A meghatározatlan jelentőségű klonális hemopoesis (CHIP) aktualitásai

Hematológia kötelező szintentartó tanfolyam

Pécs

2025. február 26.

Dr Egyed Miklós

Somogy Vármegyei Kaposi Mór Oktató Kórház

Hematológiai Osztály

A klonális vérképzés, amelyet a hemopoietikus őssejtek mutációi hozzák létre, veszélyes betegségek kialakulását indíthatja el.

Az egymást triggerelő, kórképekként és betegenként is eltérő

inflammatoros és neoplasiás kétarcúság,

korai ér- és myeloproliferatív betegségekhez,

illetve a neopláziás folyamatok

malignus transzformációjához vezethet.

Pub Med nagyszámú közlemény -2014 óta !!! cardiológia/hematológia

Igen magas imp.fact újságok, elismert egészségügyi központok !!!

CHIP Clonal hematopoiesis of indeterminate potential

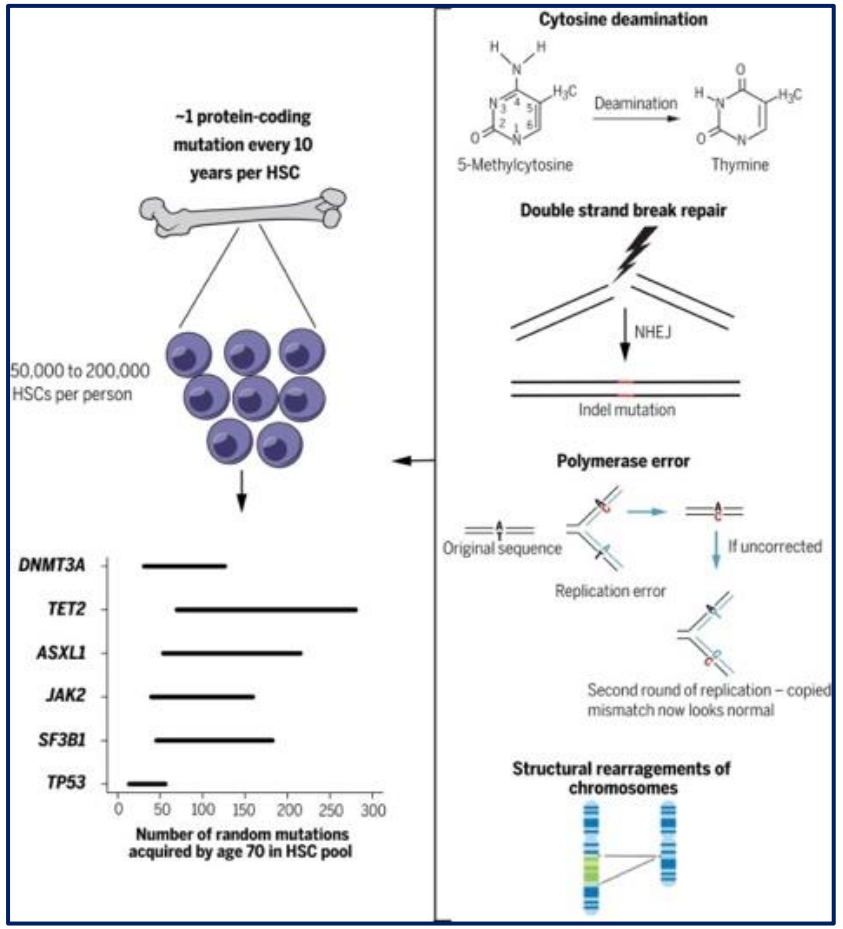
- A clonalitás fogalma, clonalis hemopoesis
- CHIP fogalma, jellegzetességei
- 2014-es év 3 nagy populáción alapuló genetikai vizsgálata
- CHIP az új, érzékenyebb genetikai vizsgálómódszerek tükrében
- CHIP és hematológiai malignus betegségek összefüggései
- CHIP és Cardiovascularis betegségek összefüggései
- CHIP és nem hematológiai malignus betegségek
- CHIP és nem malignus betegségek

- A szöveteinkben gyakori a szomatikus mutáció,
- általában következmény nélküli, kivéve ⟶ proliferációs előny
- Progresszív expanzió ⟶ klón
- Ha a HSC-ben megy végbe ⟶ clonalis hematopoiesis
- Egészséges emberben:

  50-200 ezer HSC

  1 HSC: 1mut/10 év

  70 életkor: 350 ezer-1,4 Millió mut.,betegség nélkül



H. Lee-Six, N. F. Øbro, M. S. Shepherd, et al.Population dynamics of normal human blood inferred from somatic mutations. Nature **561**, 473–478 (2018).

Jaiswal. S:clonal hematopoiesis in human aging and disease,Science,01,Nov 2019

Clonalis hematopoiesis

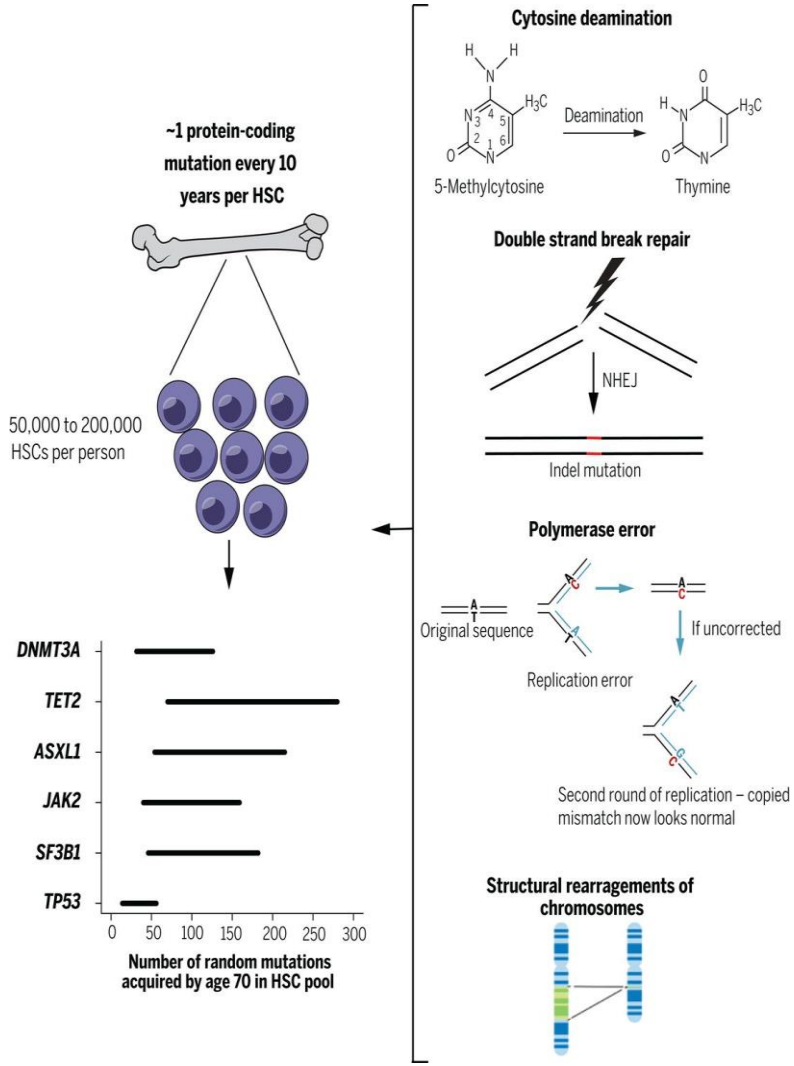
A mutációk zöme metylcytosine-thymin átalakulás ezáltal

Cytosin-Guanin……Thymin-Adenin baziscsere.

Insertio/deletio ritkán nagyobb chromatinszakaszok

insertio/deletio-translocatio-ja történik

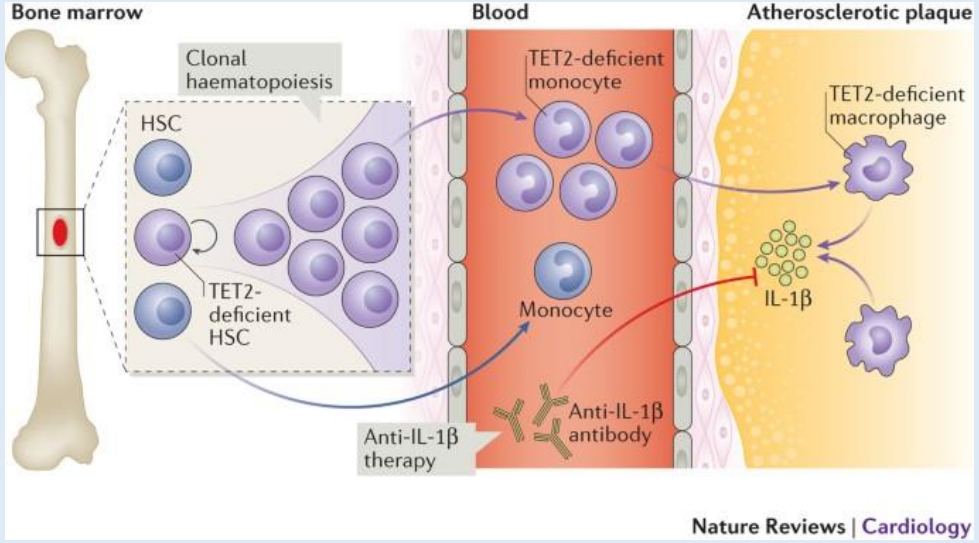
(ezek már többnyire betegséget okozva)

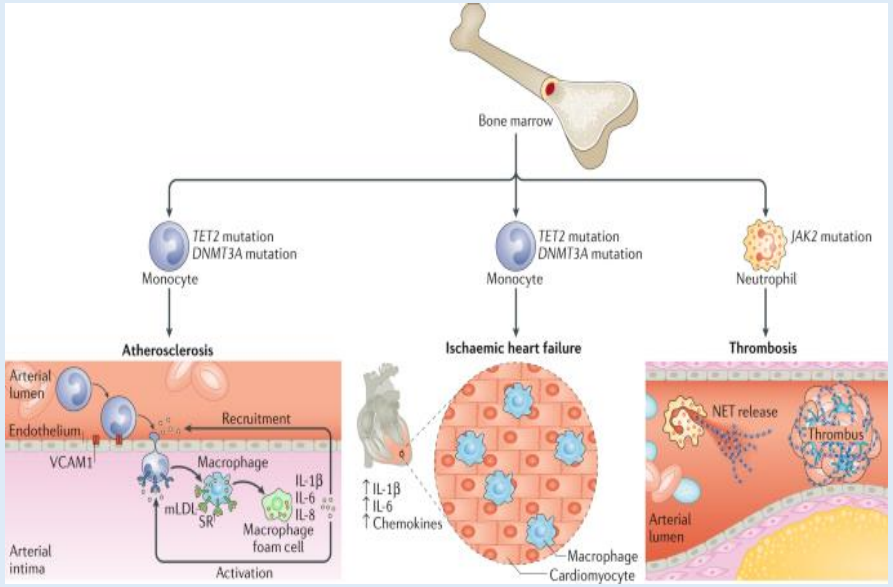


B. K. Duncan, J. H. Miller Mutagenic deamination of cytosine residues in DNA. Nature **287**, 560–561 (1980).

Clonalis hematopoiesis (CH)

- Egy adott szövetben kialakuló mutációk következményei az adott szövetben jönnek létre (bőr, nyh.k)
- A HSC mutáció és CH az egyéb szövetektől eltérően szinte minden szövetben képes hatást kifejteni

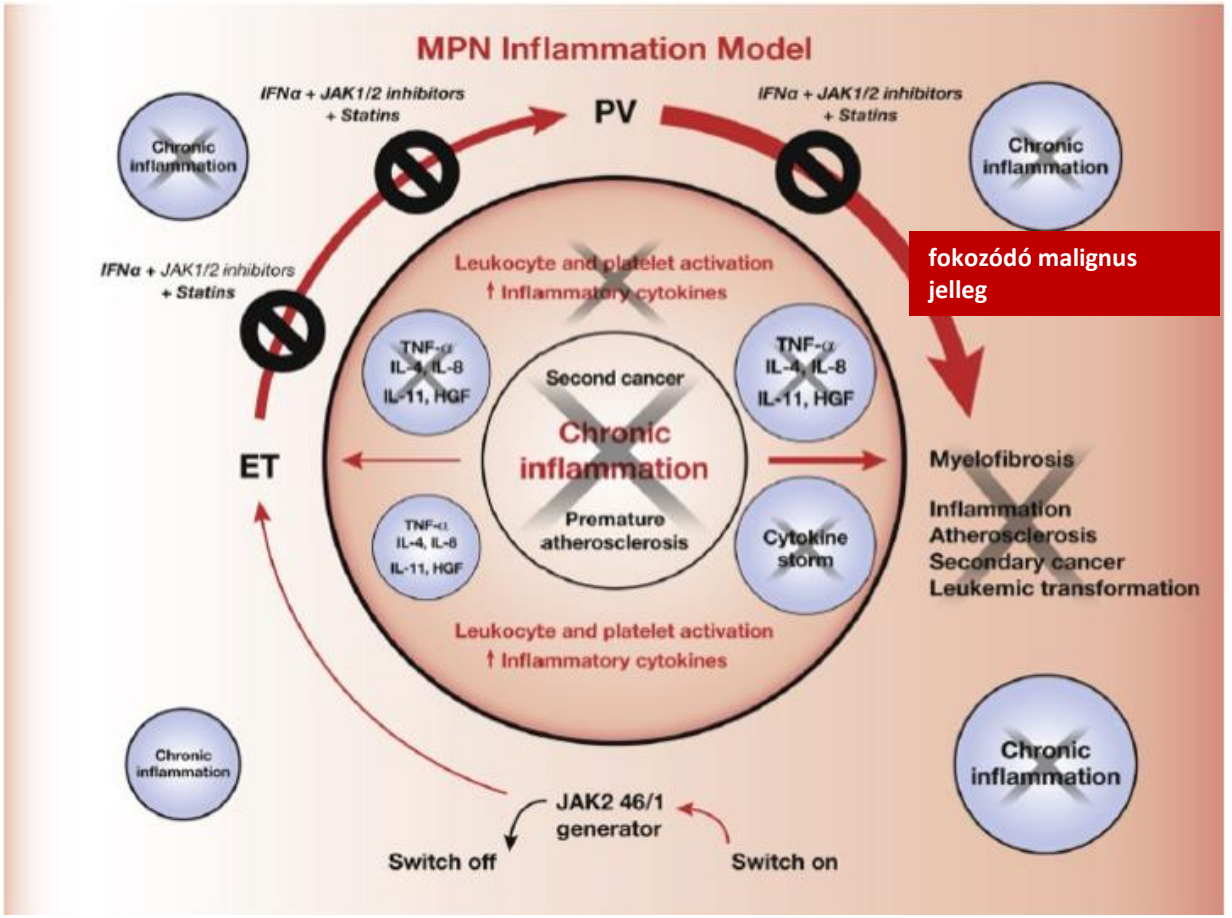




CHIP/MPN Chronic inflammation

CHIP zömében inflammatio

MPN: átmenet
- fokozódó malignus jelleg



Hasselbalch et al. Blood 2019;134(5):469-479

Clonalis hematopoiesis (CH)

- 1990-es években CML-es nőkben igazolták a CH-t Fialkov XCI (x kromoszóma inaktiváció)
- Barr RD, Fialkow PJ Clonal origin of chronic myelocytic leukemia.N Engl J Med. 1973 Aug 9; 289(6):307-9.

  M. F. Fey, S. Liechti-Gallati, A. von Rohr, et al Clonality and X-inactivation patterns in hematopoietic cell populations detected by the highly informative M27 beta DNA probe. Blood 83, 931–938 (1994).

- Később nem mal.beteg nőkben is igazolták a XCI-t és CH-t
- K. M. Champion, J. G. Gilbert, F. A. Asimakopoulos,et al. , Clonal haemopoiesis in normal elderly women: Implications for the myeloproliferative disorders and myelodysplastic syndromes. Br. J. Haematol. 97, 920–926 (1997).

- 2012-ben XCI nők 5%-ában TET2 mutációt igazoltak
- L. Busque, J. P. Patel, M. E. Figueroa, et al, Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat. Genet. 44, 1179–1181 (2012).

- AML betegekben a leukemia kialakulása többlépcsős folyamat (multiple hit) és a remisszióba jutott betegekben gyakran kimutatható az iniciáló mutációval jellemezhető CH

  T. Miyamoto, L. Weissman, K. Akashi et al, AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc. Natl. Acad. Sci. USA. 97, 7521–7526(2000).

