FMF: Evidence for a functional effect of E148Q when combined with M694V

A New Solution to an old Problem

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Familial Mediterranean Fever (FMF)

Acute attacks of fever accompanied by:

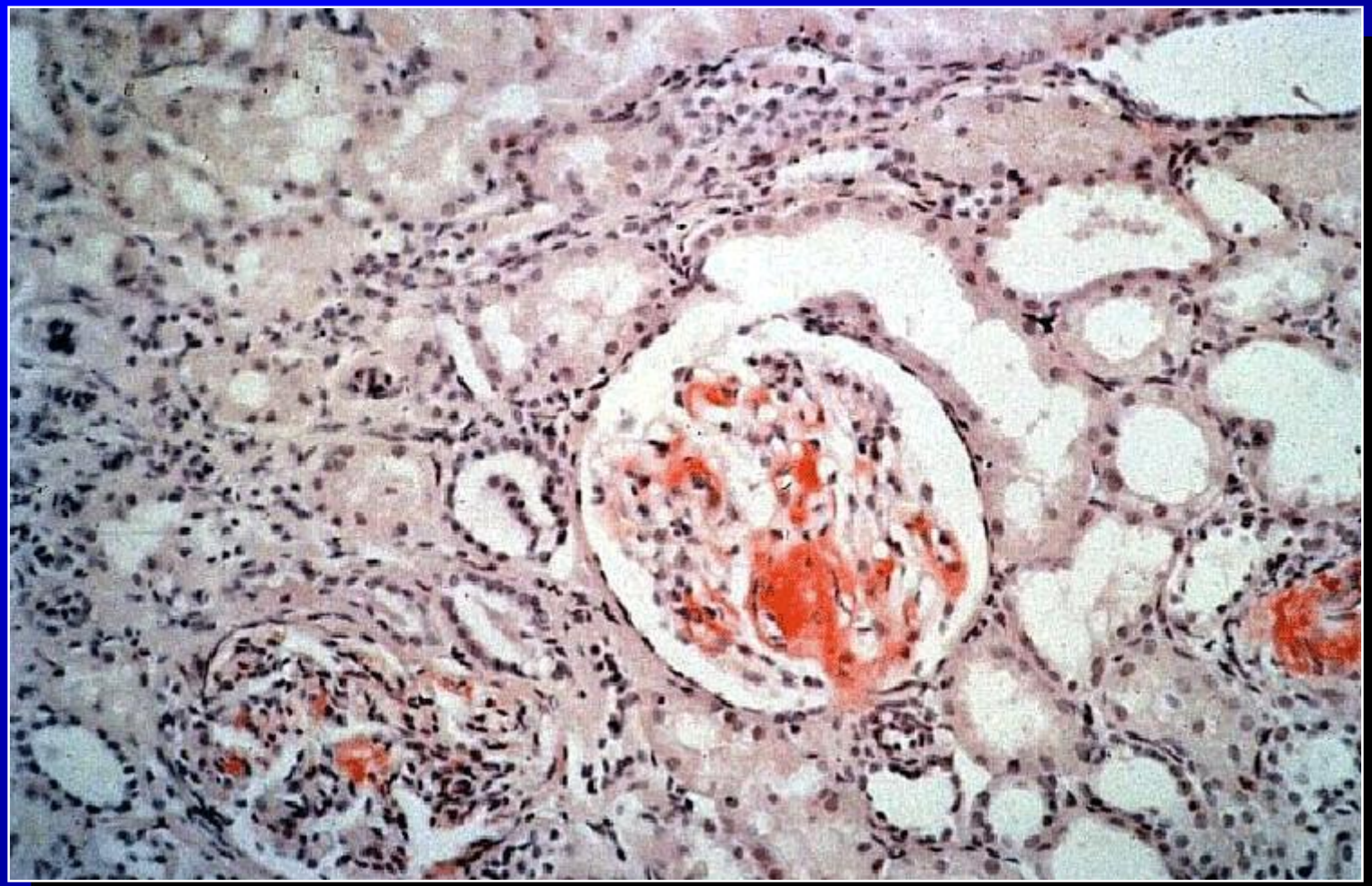
- **Peritonitis**
- **Pleuritis**
- **Arthritis**
- **Erysipelas like erythema**

