# LA FAMIGLIARITA' IN AMBITO ONCO-EMATOLOGICO

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# AGENDA

- Defintion of «Hereditary» and «Familiarity»
- The Hereditary Cancer Syndromes
- The WHO classification of AML/MDS with germline predisposition
- The genetic counselling and NGS
- The familiarity in MPNs
- CLL and related involved genes

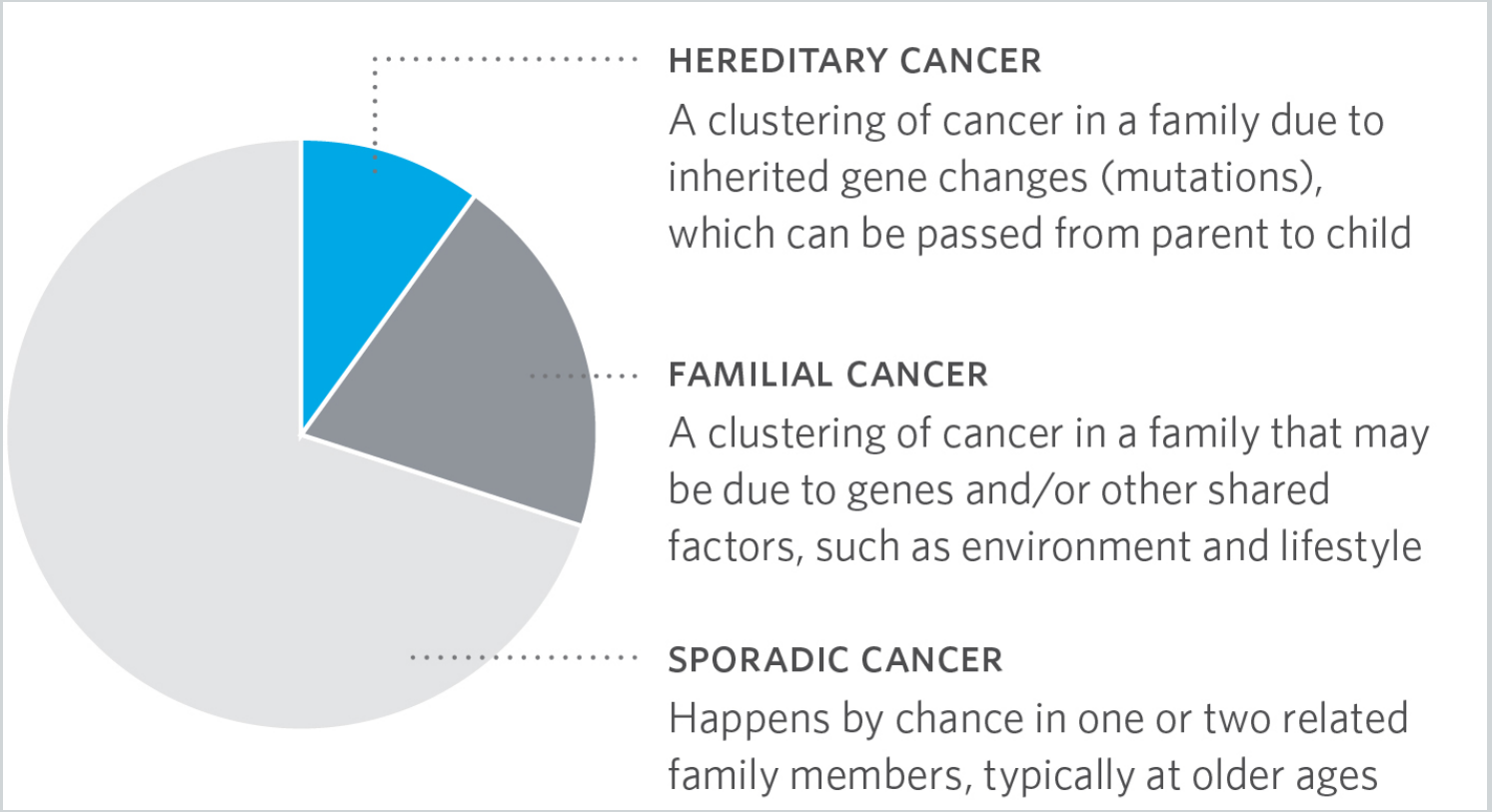
# CANCER AND HEREDITY

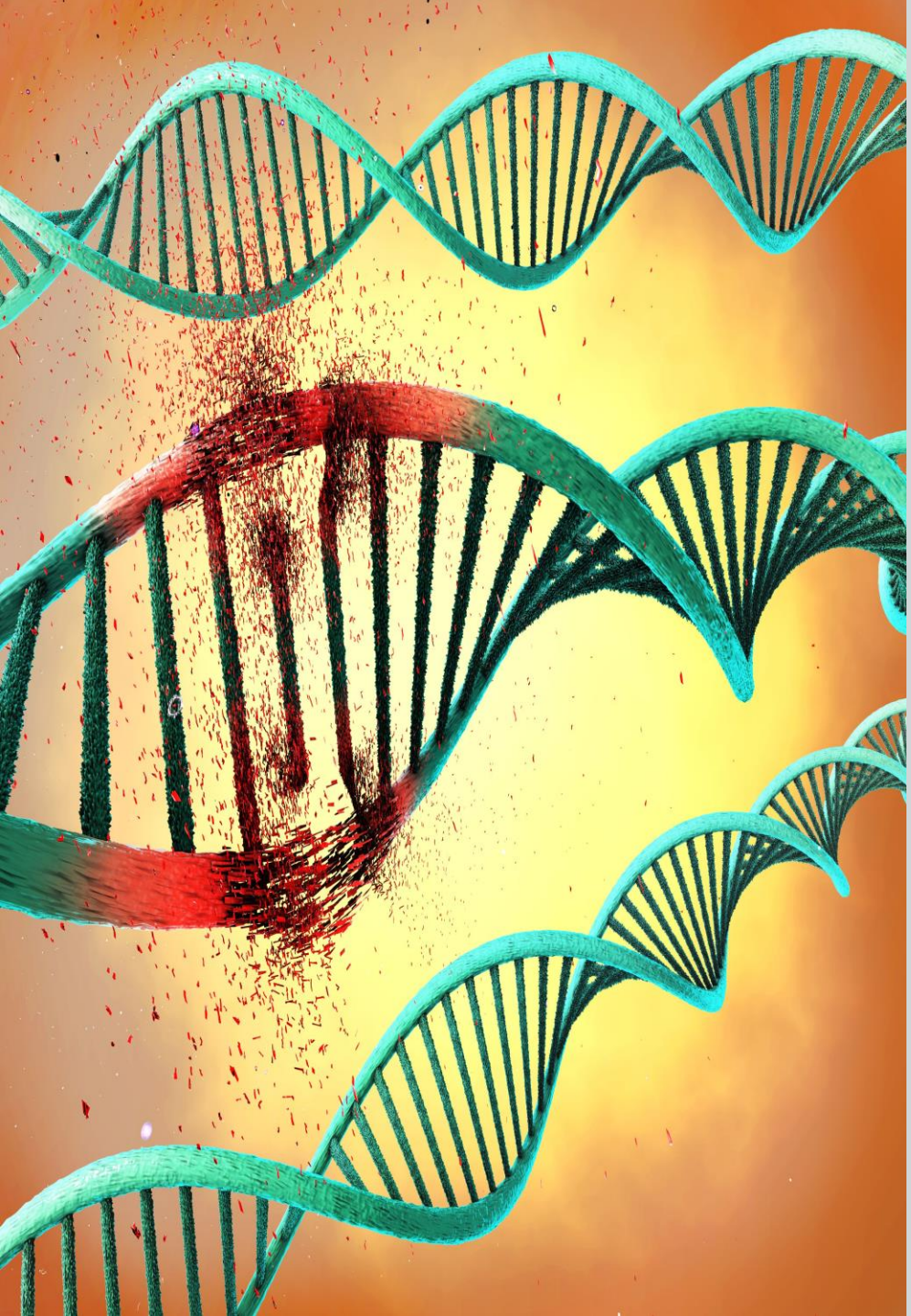
**Mutations in genetic material that increase a person's chances of developing cancer.**

**Genetic predisposition supposes an increased risk to develop a pathology in one person compared to an average risk estimated in general population.**

**The study of inherited predisposition provides an opportunity to identify key driver genes, which may lead to pathogenesis.**

# «HEREDITARY» AND «FAMILIARITY»

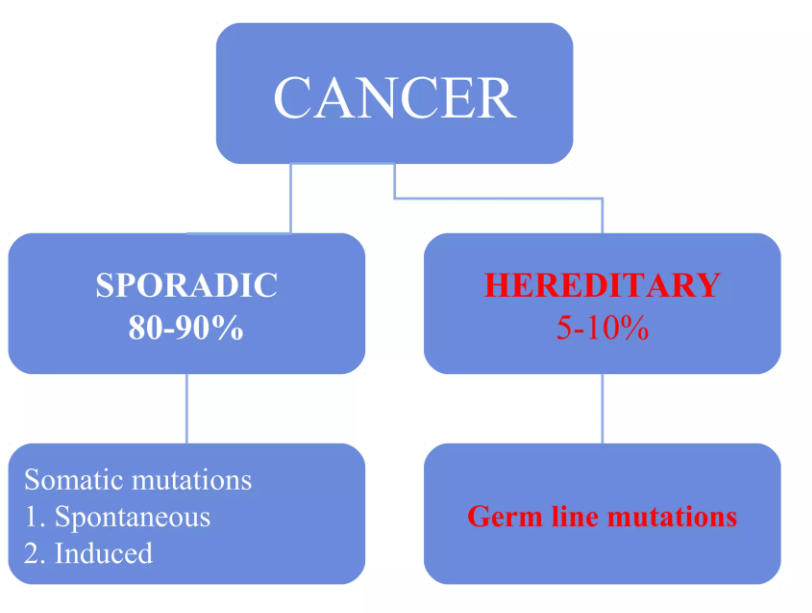




# CANCER AND HEREDITY:

## HEREDITARY CANCER SYNDROMES

# HEREDITARY CANCER SYNDROMES

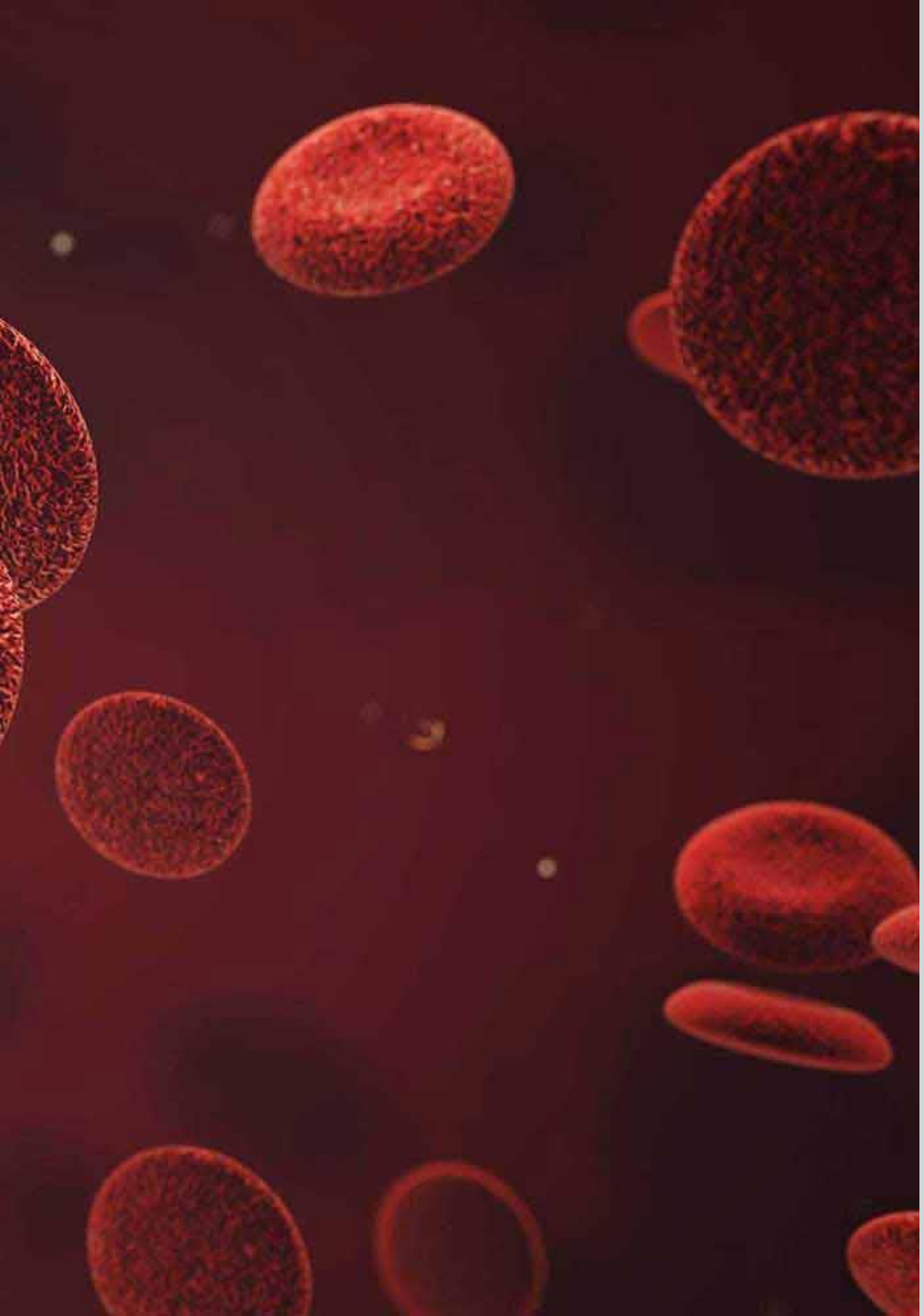



- Early onset of cancer, higher severity.
- Arising from mutations that confer an elevated susceptibility to cancer development.
- Exhibit an autosomal dominant inheritance pattern, with a 50% risk of transmission to offspring.
- Probability of occurrence of multiple tumors in the same individual.
- Occurrence of cancers in one family, which are known to be genetically related (such as breast and ovarian cancer, or colon and uterine cancer).

