Inherited Bone Marrow Failure Syndromes

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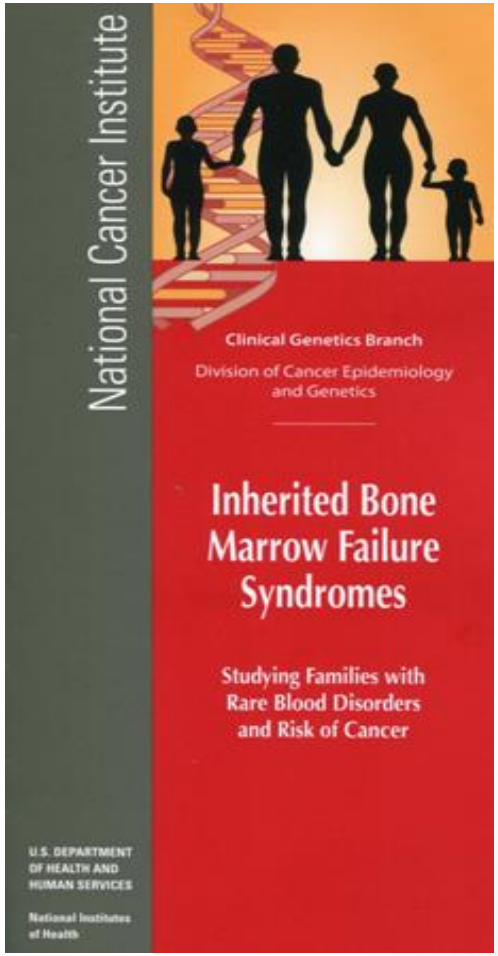
Bone marrow failure syndromes

'*Heterogeneous group of diseases characterized by progressive bone marrow failure & increased predisposition to cancer*'

- **Idiopathic (70%)** – of unknown etiology
- **Inherited (20%)**
- **Secondary (10%)**
  - ✓ Radiation / medication
  - ✓ Viral agents
  - ✓ Autoimmune diseases (SLE)

Inherited bone marrow failure syndromes (IBMFs)

- Eterogenous group of diseases characterized by:
  - Progressive BM failure
  - Predisposition to malignancies (leukemia, solid tumors)
  - Morphological stigmata
- Several well – described syndromes
- Others are harder to classify



IBMFs

- They are considered pediatric diseases
- Rarely the diagnosis is made in adulthood.
- Inability to determine their exact incidence
- >25% of children and 10% of young adults characterized as "acquired" aplastic anemia have IBMFS
- HSCT: AA (5%), MDS (14%) germline mutations IBMF

- Different severity of clinical manifestations even in the members of the same family
- 40% of patients do **NOT** have morphological stigmata

IBMFs

- Fanconi anemia (FA)
- Dyskeratosis Congenita (DC)
- Shwachman – Diamond syndrome (SDS)
- Diamond - Blackfan anemia (DBA)
- Congenital Amagakaryocytic thrombocytopenia (CAMT)
- TAR syndrome (**T**hrombocytopenia –**A**bsent **R**adius)
- Severe congenital neutropenia (SCN)
- Congenital dyserythropoietic anemias (CDA)

Diagnosis

- Patients with:
  - ✓ characteristic findings from the clinical examination and blood test the
- Patients with:
  - ✓ "Acquired" aplastic anemia MDS/AML
  - Cancer at a young age
- Definite diagnosis: Presence of characteristic mutations

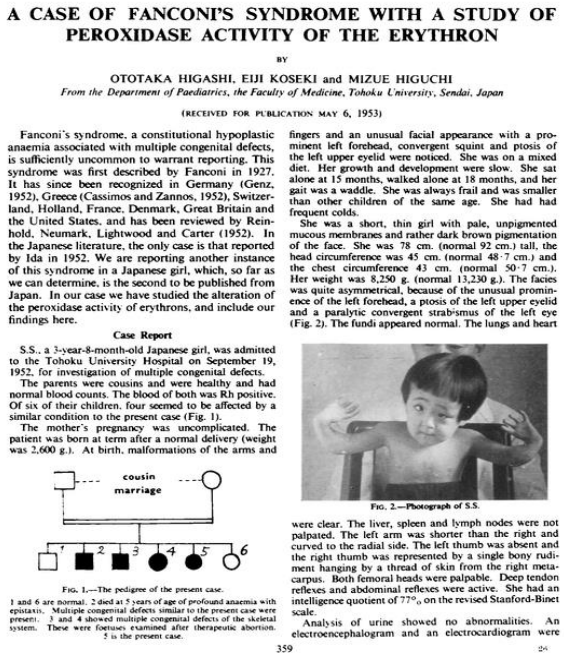
Fanconi anemia

Guido Fanconi

(Swiss pediatrician,1892-1979)

1927, 3 siblings:

- Pancytopenia
- Short stature
- café au lait spots



A CASE OF FANCONI'S SYNDROME WITH A STUDY OF PEROXIDASE ACTIVITY OF THE ERYTHRON

BY

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(RECEIVED FOR PUBLICATION MAY 6, 1953)

Fanconi's syndrome, a constitutional hypoplastic anaemia associated with multiple congenital defects, is sufficiently uncommon to warrant reporting. This syndrome was first described by Fanconi in 1927. It has since been recognized in Germany (Genz, 1952), Greece (Cassimos and Zannos, 1952), Switzerland, Holland, France, Denmark, Great Britain and the United States, and has been reviewed by Reinhold, Neumark, Lightwood and Carter (1952). In the Japanese literature, the only case is that reported by Ida in 1952. We are reporting another instance of this syndrome in a Japanese girl, which, so far as we can determine, is the second to be published from Japan. In our case we have studied the alteration of the peroxidase activity of erythrons, and include our findings here.

Case Report

S.S., a 3-year-8-month-old Japanese girl, was admitted to the Tohoku University Hospital on September 19, 1952, for investigation of multiple congenital defects.

The parents were cousins and were healthy and had normal blood counts. The blood of both was Rh positive. Of six of their children, four seemed to be affected by a similar condition to the present case (Fig. 1).

The mother's pregnancy was uncomplicated. The patient was born at term after a normal delivery (weight was 2,600 g.). At birth, malformations of the arms and fingers and an unusual facial appearance with a prominent left forehead, convergent squint and ptosis of the left upper eyelid were noticed. She was on a mixed diet. Her growth and development were slow. She sat alone at 15 months, walked alone at 18 months, and her gait was a waddle. She was always frail and was smaller than other children of the same age. She had had frequent colds.

She was a short, thin girl with pale, unpigmented mucous membranes and rather dark brown pigmentation of the face. She was 78 cm. (normal 92 cm.) tall, the head circumference was 45 cm. (normal 48·7 cm.) and the chest circumference 43 cm. (normal 50·7 cm.). Her weight was 8,250 g. (normal 13,230 g.). The facies was quite asymmetrical, because of the unusual prominence of the left forehead, a ptosis of the left upper eyelid and a paralytic convergent strabismus of the left eye (Fig. 2). The fundi appeared normal. The lungs and heart were clear. The liver, spleen and lymph nodes were not palpated. The left arm was shorter than the right and curved to the radial side. The left thumb was absent and the right thumb was represented by a single bony rudiment hanging by a thread of skin from the right metacarpus. Both femoral heads were palpable. Deep tendon reflexes and abdominal reflexes were active. She had an intelligence quotient of 77% on the revised Stanford-Binet scale.

Analysis of urine showed no abnormalities. An electroencephalogram and an electrocardiogram were

cousin marriage

1 2 3 4 5 6

FIG. 1.—The pedigree of the present case.

1 and 6 are normal. 2 died at 5 years of age of profound anaemia with epistaxis. Multiple congenital defects similar to the present case were present. 3 and 4 showed multiple congenital defects of the skeletal system. These were foetuses examined after therapeutic abortion. 5 is the present case.

FIG. 2.—Photograph of S.S.

359

Alter, FA101 (2006)

MILESTONES IN THE KNOWLEDGE OF FANCONI'S ANEMIA

- 1927: The Swiss pediatrician Guido Fanconi first described Fanconi anemia as a condition similar to pernicious anemia.

Before 1960

- 1931: The hematologist Otto Naegeli proposed the name Fanconi anemia.

1960s

- High spontaneous chromosomal breakage was observe in FA cells. Chromosomal instability syndrome was suspected.

1970s

- High sensitivity of FA cells to DNA crosslinking agents was observed and alteration of repair genes was suspected.
- Identification of adverse effects of allogenic hematopoietic cell transplantation applying standard protocols.

1980s

- Development of successful special regimens for conditioning FA patients for transplantation.
  - 1982: The international Fanconi Anemia Registry (IFAR) was created in Rockefeller University
  - 1988: First successful cord blood transplant in FA patient

1990s

- Identification of 7 complementation groups (A, B, C, D, E, F, G) by somatic cell hybridization studies, and four FANC genes (A, C, F, G) through cloning assays.
  - 1992: The first FA gene was identified: *FANCC*.
  - 1996: The *FANCA* gene was cloned.

2000s

- The name FA/BRCA was given to the repair pathway
- *FANCD1/BRCA2, FANCB, FANCL, FANCM, FANCJ/BRIF1, FANCI* were associated with FA
  - 2002: FA-D1 was identified as being the same as *FANCD1/BRCA2*, a cancer predisposition gene.