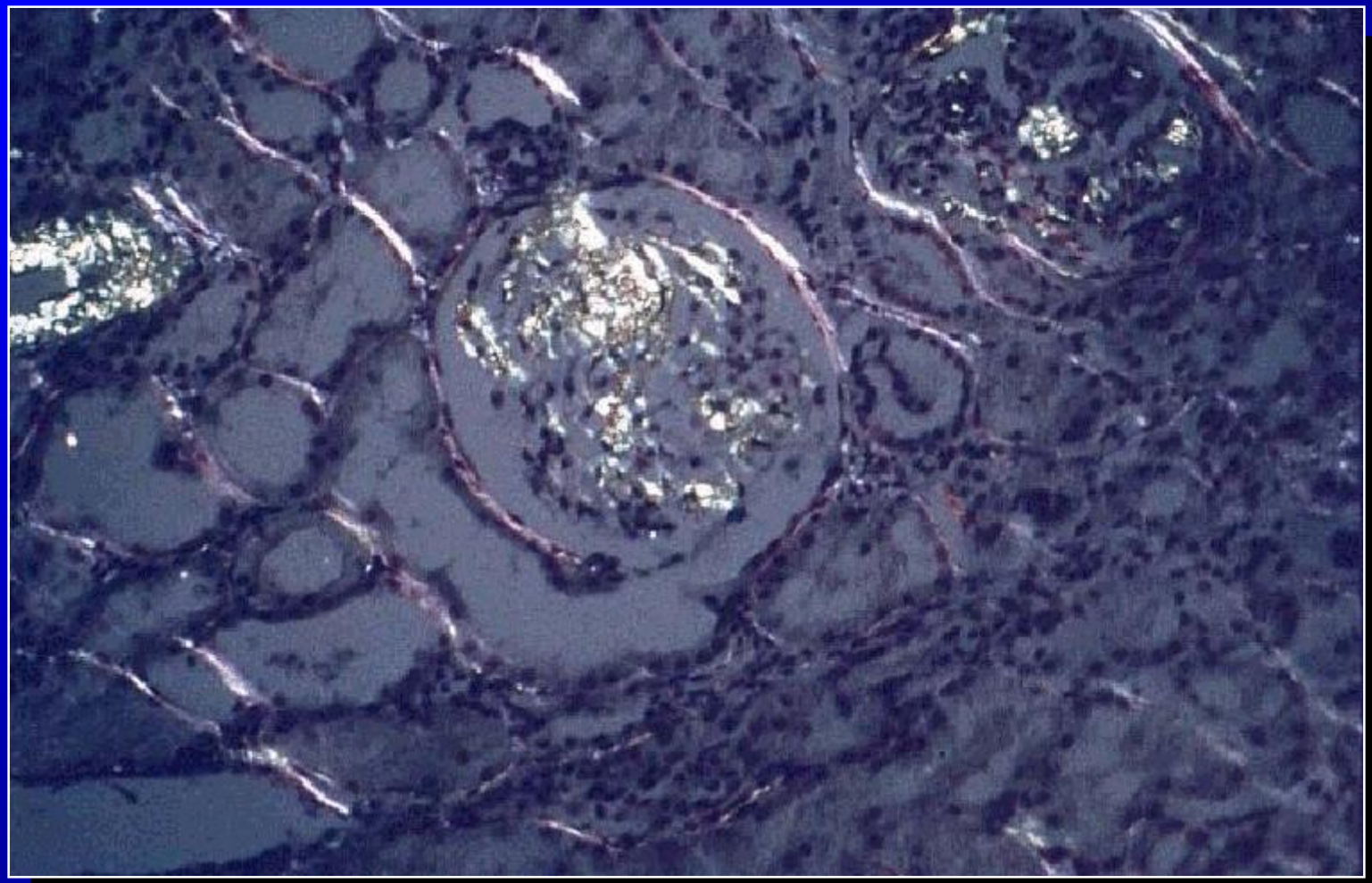
Frequency of Attacks

From twice a week to once every couple of years

Precipitating Factors

Infections, trauma, physical activity, menstrual period, mental stress, unknown factors