# HEREDITARY CANCER SYNDROMES

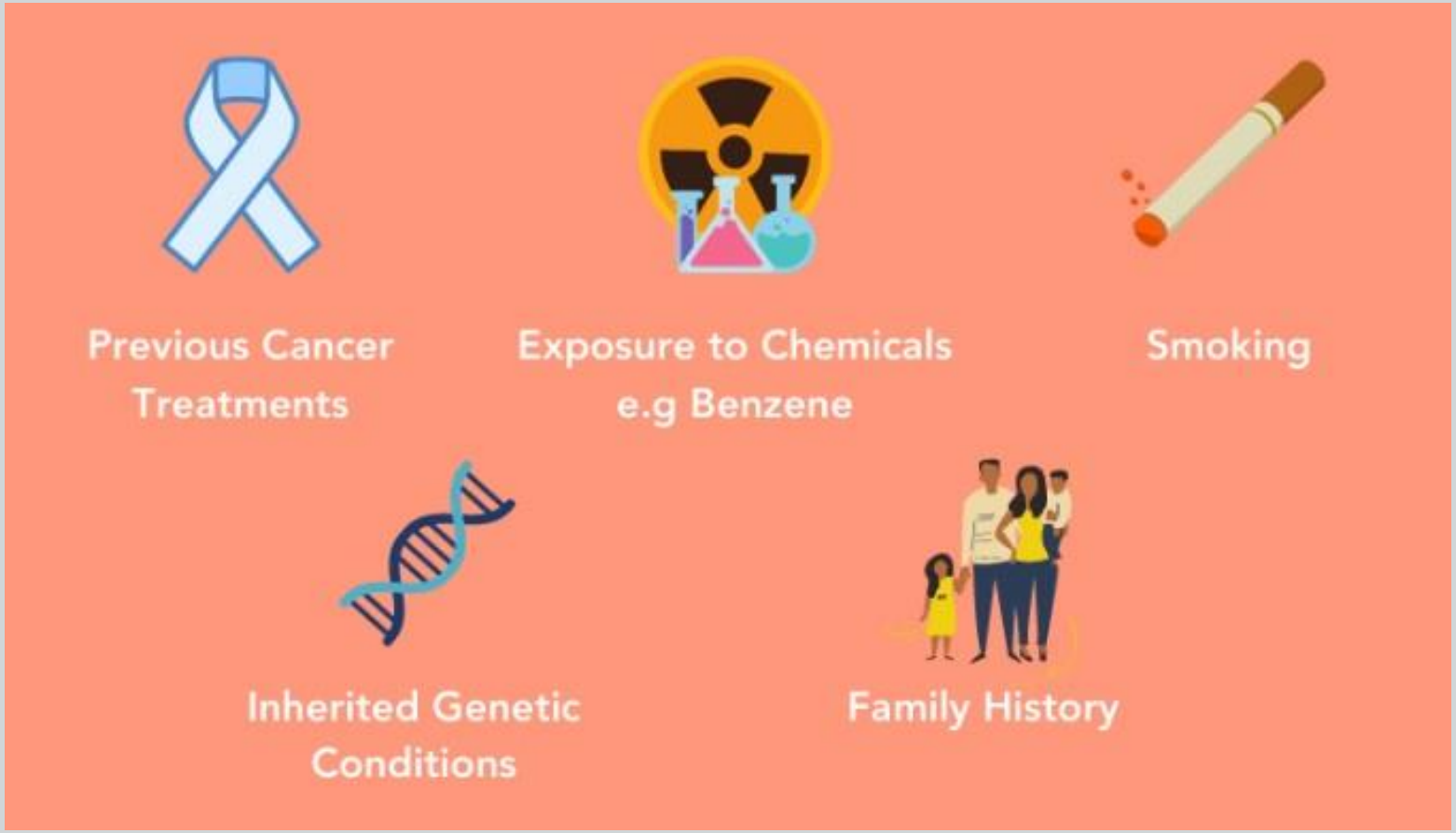
| Disease | Clinical characteristics | Gene mutated | Prevalence of disease | Pattern of inheritance | Penetrance |
|---|---|---|---|---|---|
| MEN 2A (multiple endocrine neoplasia syndromes) | Medullary thyroid carcinoma (MTC), pheochromocytoma (PC), parathyroid disease | *RET* | 1/40.000 | AD | • MTC 100%<br>• PC 50%<br>• Parathyroid disease 30% |
| VHL syndrome (von Hippel-Lindau disease) | Clear-cell renal carcinoma (cRCC), pheochromocytoma (PC), spinal hemangioblastoma (SHB) | *VHL* | 1/36.000 | AD | • cRCC 75%<br>• PC 30%<br>• SHB 50% |
| FAP (familial adenomatous polyposis) | Colorectal, duodenal, pancreatic and papillary thyroid cancer | *APC* | 1/8.000 | AD | • Colorectal cancer 100%<br>• Duodenal cancer 4-12%<br>• Pancreatic cancer 2%<br>• Thyroid cancer 1-2% |
| Hereditary breast cancer | Breast, ovarian, male breast, pancreatic cancer and melanoma | *BRCA1, 2* | 1-5/10.000 | AD | • Breast cancer 50-85%<br>• Ovarian cancer 10-60% |

## Available genetic tests

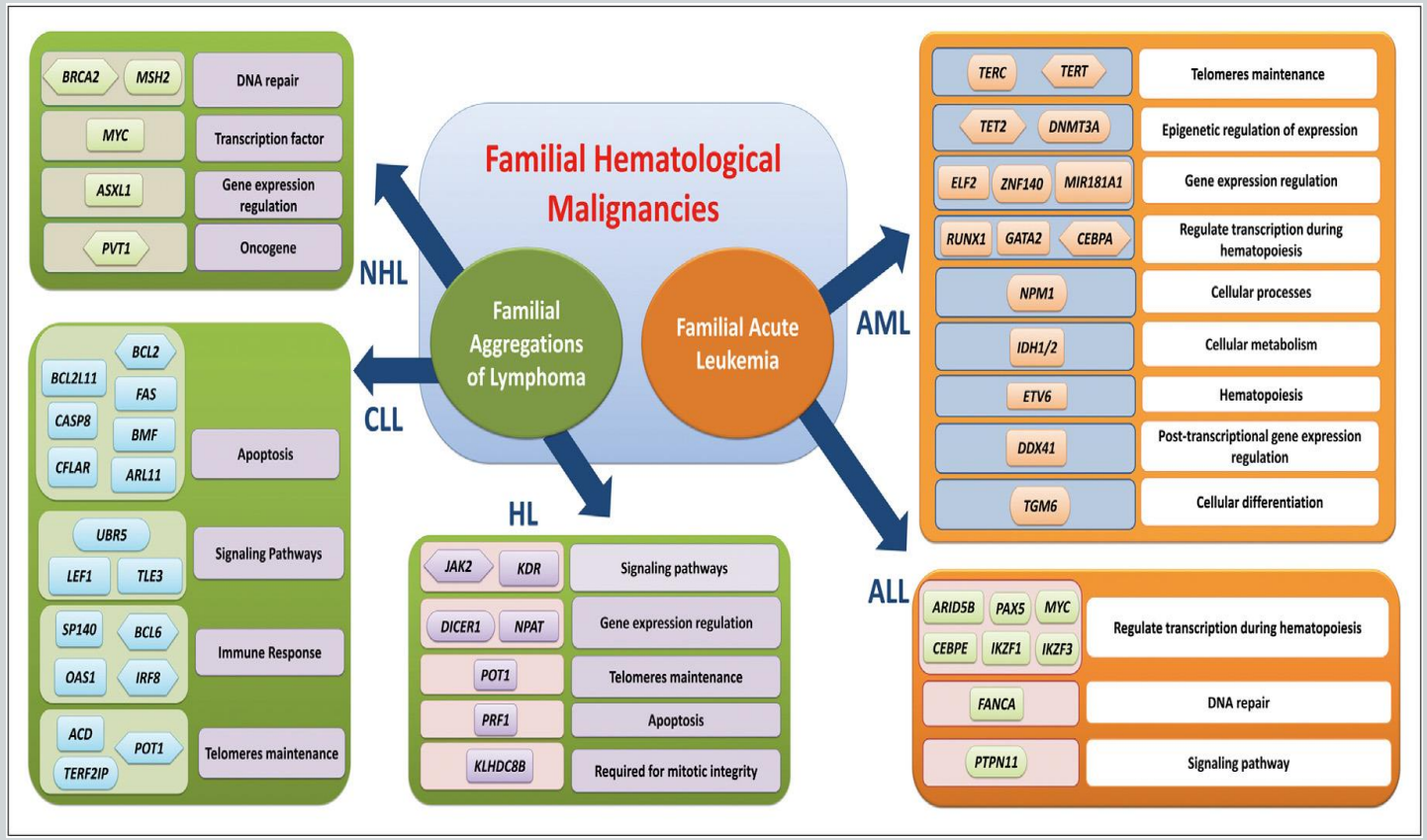


# HEMATOLOGICAL MALIGNANCIES WITH FAMILIAR PREDISPOSITION

# The risk factors for LEUKEMIA



# Predisposing genes to different subtypes of Familial aggregation of *hematological malignancies* and their function




- Malignancies associated with genetic predisposition related with rare germline mutations.
- High incidence of cases of leukemias and/or solid tumors in family members within 3° grade.
- Commonly, the inheritance is AD.

# MYELOID NEOPLASM WITH GERMLINE PREDISPOSITION

| Category | Causative Genes | Pattern of Inheritance | Germline Genetic Alterations |
|---|---|---|---|
| Myeloid neoplasms with germline predisposition without a preexisting disorder or organ dysfunction | | | |
| AML with germline *CEBPA* mutation | *CEBPA*[2] | AD | N-terminal frameshift or nonsense mutation |
| Myeloid neoplasms with germline *DDX41* | *DDX41*[21,23] | AD | Majority p.D140Gfs*2 |
| Myeloid neoplasms with germline predisposition and preexisting platelet disorders | | | |
| Myeloid neoplasms with germline *RUNX1* mutation | *RUNX1*[31,33] | AD | Frameshift, nonsense mutations, or deletion cluster to *RUNX1* N-terminal region and less frequently C-terminal region |
| Myeloid neoplasms with germline *ANKRD26* mutation | *ANKRD26*[37,38,42] | AD | Single-nucleotide substitutions in 5′ untranslated region |
| Myeloid neoplasms with germline *ETV6* mutation | *ETV6*[47,48] | AD | Frameshift, missense, and nonsense mutations in the DNA-binding and central domains |
| Myeloid neoplasms with germline predisposition and other organ dysfunction | | | |
| Myeloid neoplasms with germline *GATA2* mutation | *GATA2*[49,50] | AD | Truncating or missense mutations in second zinc finger domain, or mutations in the noncoding regulatory region |
| Myeloid neoplasm associated with inherited bone marrow failure syndromes and telomere biology disorders | | | |
| Fanconi anemia | *FANCA, FANCC, FANCG, FANCD1/BRCA2* [64,65] | AR, XL | Null mutations as results of frameshift, stop codon, and large deletions; altered protein mutations as results of missense, in-frame deletions, or C-terminus truncation mutations |
| Dyskeratosis congenita | *DKC1*,[66] *NOP10, NPH2, TCAB1, C16orf57, RTEL1*,[67,68] *TERC, TERT, TINF2*[69] | XL, AR, AD | Large and small deletions, insertions, and missense mutations throughout the coding regions |
| Telomere biology disorder | *TERT, TERC*[70] | AD, AR (*TERT*) | Large and small deletions, insertions, and missense mutations throughout the coding regions |

Abbreviations: AD, autosomal dominant; AML, acute myeloid leukemia; AR, autosomal recessive; MDS, myelodysplastic syndrome; NK, natural killer; XL, X linked.

## CLASSIFICAZIONE WHO 2016

*Inherited of the novo mutations within the germline that markedly increase the development of a myeloid neoplasm*

Arber DA, et al Blood. 2016

**CEBPA**
**DDX41**
**RUNX1**
**ANKRD26**
**ETV6**
**GATA2**
**TERT**
**TERC**

# MYELOID NEOPLASM WITH GERMLINE PREDISPOSITION: CHARACTERISTICS

- Autosomic dominant defect.
- Complete/incomplete penetrance.
- Earlier ages of cancer diagnosis.
- Early onset in the successive generations.
- Prognostic System not suitable at all.
- Hypocellular marrow not predictive of response to immunosuppressive therapy.
- Specialized approaches to therapy (e.g. HSCT earlier).
- Frequently associated with unique nonhematopoietic manifestations.

# MYELOID NEOPLASM WITH GERMLINE PREDISPOSITION: CATEGORIES

**Without a preexisting disorder or organ dysfunction (1)**

**Preexisting thrombocytopenia (2)**

**Other-organ dysfunction (3):**

Telomere biology disorders
+
BMF syndromes

| Mutated gene | Region | Inheritance | 1st report | Median age at diagnosis (range), years | Low platelets | Other organ dysfunction | Type of neoplasm |
|---|---|---|---|---|---|---|---|
| *CEBPA* | 19q13.1 | AD | 2004 | 25 (2–46) | no | no | AML |
| *DDX41* | 5q35.3 | AD | 2015 | 62 (40–85) | no | no | AML, MDS, rarely CML, CMML, lymphoma, myeloma |
| *RUNX1* | 21q22.12 | AD | 1999 | 39 (7–53) | yes | no | AML, MDS, rarely CMML, T-ALL, hairy-cell leukemia |
| *ANKRD26* | 10p12.1 | AD | 2011 | 38 (1–84) | yes | no | AML, MDS, rarely CML, CMML, CLL |
| *ETV6* | 12p13.2 | AD | 2015 | uncertain | yes | no | B-ALL, AML, MDS, CMML, myeloma, PV, solid tumors |
| *GATA2* | 3q21.3 | AD | 2010 | 20 (<1 to 78) | no | yes | AML, MDS, CMML, aCML |
| *SAMD9/SAMD9L* | 7q21.2 | AD | 2016 | uncertain | yes | yes | MDS, AML |

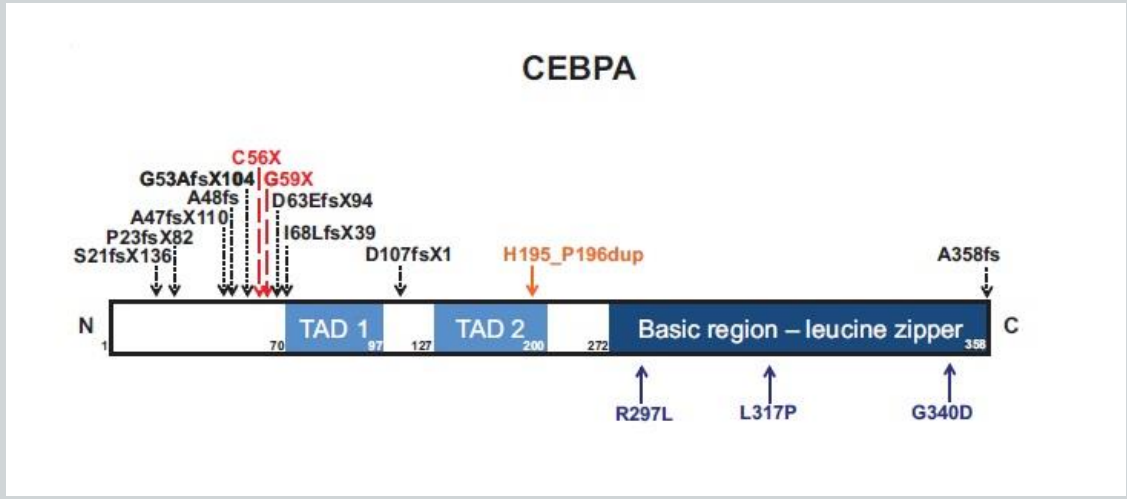
AD, autosomal dominant transmission; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; ALL, lymphoblastic leukemia/lymphoma; CLL, chronic lymphocytic leukemia; PV, polycythemia vera; aCML, atypical chronic myeloid leukemia.

