



# "Calreticulin Mutation Analysis in Iranian patients suffering from Essential thrombocythemia and Primary Myelofibrosis"

Dr Behzad Poopak, DCLS PhD.

Associate professor of hematology

Islamic Azad University Tehran Medical Science Branch

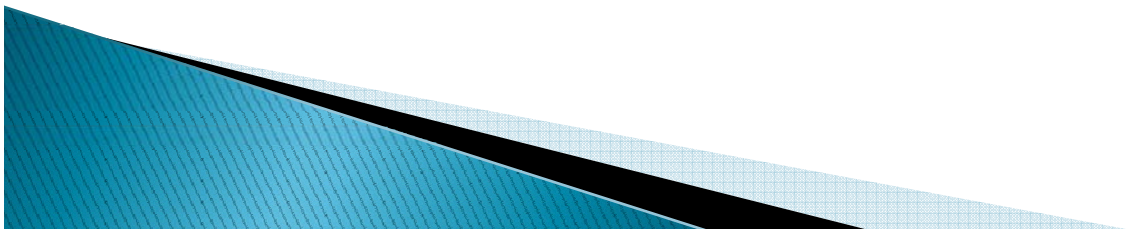
Zahra Amiri MSc student of Genetics

Islamic Azad University Tehran Medical Science Branch

Amiri.z@yahoo.com

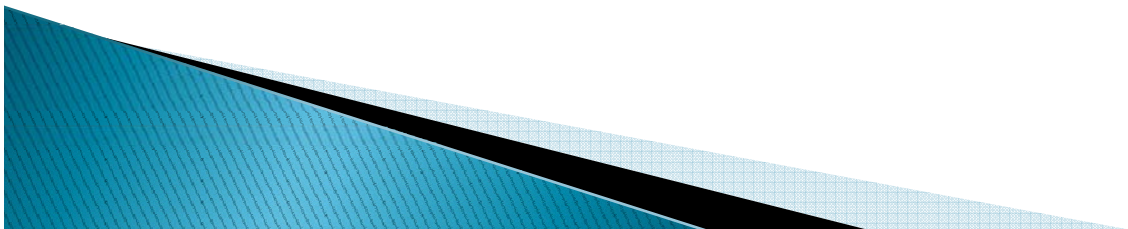
# MyeloProliferative Neoplasms Classification (MPN)

- ▶ **Philadelphia chromosome positive:**
  - ▶ Chronic myelogenous leukemia (CML)
- ▶ **Philadelphia chromosome negative:**
  - ▶ Chronic Neutrophilic Leukemia(CNL)
  - ▶ Polycythemia Vera (PV)
  - ▶ **Primary Myelofibrosis (PMF)**
  - ▶ **Essential Thrombocythemia (ET)**
  - ▶ Chronic Eosinophilic Leukemia(CEL)
  - ▶ Mastocytosis
  - ▶ Myeloproliferative Neoplasm Unclassifiable



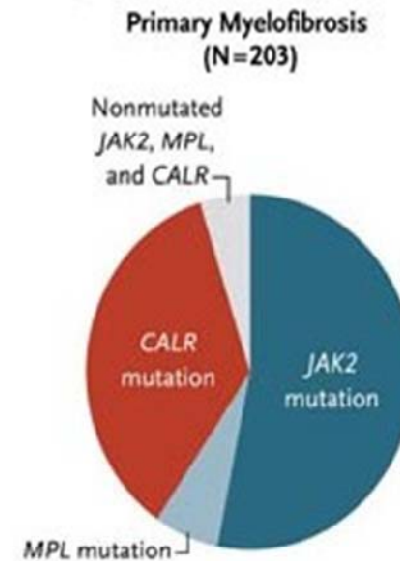
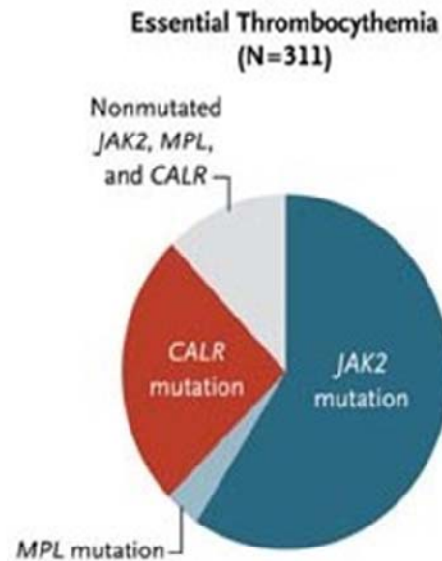
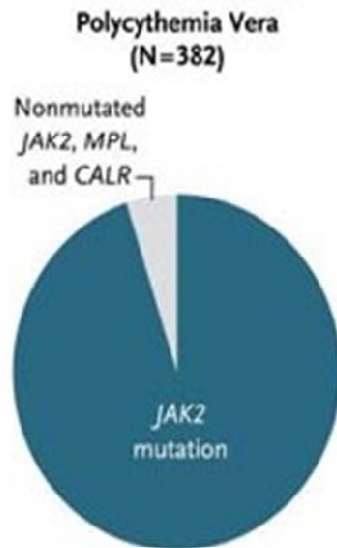
# Mutations in MyeloProliferative Neoplasms