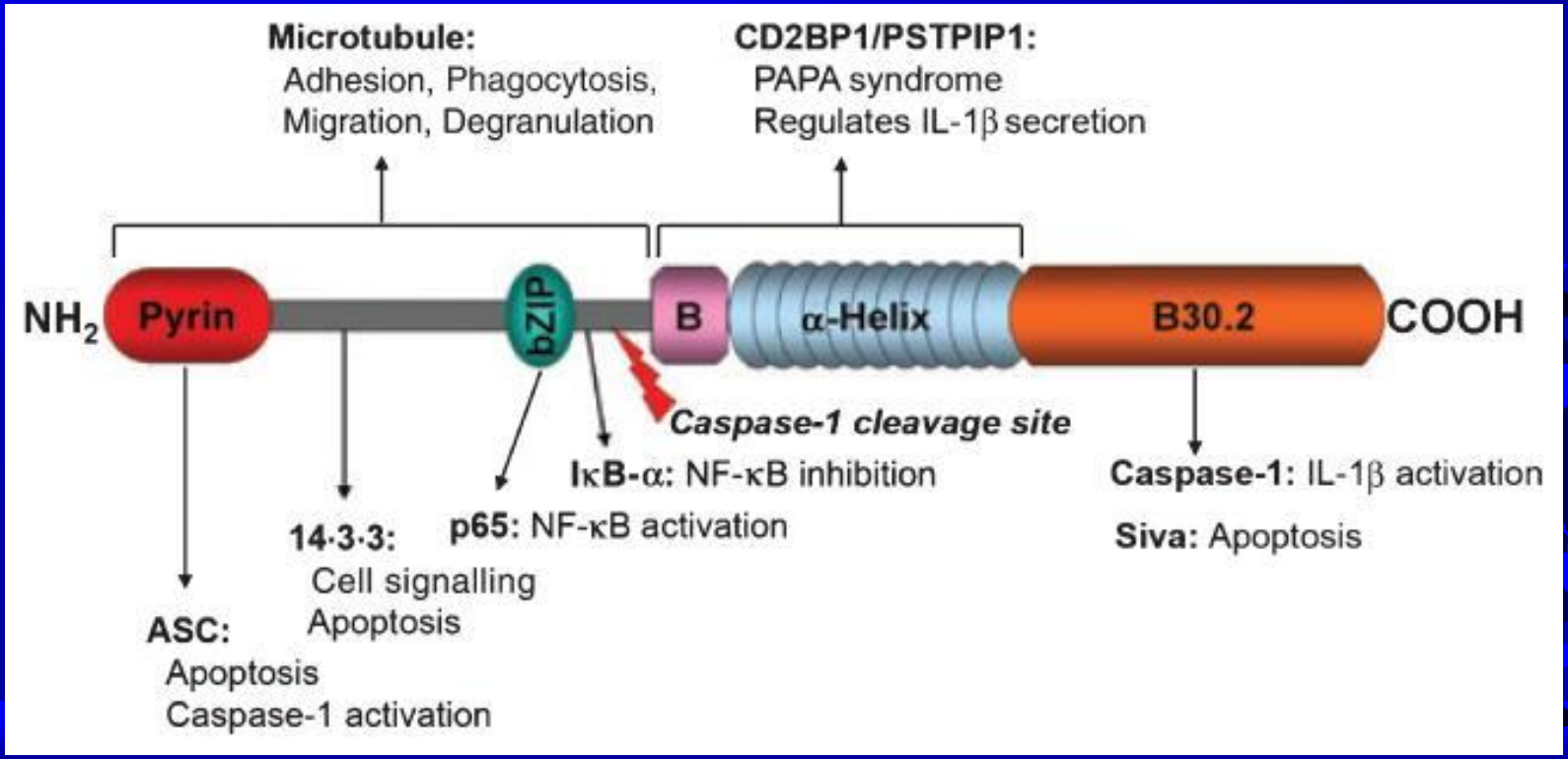


Disease Distribution

- North African Jews
- Iraqi Jews
- Armenians
- Turks
- Middle eastern Arabs
- Ashkenazi Jews

The FMF Gene (MEFV)

- mRNA-3700 bp long
- Encodes a 781 amino acid protein (Pyrin)



MEFV Mutations

- M694V
- V726A
- E148Q
- E167D
- P369S
- M680I
- M694I
- K695R
- A744S
- V704I
- G687E
- T267I
- F479L
- I692Del
- R42W
- E230K
- E148V
- L110P
- R408Q
- I591T
- R761H
- Y688X
- T681I
- M680L
- S675N
- R653H
- M640Del

E148Q a Mutation or a Polymorphism ?