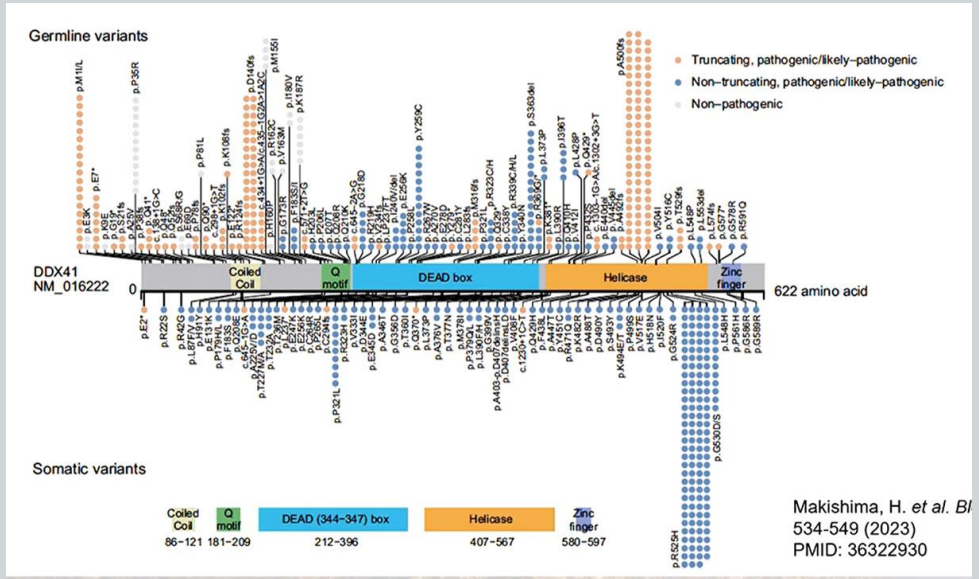
Classificazione WHO 2016

# GENES MUTATED IN MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION WITHOUT OTHER ORGAN DYSFUNCTION (1)

CEBPA (CCAAT/enhancer binding protein)
- Granulocyte differentiation transcription factor, regulates genes involved in myeloid differentiation.
- Biallelic mutations, with 1 mutation occurring in the germline.
- Penetrance close to 100%.
- Patients prone to relapse, no distinctive morphologic features.

DDX41 (DEAD/H-box helicase gene)
- RNA helicase with a role in the spliceosome.
- Biallelic mutations, with 1 mutation occurring in the germline.
- Late onset, advanced disease, normal karyotype, and poor prognosis (*loss-of-function mutants*).
- Early onset and predisposition to other hematologic malignancies, i.e. non-Hodgkin lymphoma, Hodgkin disease and multiple myeloma (*missense mutants*).



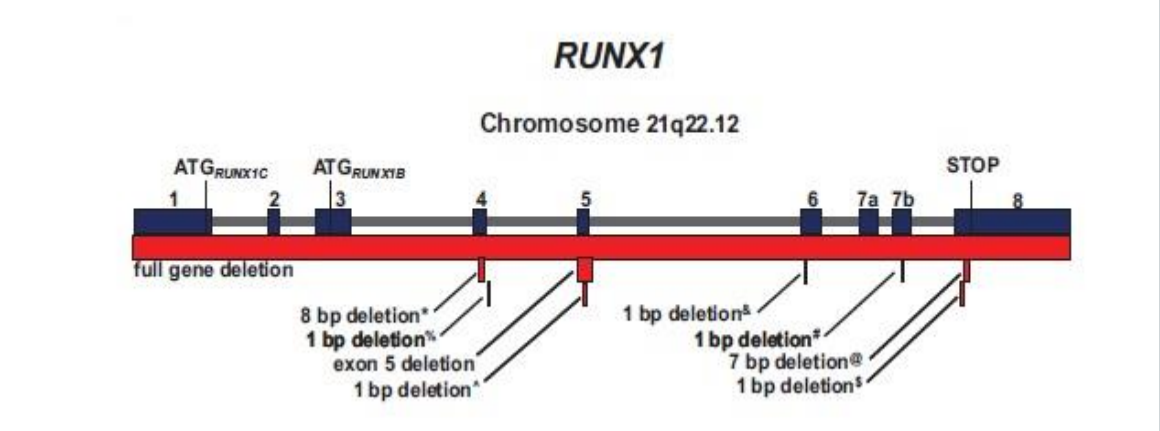

Nickels EM, et al. Ther Adv Hematol. 2013
Churpek JE et al, GeneRev, 2021

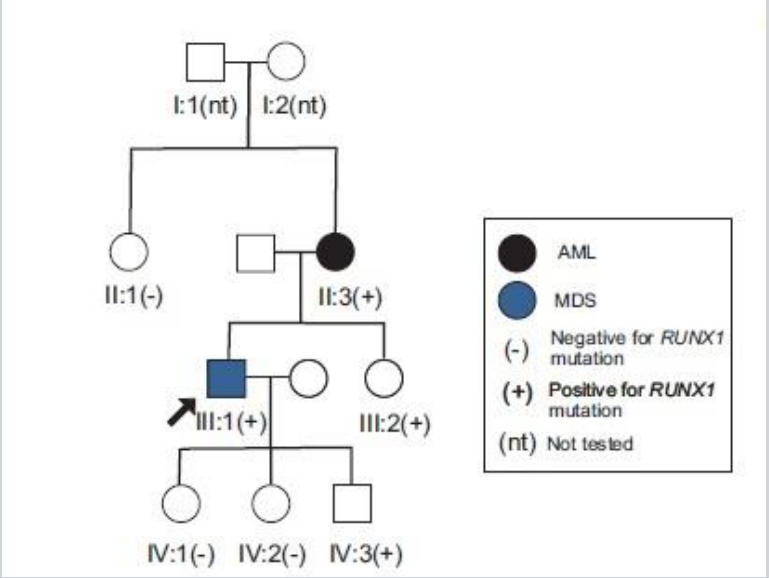
# GENES MUTATED IN MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION AND ASSOCIATED WITH PLATELET DYSFUNCTION (2)

RUNX1 (DNA-binding subunit of the core binding factor (CBF) transcription complex)
- Transcription factor required for the normal hematopoiesis.
- Monoallelic mutations.
- Complete penetrance.
- Clinical presentation is highly variable, early onset.
- Functional platelet defects.

ANKRD26 (Ankirin Repeat Domain 26)
- Expressed in megakaryocytes, and, to a lesser extent, in erythroid cells.
- Cause thrombocytopenia 2 (THC2), an autosomal dominant form of inherited thrombocytopenia.
- Mutations affect megakaryopoiesis and platelet production.

ETV6 (ETS Variant Transcription Factor 6)
- Missense mutations cause DNA-binding properties altered and autosomal dominant thrombocytopenia.
- Complete penetrance.
- Early onset of MDS, CMML, ALL and multiple myeloma.
- Familial predisposition to solid tumor (colorectal, breast, kidney, and skin cancers, and meningioma).

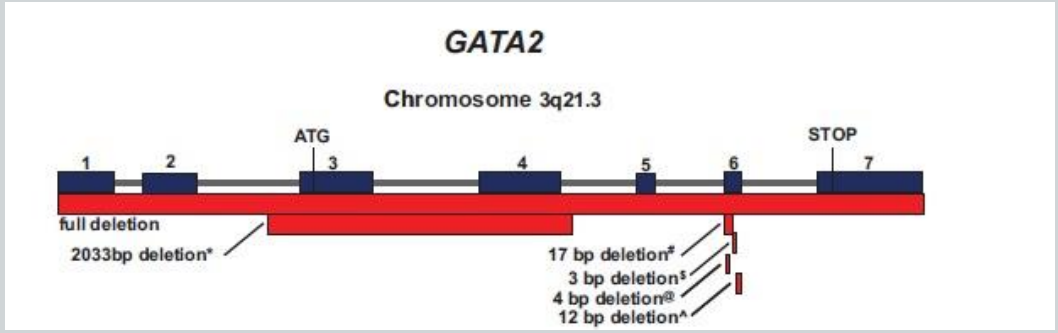


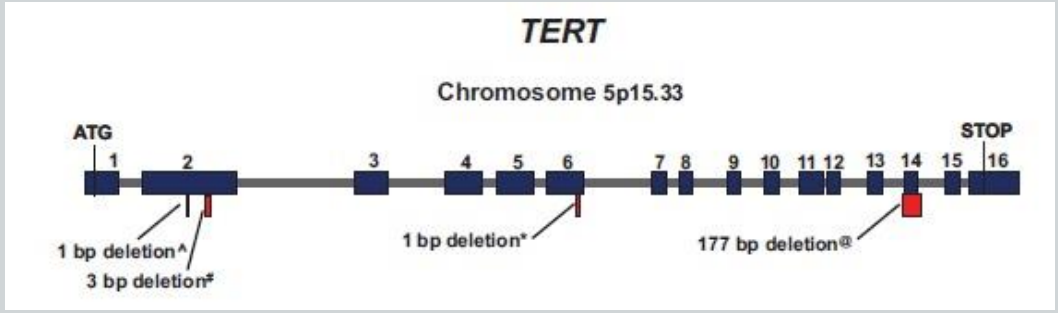



# GENES MUTATED/AMPLIFIED IN MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION AND ASSOCIATED WITH OTHER ORGAN DYSFUNCTION (3)

GATA2 (transcription factor that bind to the DNA sequence “GATA”)
- Regulates hematopoiesis, autoimmunity, and inflammatory and developmental processes.
- No genotype–phenotype correlations.
- 70% penetrance.
- Lymphedema, atypical infections, immune deficiencies.
- Earlier age at onset.
- Poor prognosis, HSCT required.

Telomere biology disorders (TERT or TERC)
- Ribonucleoprotein complex that is responsible for maintaining telomeres.
- Mutations cause excessive telomere shortening and genomic instability.
- Each generation inherits shorter telomeres and those with the shortest telomeres are affected at earlier ages and with more severe phenotypes.
- Variable clinical manifestations (skin pigmentation, oral leukoplakia, idiopathic pulmonary fibrosis, unexplained liver disease ) and incomplete penetrance.

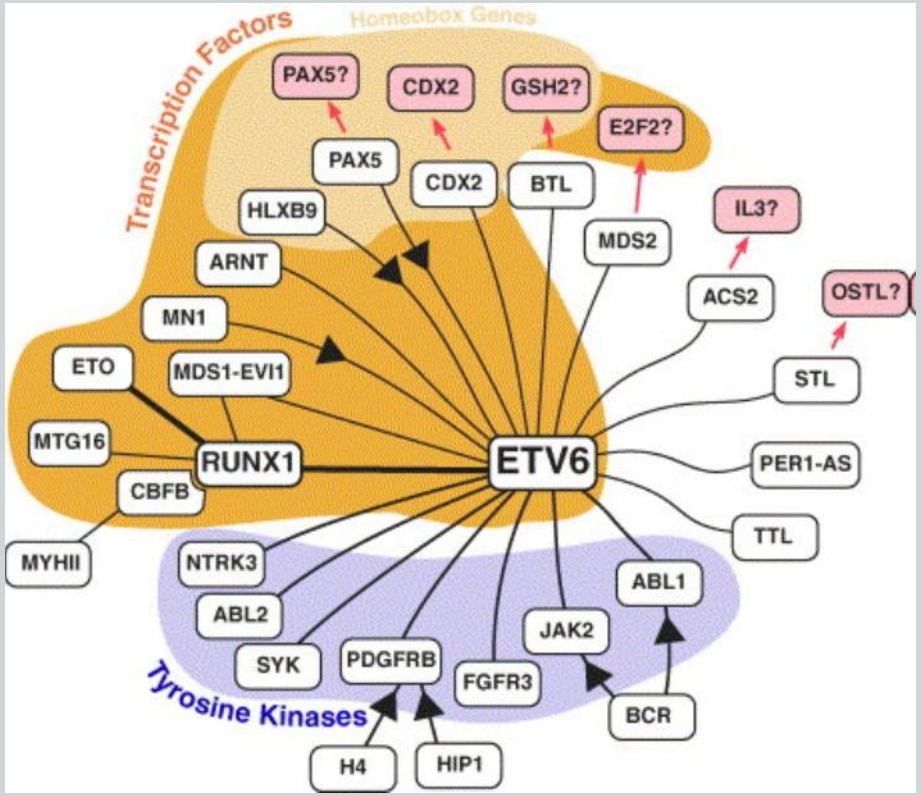


# THE CASE OF ETV6

GERMLINE PREDISPOSITION TO MYELOID MALIGNANCIES

# ETS VARIANT TRANSCRIPTION FACTOR 6 (ETV6)

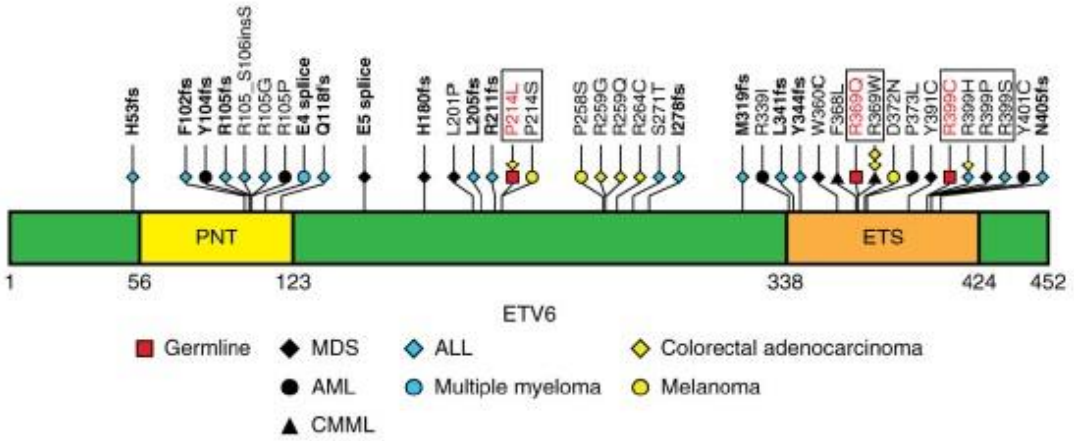



**Fusion Gene Partner of ETV6**

Main hotspot mutations in the *ETV6* gene and their impact on the protein.

| Mutation | Domain | Effect |
|---|---|---|
| P214L | Central regulatory domain | (i) Repression of DNA binding by the ETS domain<br>(ii) Defective proplatelet formation and megakaryocyte maturation<br>(iii) Alteration of proplatelet spreading<br>(iv) Down regulation of several cytoskeletal proteins<br>(v) *ETV6* delocalization |
| N385Vfs | ETS | (i) Reduction in repressive activity<br>(ii) Targeted proteins downregulation |
| Y401N | ETS | (i) Impaired interaction with corepressor<br>(ii) Defective proplatelet formation and megakaryocyte maturation |
| R369W/R369Q | ETS | (i) Reduction in repressive activity<br>(ii) Targeted protein downregulation<br>(iii) *ETV6* delocalization |

ETS = highly conserved C-teminal DNA-binding domain.