2010s

- *FANCO/RAD51C, FANCP, FANCQ, FANCR, FANCS/BRCA1, FANCT, FANCU, FANCV, FANCW* genes were associated with FA.
- Gene therapy clinical trials began to improve bone marrow function in FA patients.

Current

- Research into new therapies for treatment of solid tumors of the head and neck in FA patients.
- Trials of gene therapy for marrow failure in stage III.

Fanconi anemia

- Inherited with:
  - Autosomal recessive trait
  - X-linked recessive trait (rare)
  - Autosomal dominant trait
- It is characterized by ***chromosomal instability*** and an ***increased predisposition to malignancy***
- Diagnosis: the first decade of life (6.5 years)
- Prevalence 1 – 5/10^6 population
- Europ Registry: Prevalence 4 – 7/10^6 live births
- M/F: 1.2/1

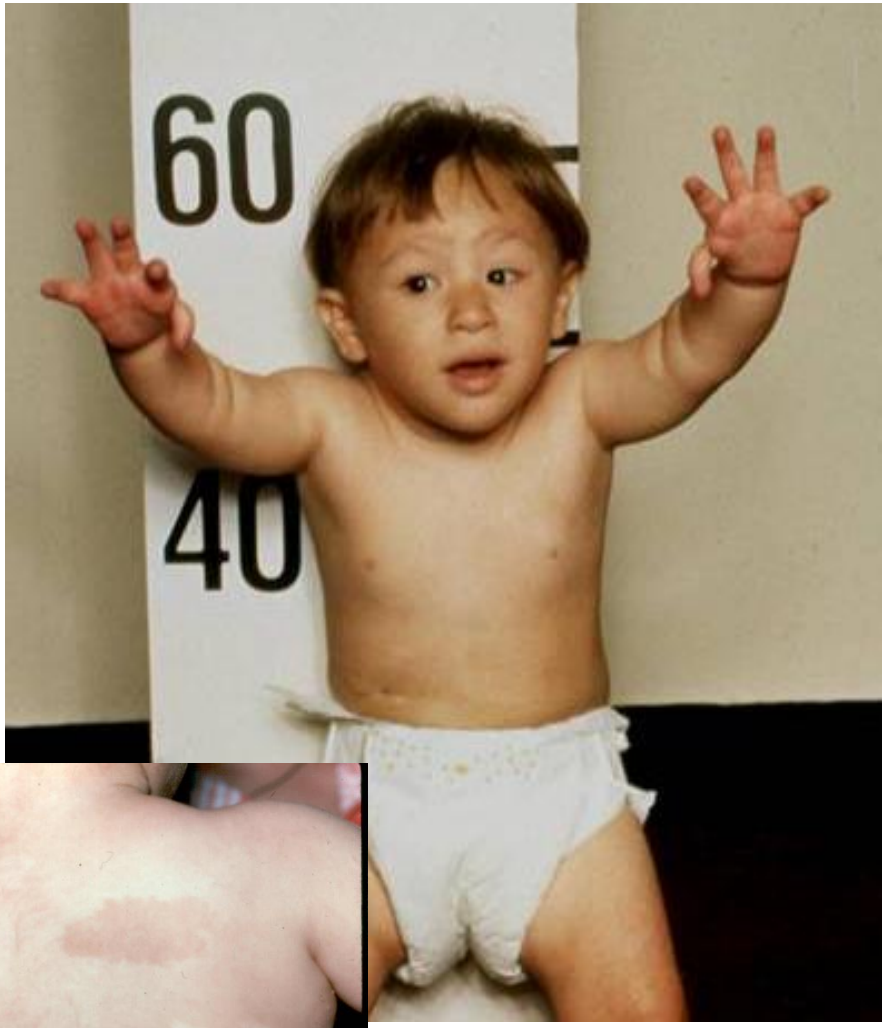
Clinical characteristics (60% of the pts)

- Café au lait spots
- Short stature
- Abnormal thumbs +/- radial hypoplasia
  Microcephaly/microophthalmia
- Hypogonadism, GH deficiency, insulin resistance, metabolic syndrome, hypothyroidism
- Horseshoe kidney,
- Duodenal atresia
- Cardiac abnormalities

VACTERL-H (5-10% FA)

- Vertebral anomalies
- Anal atresia
- Cardiac anomalies
- Trachea-esoph.fistula
- Esoph. atresia
- Renal anomalies
- Limb abnormalities
- Hydrocephalus