CHIP definició

A CHIP *Clonal hematopoiesis of indeterminate potential* definiciója

A. J. Silver, S. Jaiswal Clonal hematopoiesis: Pre-cancer PLUS. Adv. Cancer Res. **141**, 85–128 (2019).

- "öregedő vérképzés"

- olyan szomatikus mutáció jelenléte, amely általában hematologiai neoplasmával társul és legalább2% allélfrekvenciájú (VAF)
- kizárhatók a malignus hematologiai neoplasmák; MPN, MDS/AML
- kizárható a PNH, MGUS és MBL
- cytopenia jelen lehet a PB ben de nem része a CHIP definiciónak

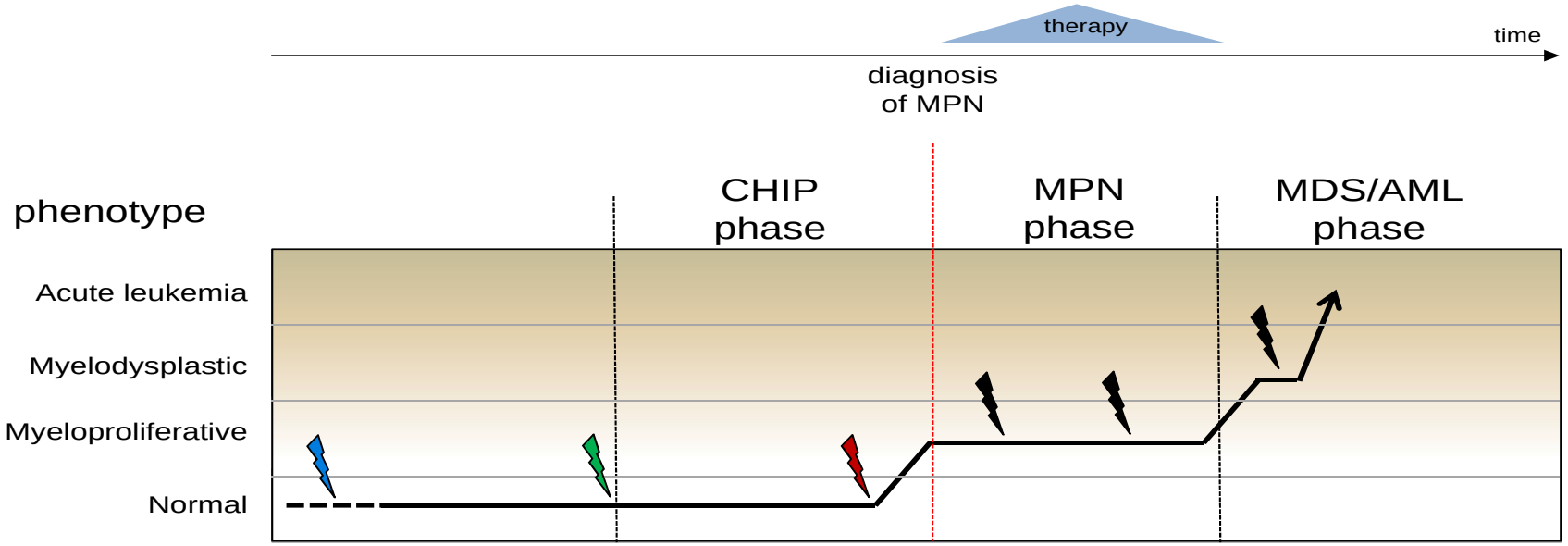
- CHIP definition of WHO 2022 (WHO 2022)
- Detection of one or more somatic mutations with variance allele frequency (VAF) ≥2% (≥4% for X-linked gene mutations in males) in DNA from blood or bone marrow cells involving selected genes
- Absence of unexplained cytopenias
- Absence of diagnostic criteria for defined myeloid neoplasms

A CHIP a myeloproliferativ neoplaziák előszobája, a CVD melegágya.

Steensma R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.

Myeloproliferativ neoplasiák többlépcsős “multiple hit” képződése

Genetic changes in MPN



Germline predispositions	Clonal drivers	MPN driver mutations	Progression associated mutations
JAK2-GGCC	*del20q*	*JAK2*	*TP53 loss, MDM4 amplification*
TERT	*del13q*	*MPL*	*PRC2 complex loss of function*
RBBP6	*TET2*	*CALR*	*ASXL1 loss*
LNK	*DNMT3A*		*CUX1 deletions*
ATG2B GSKIP			*RUNX1 loss of function*
del-IL1RAP			*SF3B1, Splicing factor mutations*

Myeloproliferativ neoplasiák többlépcsős “multiple hit” képződése

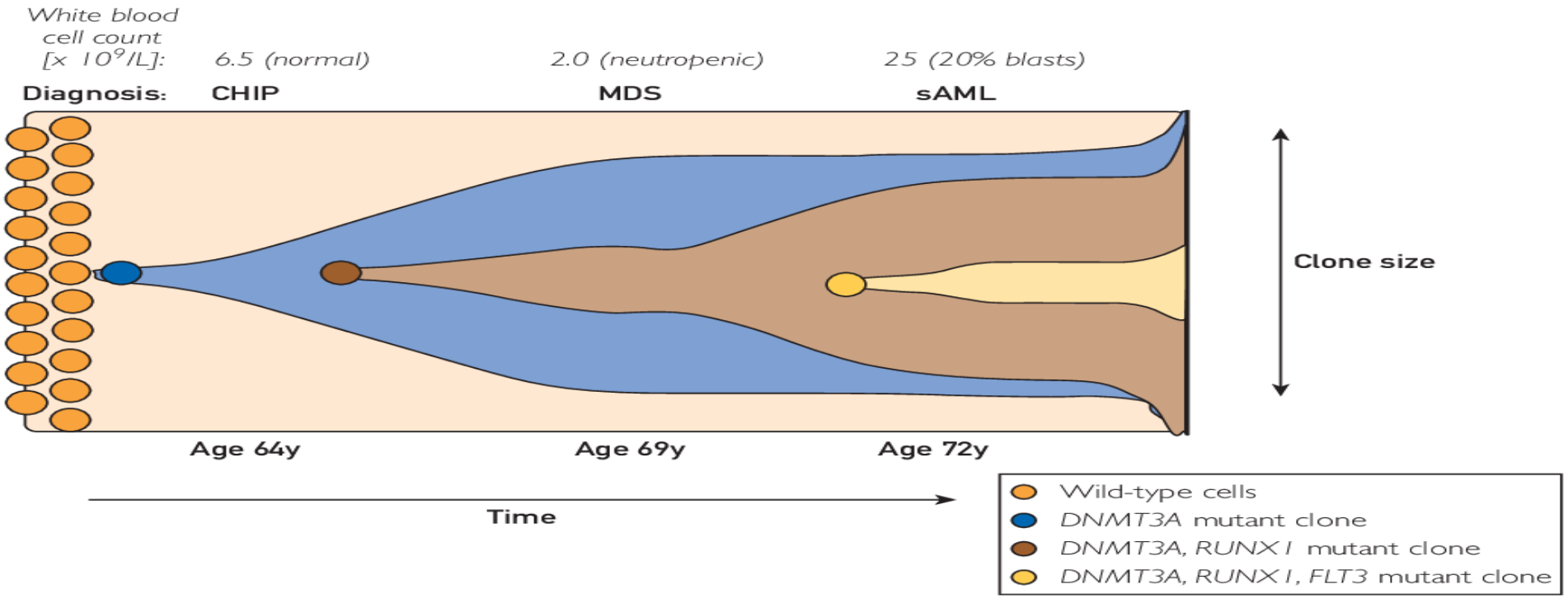


FIGURE 1. Fish or whale plot summarizing hematopoietic clonal progression in a patient who died of complications of secondary acute myeloid leukemia (sAML) after unsuccessful therapy for myelodysplastic syndrome (MDS). The clonal process began with clonal hematopoiesis of indeterminate potential (CHIP) associated with a single driver mutation in the *DNMT3A* gene, *DNMT3A R882H*, the most common CHIP-associated mutation. At the time of CHIP (which was diagnosed retrospectively from archival samples), the patient had a normal blood cell count. Several years later, a cell in this *DNMT3A* mutant clone acquired a second mutation—a loss of function mutation in the *RUNX1* transcription factor—and pancytopenia evolved and the patient met World Health Organization diagnostic criteria for MDS. After several more years, the patient developed leukocytosis and circulating blasts and a new internal tandem duplication mutation in *FLT3* was discovered in a subclone.

Clonalis Hematopoiesis az új genetikai vizsgálómódszerek idején- 2014-es publikációk

G. Genovese, A. K. Kähler,R. E. Handsaker, et al.Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med. **371**, 2477–2487(2014).

M.Xie, C. Lu, J. Wang, et al Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat. Med. 20, 1472–1478 (2014)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

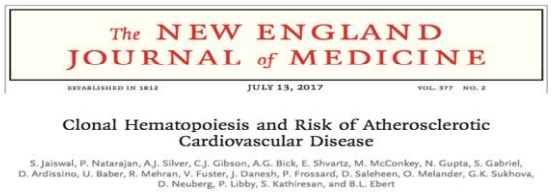
Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

Siddhartha Jaiswal, M.D., Ph.D., Pierre Fontanillas, Ph.D., Jason Flannick, Ph.D., Alisa Manning, Ph.D., Peter V. Grauman, B.A., Brenton G. Mar, M.D., Ph.D., R. Coleman Lindsley, M.D., Ph.D., Craig H. Mermel, M.D., Ph.D., Noel Burtt, B.S., Alejandro Chavez, M.D., Ph.D., John M. Higgins, M.D., Vladislav Moltchanov, Ph.D., Frank C. Kuo, M.D., Ph.D., Michael J. Kluk, M.D., Ph.D., Brian Henderson, M.D., Leena Kinnunen M.Sc., Heikki A. Koistinen, M.D., Ph.D., Claes Ladenvall, Ph.D., Gad Getz, Ph.D., Adolfo Correa, M.D., Ph.D., Benjamin F. Banahan, Ph.D., Stacey Gabriel, Ph.D., Sekar Kathiresan, M.D., Heather M. Stringham, Ph.D., Mark I. McCarthy, M.D.,* Michael Boehnke, Ph.D.,* Jaakko Tuomilehto, M.D., Ph.D., Christopher Haiman, Sc.D., Leif Groop, M.D., Ph.D., Gil Atzmon, Ph.D., James G. Wilson, M.D., Donna Neuberg, Sc.D., David Altshuler, M.D., Ph.D.,* and Benjamin L. Ebert, M.D., Ph.D.†

Jaiswal, S., Fontanillas, P., Flannick, J., et al. (2014). Age-related clonal hematopoiesis associated with adverse outcomes. New England Journal of Medicine, 371(26), 2488-2498.

- 2014 CHIP áttörés

CHIP and cardiovascular disease


The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 13, 2017 VOL. 377 NO. 2

Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease

S. Jaiswal, P. Natarajan, A.J. Silver, C.J. Gibson, A.G. Bick, E. Shvartz, M. McConkey, N. Gupta, S. Gabriel, D. Ardissino, U. Baber, R. Mehran, V. Fuster, J. Danesh, P. Frossard, D. Saleheen, O. Melander, G.K. Sukhova, D. Neuberg, P. Libby, S. Kathiresan, and B.L. Ebert

- Három tanulmány több mint 30 000, (no mal. hematol. bet.) PB. DNS alapú mutáció-analízisét végezték el (exom szekv.).

(COSMIC-adatbázisból (Catalog of Somatic Mutations in Cancer) ismert

160 hematológiai malignitással összefüggő mutációt vizsgálták.)

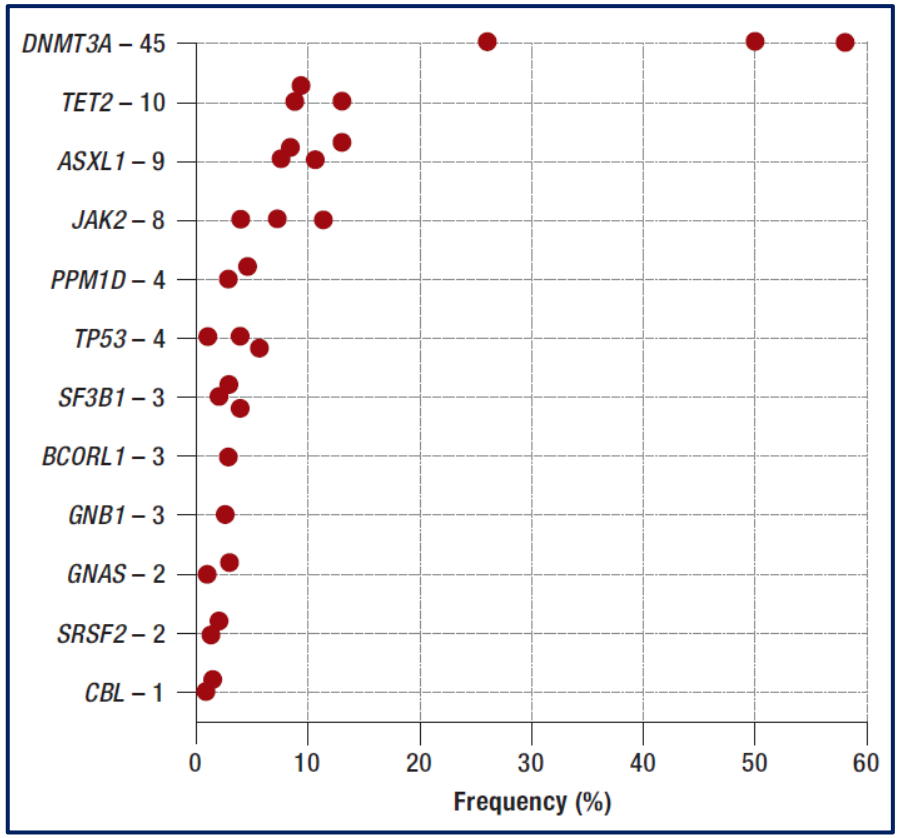
3 fontos megállapítást fogalmaztak meg:

1.) CHIP esetek 2/3- ában néhány mutáció :

DNS metiláció 2 génje DNMT3 és TET2 funkcióvesztése

chromatin reguláló ASXL és mRNS érés „splicing génjei”

CHIP-ben előforduló mutációk



(Heuser, M., Thol, F., & Ganser, A. (2016). Clonal hematopoiesis of indeterminate potential: a risk factor for hematologic neoplasms. Deutsches Ärzteblatt International, 113(18), 317.)

M.Xie, C. Lu, J. Wang, et al.. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat. Med. **20**, 1472–1478 (2014

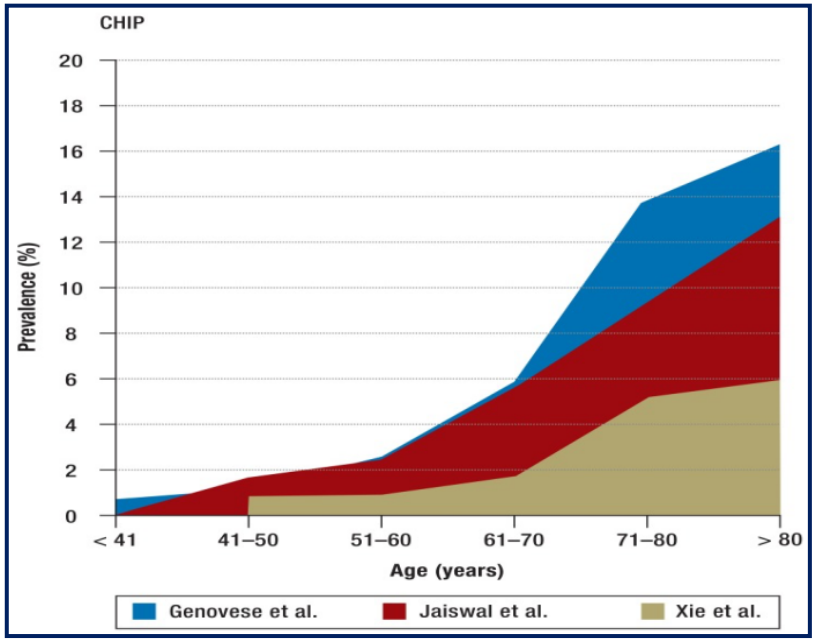
G. Genovese, A. K. Kähler,R. E. Handsaker, et al...Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med. **371**, 2477–2487(2014).