Hang et al. Nat Genet. 2015

*Case Report*

# *ETV6*: A Candidate Gene for Predisposition to "Blend Pedigrees"? A Case Report from the NEXT-Famly Clinical Trial

Simona Bernardi,[1,2] Mirko Farina,[1] Camilla Zanaglio,[1,2] Federica Cattina,[1] Nicola Polverelli,[1] Francesca Schieppati,[3] Federica Re,[1,2] Chiara Foroni,[1,2] Michele Malagola,[1] Andrew J. Dunbar,[4] and Domenico Russo[1]

70-year-old woman
Progressive thrombocytopenia,
Monocytosis
MDS with multilineage Dysplasia
Translocation (3; 21)
Died for AML

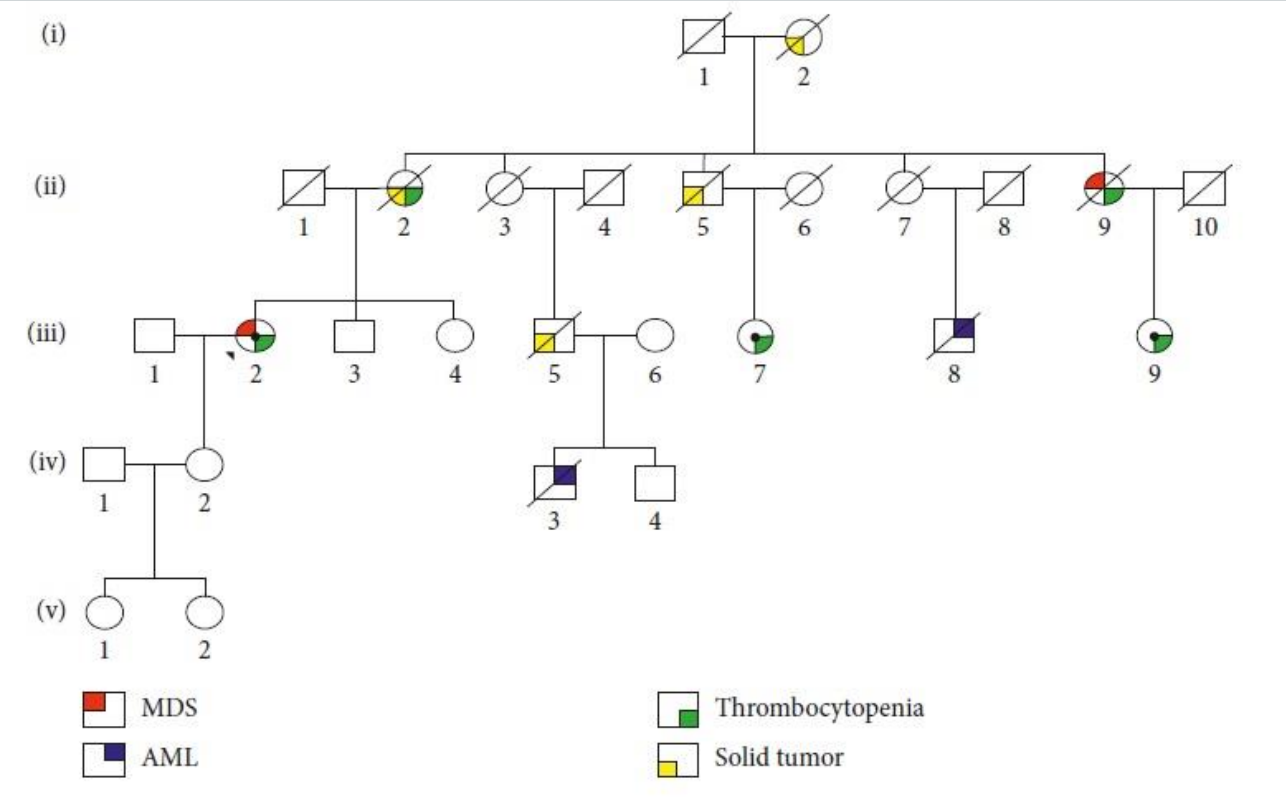
MiSeq Illumina NGS platform for mutations in 25 genes associated to myeloid neoplasms (PBMCs at time of diagnosis)

**ETV6 mutated**

Sanger Sequencing
(Germinal DNA from epithelial buccal cells)
both in index case and affected relatives still living

**NEXT-Famly Clinical Trial**

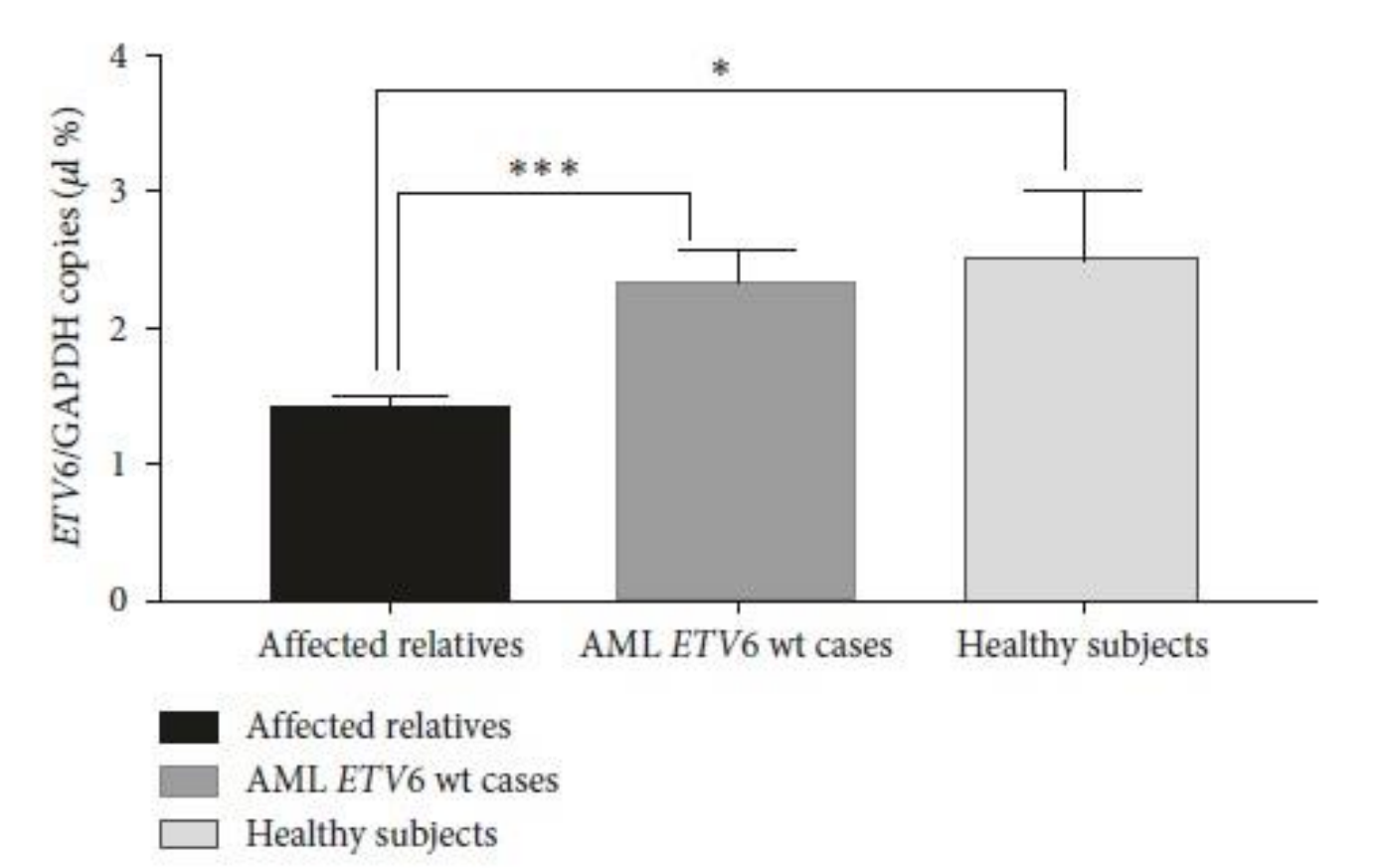
# ETV6 IN « BLEND PEDIGREES »




**c.514C>T of ETV6 3'UTR on both the tumor and germline DNA of the index case**

NEXT-Famly Clinical Trial

# DOWNREGOLATION OF ETV6 TRANSCRIPT




## Analysis of the ETV6 mRNA

ETV6 mRNA

↓

cDNA ETV6

↓

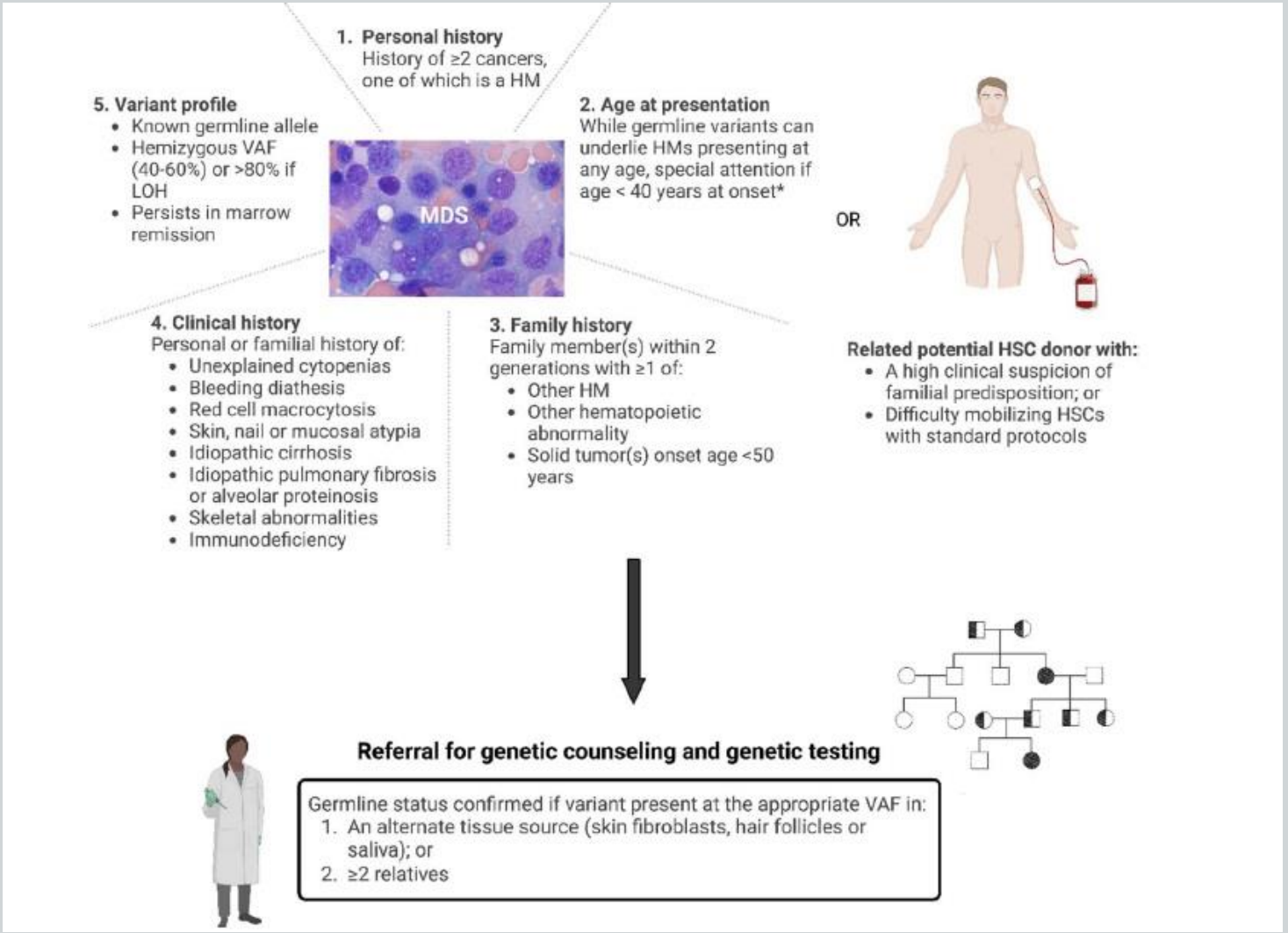
dPCR

**NEXT-Famly Clinical Trial**

**Recognition of familial predisposition is necessary for the management of patients, especially for AML/MDS**

**Genetic counseling**

# GENETIC COUNSELING PROCESS FOR FAMILIAL CANCER




**Indicators of potential *germline predisposition*:**

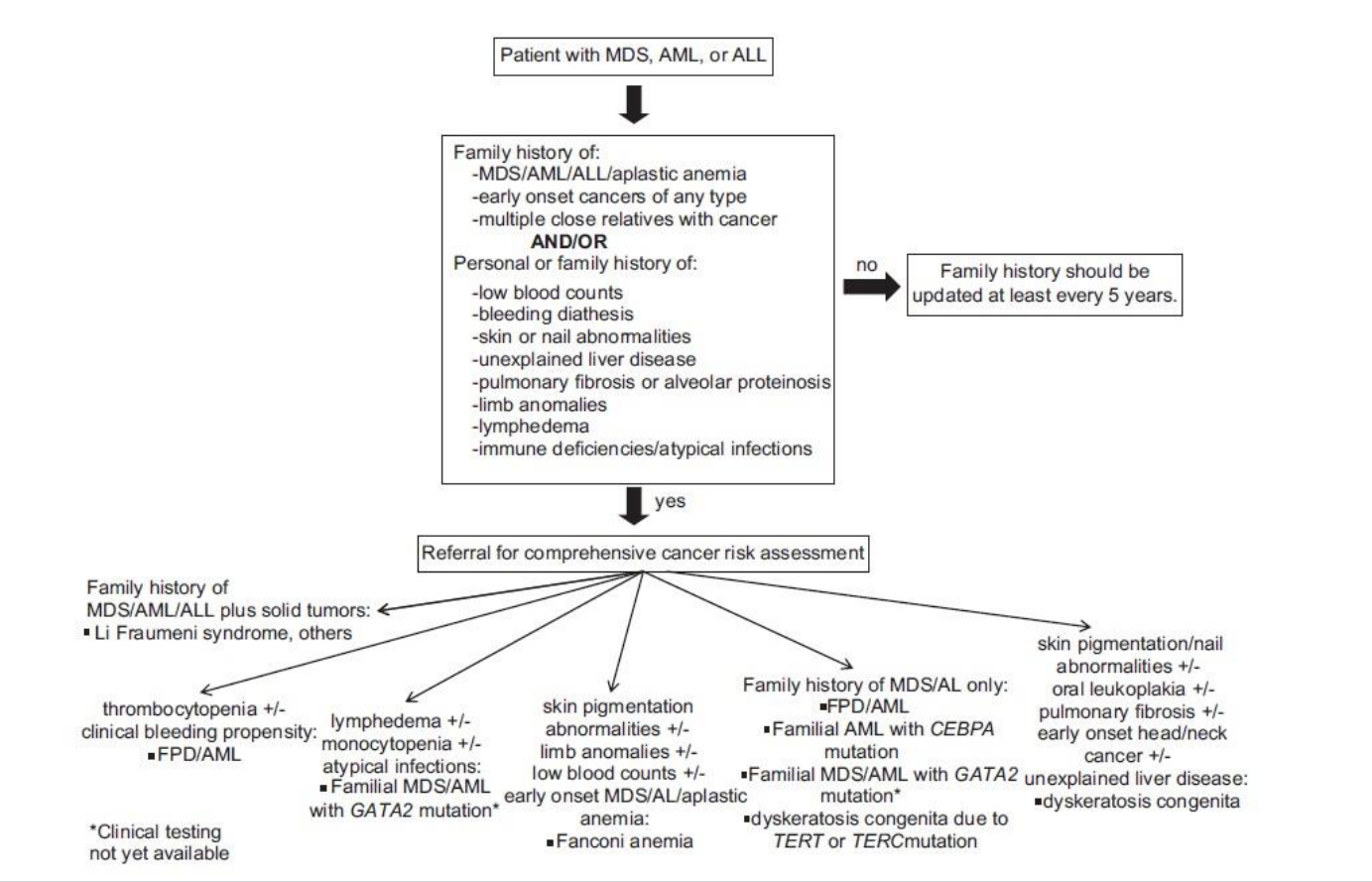
**- variant-specific, including known inheritance pattern;**