- ***40% without clinical stigmata***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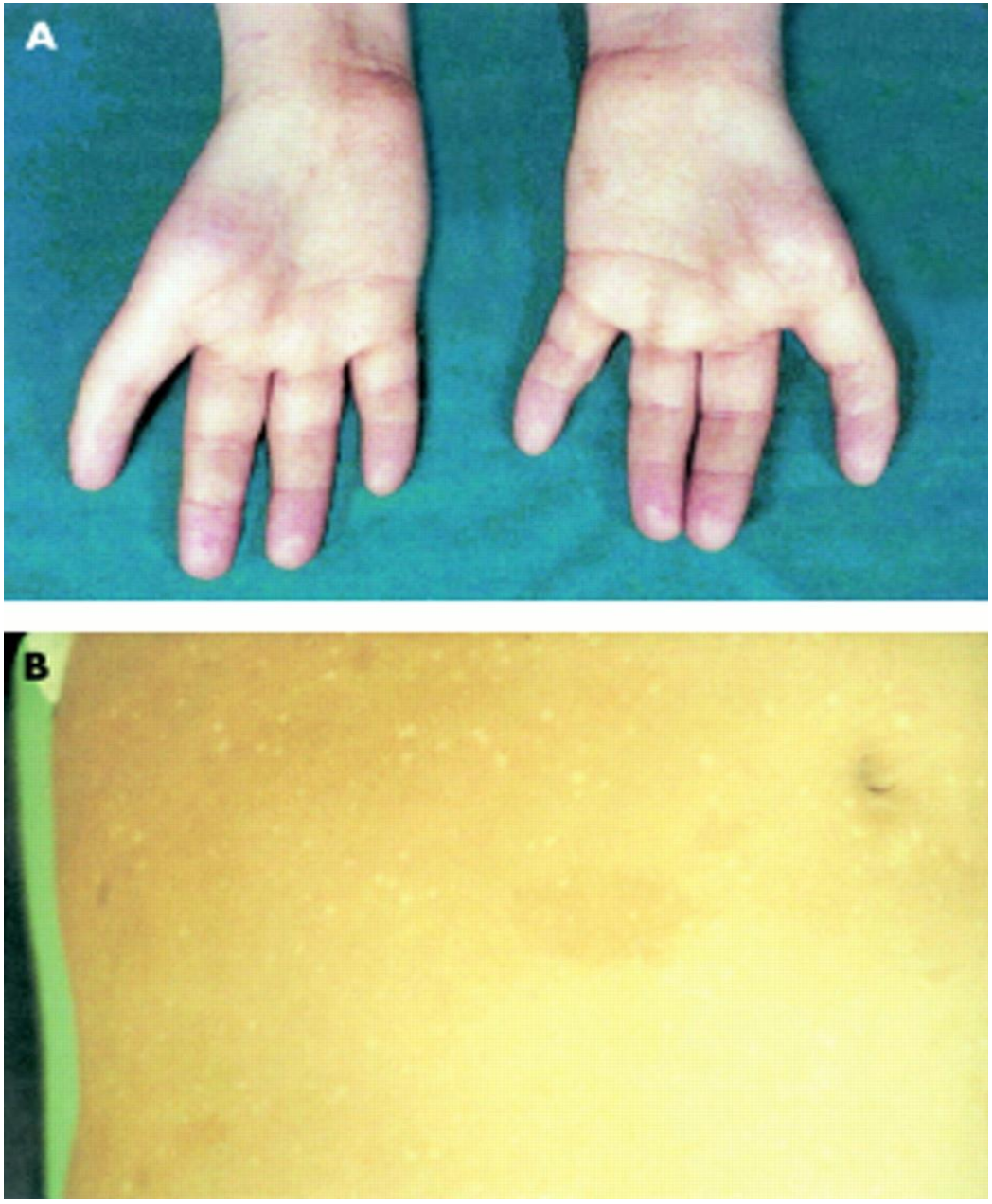
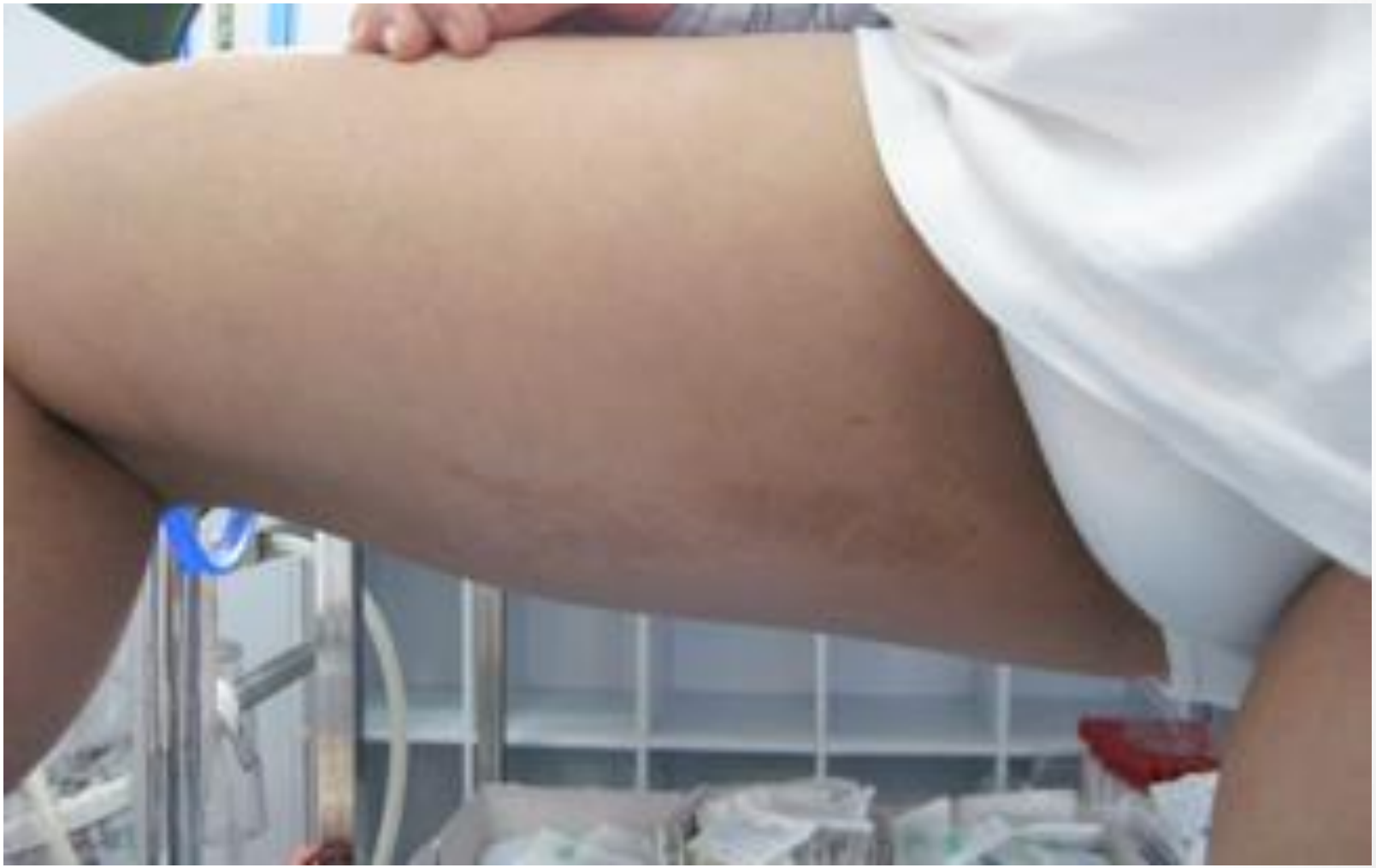
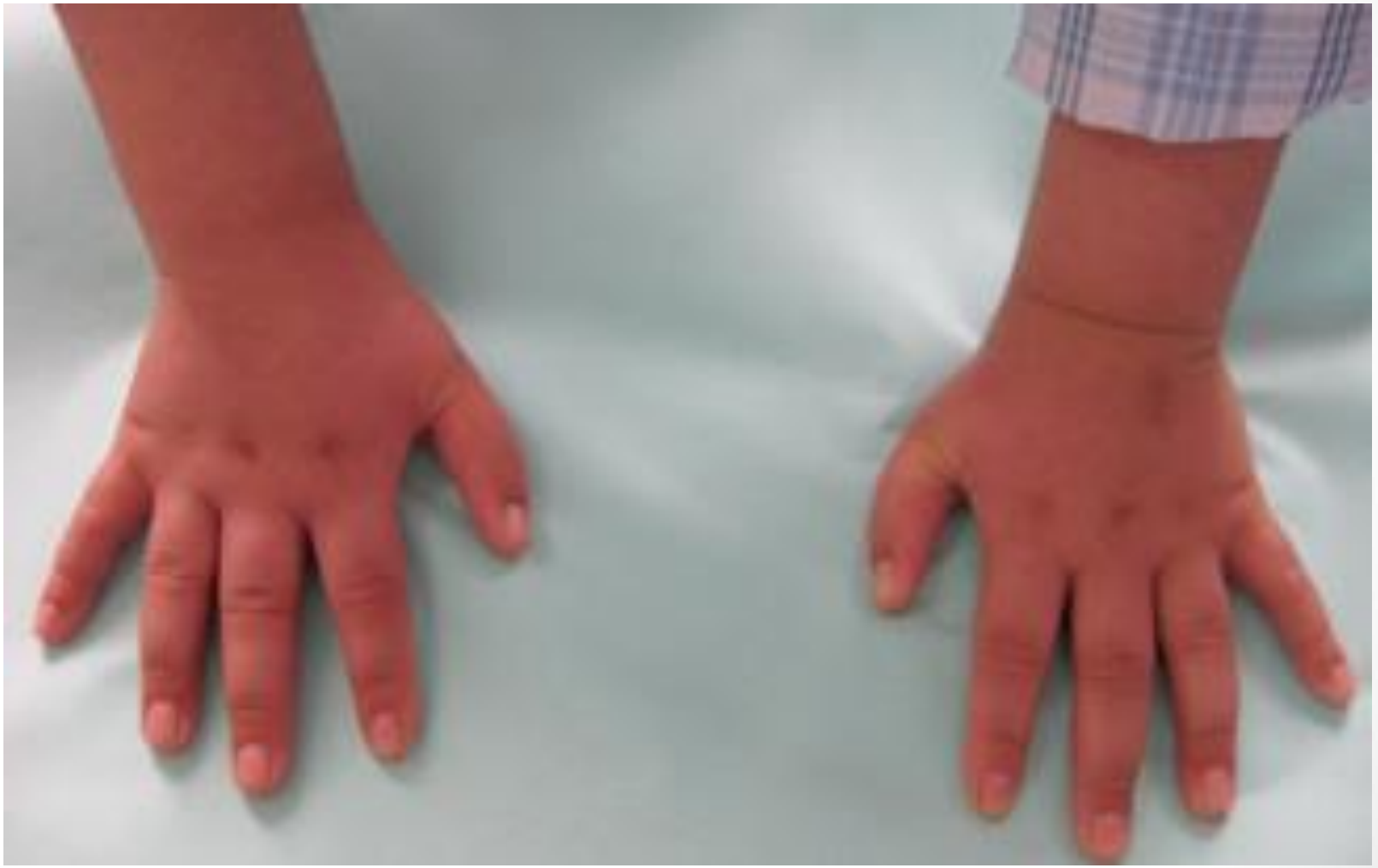
SUNSHINE
Volunteer
Sadie


A
B





Pathophysiology FA

Chromosomal instability (fragility of chromosomes)
Abnormal cell cycle kinetics (prolonged G2 phase)
Increased apoptosis

Heterozygotes' frequency: 1/300 -1/181 (US) (1/100 in Ashkenazi)

Complementation studies:

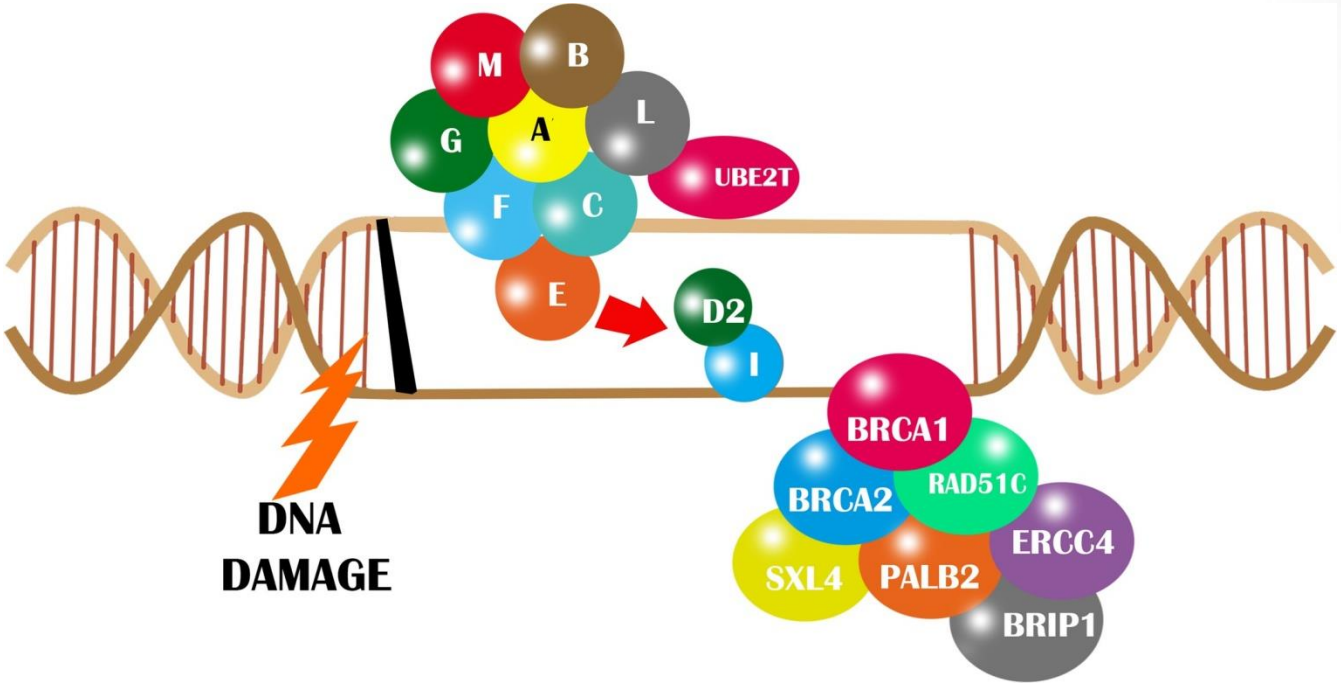
23 groups (A-Q, D1-D2)