2.) A CH életkorral összefüggő jelenség 40 éves kor alatt ritka volt a CHIP (1%),

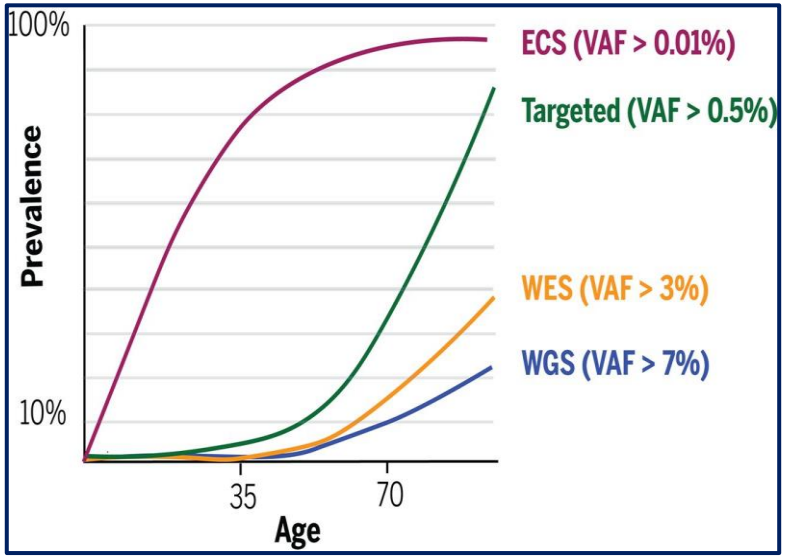
70-79 között 9,5% (219/2300),

80-89 között 11,7%(37/317),

90-108 között 18,4%(19/103) ha 2% allélfrekvenciát fogadtak el a CHIP Dg kritériumának



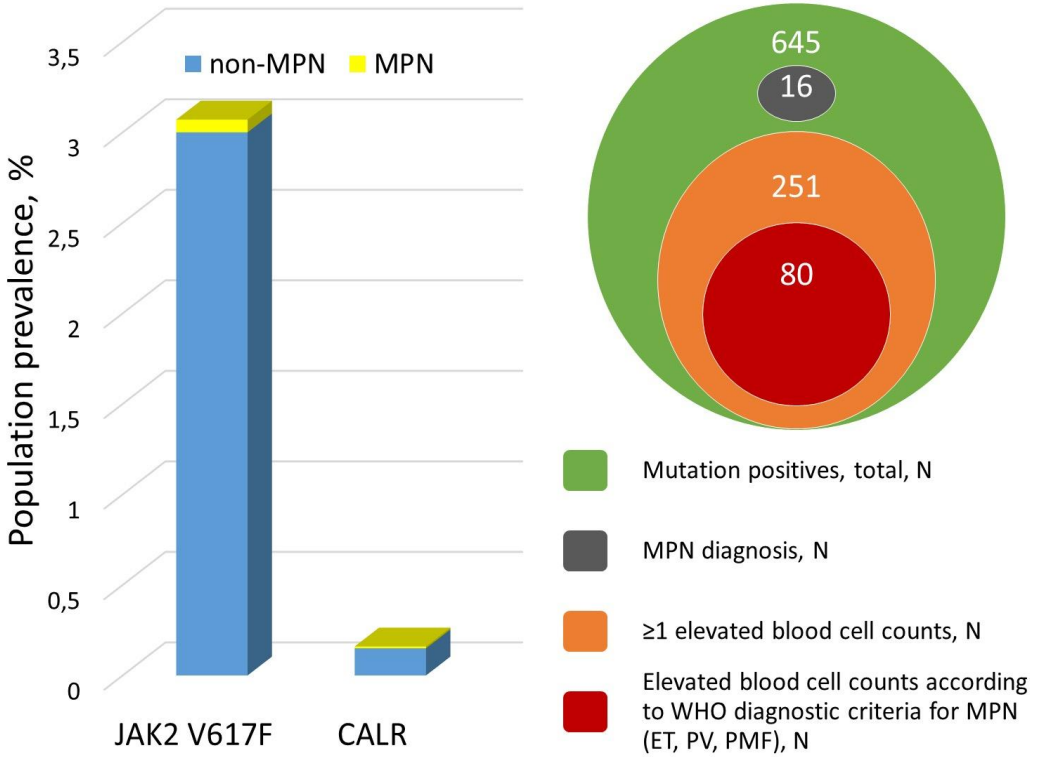
3.) CHIP prevalencia függ az alkalmazott módszer érzékenységétől a teljes genom ill exom szekvenálás helyett alkalmazott un “Error corrected sequencing ECS“ módszer 70 év felett szinte minden vizsgáltban CH-t igazolt, (de már 50 felettiekben is)



CHIP/ MPN prevalencia

A CHIP/ MPN jelentősen aluldiagnosztizált de életet veszélyeztető thrombózis és hirtelen halál rizikótényező

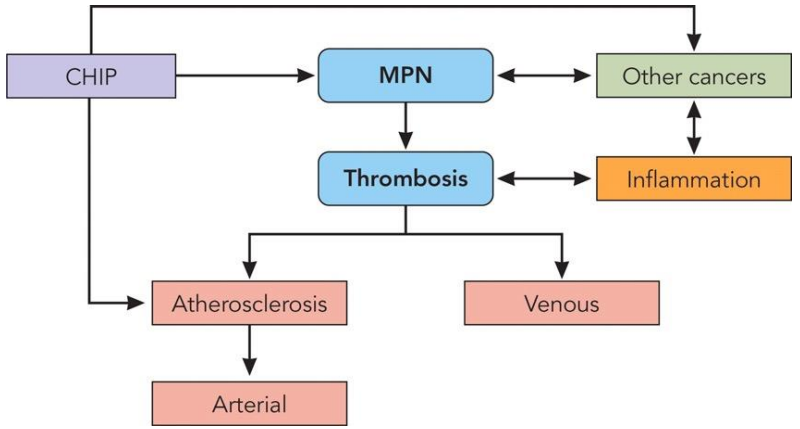
Prevalence and phenotypes of *JAK2* V617F and *Calreticulin* mutations in a Danish general population



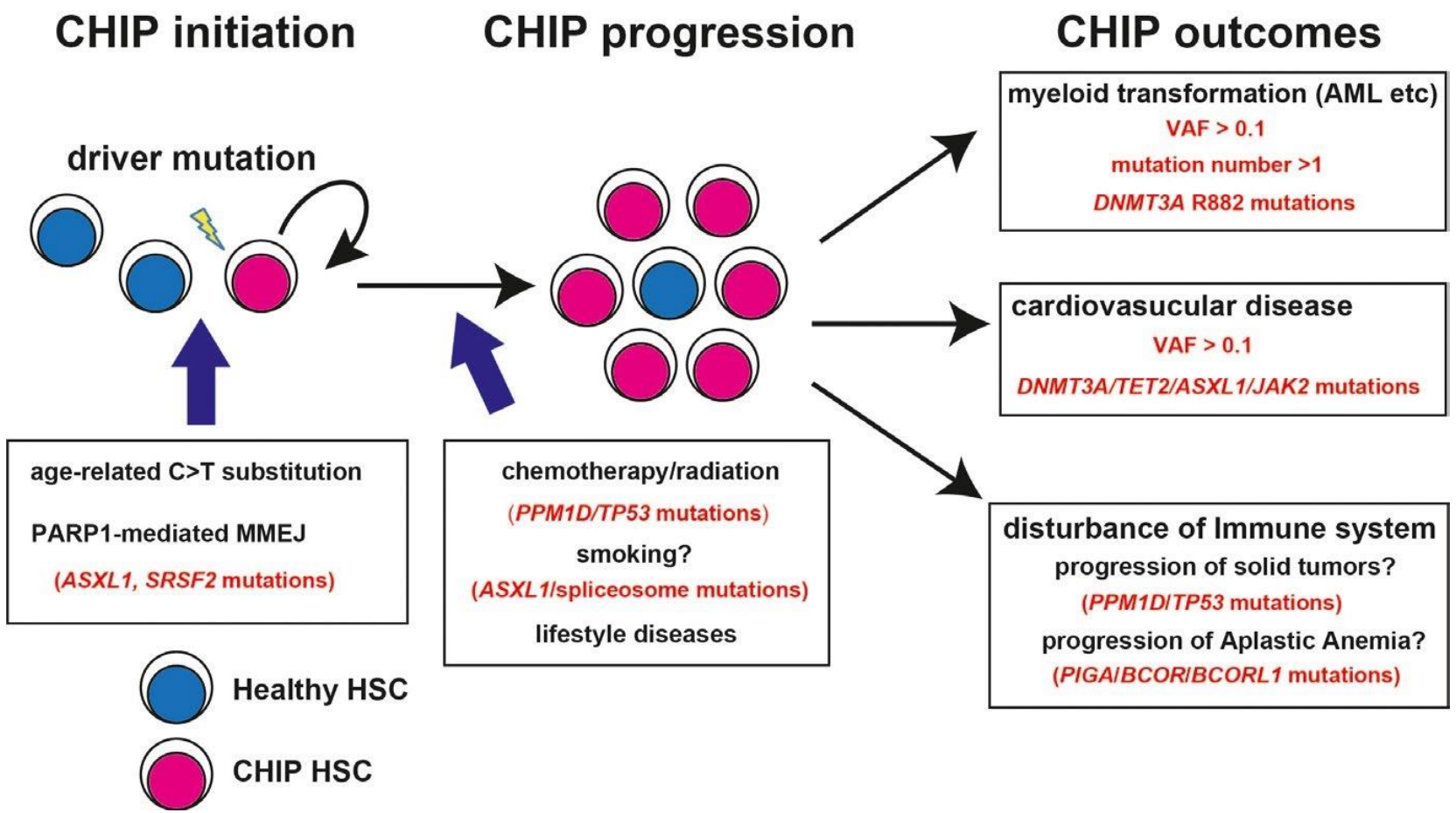
(21206 Lakos) Danish General Suburban Population Study (GESUS)

Droplet digital PCR- sens. kisebb o,o1%
JAK2V617F mut 613..(2,9%)

Dániában 10 ezer nem diagnosztizált MPN beteg
USA kb 550 ezer!

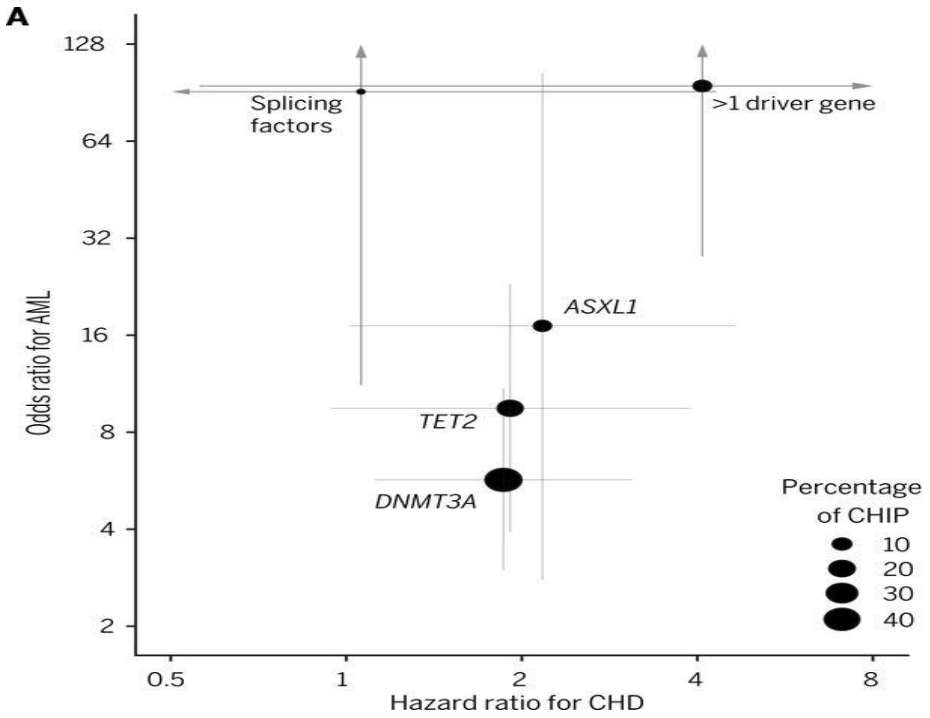


Hasselbalch et al. Blood 2019;134(5):469-479



A CHIP ellentétben az egyéb premalignus állapotokkal (MGUS, MBL..)súlyos veszélyt jelent CV risk. !

CHIP: a hematológiai malignomák és a cardiovascularis rizikó valószínűsége



B

	HR (95% CI)
Age 50-59	**2.2** (1.3-3.7)
Age 60-69	**2.4** (1.4-4.0)
Age ≥70	**6.3** (3.8-10.4)
Female	**0.7** (0.5-0.9)
Has T2D	**2.2** (1.6-3.0)
Former or current smoker	**1.4** (1.0-1.9)
Hypertension stage II-IV	**1.4** (1.0-1.9)
TC >200 mg/dL	**1.4** (1.0-1.9)
HDL<35 mg/dL	**1.4** (1.0-2.2)
HDL>60 mg/dL	**0.8** (0.5-1.1)
CHIP present	**1.8** (1.1-2.9)

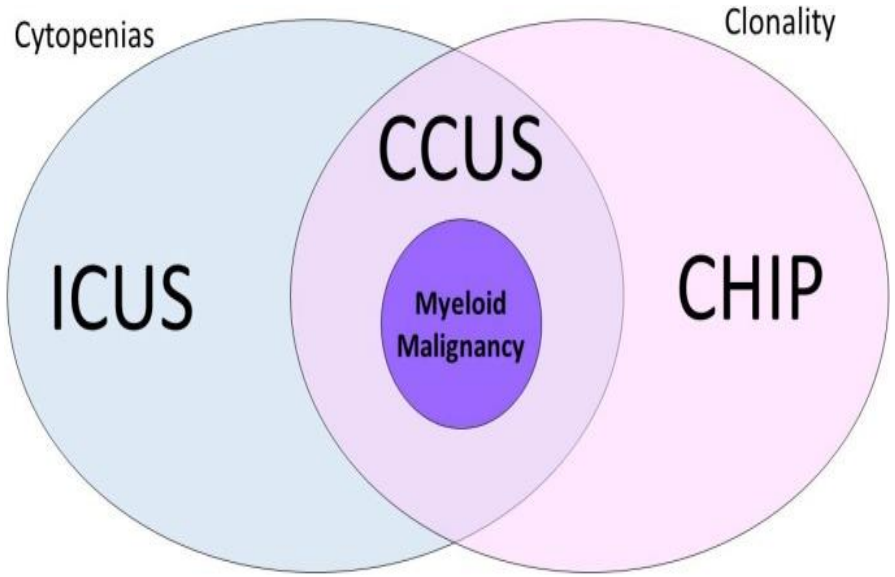
CHIP is associated with increased risk of acute myeloid leukemia and coronary heart disease.

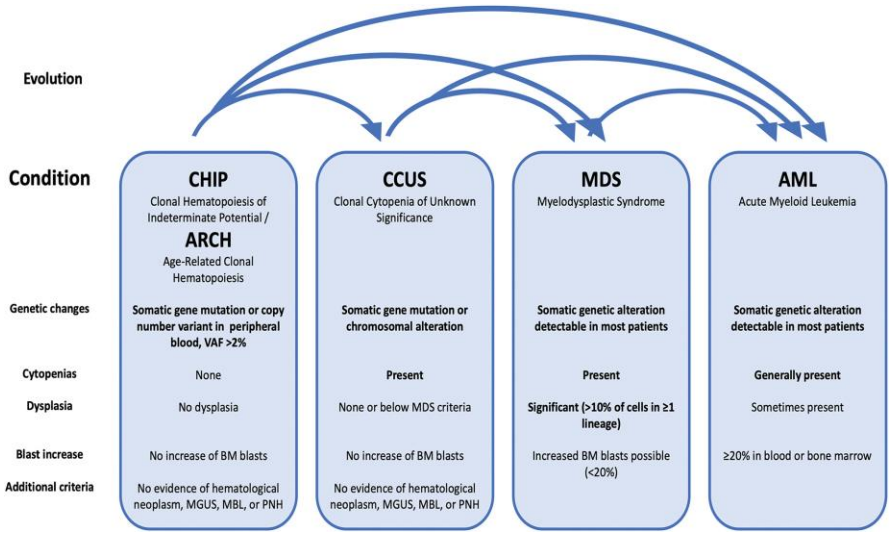
(A) Forest plots for risk of developing acute myeloid leukemia (AML) and coronary heart disease (CHD) in individuals with mutations in the genes listed. Only those mutations meeting the definition of CHIP were included. Individuals with mutations in more than one driver mutation are shown in the figure as a separate category (>1 driver gene). Lines represent the 95% confidence interval for odds or hazard ratios, and the sizes of the dots reflect the percentage of total CHIP mutations that are accounted for by each gene.

(B) (**B**) Hazard ratio (HR) and 95% confidence interval (CI) for developing CHD based on Framingham risk factors plus presence of CHIP mutations. Data are taken from population-based cohorts unselected for CHD status

Jaiswal S, Natarajan P, Silver AJ, et al Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. N Engl J Med. 2017 07 13; 377(2):111-121.