- ▶ *JAK2-V617F*
- ▶ *Calreticulin*
- ▶ *MPLW 515K/L mutation*
- ▶ *JAK2-exon12*
- ▶ *BCR-ABL*
- ▶ *ABL Kinase*
- ▶ *c-KIT*



# Frequency of mutations in MPN

Distribution of *JAK2*, *MPL*, and *CALR* Mutations in Philadelphia Chromosome–Negative Myeloproliferative Neoplasms



**PV:**

*JAK2V617F*: 95%  
*JAK2EXON 12*: 2–3%

**ET:**

*JAK2V617F*: 50–60%  
*Calreticulin*: 25–35%  
*MPL* exon 10: 5%

**PMF:**

*JAK2V617F*: 50–60%  
*Calreticulin*: 35%  
*MPL* exon 10: 5–10%

Ref: N Eng J Med 2013;369(25):2379–2390

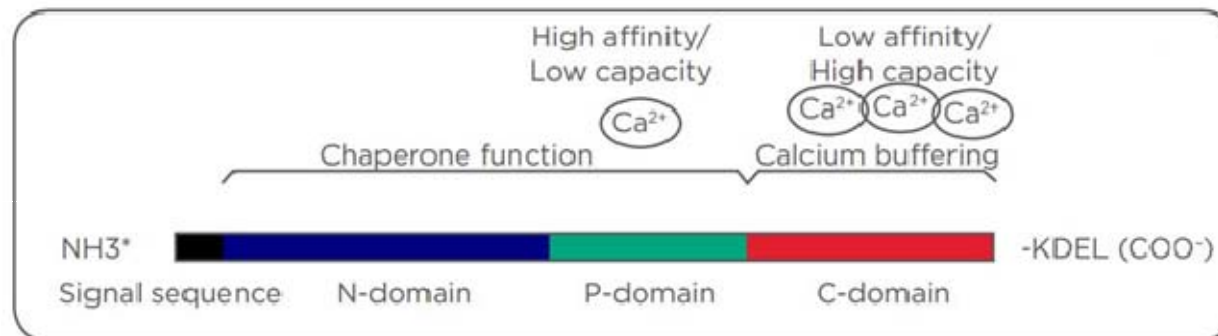
# Calreticulin protein & gene

- ✓ Gene Location: chromosome 19p13.2
- ✓ It consists 9 exons & 8 introns
- ✓ Chaperon protein, calcium hemostasis
- ✓ Control of cell proliferation, phagocytosis & apoptosis

1-180 residues: A high conserved aa sequence → Interaction with other proteins

181-290 residues: Calcium binding → high affinity

291-400 residues: Calcium binding → low affinity



# Calreticulin mutations

- Types:

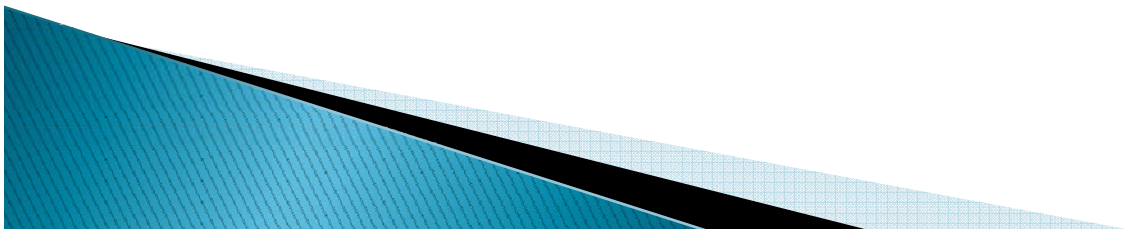
- ▶ Deletion
- ▶ Insertion
- ▶ Insertion & Deletion

- The most frequencies (more than 80%) :

Deletion 52bp (type1) & Insertion 5bp (type 2)

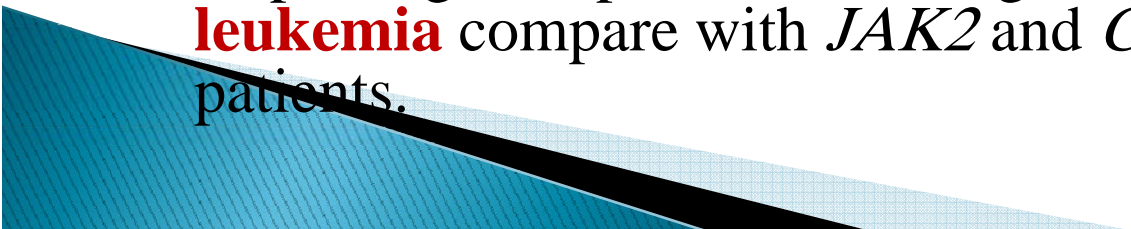
- Result proteins:

New C-domain without KDEL sequences



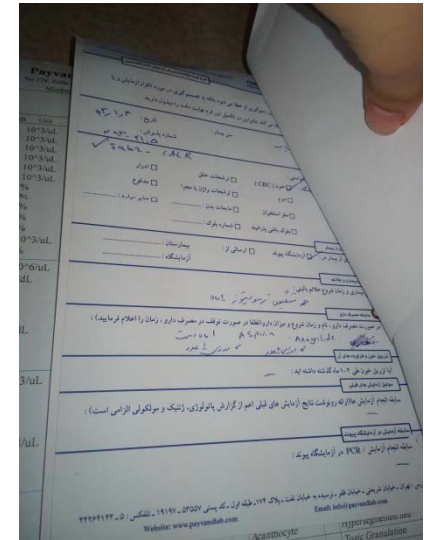


# Significance of *CALR* mutations assessment

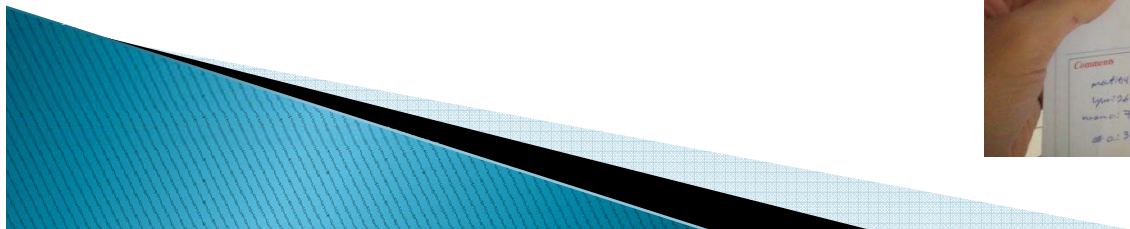
- ▶ Diagnosis of **clonality** in *JAK2-V617F* & *MPL* negative MPNs
  - ▶ MPNs versus Reactive cases.
  - ▶ *CALR* mutation assays increase our ability in Dx of MPN up to **95%**
  - ▶ *Calreticulin* mutant patients in comparison with *Jak2+ / MPL+* Patients have:
    - Lower risk for thrombocytopenia
    - Lower risk for sever leukocytosis and anemia
    - Lower risk of Thrombosis
    - longer overall survival
  - ▶ The **survival rate** of *calreticulin* mutation is more than *JAK2V617F*, *MPL* and triple negative mutations.
  - ▶ Triple negative patients have higher risk of transformation to **leukemia** compare with *JAK2* and *Calreticulin* positive patients.
- 

# Method & materials

- ▶ Patients admission, demographic data & history taking (all pts were JAK2 Neg.)