**- variant allele frequency between 40% and 60% or >80% in the case of loss of heterozygosity;**

**- persistence in post-remission bone marrow samples.**

Gener-Ricos G. et al. Cancer J. 2023

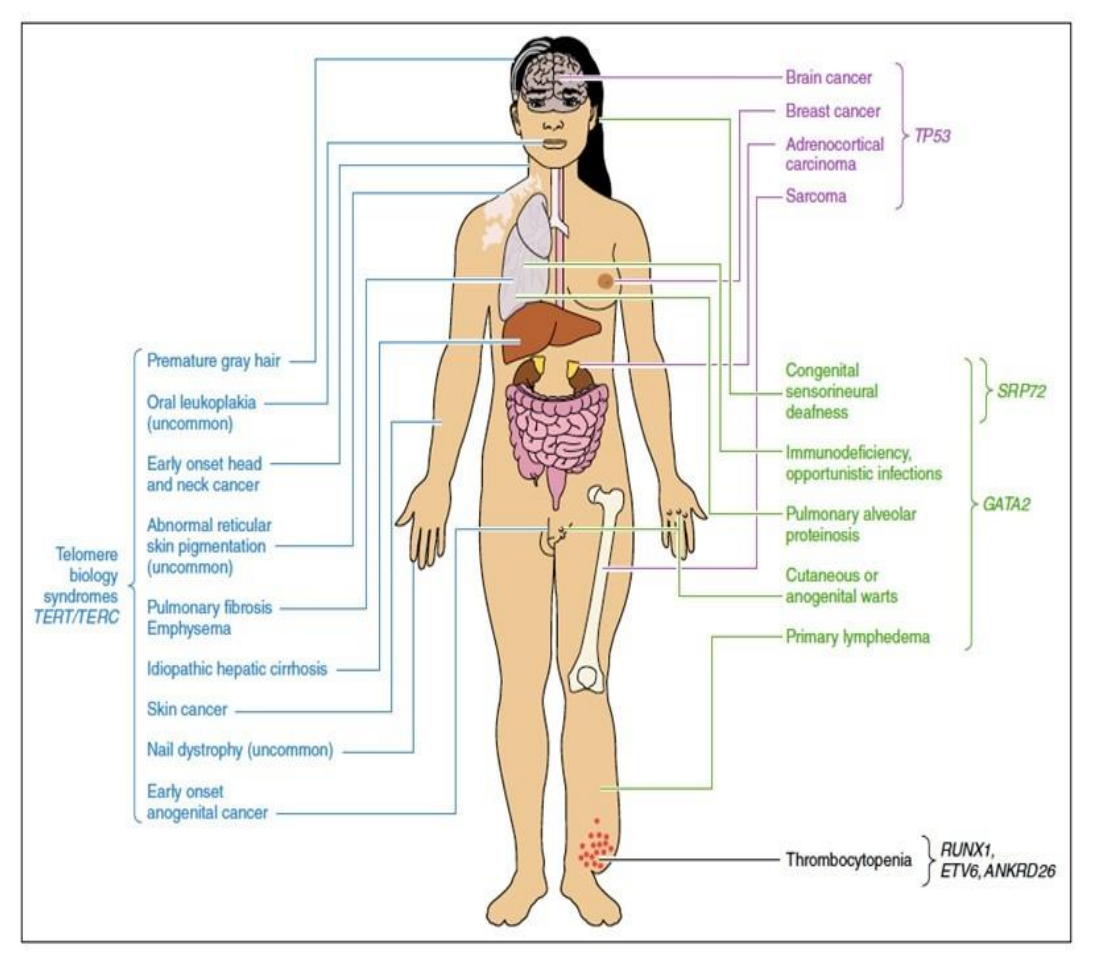
# MANAGEMENT OF MYELOID NEOPLASMS WITH FAMILIAL PREDISPOSITION




(1) a first- or second-degree family member who has a diagnosis of acute leukemia (AML or ALL), MDSs or other myeloid neoplasms;
(2) a first- or second-degree family member who has a diagnosis of other hematologic neoplasms;
(3) a first- or second-degree family member who has a diagnosis of solid tumor that has arisen in age < 40 years;
(4) Presence of signs, symptoms or laboratory tests that resemble one of the familial myeloid neoplasms predisposition syndromes

Churpek et al., Leukemia & Lymphoma 2013

# THE RED FLAGS

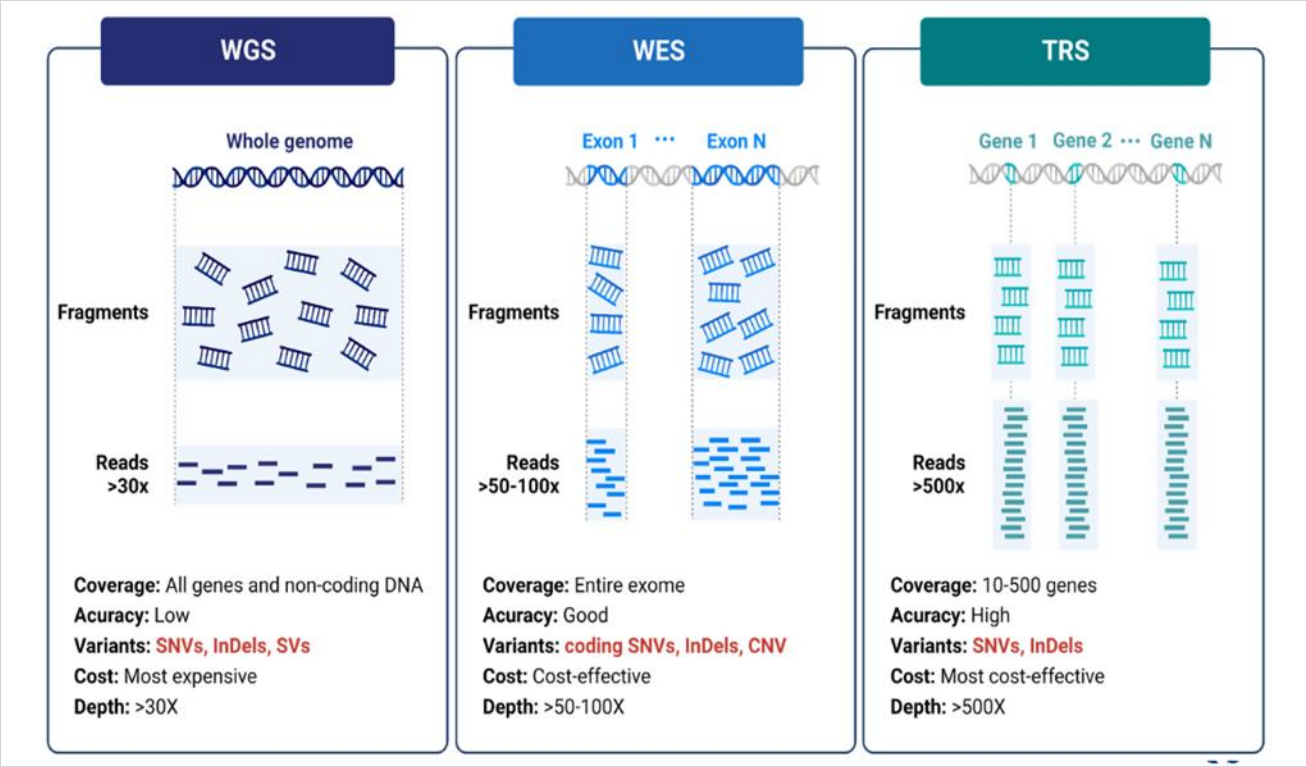



- History of thrombocytopenia and/or clinical bleeding propensity (as in *RUNX1*, *ANKRD26* or *ETV6* germline mutations)

- Abnormal nails or skin pigmentation, oral leukoplakia, idiopathic pulmonary fibrosis, unexplained liver disease (as in *TERT* and *TERC* germline mutations)

- Lymphedema, atypical infections, immune deficiencies (as in *GATA2* germline mutations)

# NGS APPROACH FOR MDS/AML FAMILIAL PREDISPOSITION STUDY

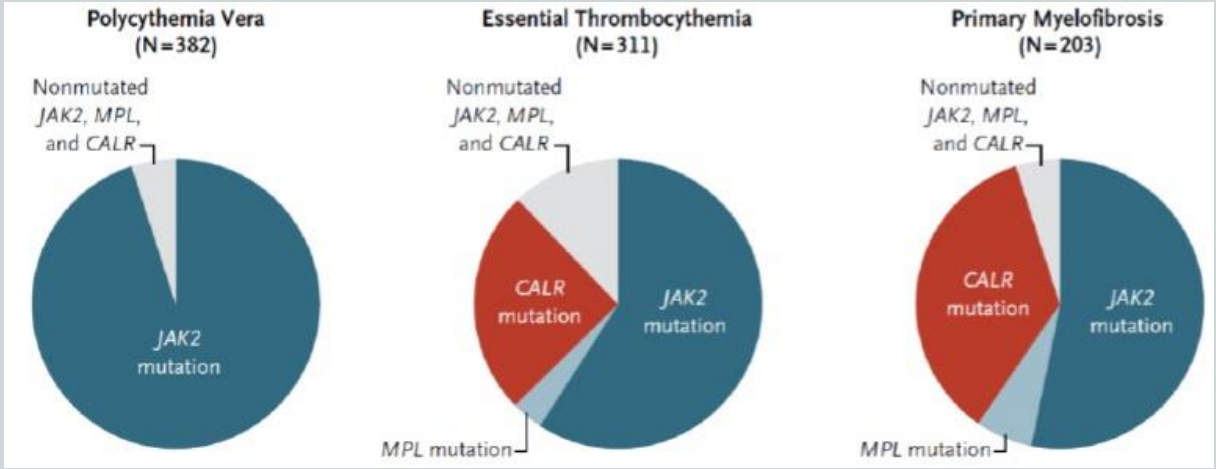



- **Identification of a family history of cancer.**
- **Help identify those at-risk.**
- **Enhanced surveillance.**
- **Early detection.**
- **Identification of novel targeted therapies.**

Single nucleotide variants (SNVs)
Duplications, Insertions, Deletions
Exon and gene copy number changes
Structural variants (SVs)

# MPNs AND FAMILIAL PREDISPOSITION

| NMC Philadelphia - | NMC Philadelphia + |
|---|---|
| Policitemia vera (PV) | Leucemia Mieloide Cronica (LMC) |
| Trombocitemia essenziale (TE) | |
| Mielofibrosi idiopatica o primaria (MF) | |



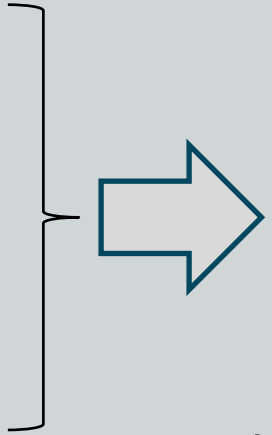

*Nangalia J et al. N Engl J Med 2013;369:2391-2405. Klampfl T et al. N Engl J 2013;369:2379-2390.*

# MPNs AND FAMILIAL PREDISPOSITION

**7% of apparently sporadic MPN are familial**

- Relatives of MPN patients have a five- to seven-fold increased risk of developing MPN.

***Evidences of Familial Predisposition***:
- Some MPN patients are shown to be "biclonal"
- Phenotypic diversity of MPN
- Different subtypes of the disease (PV, TE and MF) in patients who carry the same mutations



INFLUENCE BY A GERMINAL VARIANT

Rumi et al, 2007; Landgren et al, 2008

# MPNs WITH FAMILIAL PREDISPOSITION

- What is truly inherited is a genetic predisposition to acquire one of the three different MPN-specific mutations (JAK2, MPL, CALR).

- Predisposition would be inherited as an autosomal-dominant trait with variable expression and incomplete penetrance.

- Clinical features of Familial MPNs are the same of Somatic MPNs: the genetic test is not required since syntoms, reponse to treatment and disease development are superimposable with ones of sporadic MPNs.

- Survival is generally similar in familial and sporadic MPN (exception for ATG2B).

- Somatic mutagenesis is increased in familial MPNs.

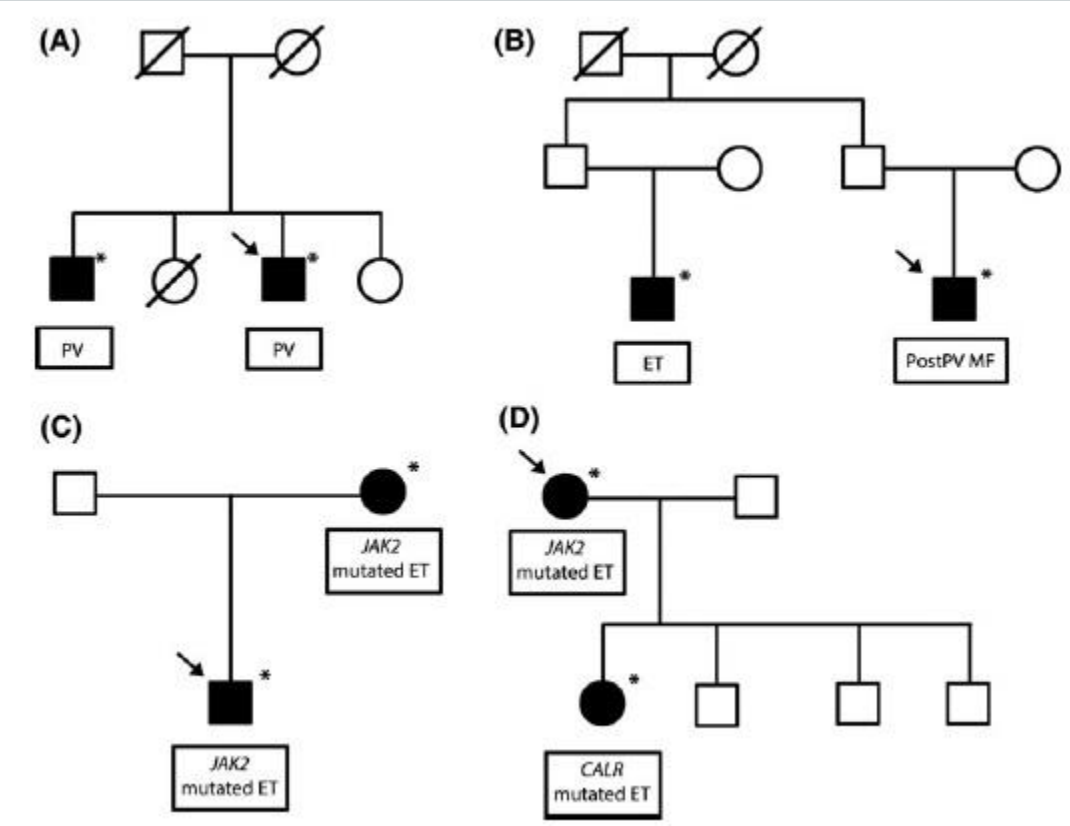
Genetic factors predisposing to myeloproliferative neoplasms.

| Genetic factor | Role |
|---|---|
| **Predisposing SNP** | |
| *JAK2* GGCC | Associated with an increased risk of developing *JAK2* V617F-mutated MPN (hypermutability hypothesis) or MPN in general (fertile ground hypothesis). Not responsible by itself for familial clustering of MPN. |
| *TERT* rs2736100_C | Associated with an increased risk of developing MPN. May be responsible for a substantial part of familial clustering in MPN. |
| SH2B3 rs3184504 | Increased risk of *JAK2*-mutated MPN |
| *MECOM, HBS1L*-MYB, *SH2B3, TET2, ATM, CHEK2, LINC-PINT, GFI1B* | Other predispositions alleles that predispose to both age-related *JAK2* V617F clonal haematopoiesis in the general population as well as MPN independent of V617F status. |
| **Predisposing germline mutations** | |
| Germline duplication of *ATG2B, GSKIP* | Overexpression of these two genes enhances haematopoietic progenitor differentiation by increasing progenitor sensitivity to thrombopoietin. They cooperate with acquired *JAK2, CALR* and *MPL* mutations during MPN development |
| Germline *RBBP6* mutations | *RBBP6* mutations are present in about 5% of familial MPN. |
| Germline *SH2B3* mutations | *SH2B3* mutations are present in about 2% of familial MPN |

MPN, myeloproliferative neoplasm; SNP, single nucleotide polymorphism.