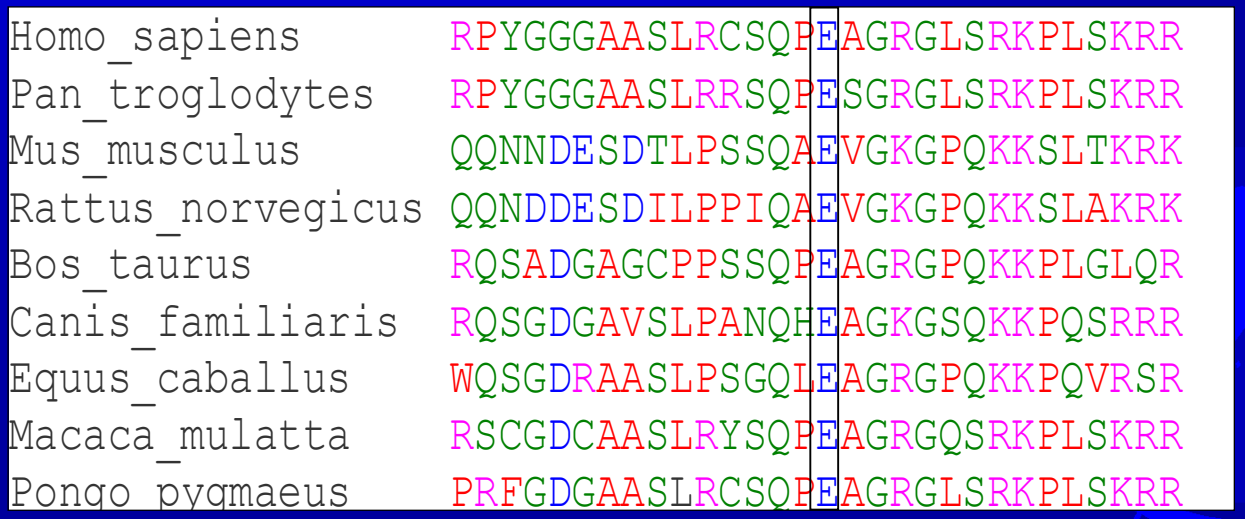
(Pros)

- Patients homozygous for E148Q have been described although they are rare and often present with atypical symptoms
- Patients with V726A-E148Q in cis have a more severe disease
- The structural effect of the E148Q MEFV mutation on the pyrin protein: a study using a quantum chemistry model (Naimushin A, Lidar M, Ben Zvi I, Livneh A)
- Association studies link E148Q to multifactorial autoimmune diseases (MS IBD)

E148Q a Mutation or a Polymorphism ?

(Pros)

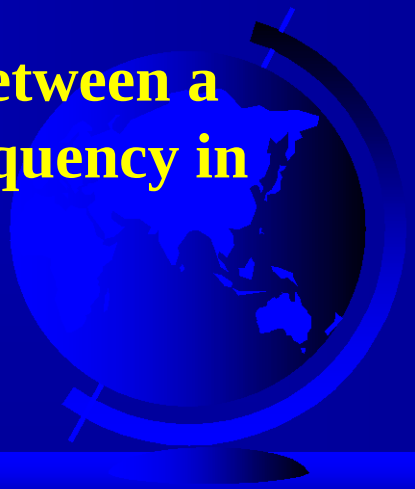
Evolutionary conserved:



E148Q In a Mutation or a Polymorphism ?