CHIP és hematológiai malignitások összefüggése





Differentiation of CHIP and CCUS from MDS

	CHIP	CCUS	Low risk MDS	High risk MDS
Clonality	+	+	+	+
Dysplasia	-	-	+	+
Cytopenia	-	+	+	+
BM Blasts	<5%	<5%	<5%	5-20%
Cytogenetic aberrations	+/-	+/-	+	++
Molecular aberrations	+	+	++	+++
Risk of progression	+	++	++	+++

Idős emberek évekig tartó követése CH/ICUS/CCUS/MDS

The Health and Anemia Study (2003-2017)

The Monzino 80+ Study (2002-2016)

The Health and Anemia Study (2003-2017)

- Prospective population-based observational study of all elderly (>65y) residents in the municipality of Biella, Italy (n=10,082)
- **Study Population (n=1043)**
- Prevalence day:
  - Hematological parameters together with clinical history
  - Screening for anemia (serum folic acid, vitamin B12, iron, ferritin and transferrin, transferrin saturation, reticulocytes, creatinine)
- Follow-up :
  - Data on hospitalization and mortality
  - Information on the development of hematological and solid cancers (provided by local tumor registry up to 2017 www.registritumori.it/cms/RTBiella).
  - 344,565 laboratory tests available

Tettamanti M et al. Haematologica 2010;95:1849

The Monzino 80+ Study (2002-2016)

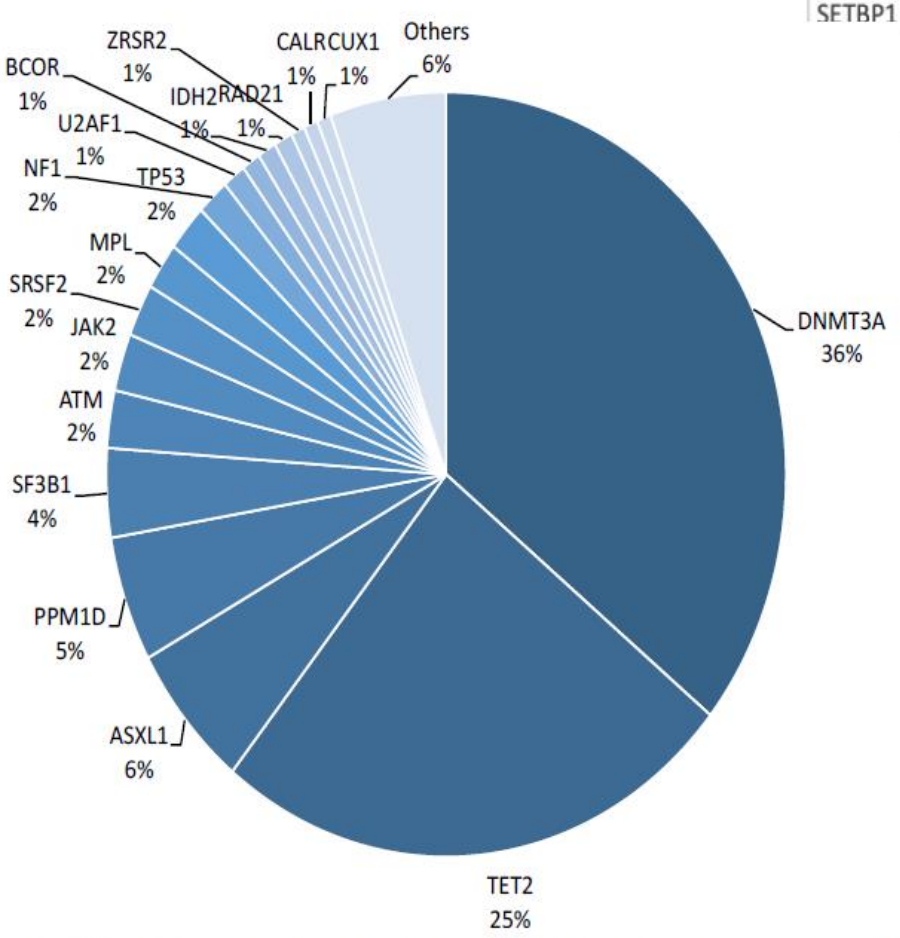
- Prospective population-based observational study of all oldestold (>80) residents in the municipality of Varese, Italy (n=2,120) aimed at investigating relationships between age, cognitive decline and dementia occurrence
- Validation (n=735)

- Prevalence day:
  - Hematological parameters together with clinical history
  - Screening for anemia (serum folic acid, vitamin B12, iron, ferritin and transferrin, transferrin saturation, reticulocytes, creatinine)
- Follow-up :
  - Data on hospitalization and mortality
  - Information on the development of hematological and solid cancers (provided by local tumor registry up to 2016

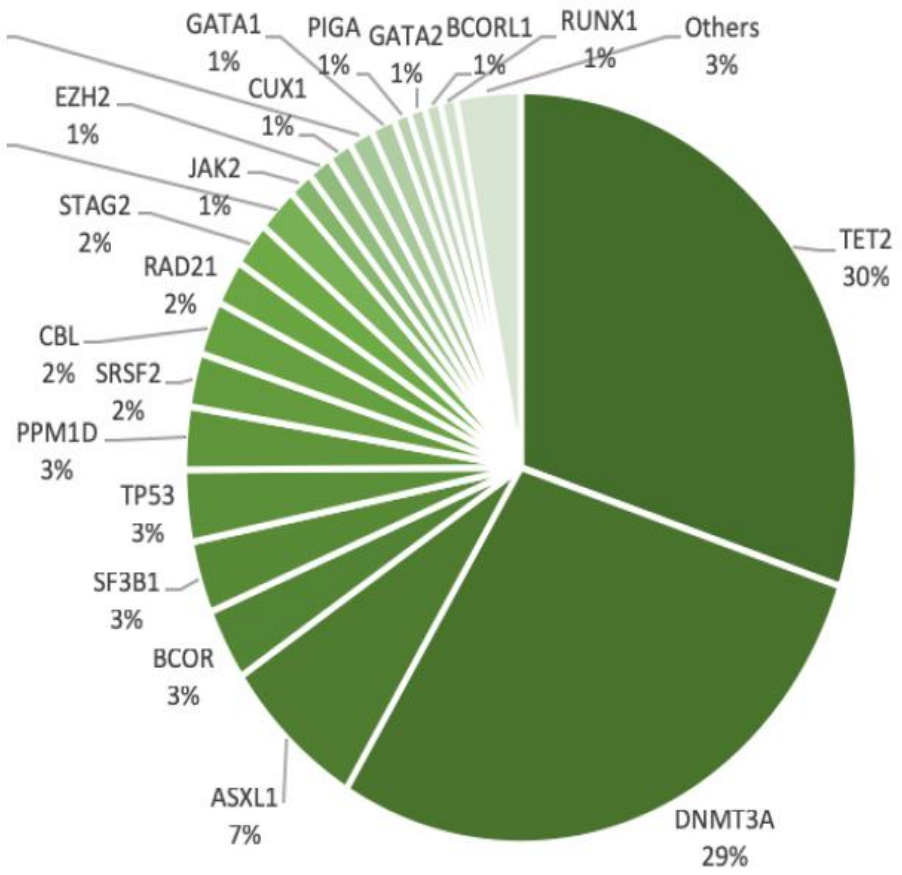
Lucca U et al. Alzheimers Dement 11:258-270, 2015

Frequency of mutations

Health & Anemia study n=1043
Median age 83 y



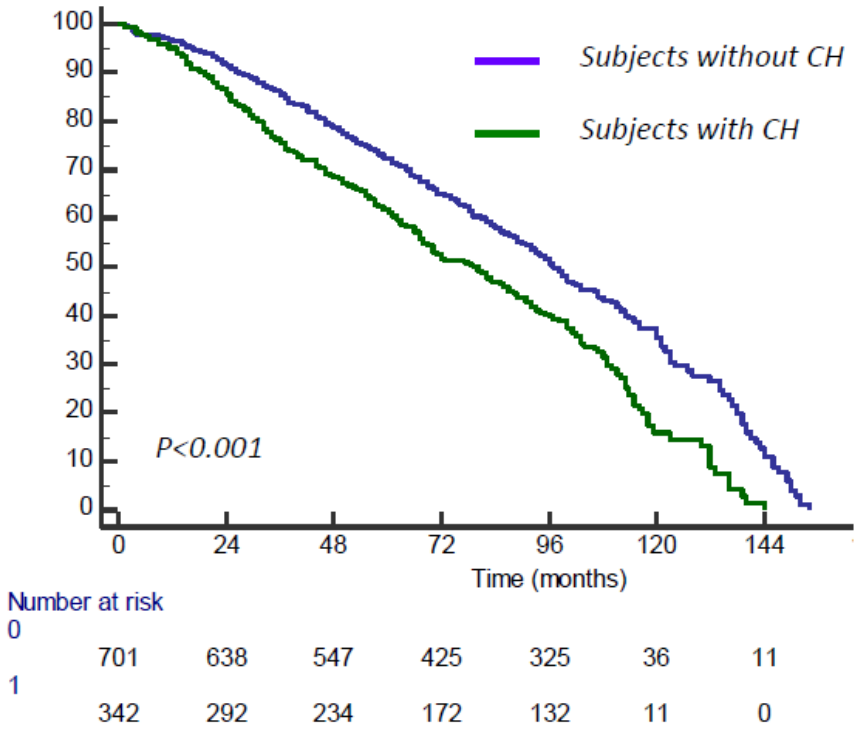
Monzino 80+ study n=735
Median age 91 y



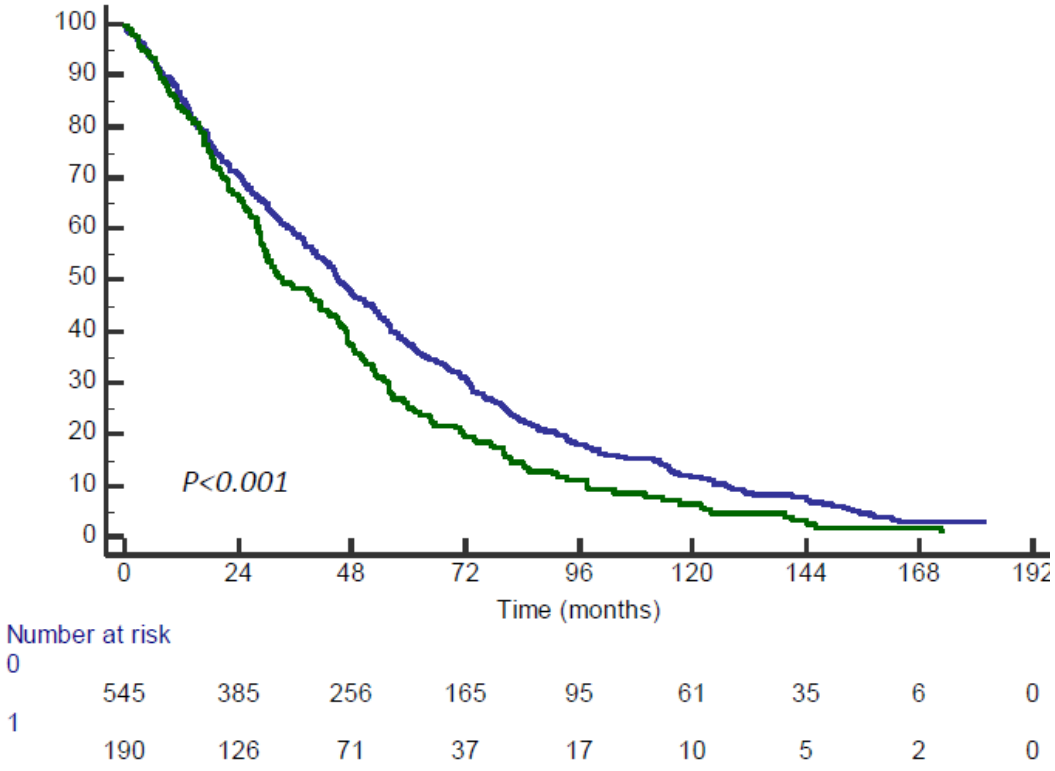
Della Porta MG, Rossi M et al., submitted

Probability of survival according to the presence of CH

Health & Anemia study



Monzino 80+ study

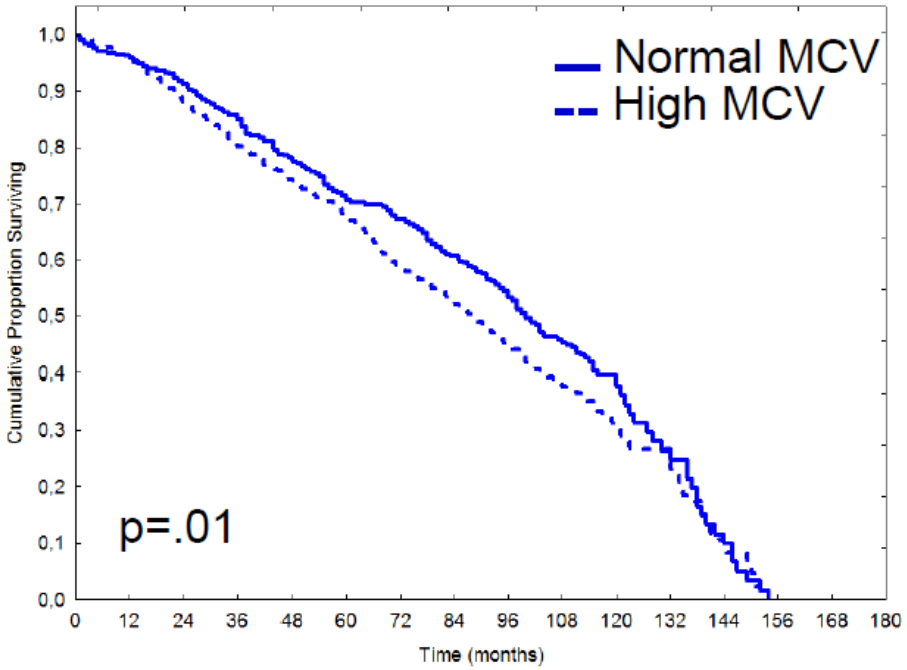


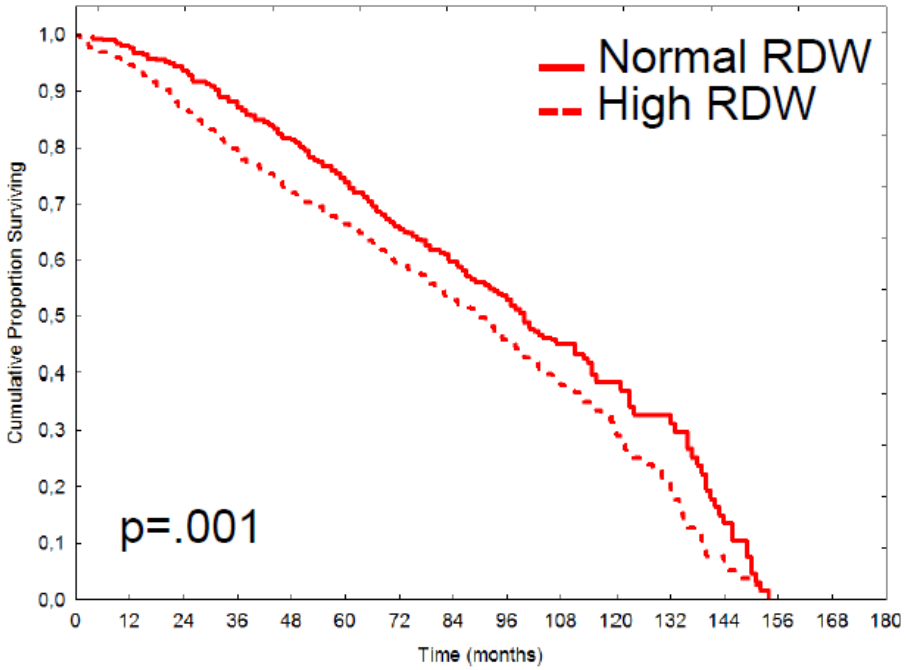
Della Porta MG, Rossi M et al., submitted

Predictive value of mutation status and hematopoietic clone size in the diagnosis of myeloid neoplasms (MDS)

- The absence of mutations in the 47 genes studied had a negative predictive value for MDS of 0.97
- Spliceosome genes (SF3B1, SRSF2, U2AF1) had the highest predictive value for MDS (0.76)
- The positive predictive value of mutations in TET2, or DNMT3A was 0,17 and 0.08, whereas that of the same mutations combined with other genetic lesions was higher (0.42 and 0.31, respectively)
- VAF ≥ 0.14 had a positive predictive value of 0,29
- Overall, mutations in spliceosome genes and comutation patterns involving TET2 and DNMT3A accounted for 74% of MDS.