- ✓ Autosomal recessive
- ✓ X-linked: FANC-B
- ✓ Autosomal dominant: FANC-R

FA genetic subtypes

Complementation group (gene)	Approximate % of patients with FA	Chromosome location	Gene product	Exons
AR				
A (*FANCA*)	65	16q24.3	FANCA	44
C (*FANCC*)	12	9q22.32	FANCC	22
G (*FANCG*)	12	9p13.3	FANCG/XRCC9	14
J (*FANCJ*)	<5	17q23.2	FANCJ/BRIP1	25
E (*FANCE*)	4	6p21.31	FANCE	10
F (*FANCF*)	4	11p14.3	FANCF	1
P (*FANCP*)	2	16p13.3	FANCP/SLX4	17
D1 (*FANCD1*)	<1	13q13.1	FANCD1/BRCA2	27
D2 (*FANCD2*)	<1	3p25.3	FANCD2	45
I (*FANCI*)	<1	15q26.1	FANCI	38
L (*FANCL*)	<1	2p16.1	FANCL	14
M (*FANCM*)*	<1	14q21.2	FANCM	25
N (*FANCN*)	<1	16p12.2	FANCN/PALB2	14
O (*FANCO*)*	<1	17q22	FANCO/RAD51C	12
Q (*FANCQ*)	<1	16p13.12	FANCQ/ERCC4	13
S (*FANCS*)*	<1	17q21.31	FANCS/BRCA1	24
T (*FANCT*)	<1	1q32.1	FANCT/UBE2T	7
U (*FANCU*)	<1	7q36.1	FANCU/XRCC2	3
V (*FANCV*)	<1	1p36.22	FANCV/REV7	10
W (*FANCW*)	<1	16q23.1	FANCW/RFWD3	18
X-linked recessive				
B (*FANCB*)	<1	Xp22.2	FANCB	17
AD				
R (FANCR)*	<1	15q15.1	FANCR/RAD51	13

The genomics of inherited bone marrow failure: from mechanism to the clinic



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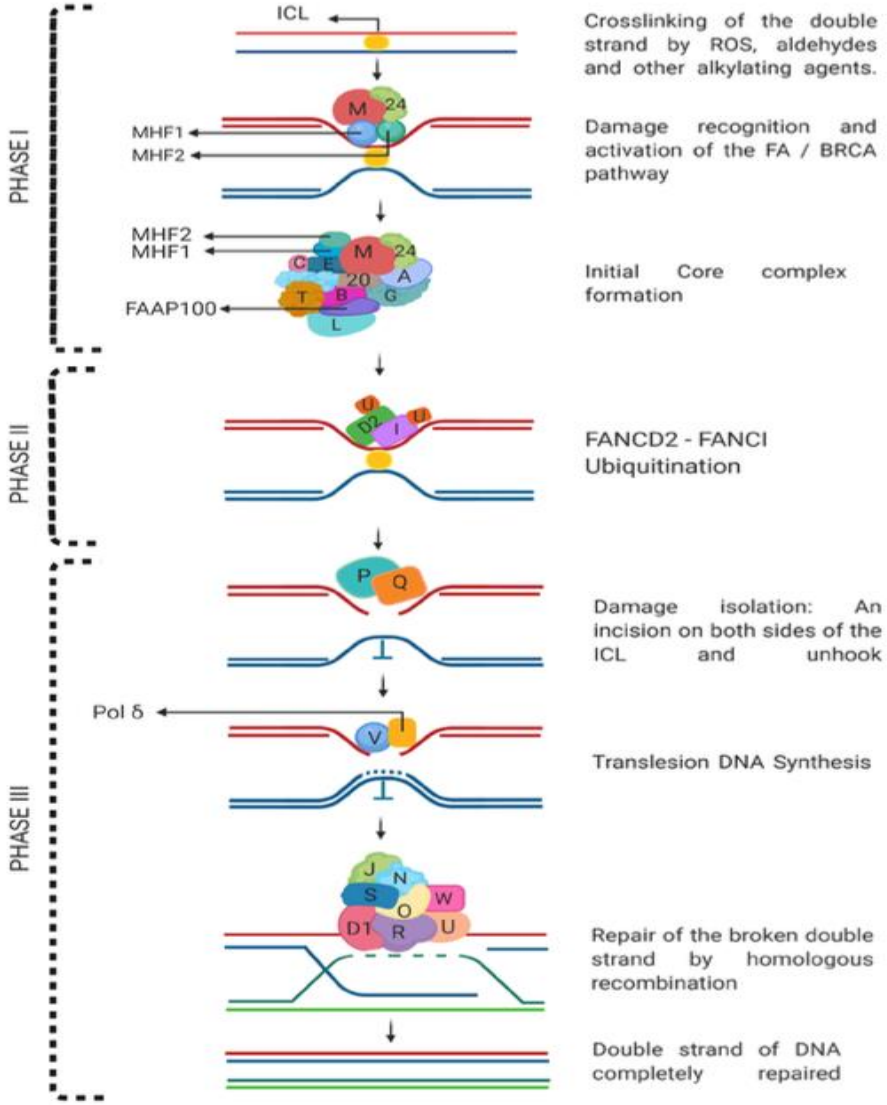
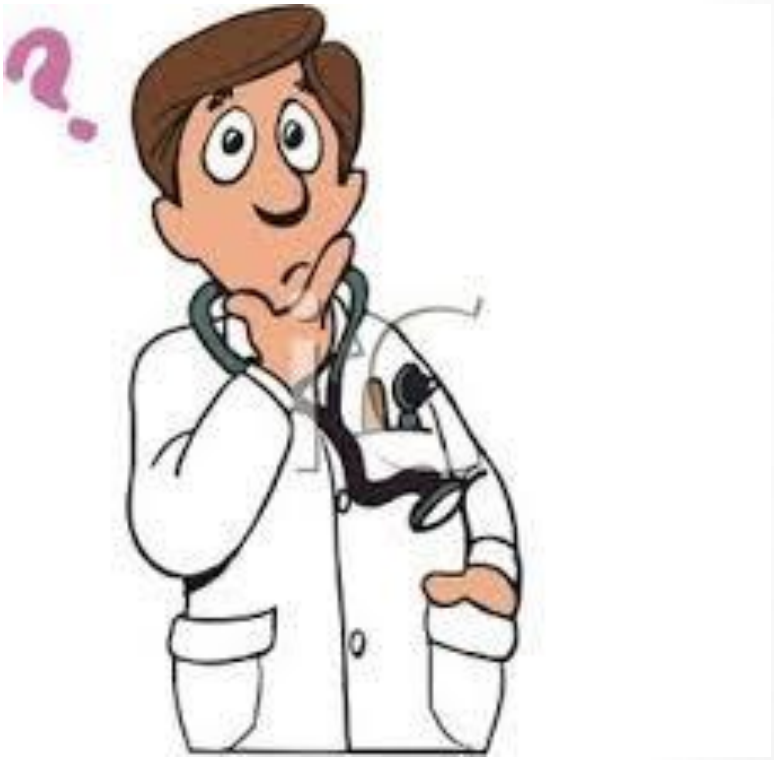