Harutyunyan et al, 2016. Rumi et al, 2016

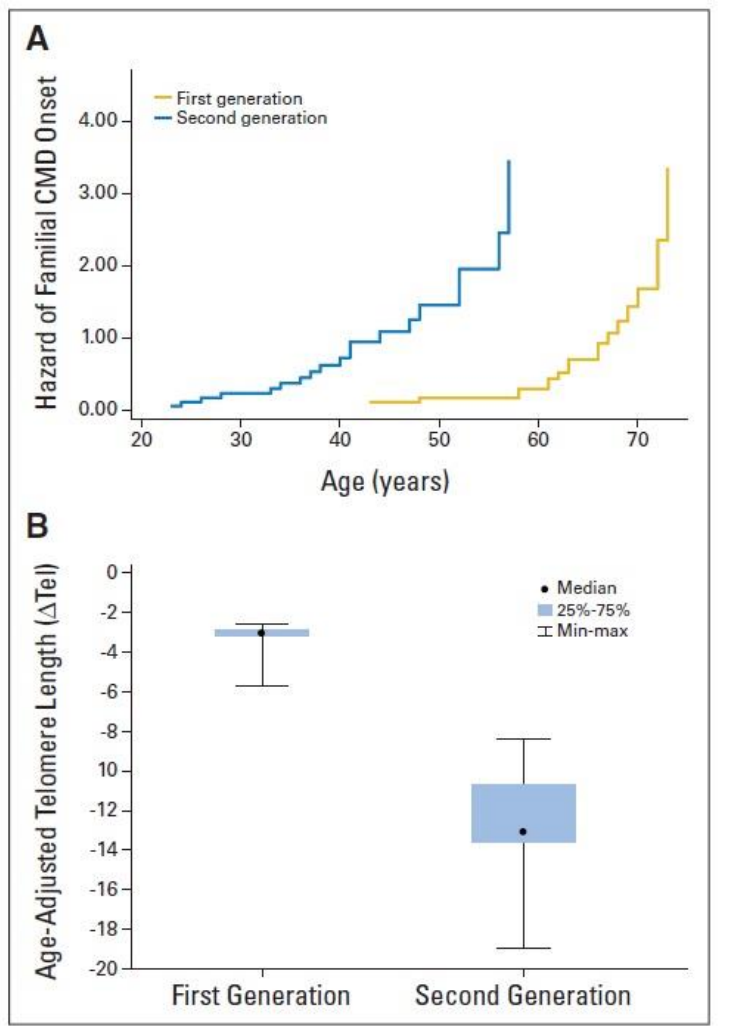
# MPNs WITH FAMILIAL PREDISPOSITION




A= Homogenous clinical phenotype
B= Heterogenous clinical phenotype
C= Homogeneous molecular status
D= Heterogeneous molecular status

Rumi et al., BJH 2017

# MPNs WITH FAMILIAL PREDISPOSITION: DISEASE ANTICIPATION

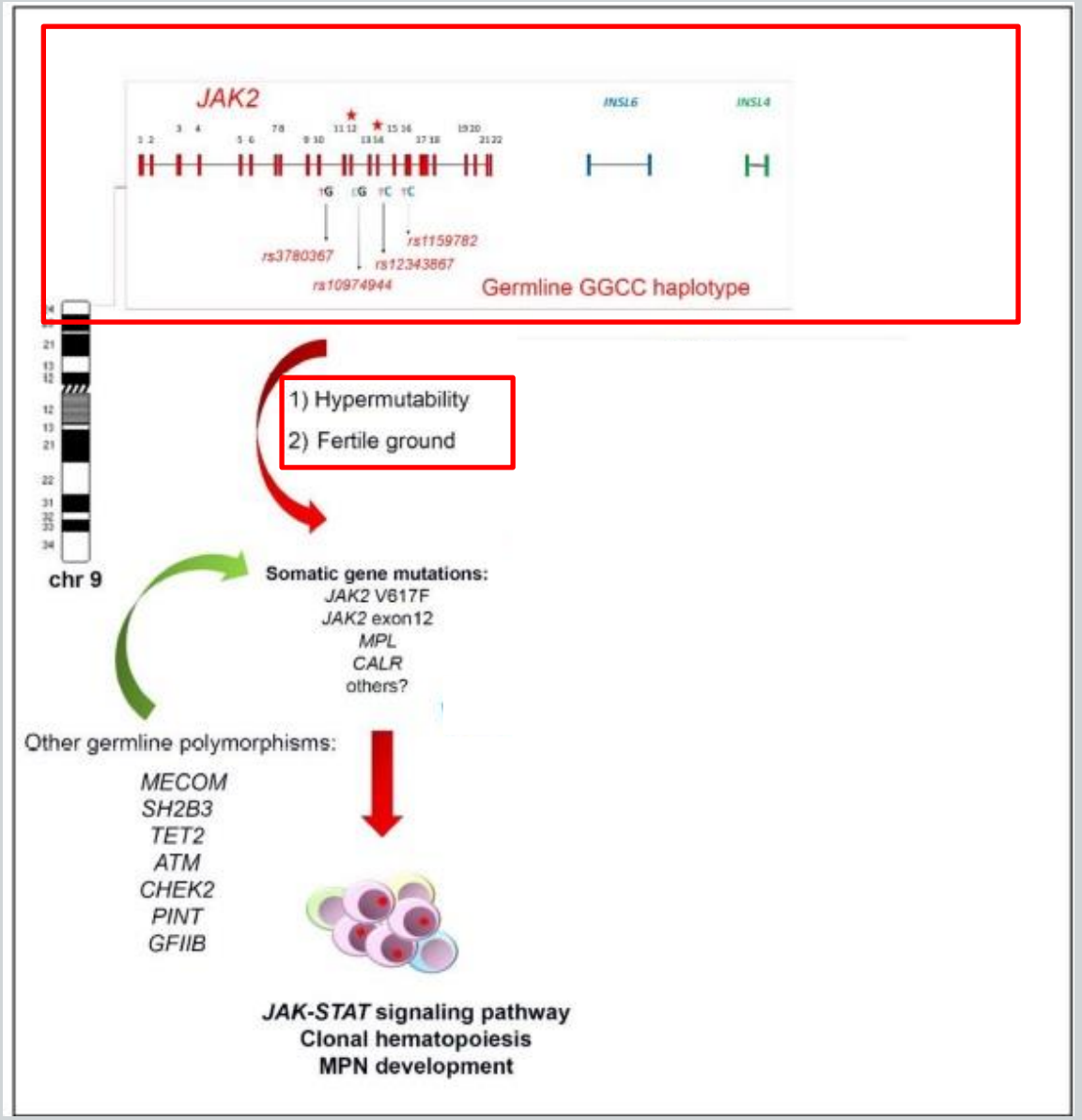



Patients of the second generation have shorter telomeres than first generation: decrease in age at diagnosis in each subsequent generation

Rumi E et al. J Clin Oncol 2007

# JAK2 GGCC

**JAK2 gene haplotype (GGCC or 46/1) confers susceptibility to JAK2 mutation-positive myeloproliferative neoplasms**




Anelli L, et al. Int J Mol Sci. 2018

# JAK2 GGCC

***JAK2* GGCC haplotype predisposes to the acquisition of *JAK2* mutations also in familial MPN, but does not underlie familial clustering.**

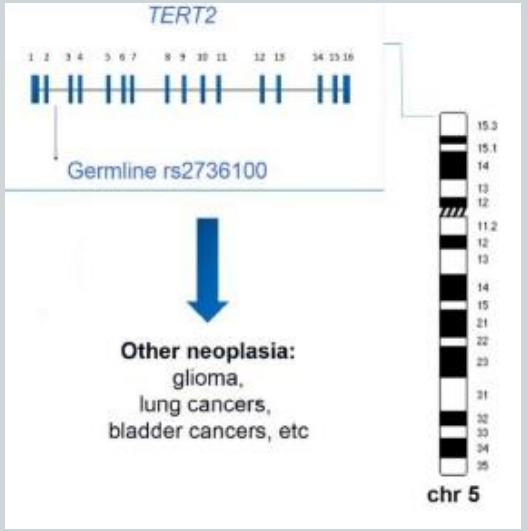
| Case population | Control population | Genotype frequency (%) case population | | | Genotype frequency (%) control population | | | Odds ratio (95% CI) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CC | GC | GG | CC | GC | GG | CC | GC | GG | *P* value |
| Familial MPN (n=88) | control (n=203) | 24 (27.3) | 51 (58.0) | 13 (14.8) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 4.36 (2.18-8.7) | 4.77 (1.9-11.99) | $1.193 \times 10^{-05}$ |
| Familial MPN V617F+ (n=61) | control (n=203) | 13 (21.3) | 37 (60.7) | 11 (18.0) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 4.8 (2.38-9.67) | 4.78 (1.88-12.2) | $4.929 \times 10^{-06}$ |
| Familial MPN V617F- (n=27) | control (n=203) | 11 (40.7) | 14 (51.9) | 2 (7.4) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 2.10 (0.90-4.89) | 1.04 (0.21-5.03) | 0.2042 |
| Sporadic MPN (n=684) | control (n=203) | 223 (32.6) | 353 (51.6) | 108 (15.8) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 2.97 (2.21-4.00) | 3.73 (2.42-5.76) | $3.27 \times 10^{-15}$ |
| Sporadic MPN V617F+ (n=481) | control (n=203) | 125 (26.0) | 268 (55.7) | 88 (18.3) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 3.54 (2.45-5.11) | 4.01 (2.32-6.94) | $7.19 \times 10^{-13}$ |
| Sporadic MPN V617F- (n=202) | control (n=203) | 98 (48.5) | 84 (41.6) | 20 (9.9) | 114 (56.2) | 69 (34.0) | 20 (9.9) | 1 | 1.42 (0.93-2.15) | 1.16 (0.59-2.29) | 0.2620 |
| Familial MPN V617F+ (n=61) | sporadic MPN V617F+ (n=481) | 13 (21.3) | 37 (60.7) | 11 (18.0) | 125 (26.0) | 268 (55.7) | 88 (18.3) | 1 | 1.33 (0.68-2.59) | 1.2 (0.51-2.81) | 0.6975 |

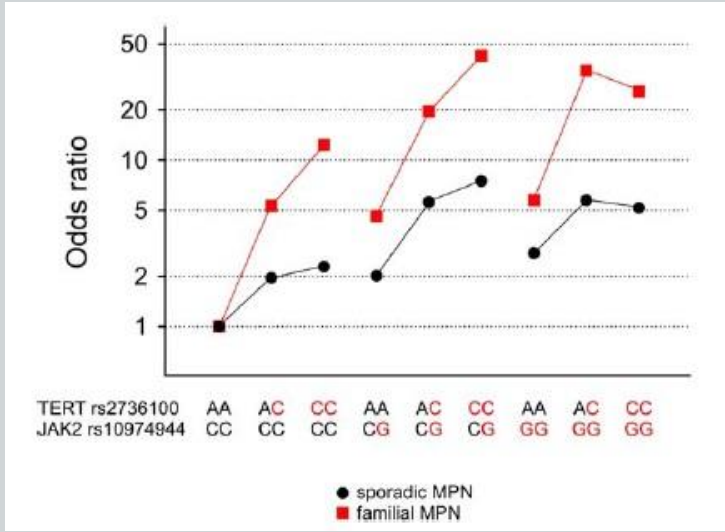
*CI, confidence interval; V617F+, JAK2-V617F-positive MPN; V617F-, JAK2-V617F-negative MPN.*

Olcaydu D et al., haematologica 2011

# TERT

**In contrast, TERT mutation is significantly enriched in familial MPN compared to sporadic MPN, suggesting that it may be responsible of familial clustering.**



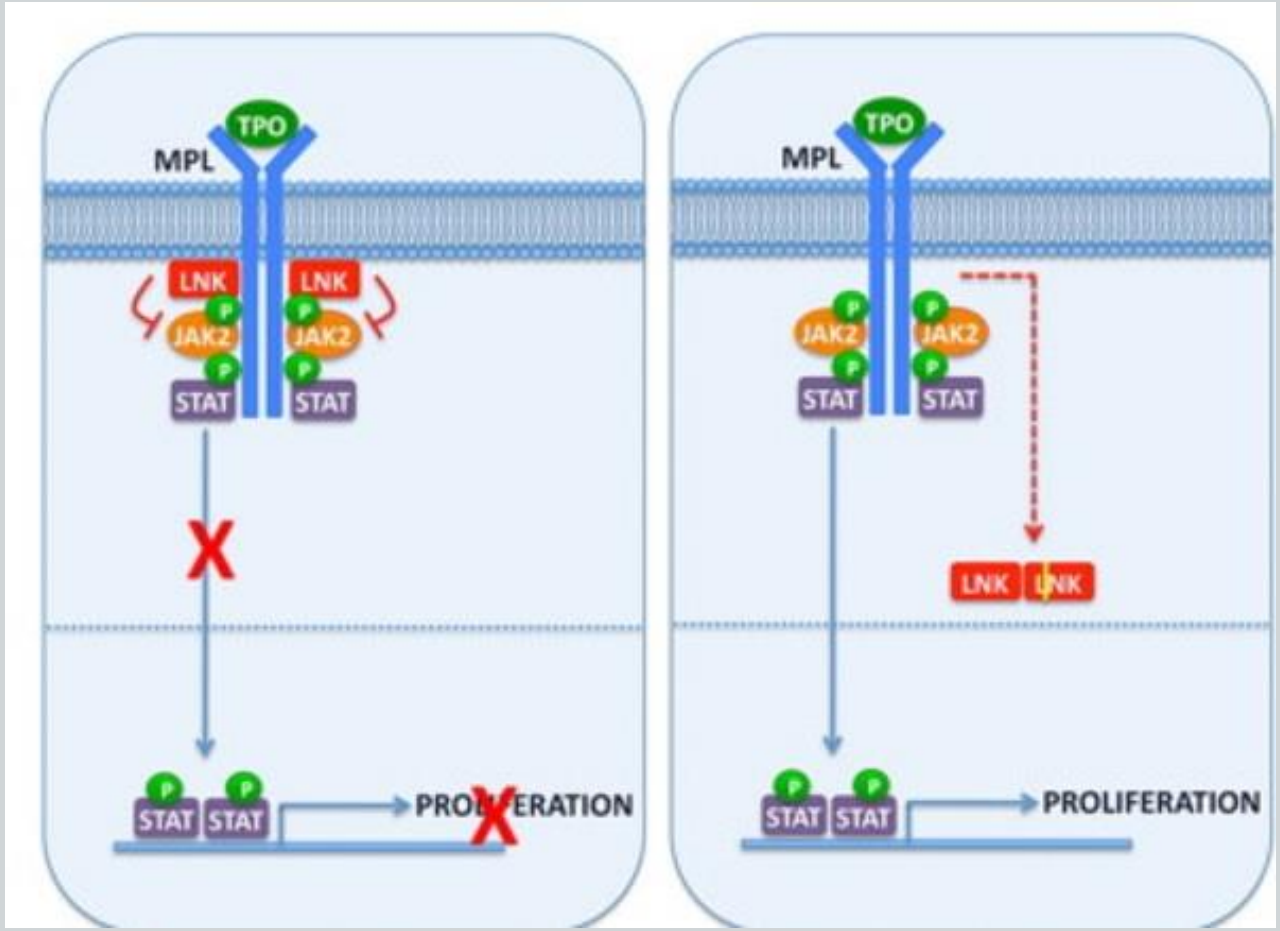

Association of *TERT* rs2736100 with Sporadic and Familial MPN and Molecular Subtypes

