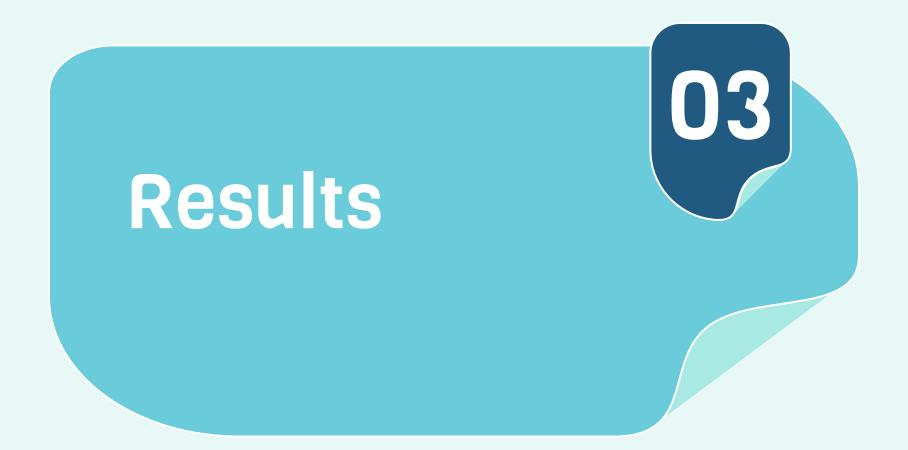






Benefits of Methods

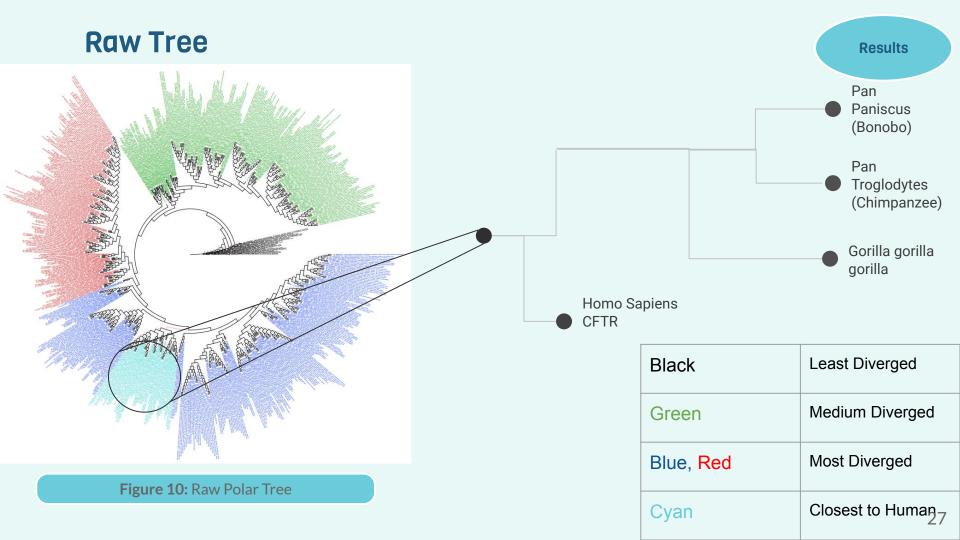




Results

3.1

Interpretation of VUS

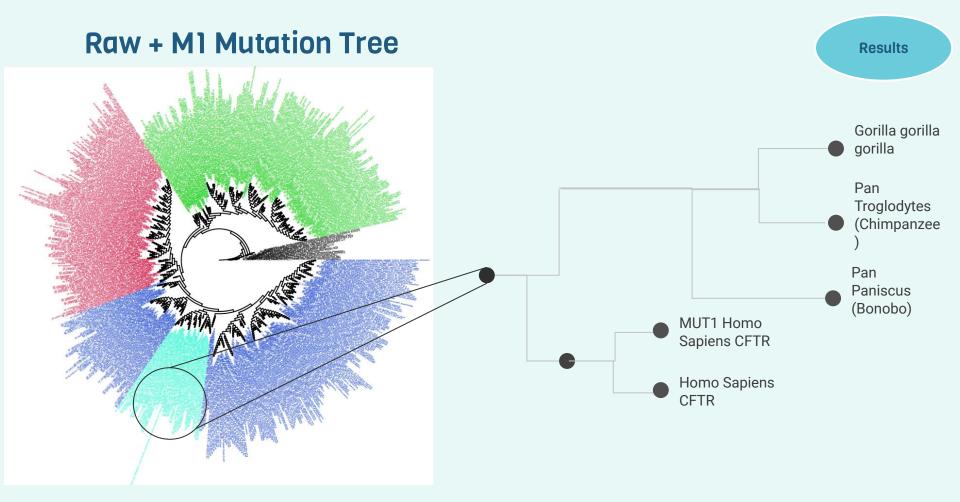


Results

M1 (VUS 1)

```
The most common characters in the profile:
-D with 70.0%
-E with 26.0%
-V with 3.0%
-T with 1.0%
Chemical properties of the most common characters:
-Acidic with 70.0%
-Acidic with 26.0%
-Aliphatic with 3.0%
-Polar uncharged with 1.0%
Substitution: N
Position in the aligned sequence: 3290
Chemical property of the substitution character: Polar uncharged
Change in entropy: 0.013867385915318664
Substitution score (BLOSUM62): 1.0
Automated prediction: Possibly (<likely) non-pathogenic
```

Figure 11: Output of the program for VUS 1



Results

M20 (VUS 20)

```
The most common characters in the profile:
-T with 45.0%
-I with 27.0%
-G with 11.0%
-V with 8.0%
Chemical properties of the most common characters:
-Polar uncharged with 45.0%
-Aliphatic with 27.0%
-Aliphatic with 11.0%
-Aliphatic with 8.0%