Moreno OM, et al.
Biomedical Reports, 2021

Diagnosis

- Physical examination
- Personal /Family history
- Laboratory evaluation

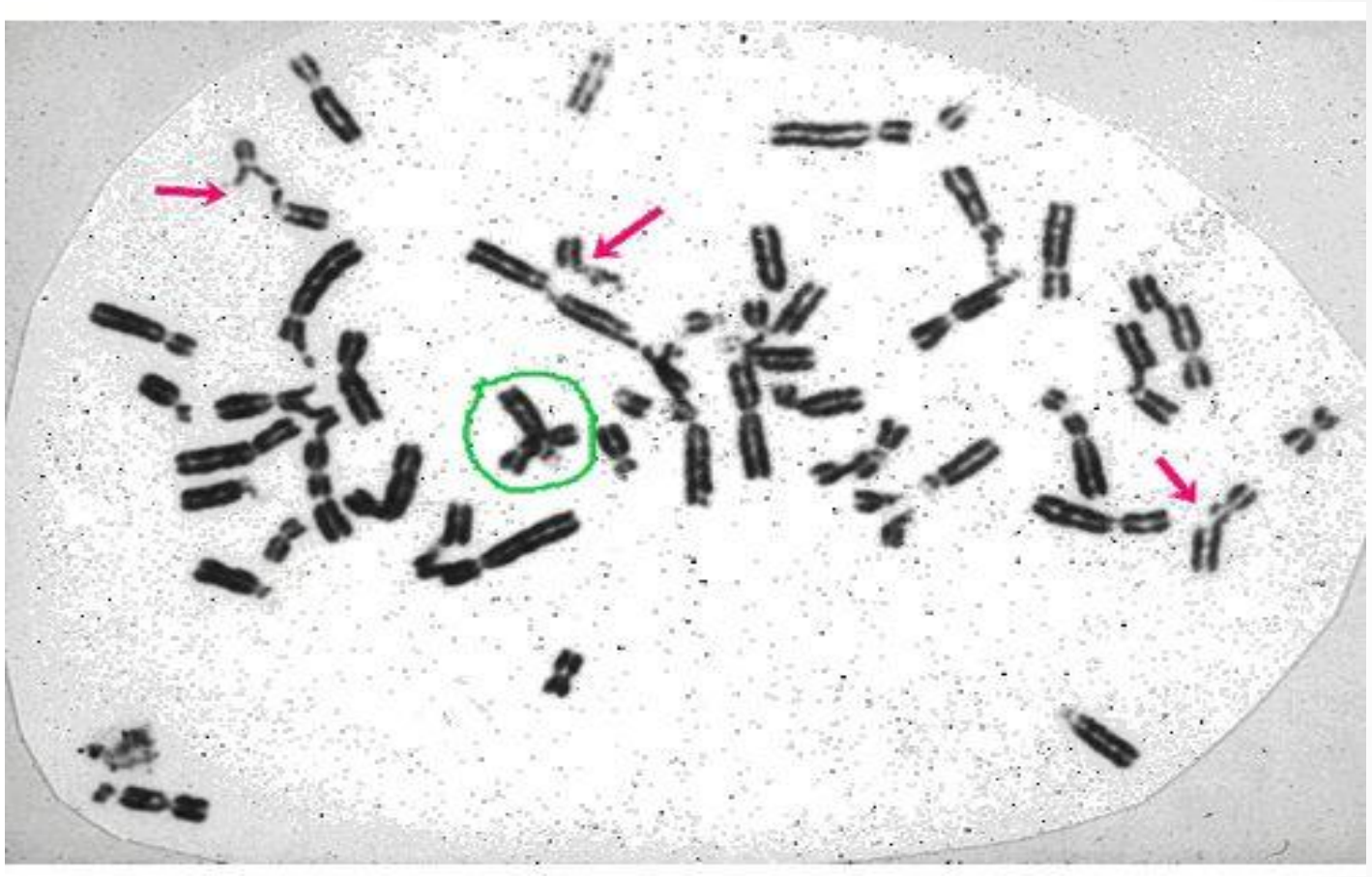


Laboratory evaluation

- **Peripheral blood**:
  - ✓ ↓PLTs, ↓WBC, AA
  - ✓ ↑Hb F, ↑a-feto, ↑i
- **BM aspirate/biopsy**
  - ✓ Decreased cellularity
- **Karyotypic analysis**

Screening tests:

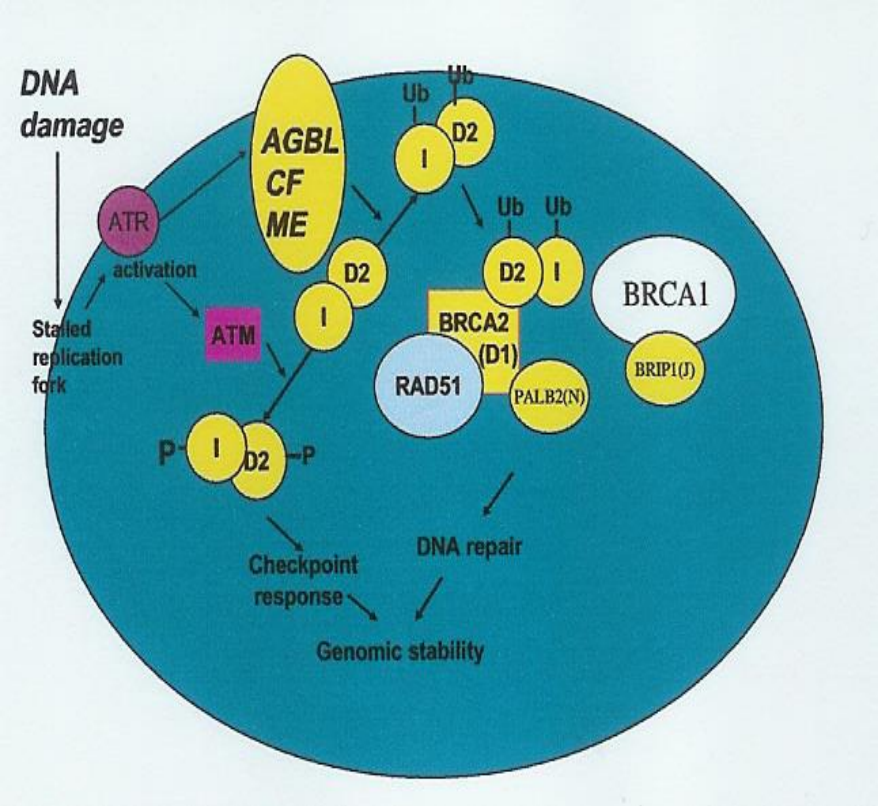
- ✓ Fragility test (DEB, MMC)(stress test) – elevated percentage of chromosomal breaks
- ✓ Flow cytometry: prolonged *G2 phase (lymphocytes)*



FA cells were treated with mitomycin C and harvested in metaphase. Typical abnormalities include radial formation (green circle) and chromosome breaks (red arrows).

Definitive diagnosis

Molecular analysis



Genotype – phenotype correlation

- ***FANC – A***:
  - Late manifestation of BMF
- ***FANC - C and G*** :
  - Severe disease
- ***FANC - D1(BRCA2)*:**

  Early manifestation of:
  - MDS/AML
  - Wilm's tumor
  - Medulloblastoma
- ***FANC-O/FANC-S:***
  - NO BMF (FA-like syndrome)

FA-D1 (BRCA2)
FA-N
FA-J
FA- G
FA- R
↓

Gynecological cancer

Disease progression

- **Marrow failure:** usually occurs in the first decade of life
  - ✓ 50% of patients with thrombocytopenia → pancytopenia (within 3-4 years)
- **Pancytopenia:** 84% of at age 20
- **MDS** (6%), **AML (**600 ×)
- **Head and neck cancer (**500 ×)

Cantu C et al, *J Hematopathol*, 2015

Disease progression

Average life expectancy: 29yrs

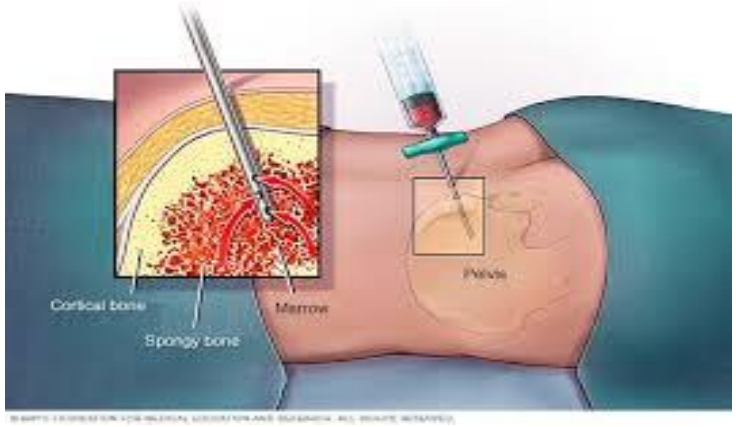
Causes of death:

- ✓ Marrow failure (CR 50%)
- ✓ AML / MDS (CR 25%)
- ✓ Solid tumors (epithelial tumors of the head, neck, gynecological cancers (HPV), cancer of the esophagus, liver, skin, etc. (CR10%)

25% of patients the solid tumor precedes the diagnosis of FA

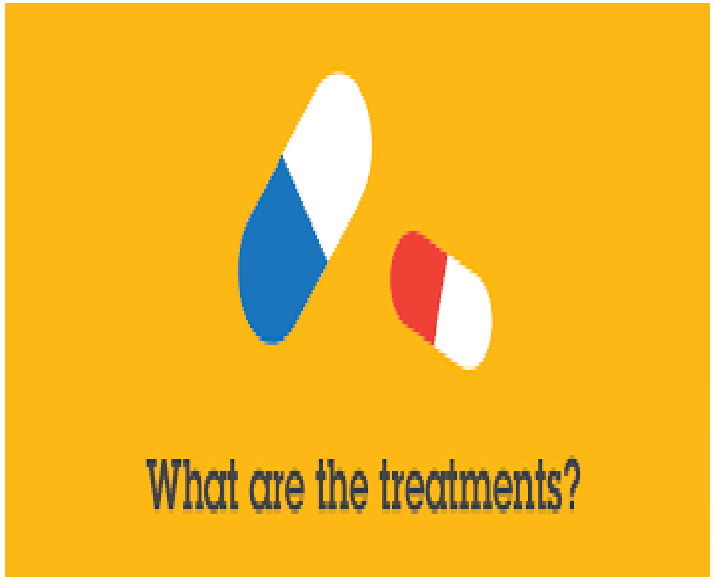
Follow up

- FBC /3 months
- BM aspirate-biopsy/yr
- BM cytogenetic analysis/yr
- ENT/ yr (>10 yrs)
- Gynecological examination / yr from the initiation of menarche