Non-mutational factors predicting the risk of MDS





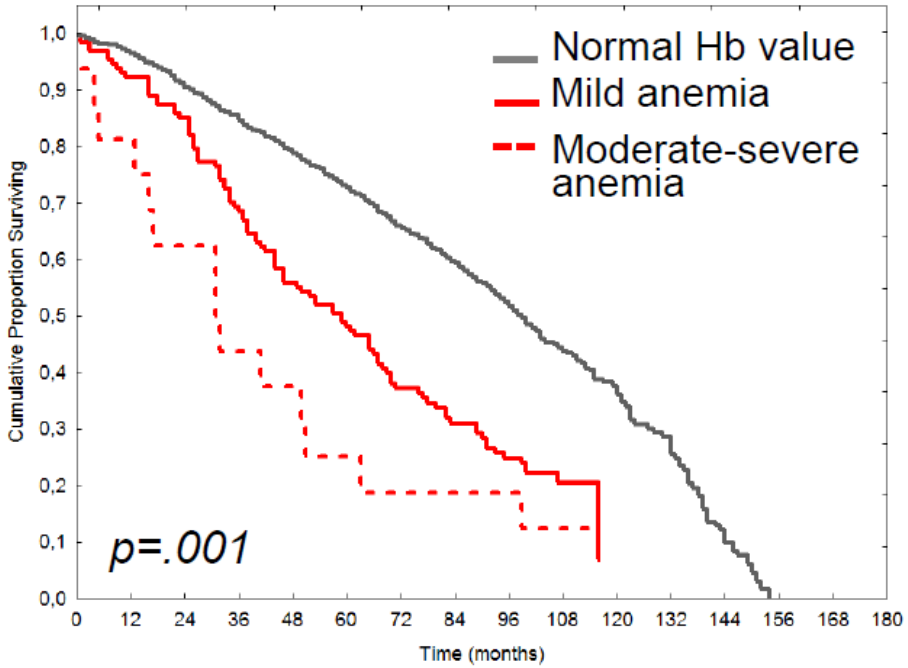
The combination of mutations and non-mutational factors (RDW and MCV, after excluding iron/vitamin depletion and thalassemia) improves the capability to capture individual risk of MDS with respect to molecular data alone (P=.01)

	mutations alone PPV	mutation+ high RDW/MCV PPV
Splicing factors	0,76	0,92
DNMT3A/TET2 + others	0,33	0,49

Unexplained Anemia (ICUS)

Prevalence of anemia in the study population
n=1778

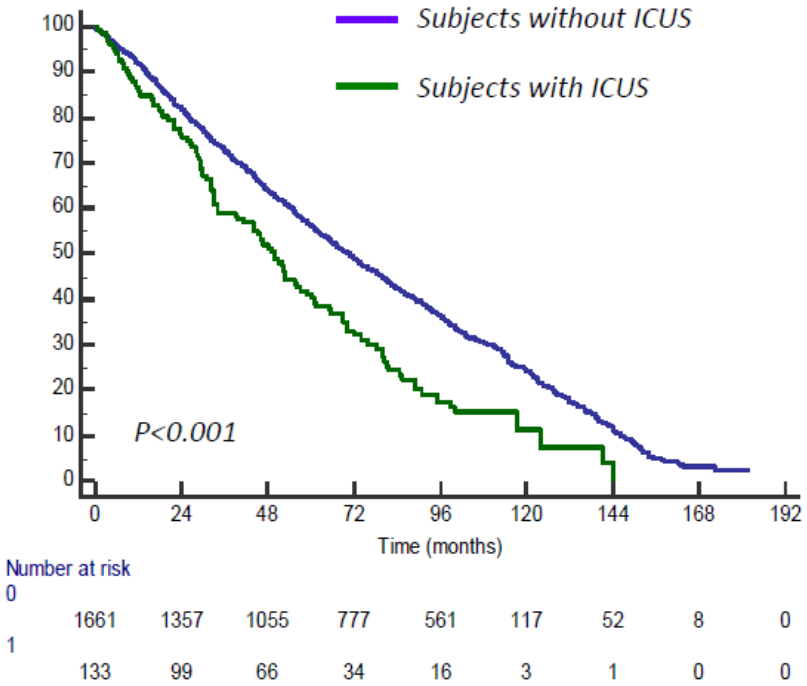
Age (y)	Prevalence of anemia (%)
80-84	18,9
85-89	26,6
90+	41,8

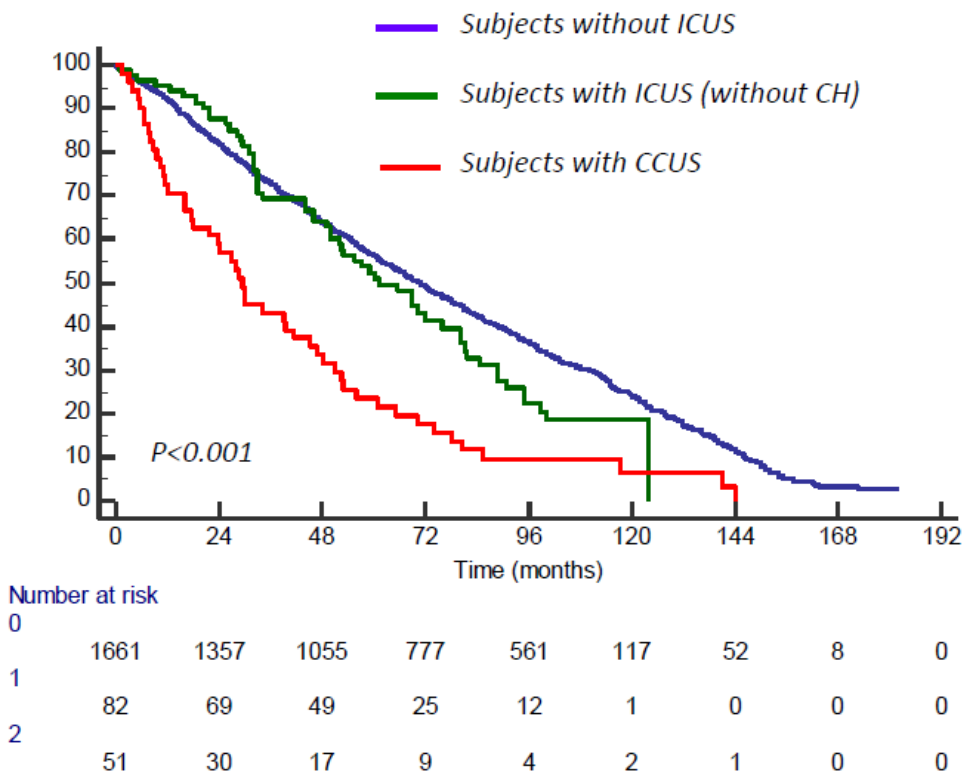


Types of anemia in the study population

Cause of anemia	%
Vitamin B12/folate/iron deficiency	26,3
Anemia of chronic disease	17,2
Renal insufficiency	15,1
Unexplained anemia (ICUS)	**28,7**

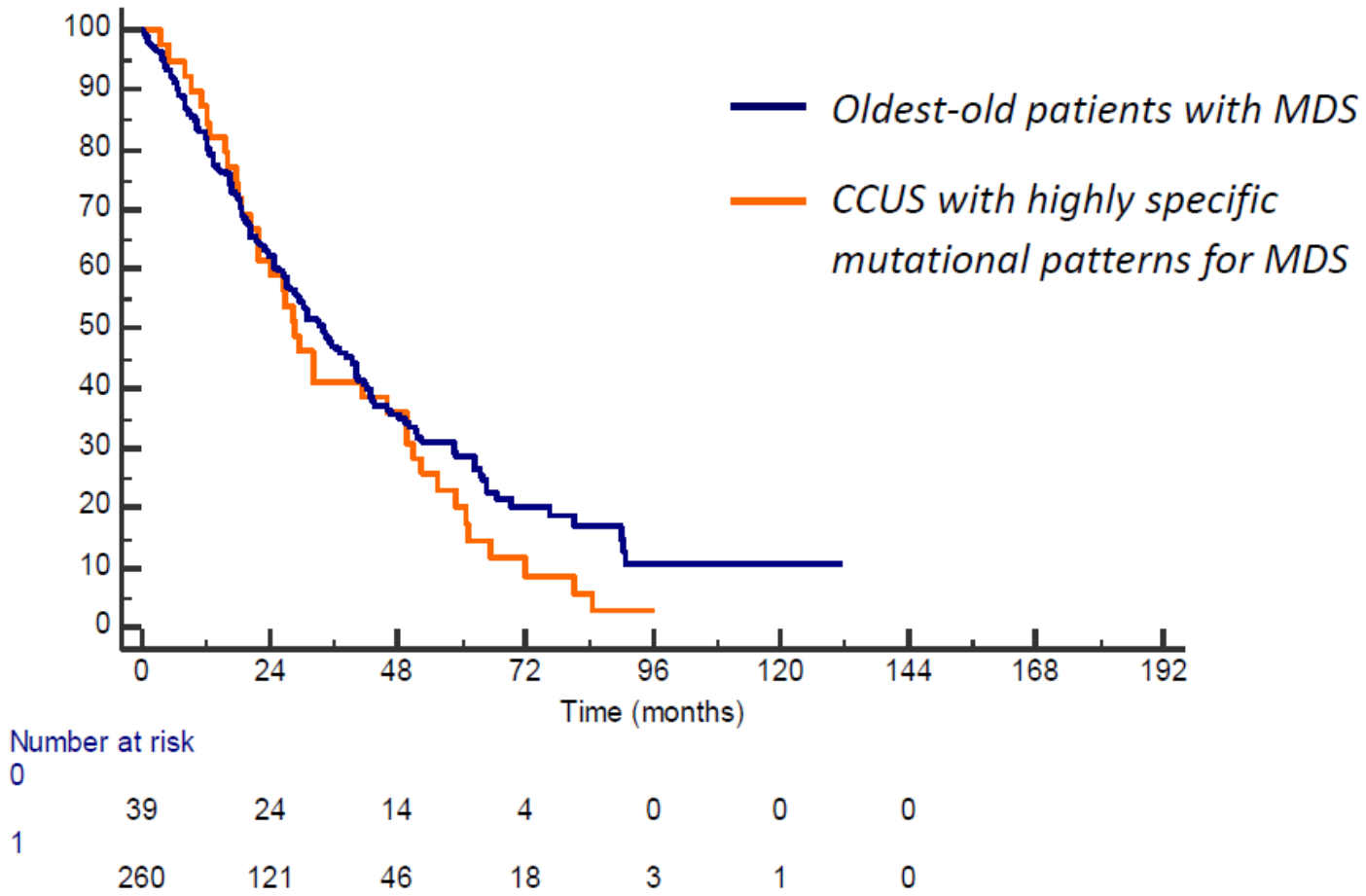
ICUS and CCUS





Della Porta MG, Rossi M et al., submitted

Overall survival of subjects with CCUS with highly specific mutational pattern vs. oldest-old MDS



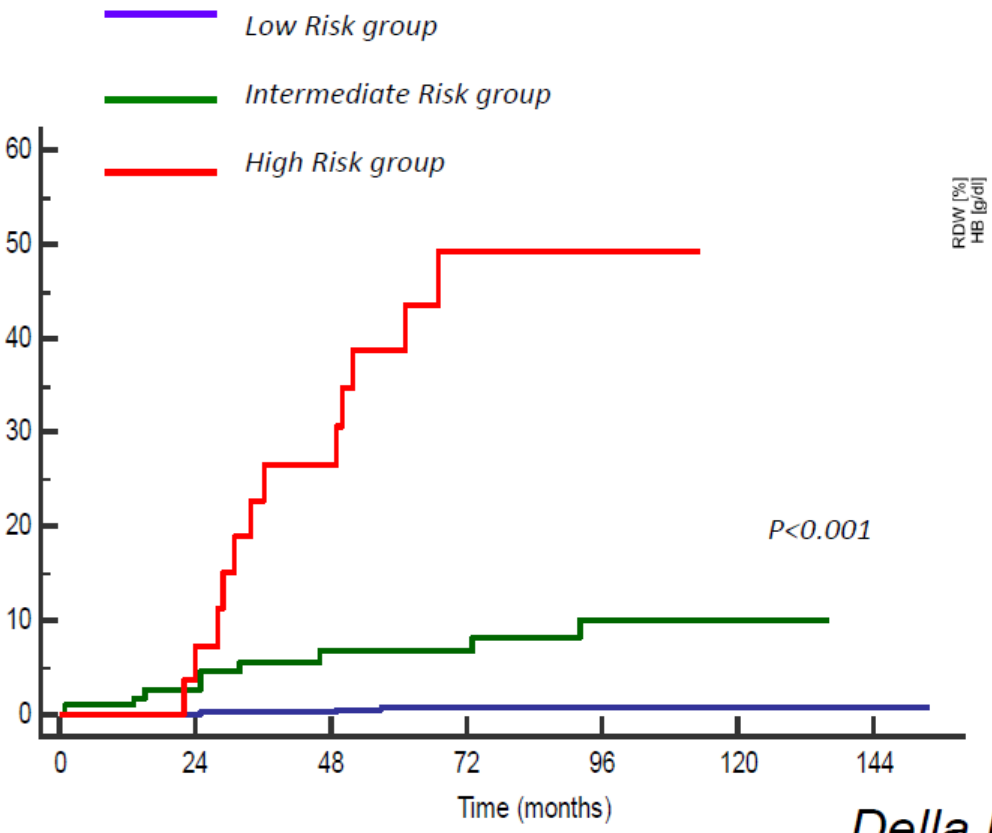
Della Porta MG, Rossi M et al., submitted

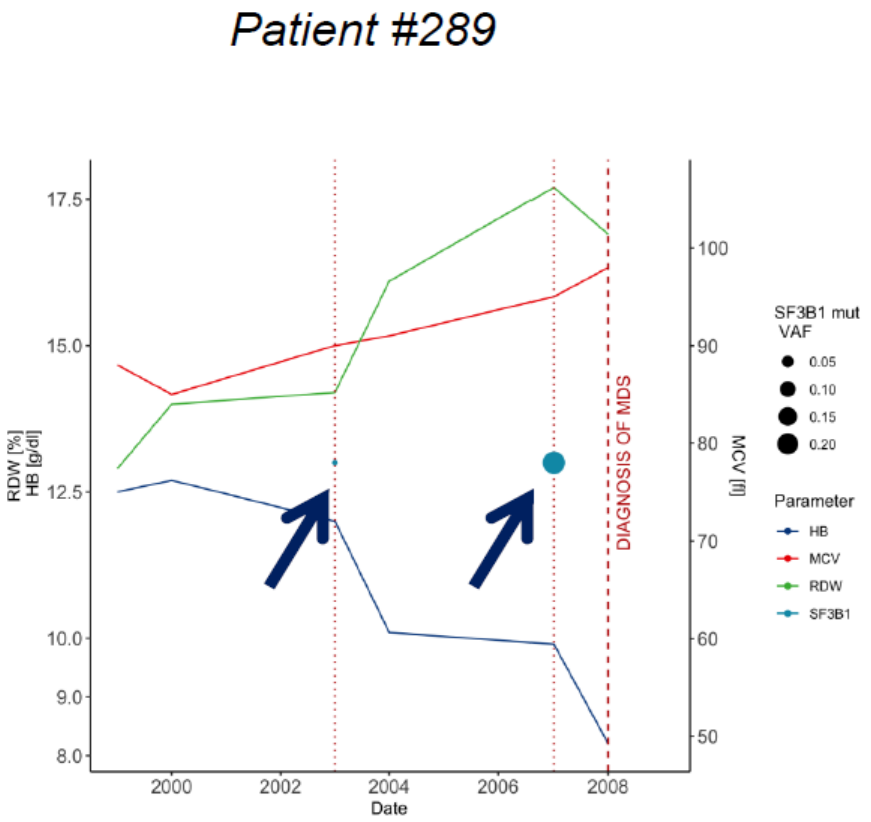
Summary

- Clonal hematopoiesis is associated with reduced survival in an oldest-old population
- Specific mutational profiles define different risks of developing MDS and inflammatory/vascular diseases.
- Carrying a somatic mutation with a variant allele frequency (VAF) ≥10%, carrying ≥2 mutations, spliceosome gene mutations and comutation patterns involving TET2, DNMT3A has a positive predictive value for MDS
- Non mutational factors, such as early changes in red blood cell indices, may improve the capability to identify patients at increased risk of developing myeloid cancers.
- In patients with unexplained anemia, carrying a somatic mutation has a positive predictive value for persistent, progressive, multilineage cytopenia (findings consistent with a MDS phenotype) and shorter survival. On this basis, 8% of all cytopenias might be undiagnosed MDS.

Clonal evolution

Parameter	Score Value*
Presence of MCV and/or RDW	1
Somatic mutation related to CH with VAF >0.14	1
Presence of TET2, DNMT3A, or ASXL1 combined with other genetic lesions	2
Presence of spicing gene mutations	5

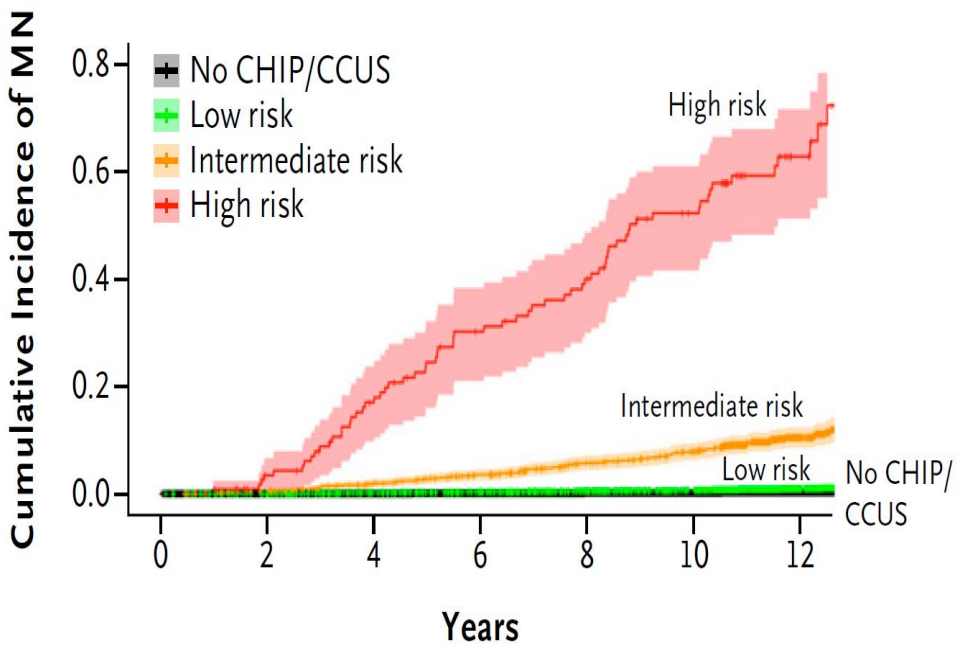




Della Porta MG, Rossi M et al., submitted

CHIP-hematológiai progresszió rizikó- CHRS

- defined high-risk mutation (*SRSF2, SF3B1, ZRSR2, IDH1, IDH2, FLT3, RUNX1, JAK2* and *TP53*)
- Detection of multiple mutations
- Clone size of at least 20% variant allele frequency (VAF)
- Red cell distribution width (RDW) of ≥15%
- Mean corpuscular volume (MCV) of ≥100 fl
- Presence of cytopenia (CCUS vs. CHIP)
- Age ≥65 years



HIGH Risk : CHRS > 12,5
IMTERMED RISK : 10-12
LOW RISK < 9,5

Table 2. CHRS Values.*

Prognostic Variable	0.5	1	1.5	2	2.5
Single *DNMT3A*	Present	Absent			
High-risk mutation		Absent			Present
Mutation number		1		≥2	
Variant allele fraction		<0.2		≥0.2	
Red cell distribution width		<15			≥15
Mean corpuscular volume		<100			≥100
Cytopenia		CHIP	CCUS		
Age (yr)		<65	≥65		

* CCUS denotes clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; and CHRS, clonal hematopoiesis risk score.