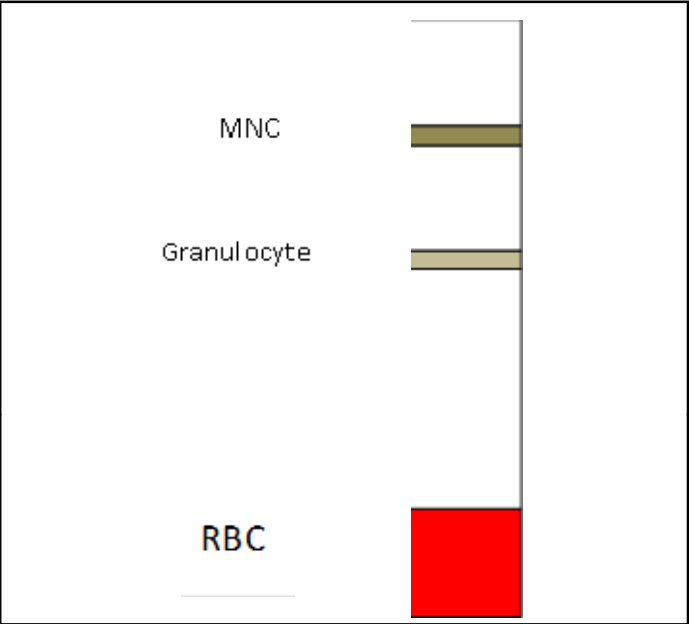


- ▶ EDTA anticoagulant blood collected for CBC & molecular studies





# Granulocytes isolation( ficoll SG: 1119, 1077)



DNA Extraction and QC

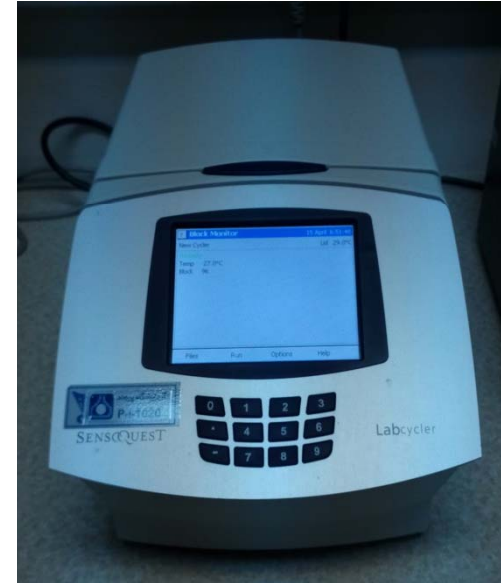


# Running PCR using specific primers

▶ **Primers sequence:**

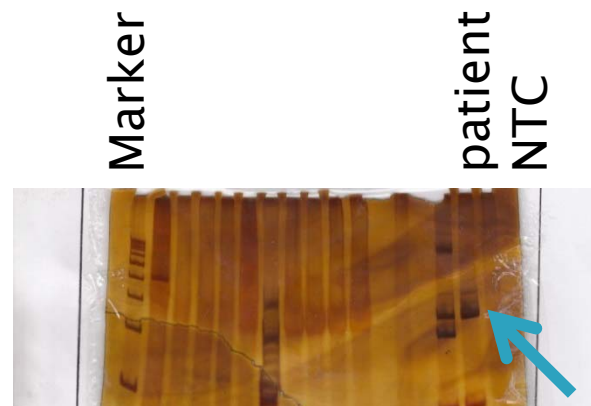
**Forward:** GGCAAGGCCCTGAGGTGT

**Reverse:** CAGGGCTGGACTGAGGCC

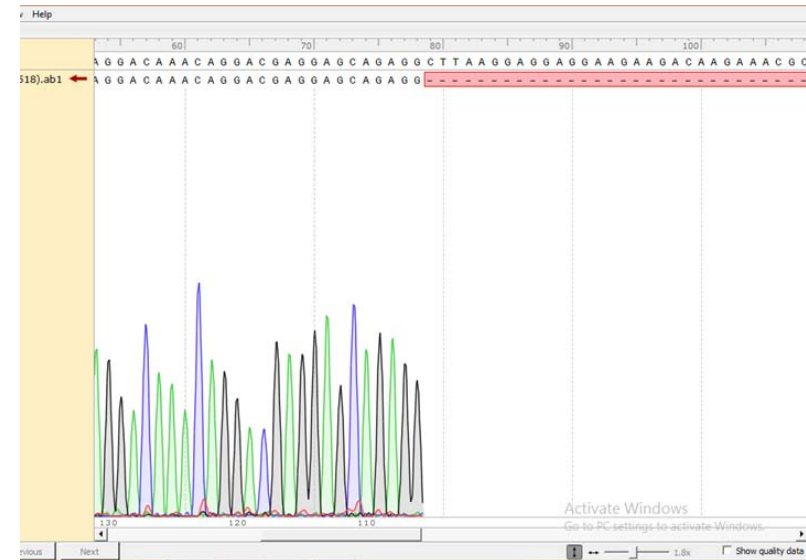


|                 | Cycles | Time   | Temperature |
|-----------------|--------|--------|-------------|