| | | Genotype frequency (%) case population | | | Genotype frequency (%) control population | | | Odds ratio (95% CI) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Case population | Control population | A/A | A/C | C/C | A/A | A/C | C/C | A/A | A/C | C/C | *P* value |
| Sporadic MPN($n = 717$) | Control($n = 202$) | 11.3(81) | 46.2 (331) | 42.5 (305) | 23.3 (47) | 43.6 (88) | 33.2 (67) | 1 | 2.18 (1.42–3.35) | 2.64 (1.69–4.13) | $1.15 \times 10^{-4}$ |
| Sporadic MPN JAK2+ ($n = 516$) | Control ($n = 202$) | 10.7(55) | 44.8 (231) | 44.6 (230) | 23.3 (47) | 43.6 (88) | 33.2 (67) | 1 | 2.24 (1.42–3.55) | 2.93 (1.82–4.72) | $5.55 \times 10^{-5}$ |
| Sporadic MPN CALR+ ($n = 126$) | Control ($n = 202$) | 11.9 (15) | 46.8 (59) | 41.3 (52) | 23.3 (47) | 43.6 (88) | 33.2 (67) | 1 | 2.10 (1.08–4.10) | 2.43 (1.23–4.82) | 0.0270 |
| Familial MPN ($n = 121$) | Control ($n = 202$) | 5.0 (6) | 39.7 (48) | 55.4 (67) | 23.3 (47) | 43.6 (88) | 33.2 (67) | 1 | 4.27 (1.7–10.72) | 7.83 (3.14–19.55) | $1.10 \times 10^{-6}$ |
| Familial MPN probands ($n = 75$) | Control ($n = 202$) | 5.3 (4) | 36.0 (27) | 58.7 (44) | 23.3 (47) | 43.6 (88) | 33.2 (67) | 1 | 3.61 (1.19–10.92) | 7.72 (2.60–22.94) | $2.65 \times 10^{-5}$ |
| Familial MPN ($n = 121$) | Sporadic MPN ($n = 717$) | 5.0 (6) | 39.7 (48) | 55.4 (67) | 11.3 (81) | 46.2 (331) | 42.5 (305) | 1 | 1.96 (0.81–4.73) | 2.97 (1.24–7.08) | 0.0090 |
| Familial MPN probands ($n = 75$) | Sporadic MPN ($n = 717$) | 5.3 (4) | 36.0 (27) | 58.7 (44) | 11.3 (81) | 46.2 (331) | 42.5 (305) | 1 | 1.65 (0.56–4.85) | 2.92 (1.02–8.37) | 0.0180 |

Jager et al, AJH 2014

# SH2B3 (LNK)

- Negative regulator of JAK-STAT signaling.
- 2% of MPN families: germ line SH2B3 mutations rarely occur in familial MPNs and do not segregate with the disease phenotype.
- Mutations in SH2B3, either germ line or acquired, may cooperate with acquired driver mutations in JAK2, CALR, or MPL to determine disease phenotype in MPNs.




Rumi et al, Blood 2016
Oh S.T. et al, Blood 2010

# RBBP6

- 5% of MPN families.
- Low penetrance associated with RBBP6 mutations.
- Common germline predisposition factors, such as JAK2 GGCC haplotype and TERT rs2736100 SNP, seem to have an additive effect on the MPN risk in RBBP6 mutation carriers.

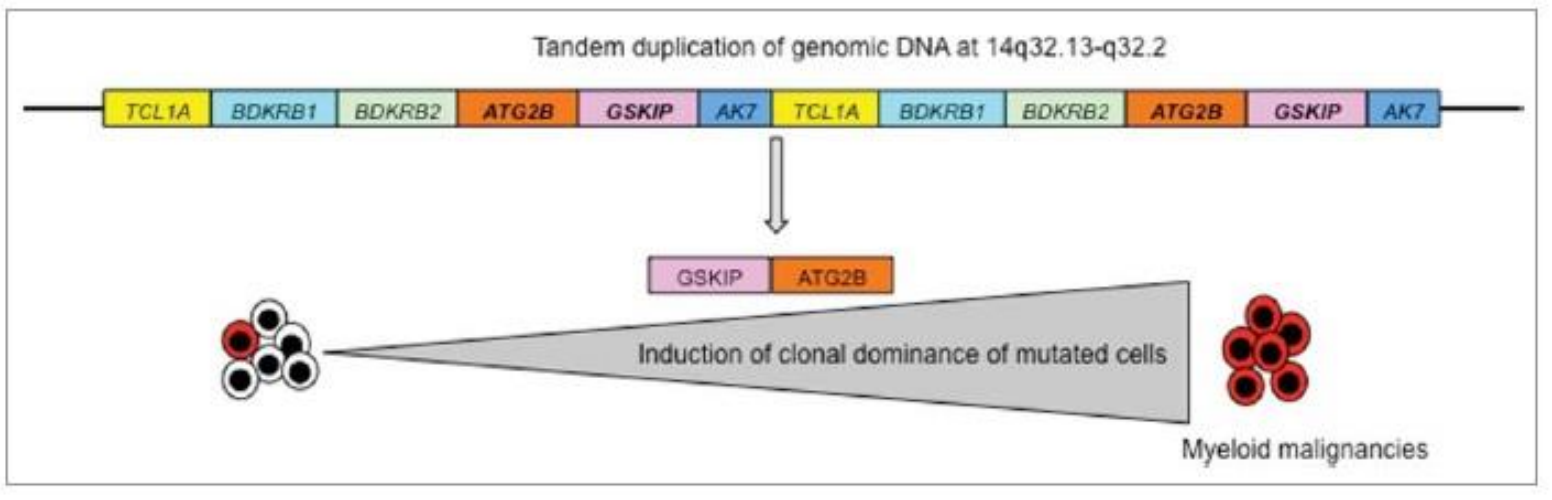

Summary of unique *RBBP6* variants in familial and sporadic MPN cases

| Pedigree | Sample | Diagnosis | *JAK2/MPL/CALR* | cDNA change | Amino acid change | Polyphen 2 score | Polyphen 2 prediction | In healthy controls |
|---|---|---|---|---|---|---|---|---|
| 1 | MPD214 | ET | *MPL*-W515L | c.4706G>T | R1569H | 0.766 | Possibly damaging | 0/715 |
| | MPD219 | PMF | *CALR*-Type 1 | c.4706G>T | R1569H | — | — | — |
| | MPD227 | PMF | *JAK2*-V617F | c.4706G>T | R1569H | — | — | — |
| 2 | f16p1 | PMF | *JAK2*-V617F | c.4961A>G | E1654G | 0.375 | Benign | 0/649 |
| 3 | 570 | PMF | *JAK2*-V617F | c.4352G>C | R1451T | 0.942 | Probably damaging | 0/642 |
| Sporadic | H_0327 | PV | *JAK2*-Ex12del | c.4331C>T | S1444F | 0.976 | Probably damaging | 0/650 |
| Sporadic | H_0580 | ET | *JAK2*-V617F | c.4331C>T | S1444F | 0.976 | Probably damaging | 0/650 |
| Sporadic | H_0437 | PV | — | c.5018C>T | A1673V | 0.010 | Benign | 0/607 |

cDNA, complementary DNA; del, deletion; Ex12del, exon 12 deletion E543-D544.

Harutyunyan A.S. et al., Blood 2016

# ATG2B E GSKIP



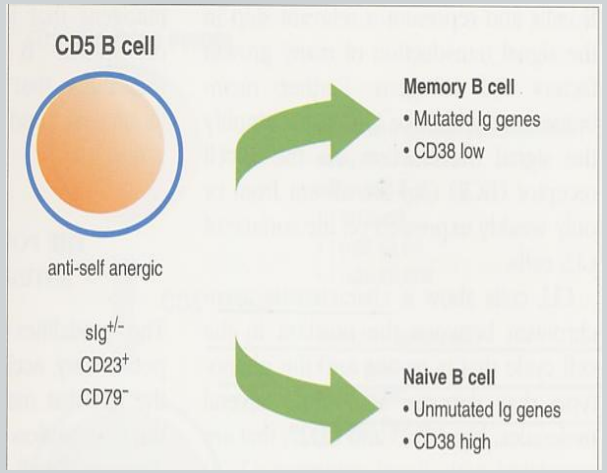

- **700-kb germline duplication.**
- **Enhances haematopoietic progenitor differentiation.**
- **Increase sensibility to TPO.**
- **Cooperate with acquired JAK2, CALR, MPL.**
- **Predispose to myeloid malignancies, most frequently TE.**
- **Poor prognosis and rapid progression to AML.**

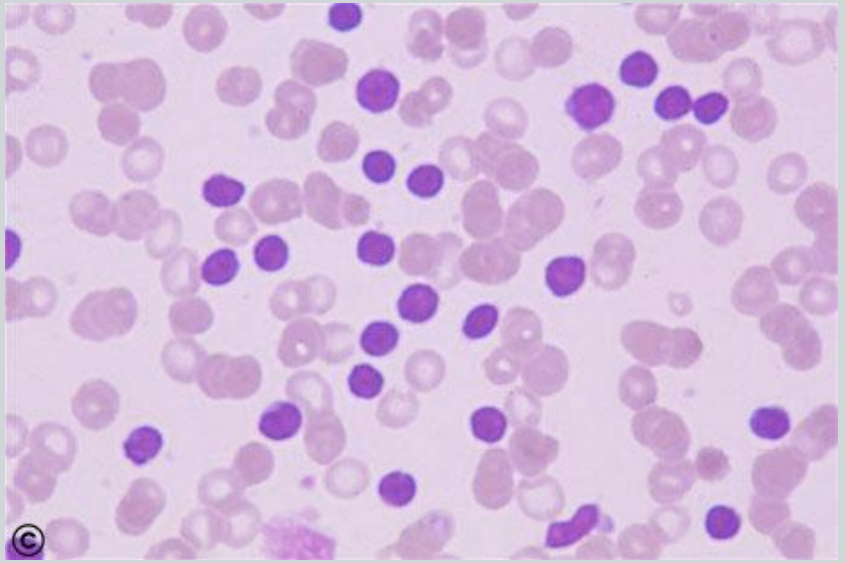
Saliba J et al. Nat Genet. 2015; Plo et al. MOLECULAR & CELLULAR ONCOLOGY 2016

# CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

- Presence of a clonal population of B-cell lymphocytes (≥5.000/μL clonal lymphocytes B ) with a characteristic immunophenotype (CD19+, CD20+, CD23+, CD79b low, FMC7- low).

- 25% of all leukemia and is the most common form of lymphoid malignancy in Western countries.

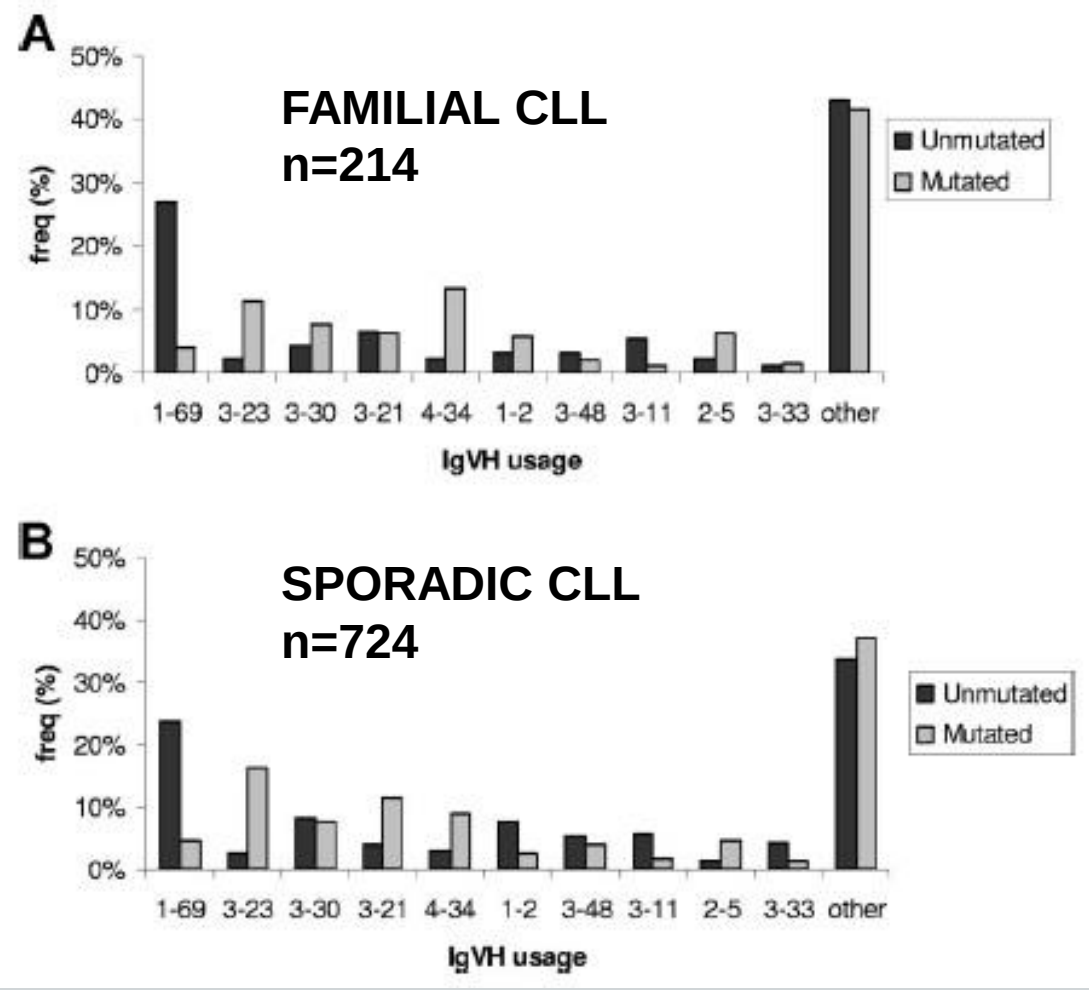


# FAMILIAL CLL

- Family history is the strongest risk factor identified in CLL.
- First-degree relatives of individuals with CLL have a three- to eightfold increased risk of CLL and smaller but still increased risks of Hodgkin's or non-Hodgkin's lymphomas.
- CLL is genetically heterogeneous, with multiple loci involved.
- Familial CLL appears like sporadic CLL in clinical features but has been reported to show more frequent somatic hypermutation of the immunoglobulin heavy-chain variable (IgHV) region.