Weeks LD et al NEJM Evid 2023:2 (5))

Miért DNMT3A és TET2 okozza a CHIP-et ? Mi a clonalis expansio mechanismusa

- DNMT3A a DNS cytosin bazis methylációját okozva lassitja a génexpressziót

  D. Schübeler Function and information content of DNA methylation. Nature **517**, 321–326 (2015).

- TET2 gyakorlatilag DNMT3A ellenesen demetylálja a cytosint

  S. Ito, L. Shen, Q. Dai, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science **333**, 1300–1303 (2011).

- Egerekben ezen mutált HSC-k jó koloniaképzők, megnőtt az un “ self renewal” kapacitásuk

  K. Moran-Crusio, L.Reavie, A. Shih, et.al Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell **20**, 11–24 (2011).

- Ma sem tudjuk miért okoznak CH-t !!

- A CHIP egyértelműen az öregedéssel függ össze, férfiakban és dohányosokban gyakoribb, Hispanikus rasszban ritkább kérdéses a genetikus predispozició és kérdéses az összefüggése a microbiommal.

DNMT3A

- DNA methyltransferase 3a (*DNMT3A)* is the most commonly mutated gene in CHIP. ***DNMT3A*** **encodes a methyltransferase enzyme that catalyzes DNA methylation at CpG sites and is a critical epigenetic regulator of gene expression.** The majority of pathogenic mutations *loss-of-function i*ncluding disruptive missense mutations in regulatory and catalytic domains, nonsense mutations, insertions-deletions, and splice site mutations.
- *DNMT3A*pathogenic mutations enhance **HSC self-renewal** and promote the expression of multipotency genes while suppressing differentiation factor expression This enables *DNMT3A* mutations to affect all hematopoietic lineages, **inducing pro-inflammatory T-cell polarization and activating the inflammasome complex.**
- This pro-inflammatory T-cell polarization signature is observed in *DNMT3A* CHIP carriers in a cohort **of aortic stenosis patients undergoing transcatheter aortic valve replacement**, with a significantly increased Th17/Treg ratio in such patients
- *DNMT3A*-mutant CHIP carriers demonstrated **significantly increased expression of inflammatory interleukins IL-1β, IL-5, IL-8, activation of the NLRP3 inflammasome, macrophage inflammatory proteins CCL3 and CCL35, and resistin.**

TET2

- The second most commonly mutated CHIP gene is DNA demethylase *TET2* (ten-eleven translocation-2).
- *TET2* ***loss of function*** enhances HSC self-renewal and preferentially leads to differentiation toward myeloid lineages
- ***TET2***-deficient macrophages show increased inflammation, both spontaneous and in response to lipopolysaccharide, further potentiating an activated pro-inflammatory state
- *TET2* deficiency is associated with higher circulating levels of IL-1β through induction of the NLRP3 inflammasome , IL-6, and IL-8
- This pro-inflammatory state potentiated by *TET2* CHIP leads to accelerated atherosclerosis
- A cohort of patients with severe degenerative aortic stenosis undergoing transcatheter aortic valve implantation (TAVI) noted an association between *TET2* CHIP carrier status and higher circulating levels of non-classical monocytes (CD14dimCD16++), which secrete higher concentrations of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-8.

ASXL

- *SXL1* (additional sex combs-like 1) is the third most commonly mutated gene in CHIP, with its gene product regulating polycomb-mediated transcriptional repression. *ASXL1* mutations are typically frameshift or nonsense mutations occurring near the 5′ end of the gene and likely lead to *gain of function* and aberrant histone modifications

- Observational studies detail a link between *ASXL1* mutations in blood cells with smoking , and among patients with HIV *ASXL1* deletion facilitates aberrant gene expression and results in myeloid transformation

- The mechanisms by which *ASXL1*mutations lead to clonal hematopoiesis are not clear.

- Similarly, the mechanism by which ASXL1 enhances inflammation and atherosclerosis is also poorly understood.

- The shift toward myeloid transformation in *ASXL1* CHIP carriers may carry similar downstream effects to *TET2* loss of function.

JAK2

- *JAK2* p.V617F gain-of-function mutations in hematopoietic cells are associated with myeloproliferative neoplasm , which are in turn associated with myocardial infarction, deep vein thrombosis and stroke.
- *JAK2* p.V617F mutations enhance formation of neutrophil extracellular traps (NET) that promote thrombosis.
- *JAK2*CHIP carrier status is associated with higher levels of IL-18, and downstream increases in IL-6 production and inflammation
- *JAK2* p.V617F mutations in CHIP tend to occur at a younger age and carry an up to a 10-fold increased risk of coronary artery disease – the strongest risk of premature cardiac disease among CHIP variants

SF3B1, SRSF2

- CHIP driver mutations in *SF3B1* and *SRSF2* are key components of the mRNA spliceosome.
- Mutations in these genes lead to defects in splicing and export of mRNAs encoding genes involved in translation.
- While these CHIP mutations are not well studied with respect to cardiovascular disease, studies have shown that patients with *SF3B1* mutant-CHIP have higher circulating levels of IL-18

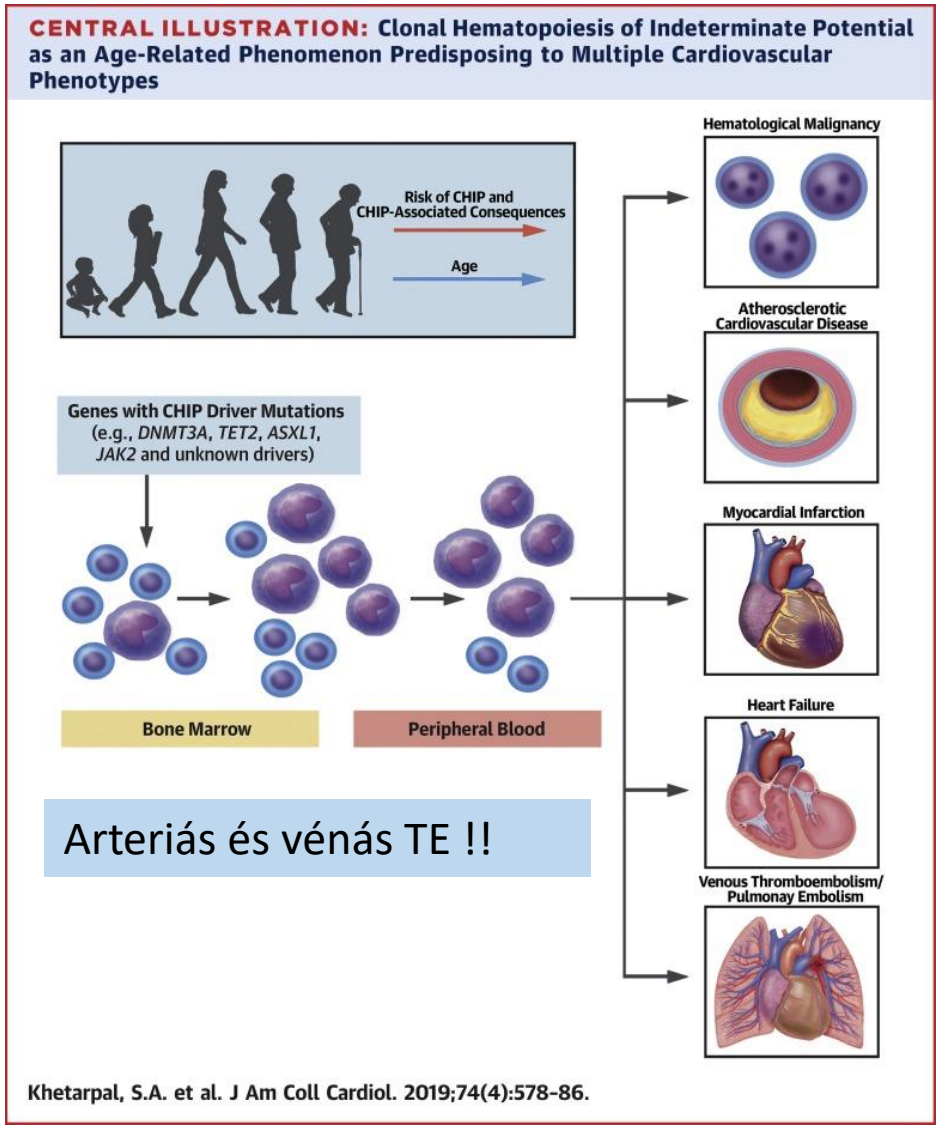
TP53, PPM1D,

PPM1D (protein phosphatase Mn2+/Mg2+-dependent 1D) is part of the DNA damage response pathway and in regulatory feedback loop with the tumor suppressor p53.

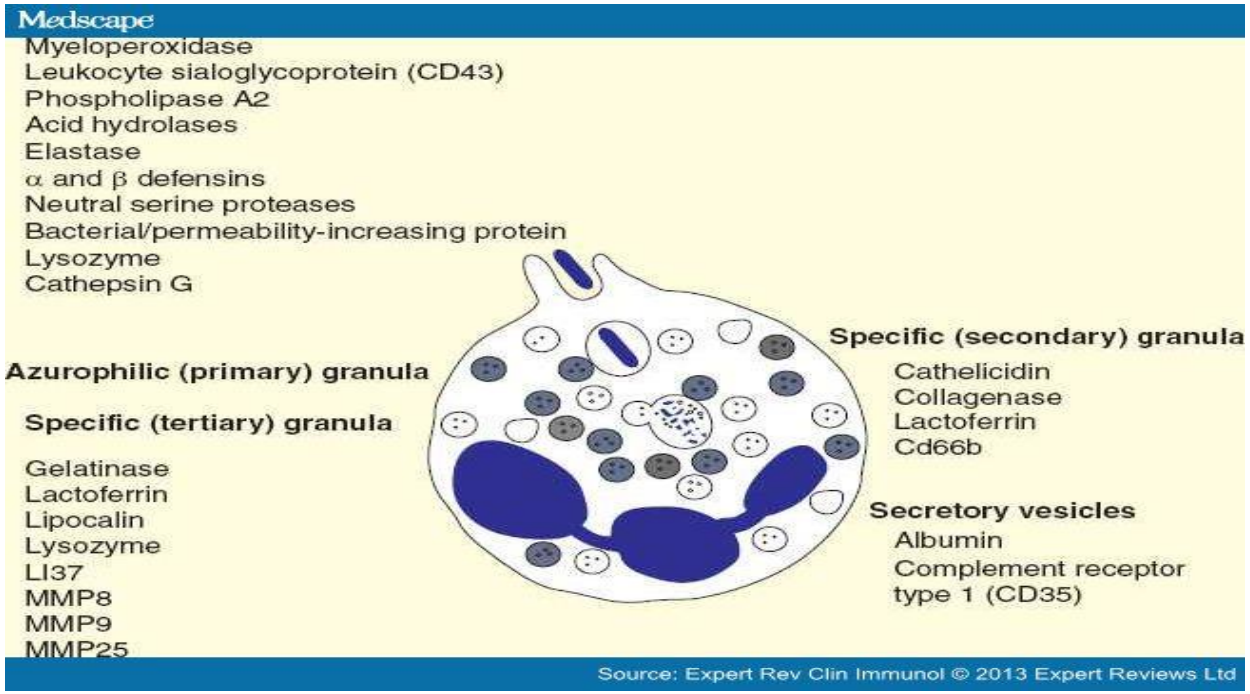
Activated p53 induces PPM1D expression, leading to downstream dephosphorylation of p53 and downregulation of apoptosis.

PPM1D loss-of-function mutations are in particular associated with CH in the context of prior exposure to cytotoxic chemotherapies such as cisplatin, etoposide and doxorubicin

CHIP és cardiovascularis betegségek



Lehetséges endothelkárositó complex



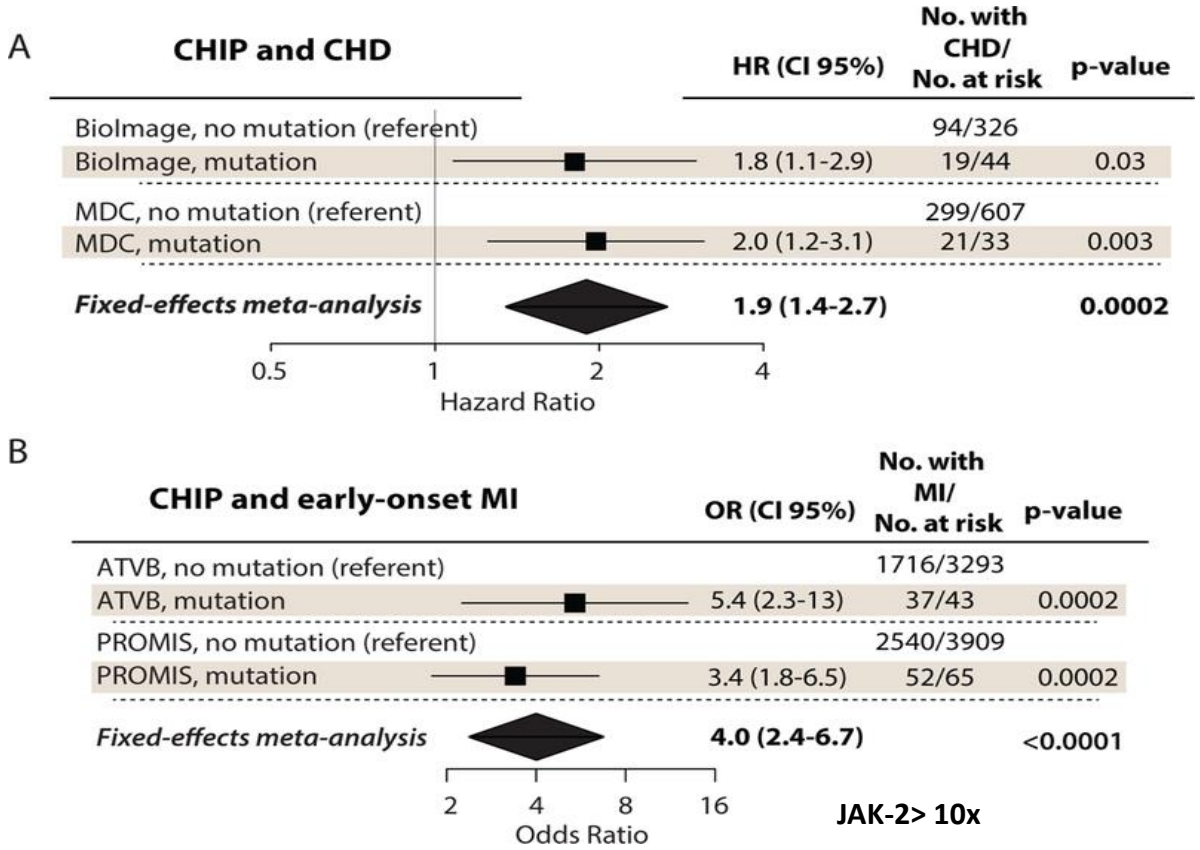
Neutrophilok: tertier granulumai → Gelatinase-NGAL-MMP-9 complex → pathologia ezen 3 komponens overexpressioja → endothel sérülést okozhat az MMP szövetkárosító hatása és az NGAL indukálta catalyticus vasterhelés miatt

Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease

S. Jaiswal, P. Natarajan, A.J. Silver, C.J. Gibson, A.G. Bick, E. Shvartz, M. McConkey, N. Gupta, S. Gabriel, D. Ardissino, U. Baber, R. Mehran, V. Fuster, J. Danesh, P. Frossard, D. Saleheen, O. Melander, G.K. Sukhova, D. Neuberg, P. Libby, S. Kathiresan, and B.L. Ebert

The new england journal *of* medicine

July 13, 2017 vol. 377 no. 2



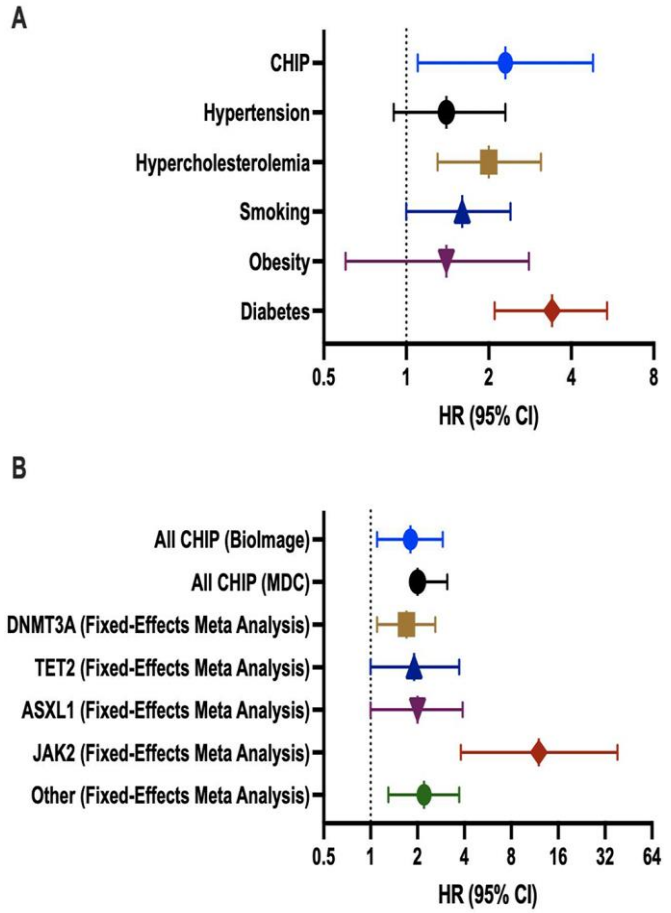
CHIP associates with coronary heart disease

A) Forest plot for association between CHD and CHIP in BioImage and MDC. Hazard ratio for having CHD in those with mutations was obtained by a Cox proportional hazards model adjusted for age, sex, type 2 diabetes, total cholesterol, high-density lipoprotein cholesterol, smoking status, and hypertension.