(Cons)

- In the Far East carrier rate of 25% in control samples
- No difference in the frequency of this variant between cohorts of patients and controls
- One of the most common ways to differentiate between a mutation and a polymorphism is to assess its frequency in patients vs. controls



	Patients (Zaks 2003)	Healthy Controls
E148Q	58	163
--------	766	2639
Total	824	2802
	7.04%	5.81%

PV=0.2280

Marek-Yagel D, J Rheumatol. 2009

	Patients (Zaks 2003)	Healthy Controls
V726A	122	141
--------	702	3877
Total	824	4018
	14.8%	3.5%

PV=0.0001

Marek-Yagel D, J Rheumatol. 2009

No functional assay for FMF mutations

IN-FEVERS DATA BASE

E148Q is defined as a variant of unknown significance (VOUS)

(http://fmf.igh.cnrs.fr/infevers)

Through out the years

We all shared the feeling that E148Q has some functional effect in FMF

FMF patients with a single mutation

Mutation analysis in FMF patients reveal:

- **60-65% two mutations**
- **20-25% one mutation**
- **5-10% no mutations**

The most common single mutation genotype found is M694V/null

E148Q has not been described in cis with M694V

The penetrance of M694V/M694V by the age of 20 is close to 100%

M694V/E148Q

M694V/null

Compare

the penetrance of

M694V/E148Q

to the penetrance of M694V/null

Penetrance

$$\frac{\text{No. of symptomatic patients with a given genotype}}{\text{Total no. of individuals with that genotype}}$$

Full Penetrance = 1.0

Calculating penetrance

1. Direct calculation: Genotype 100,000 individuals, look for the M694V/E148Q and M694V/null genotypes and see how many of them have FMF.

2. Indirect calculation

Methods

Find a population group in which FMF and these 2 mutations are very prevalent.

Assess the allele frequencies of these two variants in a large control group.

Calculate the expected frequencies of the M69V/M694V, M694V/null and M694V/E148Q genotypes.

Construct a cohort of at least 100 consecutive FMF patients who came for genetic analysis, all belonging that population group.

Assuming a penetrance of 100% for the M694V/M694V genotype:

The ratio between the calculated frequencies of the 3 genotypes and the actual frequencies of these 3 genotypes obtained from the patient cohort and use them to determine the penetrance of M694V/null and M694V/E148Q.

North African Jewish population (NAJ)

FMF is extremely prevalent

Allele frequencies for M694V and E148Q are well established

NAJ allele frequencies (from the literature)

	M694V	E148Q
Kogan et al.	16/240	9/240
Stoffman et al.	16/200	10/200
Gershoni-Baruch et al.	27/486	11/200
Sum	59/886	30/640
Allele frequency	0.066	0.0468

Expected Genotypes in the NAJ Population

- P – M694V – Mutant allele
- Q – M694V – Wild type allele
- P_1 – E148Q - Mutant allele

M694V/M694V = $P^2 = (0.066)^2$ = 0.004

M694V/E148Q = $2PP_1 = (2\times0.066X0.0468)$ = 0.006

M694V/Null = $2PQ = (2\times0.066\times0.934)$ = 0.123

Patient cohort

148 consecutive patients suspected of FMF

All were of NAJ decent

Patient who did not fulfil the
Tel Hashomer Criteria were excluded

MEFV Genotypes in NAJ FMF patients

Genotype	Number
M694V/M694V	75
M694V/Null	16
M694V/E148Q	15
M694V/726	1
Total	107

$Penetrance\ of\ M694V/E148Q$

Expected		**Observed**
M694V/M694V	= 0.004	• 75
M694V/E148Q	= 0.006	• 15

Assuming 100% penetrance for M964V homozygotes by the age of 20:

$$\frac{Expected\ M694V/M694V}{Expected\ M694V/E148Q} \div \frac{observed\ M694V/M694V}{observed\ M694V/E148Q} = Penetrance\ of\ M694V/E148Q$$

$Penetrance\ of\ M694V/Null$

Expected		**Observed**
M694V/M694V	= 0.004	• 75
M694V/Null	= 0.123	• 16

- Assuming 100% penetrance for M964V homozygotes:

$$\frac{Expected\ M694V/M694V}{Expected\ M694V/Null} \div \frac{observed\ M694V/M694V}{observed\ M694V/Null} = Penetrance\ of\ M694V/Null$$

Penetrance of $\boldsymbol{M694V/E148Q}$ & $\boldsymbol{M694V/Null}$

- *M694V/E148Q*

- $\frac{Expected\ M694V/M694V}{Expected\ M694V/E148Q} \div \frac{observed\ M694V/M694V}{observed\ M694V/E148Q} = 0.14$