# FAMILIAL CLL




**Hypermutation of IgHV in CLL**

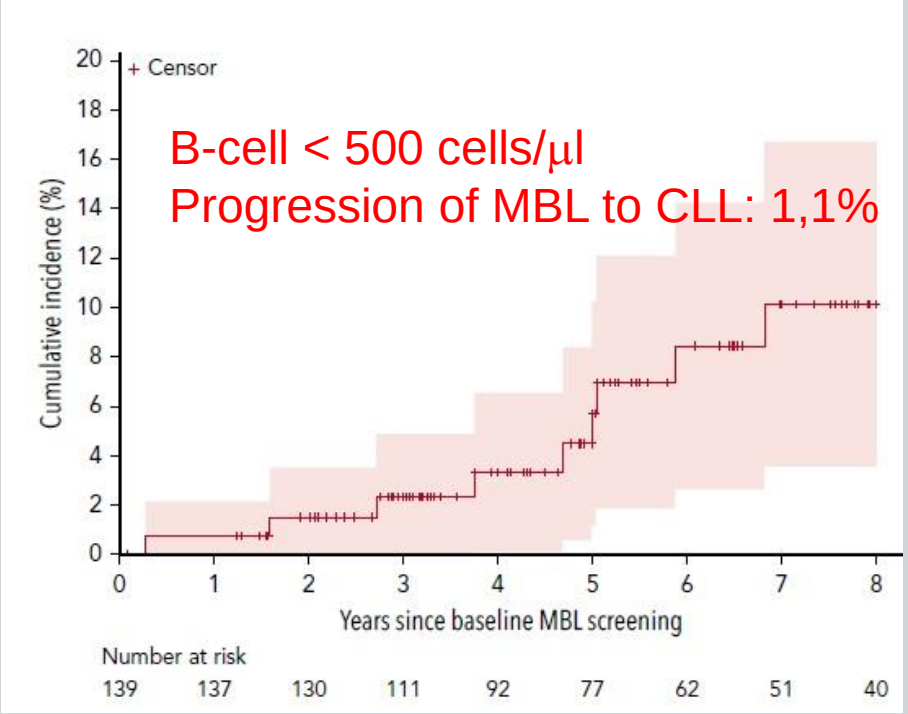
- Mutated CLL was observed in 68% of the familial cases, compared with 47% of the sporadic cases.
- Mutations in genes: VH3 (49%), VH1 (25%), and VH4 (18%).
- Mutation status was correlated within families.
- Mutation status was not related to age at diagnosis or stage.

Crowther et al. Blood 2008

# FAMILIAL CLL

**Monoclonal B-cell lymphocytosis (MBL): may provide a window into the earliest stages of CLL pathogenesis and an opportunity to identify additional gene carriers within families.**

- MBL more common in unaffected relatives in CLL families.
- 88–96% have shown mutated *IgVH* genes, with intraclonal heterogeneity like CLL.



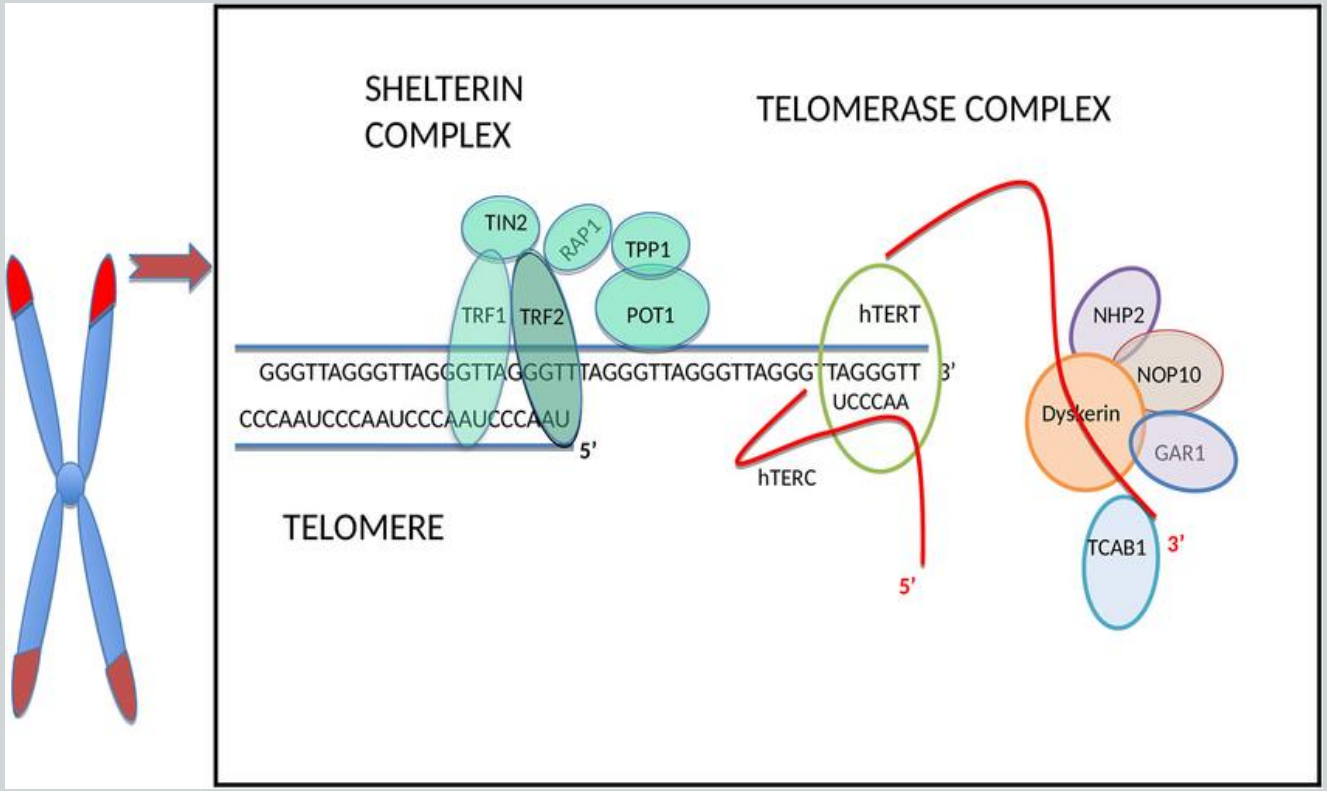

Marti GE et al., Cytometry B Clin. Cytom 2003; Vogt RF et al., Br. J. Haematol 2007; Slager SL et al., Blood 2021

# GENES INVOLVES IN FAMILIAL CLL

| | |
|---|---|
| **APOPTOSIS** | **ARL11,BMF,FAS,BCL2,BCL2L11,CASP8,CFLAR** |
| **SIGNALING PATHWAYS** | **UBR5,LEF1,TLE3** |
| **IMMUNE RESPONSE** | **SP140,OAS1,BCL6,IRF8** |
| **TELOMERES LENGHT MAINTENANCE** | **ACD,TERF2IP,POT1** |

GWAS analysis. Speedy HE et al. Nat Commun 2019

# SHELTERIN COMPLEX



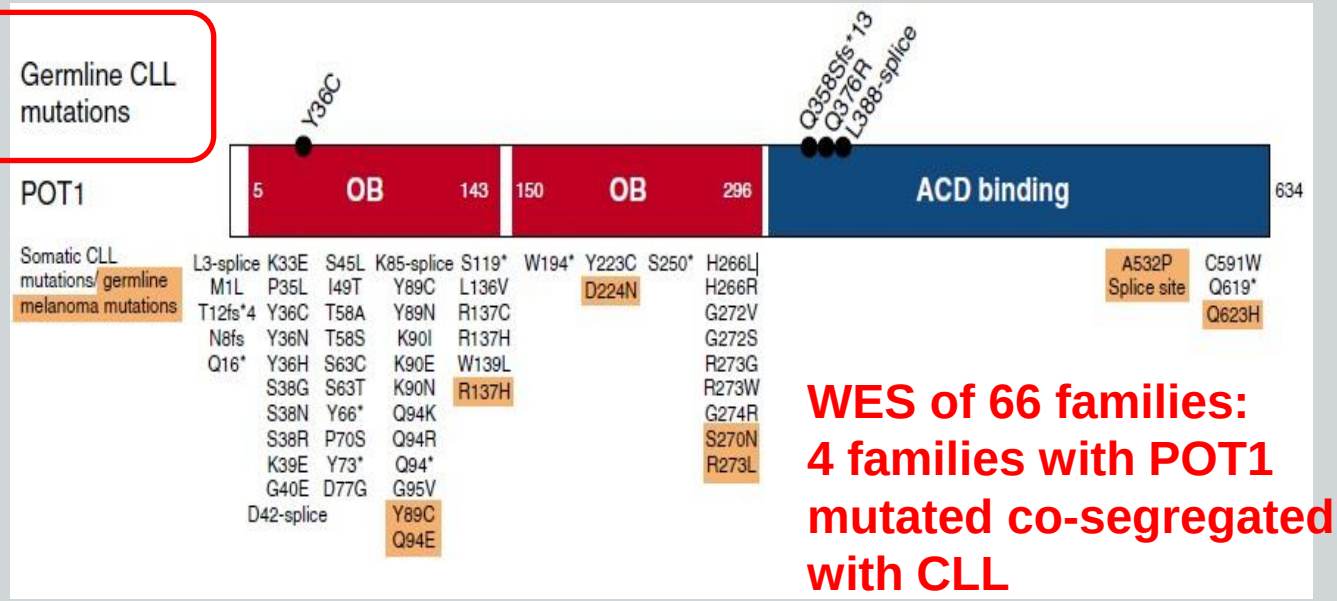

Telomere length is one of the main element affected in CLL.

The shorter are the telomere, the higher is the proliferation rate and the aggressiveness of the disease.

The Shelterin protein complex has a fundamental role in modulating the telomere replication process, in safeguarding the latter from degradation processes and aberrant recombinations, and in regulating telomerase activity.

# PROTECTION OF TELOMERES 1 (POT1)

- POT1 in 6% of the CLL families.
- Associated with an increased risk for multiple cutaneous melanomas and gliomas.
- POT1-mutated CLL cells have numerous telomeric and chromosomal abnormalities.
- A 3.6-fold increased risk for CLL was reported for individuals with POT1 germline variant p.Gln376Arg.
- This cohort also exhibited a younger average age of diagnosis than in sporadic CLL (59 years vs 70 years).




**WES of 66 families: 4 families with POT1 mutated co-segregated with CLL**

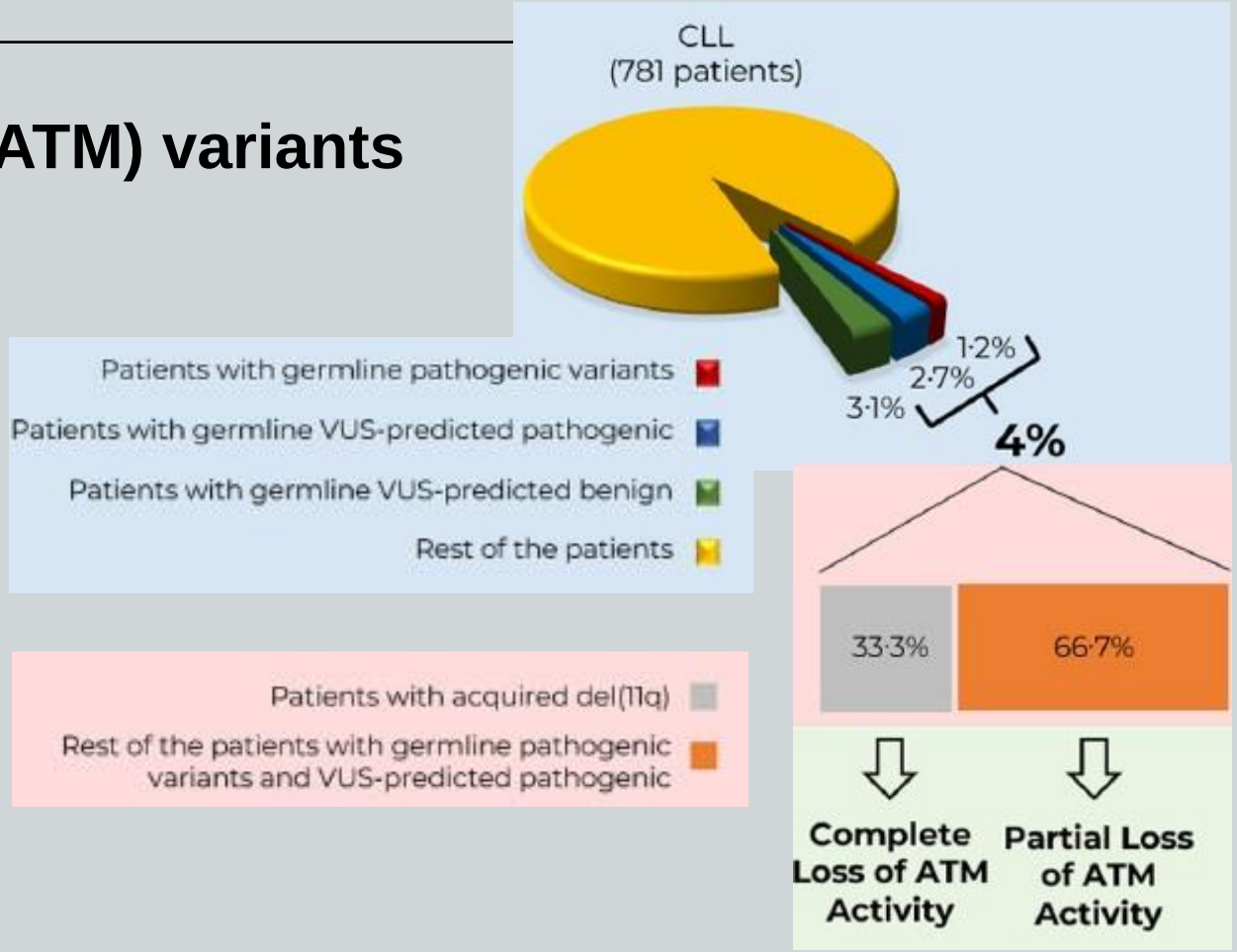
**Recommended Surveillance for Individuals with POT1**

Speedy HE et al. Blood 2016

# ATM SERINE/THREONINE KINASE GERMLINE MUTATIONS IN CLL

## Ataxia–telangiectasia mutated (ATM) variants

- Somatic disruption of ATM by (del)11q and/or mutation is common in CLL (24% of patients): voluminous adenomegaly and rapid disease progression.
- Germline ATM variants are pathogenic, VUS, and benign; heterozygous, mostly missense.
- **Somatic loss of the second ATM allele in patients with ATM germline pathogenic mutations is frequent, influencing rapid disease progression.**




Patrackova et al Br J Haematol 2022