B) Forest plot for association between MI and CHIP in ATVB and PROMIS. Odds ratio was obtained by a logistic regression model adjusted for age, sex, type 2 diabetes, smoking status. CHIP (clonal hematopoiesis of indeterminate potential), CHD (coronary heart disease), HR (hazard ratio), MDC (Malmo Diet and Cancer Study), VAF (variant allele fraction), MI (myocardial infarction), OR (odds ratio), ATVB (Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group), PROMIS (The Pakistan Risk of Myocardial Infarction Study)

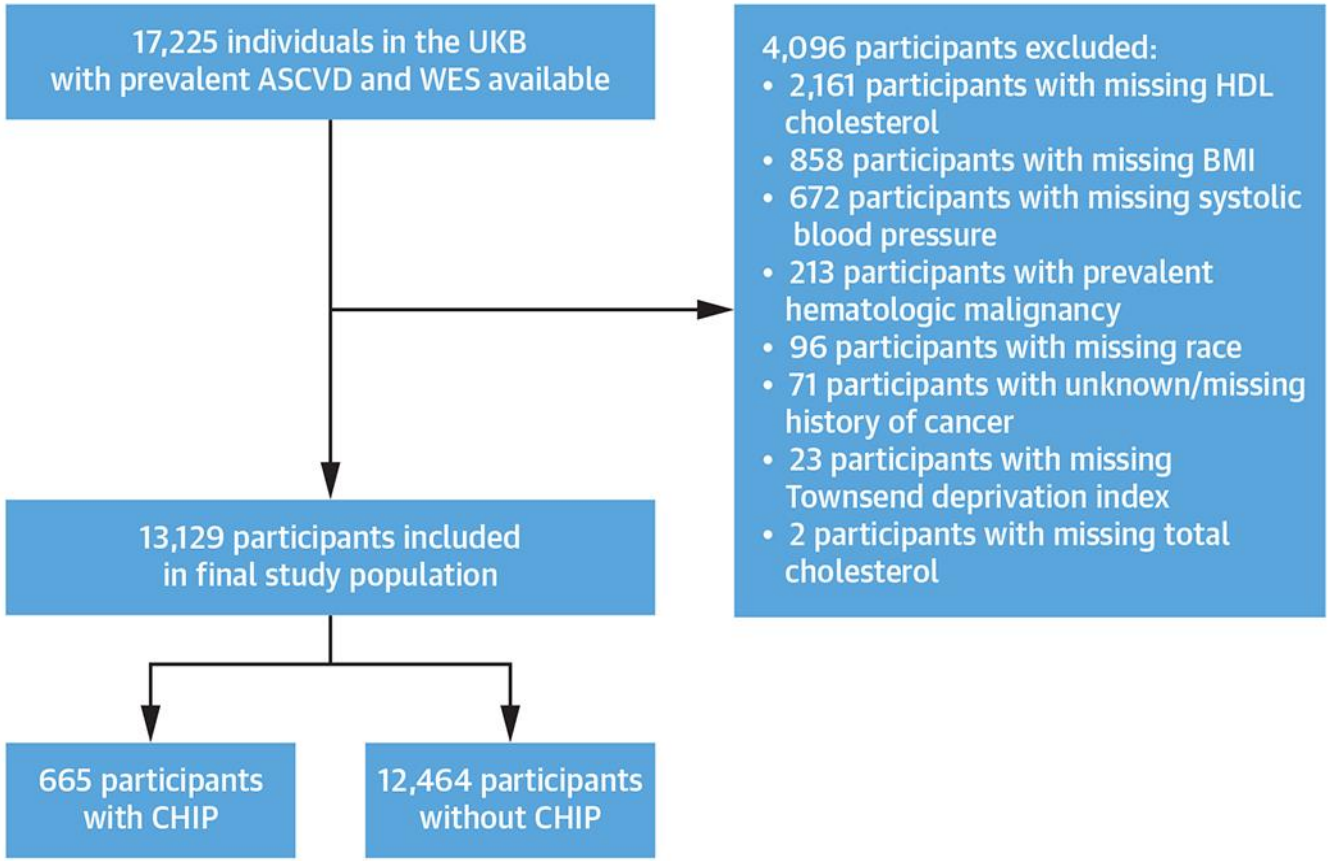
Selected hazard ratios (HR) for CHIP and incident coronary heart disease are of similar magnitude to traditional risk factors.

Candidate Driver	% of CHIP[1]	Mechanism	References
DNMT3A	**~58.5%**	Methyltransferase enzyme that catalyzes DNA methylation at CpG sites and alters epigenetic signature; tumor suppressor gene	
TET2	**~20%**	DNA demethylase *TET2* (ten-eleven t translocation-2) augments DNA methylation and affects transcription by recruiting histone deacetylases toward promoters; tumor suppressor gene	
ASXL1	**~8.0%**	Epigenetic modulator and chromatin-binding protein, function relatively unknown	
JAK2	**~3.2%**	Transmits intracellular signals downstream of cytokine receptors. *JAK2*tyrosine phosphorylates and activates *TET2* in response to cytokines, linking extracellular signals with epigenetic changes in hematopoiesis	
PPM1D, TP53	**~3.8%, 1.9%**	DNA damage response pathway in regulatory feedback loop with the tumor suppressor p53.	
SF3B1, SRSF2	**2%, 2%**	mRNA spliceosome complex components	
No candidate driver mutation		Limits of detection methods, epigenetic changes not detectable, neutral drift, or mosaic chromosomal alterations	

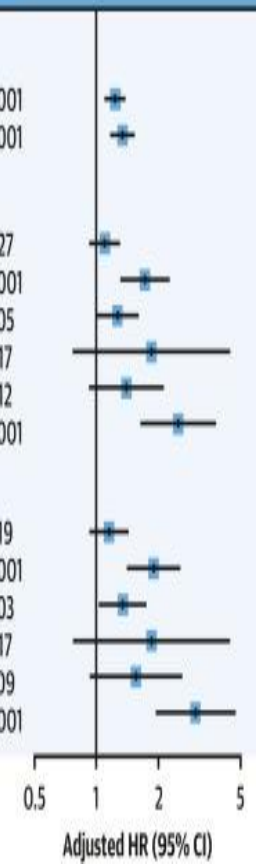


Marnell.C.S.. J Mol.Cell Cardiol 2022

CHIP-ASCVD Risk

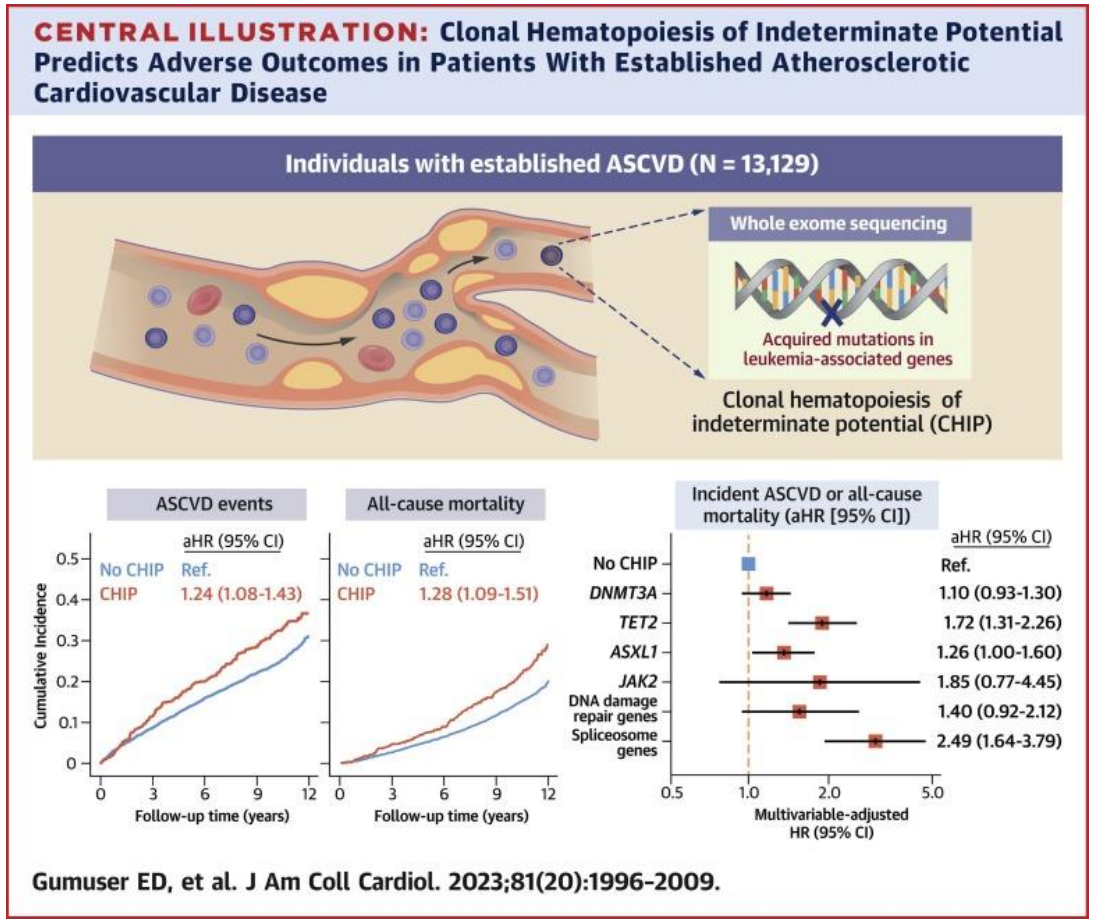


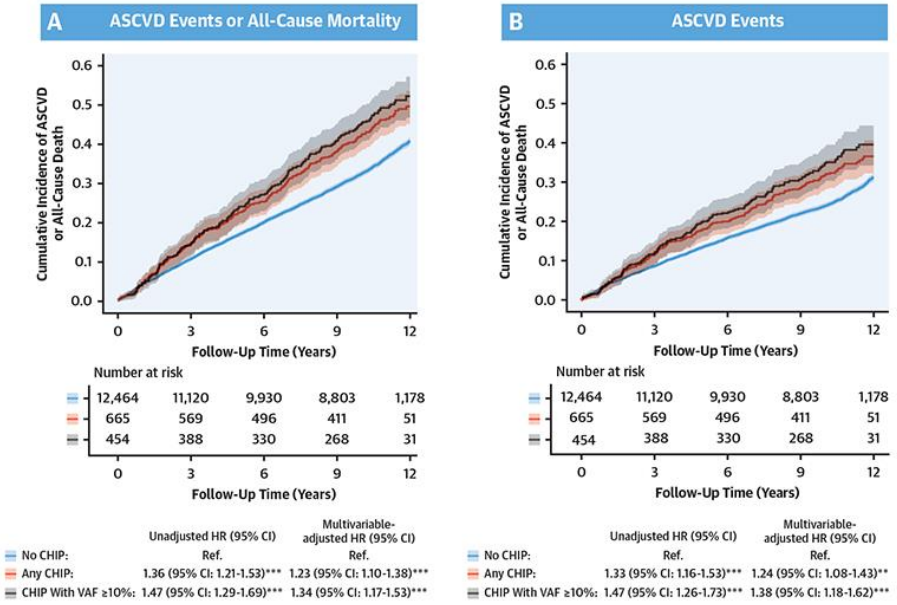
	Incidence Rate (Per 1,000 Person-Years)	Unadjusted HR (95% CI)	P Value	Adjusted HR (95% CI)	P Value
No CHIP	40.9	Ref.		Ref.	
CHIP (VAF ≥2%)	55.4	1.36 (1.21-1.53)	<0.001	1.23 (1.10-1.38)	<0.001
Large CHIP (VAF ≥10%)	59.8	1.47 (1.29-1.69)	<0.001	1.34 (1.17-1.53)	<0.001
Any CHIP (VAF ≥2%)					
DNMT3A CHIP	48.2	1.18 (1.00-1.39)	0.05	1.10 (0.93-1.30)	0.27
TET2 CHIP	72.5	1.79 (1.36-2.35)	<0.001	1.72 (1.31-2.26)	<0.001
ASXL1 CHIP	61.3	1.52 (1.20-1.92)	<0.001	1.26 (1.00-1.60)	0.05
JAK2 CHIP	69.8	1.71 (0.71-4.12)	0.23	1.85 (0.77-4.45)	0.17
PPM1D/TP53 CHIP	69.3	1.69 (1.11-2.57)	0.01	1.40 (0.92-2.12)	0.12
SF3B1/SRSF2/U2AF1 CHIP	116.6	2.96 (1.94-4.49)	<0.001	2.49 (1.64-3.79)	<0.001
Large CHIP (VAF ≥10%)					
Large DNMT3A CHIP	51.1	1.26 (1.02-1.55)	0.04	1.15 (0.93-1.43)	0.19
Large TET2 CHIP	75.8	1.87 (1.39-2.52)	<0.001	1.89 (1.40-2.55)	<0.001
Large ASXL1 CHIP	64.4	1.61 (1.23-2.10)	<0.001	1.35 (1.03-1.76)	0.03
Large JAK2 CHIP	69.8	1.71 (0.71-4.12)	0.23	1.85 (0.77-4.45)	0.17
Large PPM1D/TP53 CHIP	78.2	1.91 (1.15-3.18)	0.01	1.55 (0.93-2.58)	0.09
Large SF3B1/SRSF2/U2AF1 CHIP	135.9	3.47 (2.23-5.38)	<0.001	3.02 (1.95-4.70)	<0.001

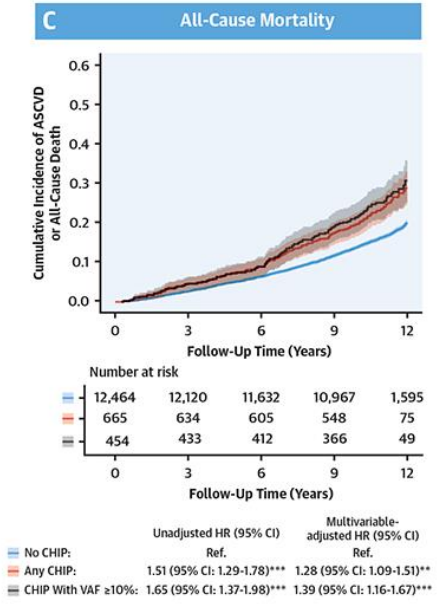


Gumuser ED J Am Coll Cardiol.2023:81

CHIP-ASCVD Risk







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Mekkora cardiovascularis rizikót jelent a JAK2-CHIP ?

- exome sequencing data from the MGB Biobank 54,000 patients 120 with *JAK2* VAF greater than 2%.
- 51 patients had a diagnosis of MPN by WHO 2016 criteria,
- 42 patients did not carry a diagnosis of MPN but were assumed to based on blood counts and were excluded from the study.
- 29 patients with *JAK2*-CHIP who had no other known lifetime hematologic disorders including MPNs and normal cell counts.
- retrospective analysis of thrombosis and CVD patients with *JAK2*-CHIP and *JAK2*-mutated MPNs.
- Rates of venous thrombosis were comparable across these groups (14.8% vs. 21%, p-value = 0.46).
- similar rates of arterial thrombosis across these groups, as evidence by ischemic stroke (11% vs. 12%, p-value =0.92) and acute coronary syndrome (7.4% vs. 5.8%, p-value=0.78).
- Conclusion: Rates of thrombosis and CVD complications were similar between patients with *JAK2*-CHIP and those with *JAK2*-mutated MPN.

Grevet J: JAK2-CHIP Iis associated with similar Cardiovascular disease risk as JAK-2 MPN Hemasphere 2023 Aug 8;7

Mekkora cardiovascularis rizikót jelent a JAK2-CHIP ?

- *Limvorapitak, W., Parker, J., Hughesman, C., McNeil, K., Foltz, L., Karsan, A. (2020).*

  No Differences in Outcomes Between JAK2 V617F–Positive Patients with Variant Allele Fraction

  VAF < 2% Versus 2-10%:

  A 6-Year Province-wide Retrospective Analysis.

  Clinical Lymphoma Myeloma and Leukemia, 20(9), e569-e578.