Treatment

- Steroids
- Androgens
- HSCT
- Gene therapy



HSCT

- Definitive treatment of bone marrow failure
- Two-year survival:
  - ✓Relative compatible donor: 70%
  - ✓Unrelated compatible donor: 20-40%
- Modified protocol
  - ✓Low dose of cyclophosphamide and radiation
  - ✓ Use of fludarabine
  - ✓Two-year survival 65-90%
- ↑ risk of cancer
- Treats bone marrow failure **ONLY**.
- It does **NOT** reduce the risk of other cancers

Gene Therapy

- Objective: To provide a proliferative advantage of the modified cells over the rest of the marrow subcellular population Encouraging results
- ***Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia.***

  - *Rio P et al. Nat Med. 2019 ;25:1396-1401*

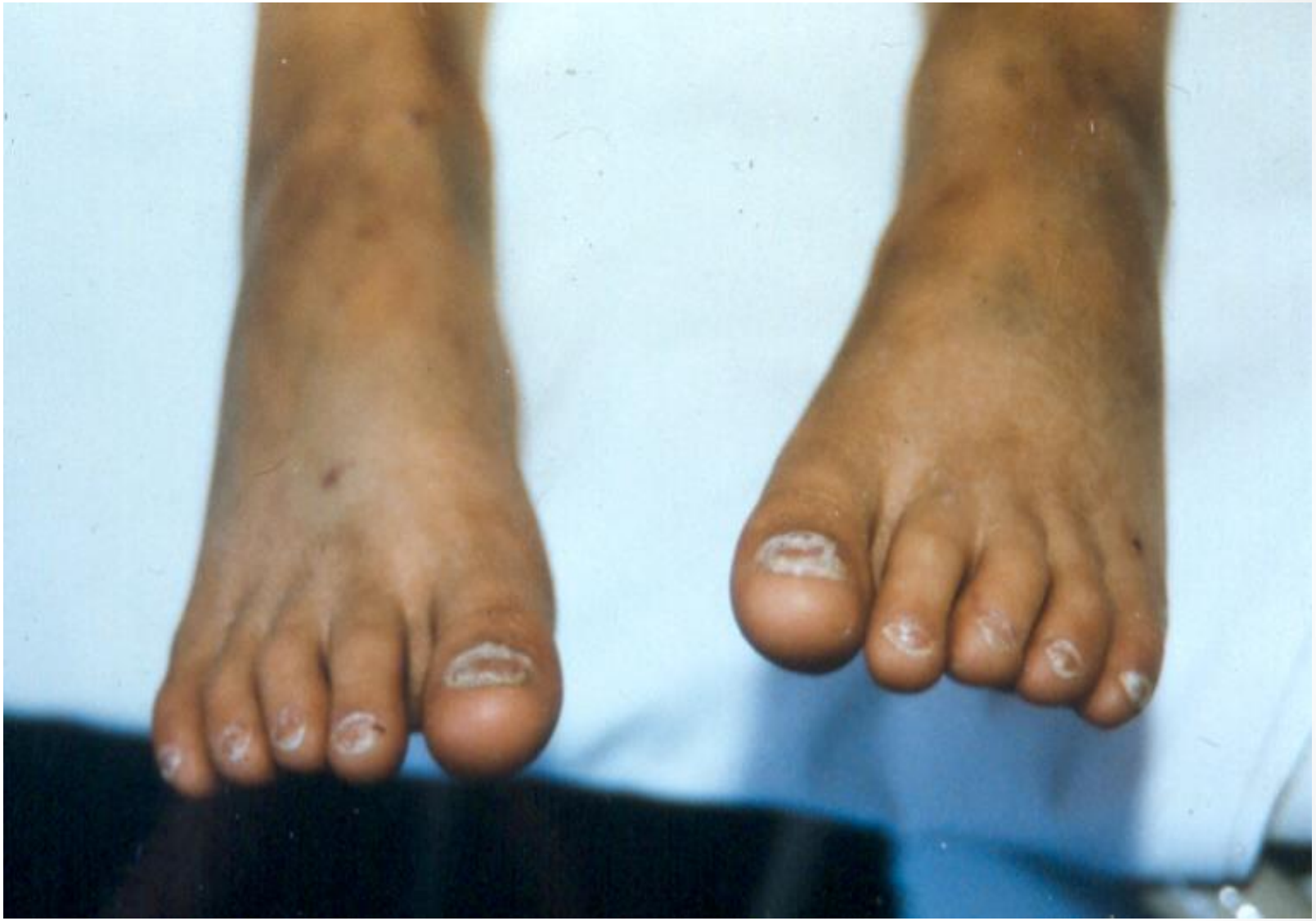
Safety issues ???

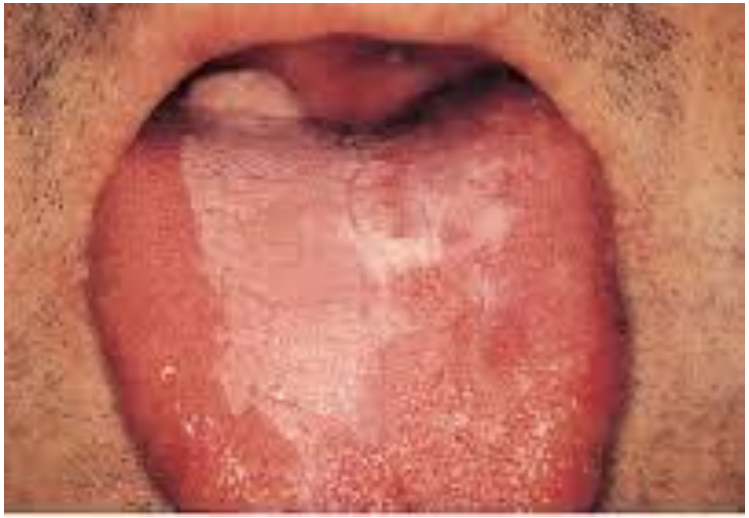
Dyskeratosis Congenita

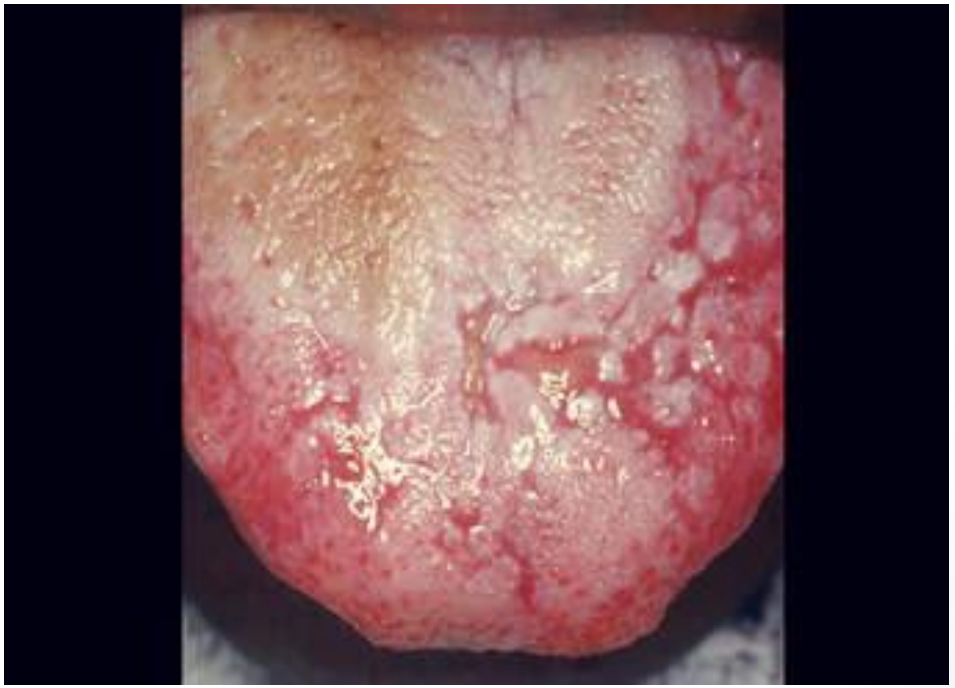
- Characteristic triad:
  - ***Skin pigmentation***
  - ***Onychodystrophy***
  - ***Oral leukoplakia***
- 1910-1930: first dermatological reports (75% of patients with DC)
- 1960: report of hematological manifestations along with skin anomalies
- Incidence: $1/10^6$ people
- M/F 3.2/1