- M694V/Null

- $\frac{Expected\ M694V/M694V}{Expected\ M694V/Null} \div \frac{observed\ M694V/M694V}{observed\ M694V/Null} = 0.0076$

18 higher

Expected vs. Observed

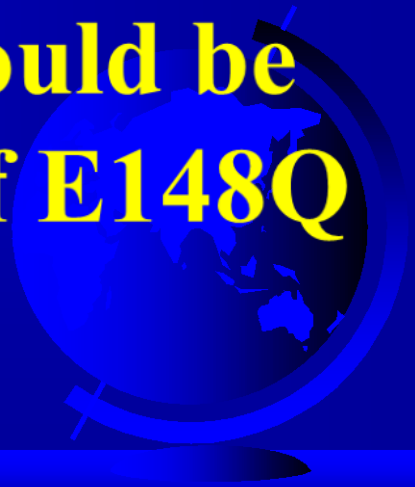
	expected	observed
M694V/E148Q	6	15
M694V/Null	123	16

P<0.0001 (Fisher Exact Test)

Conclusions

The M694V/E148Q Genotype is 18 times more penetrant than the M694V/Null genotype

The difference most probably could be attributed to a functional role of E148Q



The E148Q paradox

If E148Q is functional, Why does it appear in a similar frequency in patient cohorts and control cohorts

Hypothetical neutral substitution

Population allele frequency of 20%

Never appears on the same allele with M694V

Genotype	Number
M694V/M694V	75
M694V/Null	16
M694V/E148Q	15
M694V/726	1
Total	107

20% of 32 alleles

If E148Q was completely neutral:

We would expect it to appear in 4.7% of 32 alleles (1.5 alleles)

Genotype	Number
M694V/M694V	75
M694V/Null	16
M694V/E148Q	15
M694V/726	1
Total	107

1.5 expected vs. 15 observed
P<0.0001 (Fisher Exact Test)

A severe mutation such as M694V many times acts as a detector (magnet) of mild ones such as E148Q

The final number of E148Q alleles is determined by two opposing factors:

1. **The overwhelming abundance of M694V alleles.**
2. **The magnetic effect of single M694V alleles attracting E148Q.**

THANK YOU FOR LISTENING

ANY QUESTIONS?

Excluded genotypes

- M694V/M694V 2
- M694V/E148Q 2
- M694V/null 14
- null/null 19
- E148Q/null 3
- Penetrance:
- M694V/E148Q 0.155
- M694V/null 0.014


The End

If E148Q was neutral:

	expected	observed
E148Q	1.5	16
Others	213.5	198
Total	214	214

- 1.5 expected vs. 15 observed
- P<0.0001 (Fisher Exact Test)

Aim:

To determine the penetrance of the M694V/E148Q and M694V/null genotypes in adult FMF patients.

Functional role for E148Q in the disease

What is the penetrance of the disease in patients with the M694V/E148Q genotype

What is the penetrance of the disease in patients with the M694V/null genotype

Bernot et al. (1998) The distribution of the E148Q substitution was significantly higher in chromosomes from FMF patients(29/120) then in chromosome from healthy controls(3/131), $p<0.0001$.

Konstantopoulos et al. (2005)
35% of the patients, carried mutation E148Q. One was a homozygote and 20 carried mutation E148Q in combination with other mutations.
Compared with the results for healthy controls, E148Q mutation is significantly frequent

Tchernitchko D et al. (2003)

The frequency of the E148Q allele was found to be similar between FMF patients and controls (3.62% and 3.75%, respectively, p=0.93

This study, therefore, strongly supports the hypothesis that E148Q is a just a benign polymorphism and not a disease-causing mutation.

Ben Chetrit et al. (2000)

The relative frequency of the E148Q mutation in the control group (6.4%) and the patient group (7.8%) was not significantly different 0.25>p>0.1.

Marek-Yagel D, (2009)

Is E148Q a benign polymorphism or a disease-causing mutation?

Are there Phenotype Differences Between E148Q/M694V and M694V/0 Patients

A difference in the phenotype between the patients can prove functional role for E148Q