HUMYPRON :351 PV (csak JAK+) - TE incidencia 4,2% (év) Karádi. Eur J Hematol

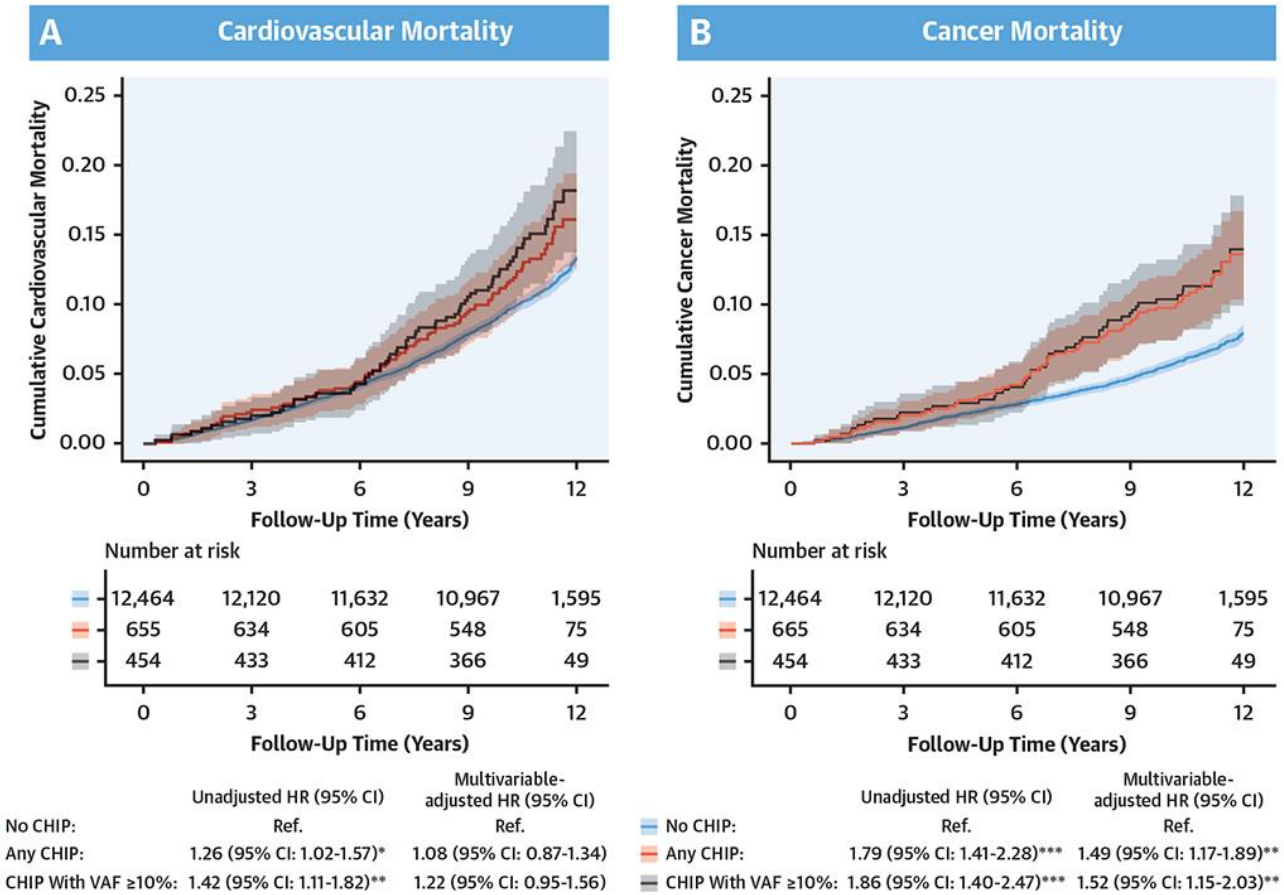
237 ET (2/3 JAK+) - TE incidencia 2,8% (év) JAK+ TE incidencia 4,2% (év) Kellner, Eur J Hematol

CHIP és egyéb malignus betegségek társulása

- CHIP-pel társult malignomak cytosztatikus kezelését követően megnő a sec MDS/AML prevalencia
  K. Takahashi, F. Wang, H. Kantarjian, D. Doss, et al , Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: A case-control study. Lancet Oncol.**18**, 100–111 (2017).

- CHIP-pel társult malignomak kezelési eredményei rosszabbak,mint a CHIP nélkülieké

  C. C. Coombs, A. Zehir, S. M. Devlin, et al, Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell **21**, 374–382.e4 (2017).



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CHIP és nem malignus betegségek társulása

- Az egyéb szövetek mutációi az adott szövetben/szervben okoznak eltérést
- A HSC mutációi más szövetekben is potenciálisan betegségkeltők ,mert a klonalis sejtek (Ly..Neutr.Gran. Mo) migrálnak

D. E. Wright, A. J. Wagers, A. P. Gulati, et al, Physiological migration of hematopoietic stem and progenitor cells. Science **294**, 1933–1936 (2001).

. A CHIP megnöveli a teljes mortalitást….2x (Stroke és Cardiovasc.)

S. Jaiswal, P. Fontanillas, J. Flannick, etz al,Age-related clonal hematopoiesis associated with adverse outcomes. N. Engl. J. Med. **371**, 2488–2498 (2014).

CHIP 4x-re fokozza a fiatalkori MI (ffi 40 és nő 50 év alatt) prevalenciát és rontja az infarctust követő szivelégtelenséget

Jaiswal S, Natarajan P, Silver AJ, et al Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. N Engl J Med. 2017 07 13; 377(2):111-121.

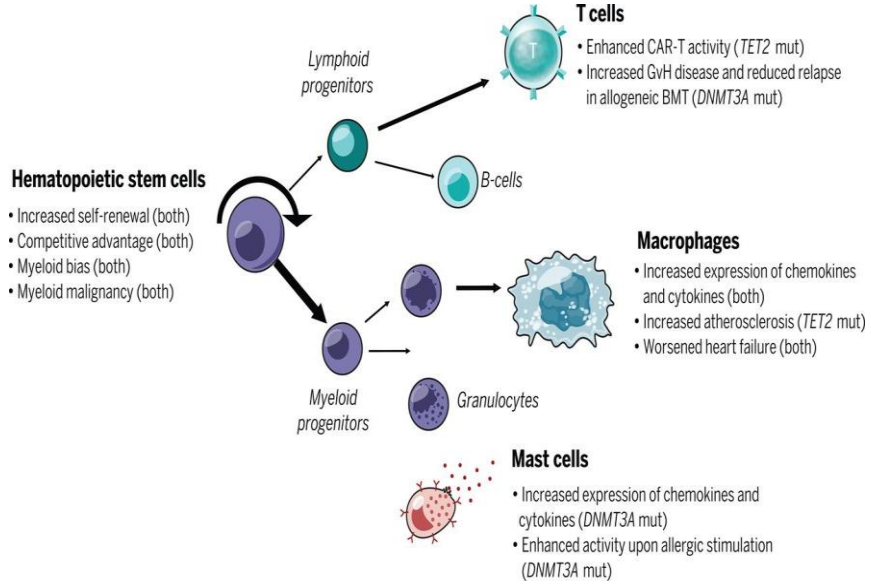
- JAK 2 mutációju CHIP 12x-re , egyéb mutáció 2x-re fokozza a VTE prevalenciát

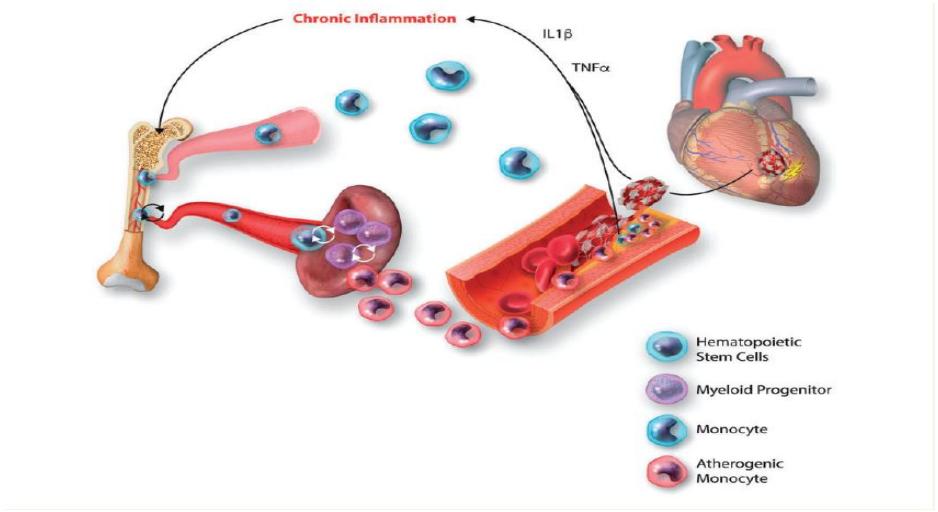
O. Wolach, R. S. Sellar, K. Martinod, et al., Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci. Transl. Med. **10**, eaan8292 (2018).

CHIP és nem malignus betegségek társulása

CHIP mutációk megváltoztatják a neutrofil granulocytak, monocytak inflammatoros hatásait

Q. Zhang, K. Zhao, Q. Shen, et al Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. Nature **525**, 389–393 (2015).





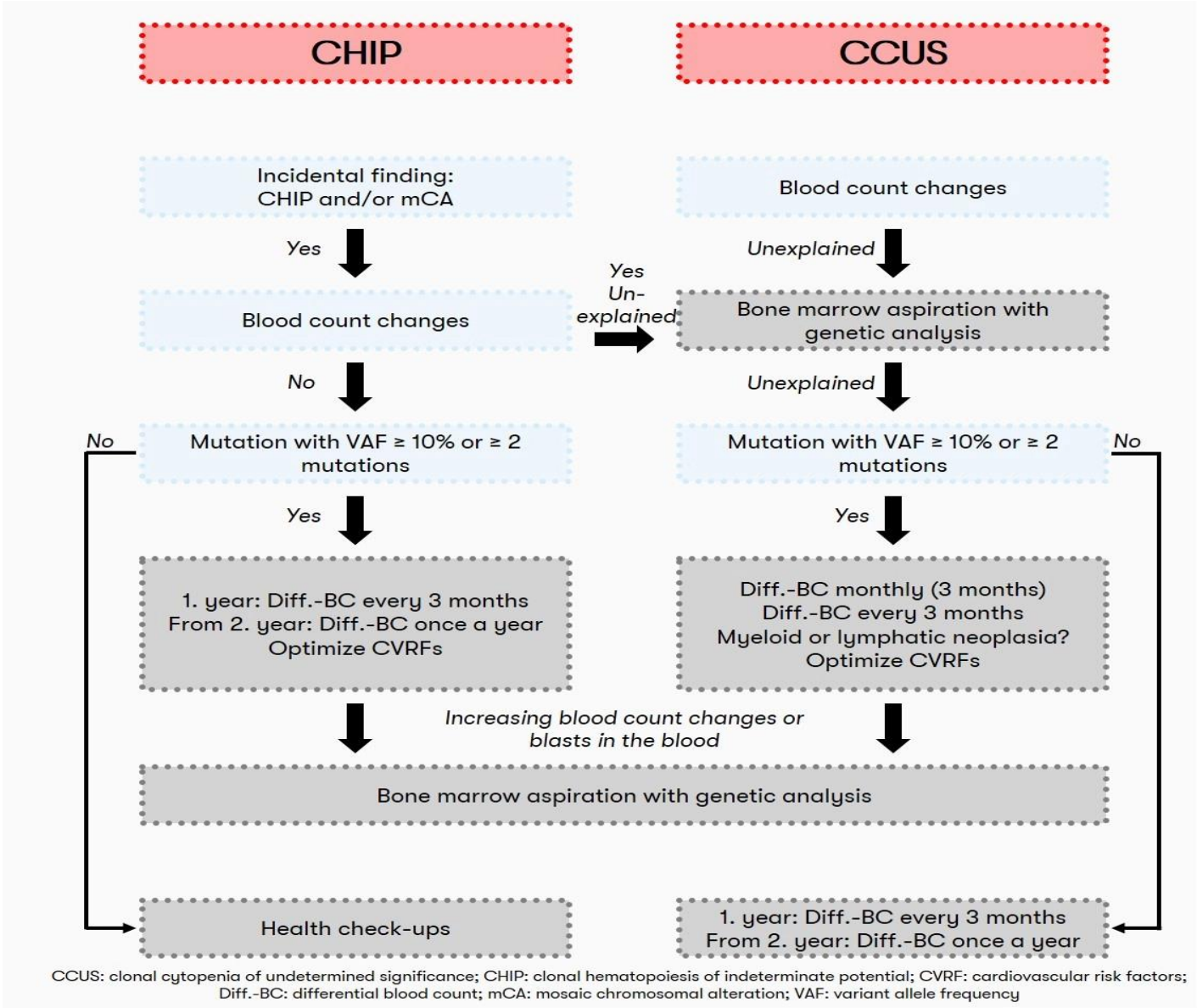
Phenotypic changes in HSCs and immune cells with *TET2* or *DNMT3A* mutations.
HSCs that lack *TET2* or *DNMT3A* display several convergent phenotypes in model systems, such as competitive advantage, enhanced self-renewal, myeloid bias in differentiation, and propensity for transformation to myeloid malignancies . The mature immune effector cells that derive from these mutated HSCs are increasingly appreciated to be functionally altered as well. Recent work has found that loss of *TET2* or *DNMT3A* increases inflammatory responses in macrophages and mast cells. Emerging work also suggests an effect of these mutations on T cell function, which may influence immune response to tumors . CAR-T, chimeric antigen receptor T cell; GvH, graft-versus-host; BMT, bone marrow transplant.

CHIP: kell-e szűrni?

- CHIP felfedezése incidentalis egyéb okból (hemat./onkol./genetic.) elvégzett DNS analizisből.
- Az eredmény értékelése multidisciplinális kell legyen. (hematol./onkol., cardiol., belgyógy., pathol./labor. clinical gen.,és bioinformatikus)
- Cardiológiai okból CHIP szűrés inkább csak bizonyos esetekben és tudományos kutatás céljából- noha ismert, hogy a tradicionális rizikótényezőkkel egyenrangú. Nincs evidedensen elismerten hatékony kezelése sem.

- Kell-e szűrni CHIP-re ? Magyarországon magas RDWés MCV bizonyos eseteiben ??
- Kell-e szűrni CHIP betegeket CV betegségre?
- Hogyan kövessük a CHIP betegeket?
- Hogyan kezeljük a CHIP betegeket?

CHIP/CCUS követés



modeled after Haferlach & Heuser 2022).

Összefoglaló I:

- A CHIP általában az MDS/AML, iniciáló mutációi hatására kialakuló CH “premalignus állapot” nem okoz cytopeniát (RDW!)
- A CHIP előfordul kimutatható genetikai eltérések nélkül…ismeretlen gén/gének okozhatják (COSMIC-ban nem szerepel)
- A CHIP fokozza a hematológiai malignomák, cardiovascularis betegségek prevalenciáját és a teljes mortalitást
- A CHIP betegek rizikója jelentősen különbözik az érintettek között a mutációk számától és minőségétől, a klón nagyságától(VAF) és annak változásától függően.
- A CHIP lényege: alapvetően megváltozott inflammatorikus folyamat …(amelyet jelenleg még nem értünk) - a klonális fvs-ek megváltozott tulajdonságai- cytokinek,kemokinek, egyébb gyulladásos molekulák okozzák, fő targetjük a vaszkuláris endothel, lehet,hogy összefügg több öregedéssel kapcsolatos betegséggel.

Összefoglaló II:

- Valószinű, hogy a csontvelői mikrokörnyezet (bone marrow microenvironment), microbiome?diéta? befolyásolják a CHIP hatásait, az inflammatoros fvs-ek képzését, az endothelsérülés progresszióját.
- Az exponenciálisan növekvő ismereteink ellenére nem értjük a CHIP klonalis expansio és inflammatio mechanizmusát
- Jó lenne terápiásan befolyásolni: inflammatorikus molekulák blokádja? Ideális lehetne a mutáns klón gátlása (suppresszio)
- Ma nincs elfogadott Tx…. (Mayo klinika:ahogy a CVD-t egyébként is kezelnénk)
- Mivel egyéb szövetek mutációi is hasonlóak, a CHIP jobb megismerése segithet egyéb malignomak és az öregedés folyamatainak megismerésében
- Az érzékenyebb szekvenálási módszerekkel az érintettek egyre nagyobb arányában igazolunk CHIP-t. Kérdéses a kicsi VAF mutációk szerepe, de valószinűleg csökkenni fog a CHIP érintettek hematológiai malignómákba való transzformáció aránya és emelkedni fog a CV betegségekben való részvétel

Akkor most mi is a CHIP ?

- A CHIP az öregedés folyamata során fellépő inflammatorikus állapot/jelenség ?
- A CHIP premalignus állapot, de egyben olyan, a vascularis endothél ellen irányuló inflammatoros jelenség is , amely az öregedés szempontjából fontos betegségeket okoz (fokoz) ?
- A CHIP az öregedés során kialakuló premalignus folyamat és egyben a vascularis endothél ellen irányuló inflammatoros állapot/jelenség, amely az öregedés szempontjából fontos betegségeket okoz(fokoz) ?

Köszönöm a felkérést és a megtisztelő figyelmet !

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