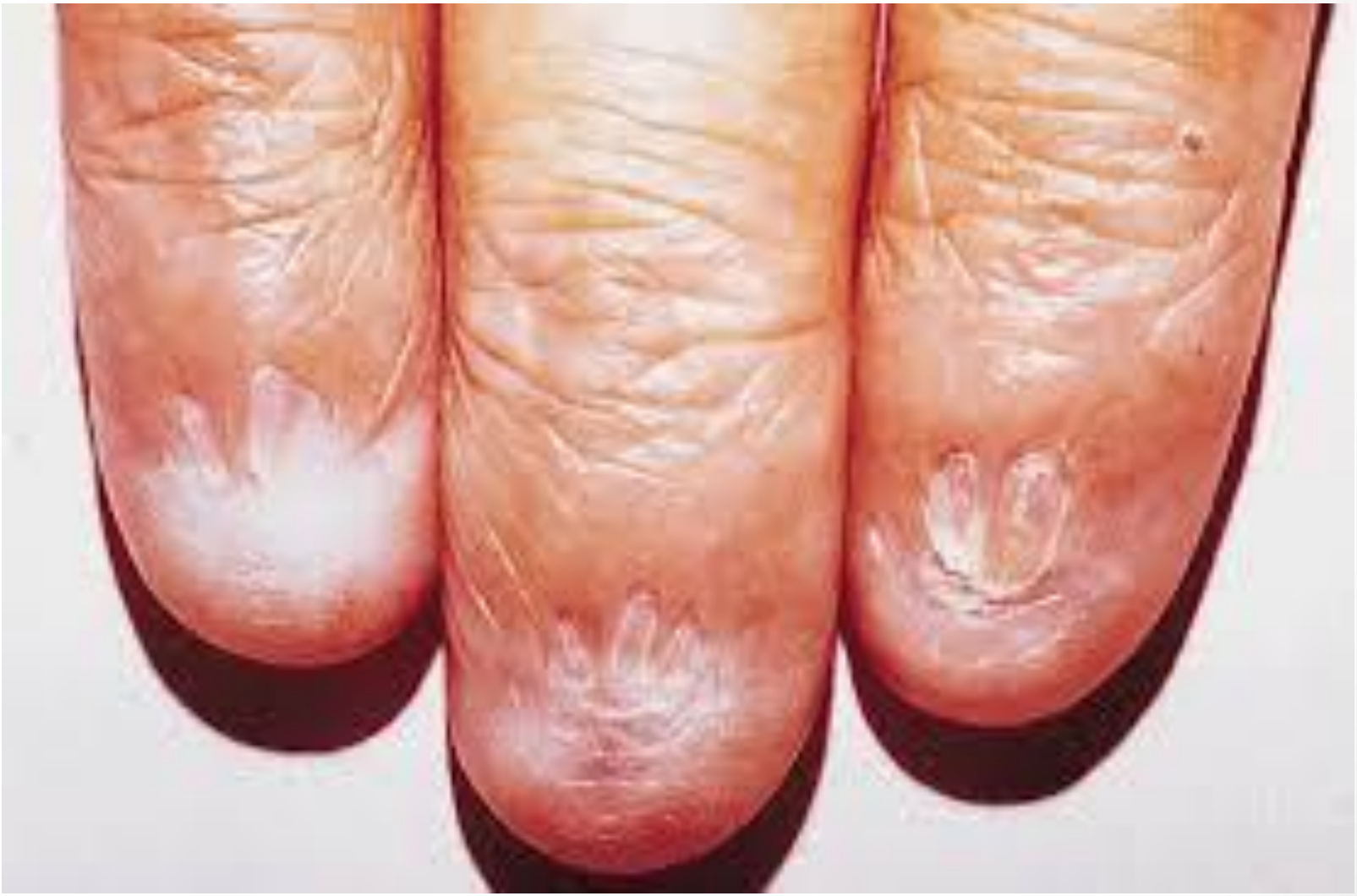
Additional clinical manifestations

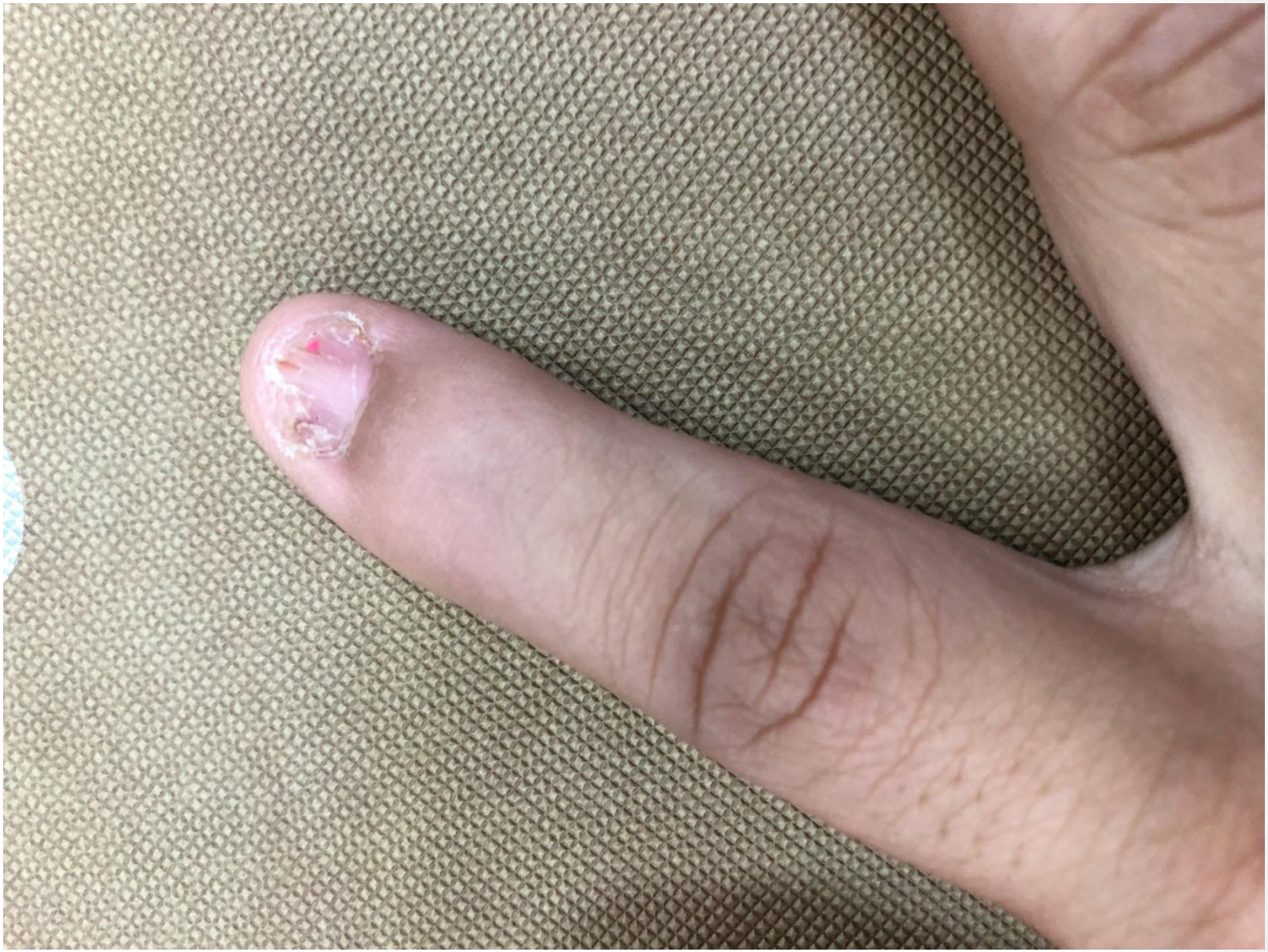
- Atresia of lacrimal ducts (30%)
- Learning disabilities, developmental / mental retardation (25.4%)
- Pulmonary disease (20.3%)
- Short stature (19.5%)
- Severe dental caries (16.9%)
- Esophageal stenosis (16.9%)
- Premature graying of hair (16.1%)
- Hyperhidrosis (15.3%)
- Malignancy (9.8%)
- Liver disease, enteropathy (7.3%)