| pre-PCR         | 1      | 10 min | 95°C        |
| Amplification   | 45     | 15 sec | 95°C        |
|                 |        | 15 sec | 59.9°C      |
|                 |        | 30 sec | 72°C        |
| Final extension | 1      | 5min   | 72°C        |

## ▶ PCR product Observation & sequencing

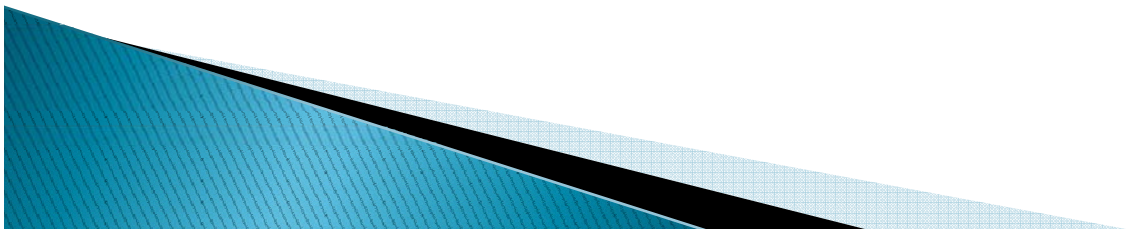


## ▶ Bioinformatics Analysis



# Results

- ▶ 30 patients(M:19=63% , F:11=36%), **mean Age: 45.85**
- ▶ All of them were referred with Dx of **MPN**
  
- ▶ 2 of 30 pts (6%) were positive for *CALR* mutation  
( **both were Male**)
  