Substitution: I
Position in the aligned sequence: 2994
Chemical property of the substitution character: Aliphatic
Change in entropy: 0.0010135713771433075
Substitution score (BLOSUM62): -1.0
Automated prediction: Likely pathogenic
```

Figure 13: Output of the program for VUS 20

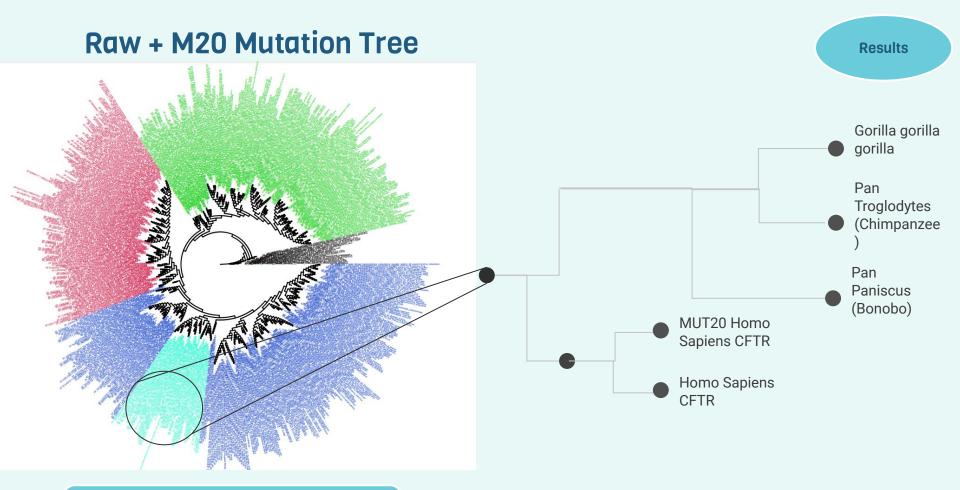
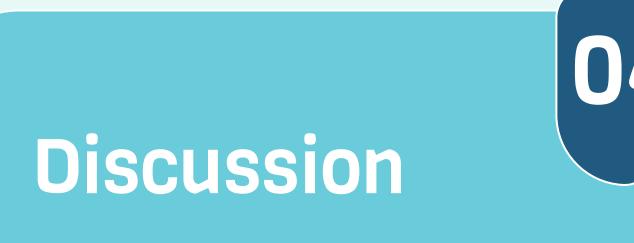


Figure 14: Raw + M20 Mutation Polar Tree







Discussion

- Not considered: Whether the substituted position is in a domain or critical residue
- Not considered: Most common amino acids in a given position for close relatives of Homo sapiens
- The risk of PSIBLAST was not considered, the reverse check should have been done.
- Difficulty of interpreting changes in Shannon's entropy
- Effectiveness of the trees in the analysis

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Thanks!

Do you have any questions?

ksalih@sabanciuniv.edu ahmetbera@sabanciuniv.edu denizm@sabanciuniv.edu

+90 (553) 013 1523

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