- ▶ Type of mutation: **deletion, insertion/deletion**



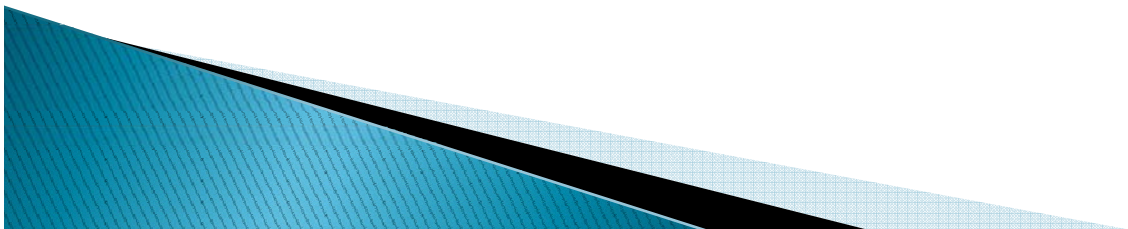
# Discussion

Calreticulin positive frequency in *JAK2-V617F* negative patients

- Klampfl (2013): 68% ET & 88% PMF (*JAK2 V617F*-neg)
- Our study: 6%

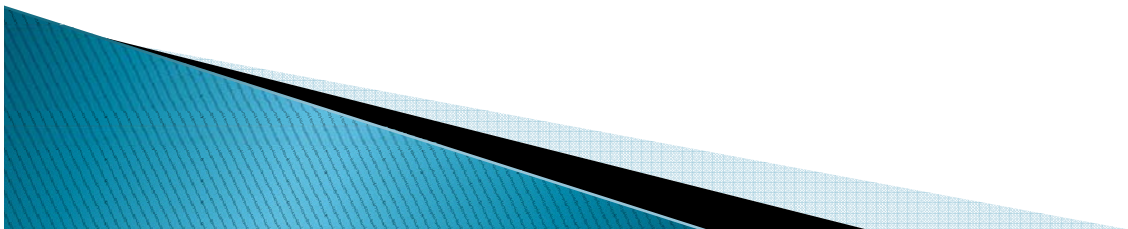
## Reasons may be:

- Some referred patients may not be MPN
- Study was done with low number
- Different frequency of the mutation in Iranian patients compare with other countries



# Summery

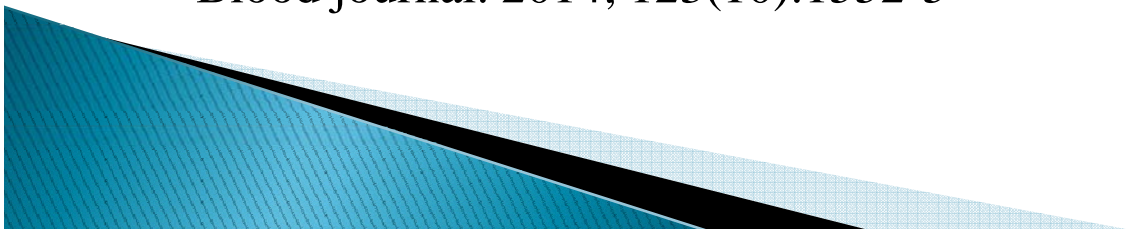
- **Introduction:** Calreticulin mutation is found in about **35%** of patients with ET and PMF.
  - ▶ Lower risk of thrombosis & longer overall survival
- **Method & materials:**
  - ▶ DNA extraction from granulocyte cells
  - ▶ Running PCR using specific primers
  - ▶ PCR product Observation & sequencing
  - ▶ Bioinformatics Analysis
- **Results:**
  - ▶ **2 of 30 (6%)** pts were positive for CALR mutation ( both were Male)





# References

1. Varricchio L, & Migliaccio A. R. Calreticulin in myeloproliferative: The other side of the alice mirror. *EMJ Hema* . 2014;(1):114-122.
2. Nangalia J, Massie C. E, Baxter E. J, Nice F. L , Gundem G, Wedge D. C, et all. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *New England Journal of Medicine* . 2013;369(25):2391-2405.
3. Klampfl T, Gisslinger H, Harutyunyan A. S, Nivarthi H, Rumi E, Milosevic J. D, et all. Somatic mutations of calreticulin in myeloproliferative neoplasms. *New England Journal of Medicine* . 2013; 369(25):2379-2390.
4. Rotunno G1, Mannarelli C, Guglielmelli P, Pacilli A, Pancrazzi A, Pieri L, Fanelli T, Bosi A, Vannucchi AM. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. *Blood journal*. 2014; 123(10):1552-5



# Acknowledgement

## □ My colleagues :

- Dr Mohammad Hassan Pouriayevali PhD of Virology  
Faculty of medical sciences, Tarbiat modars university, Tehran, Iran
- Mohammad Yaghoob taleghani MSc of Molecular Biology  
Gastroenterology and Liver Diseases Research Center, Research Institute for  
Gastroenterology and Liver Disease, Shahid Beheshti University of Medical  
Sciences, Tehran, Iran
- Fatemeh Sheikhsofla MSc of Molecular Biology  
Payvand Clinical and Specialty Laboratory