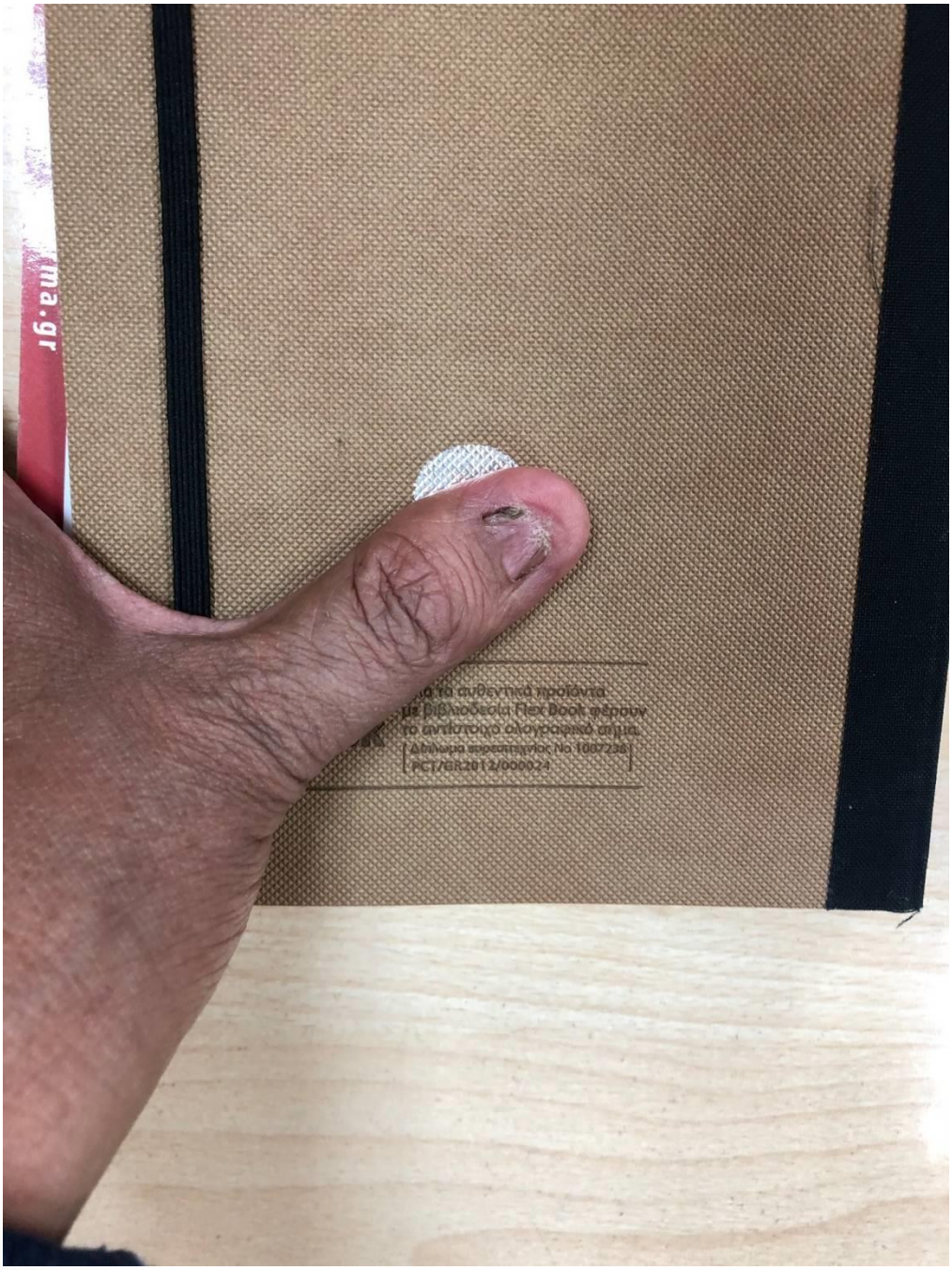








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Inheritance

Inheritance

- X-linked recessive trait (Xq28)
  - A characteristic triad of clinical manifestations
- Autosomal dominant trait
- Autosomal recessive trait
- Sporadic mutations

Progression of the disease

- First decade: nail-skin disorders
- Second decade: bone marrow failure (90% of patients by age 30)
- Rarely, bone marrow failure precedes skin manifestations
- They do not have an increased risk of AA during adolescence but the risk increases with age (CR AA 50% at age 50)

Prognosis

- Average life expectancy: 49 years
- Cause of death:
  - Marrow failure (70% - third decade)
  - Development of malignancies (10 - 15%)
  - Pulmonary disease (10%)

Pathophysiology

Short telomeres

↓

Activation of p53

↓

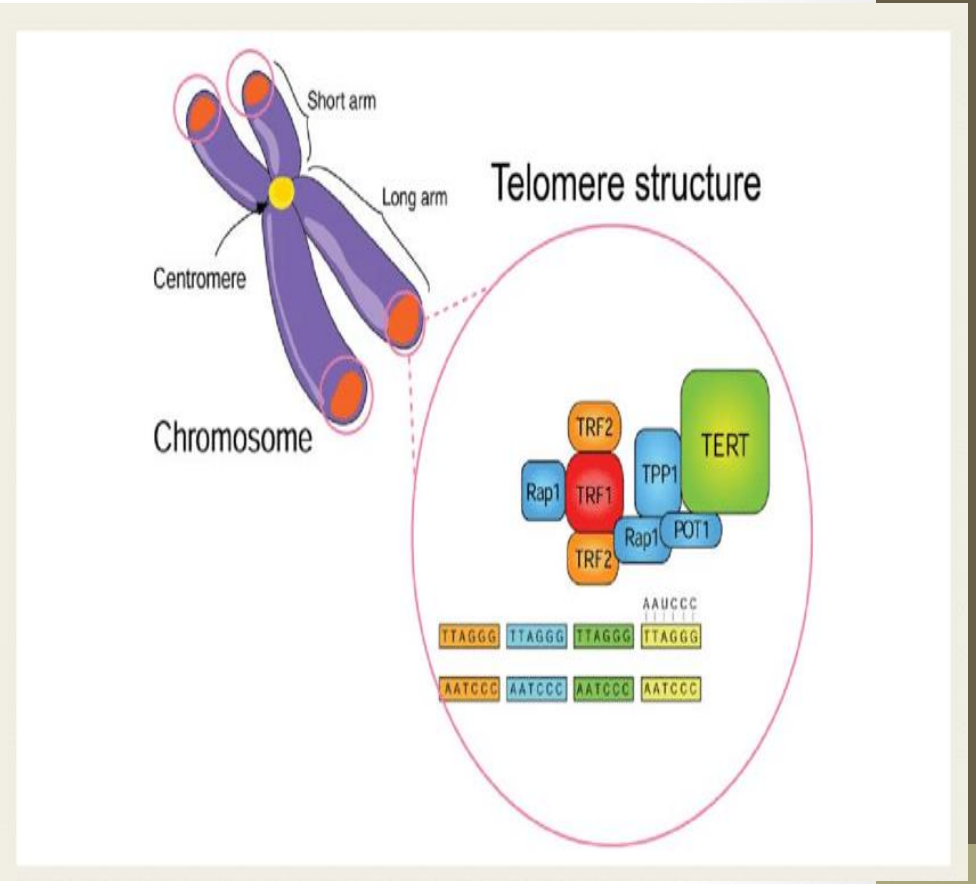
Early apoptosis

New somatic mutation → malignancy

Reduced number of hematopoietic progenitor cells even in cases where peripheral blood measurements are within normal limits

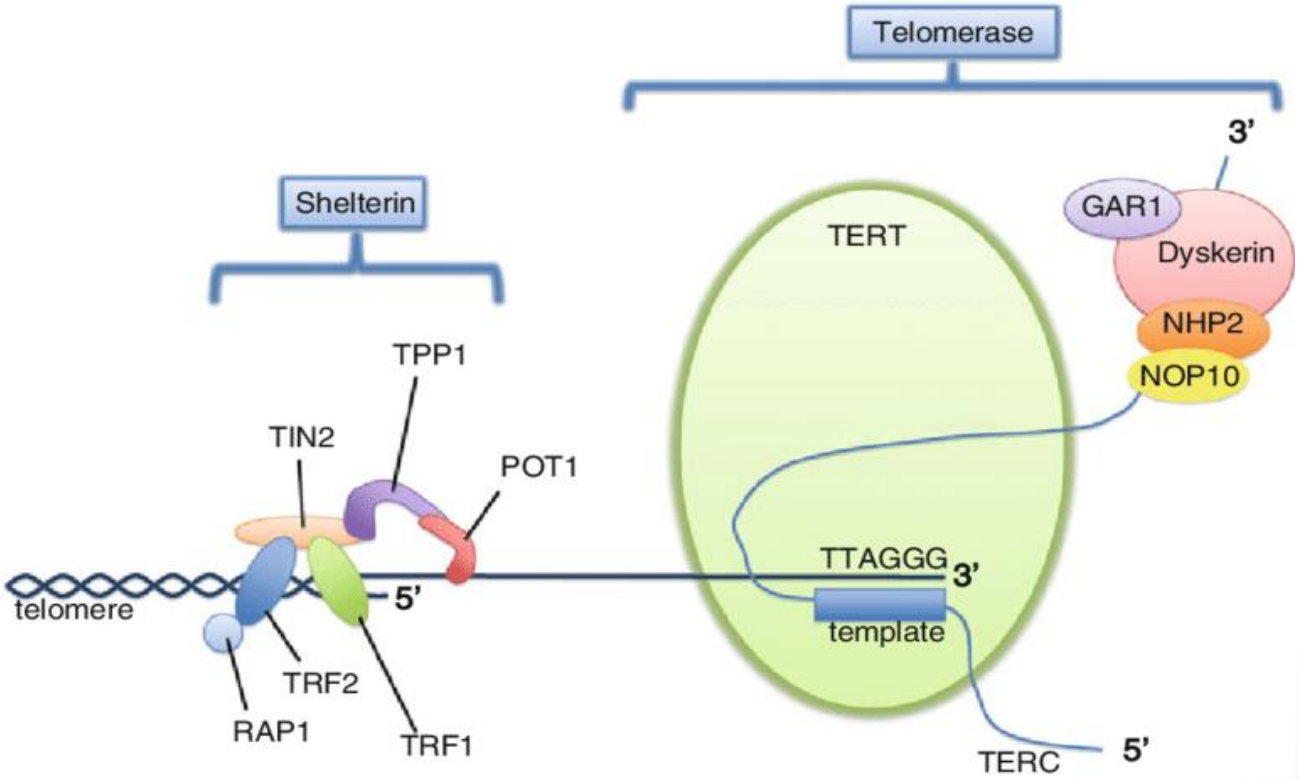
Telomeres

- Characteristic amino acid repeats (TTAGGG) at chromosome ends
- They protect the integrity of chromosomes during the replication phase
- Telomere length is kept unaffected by the action of telomerase

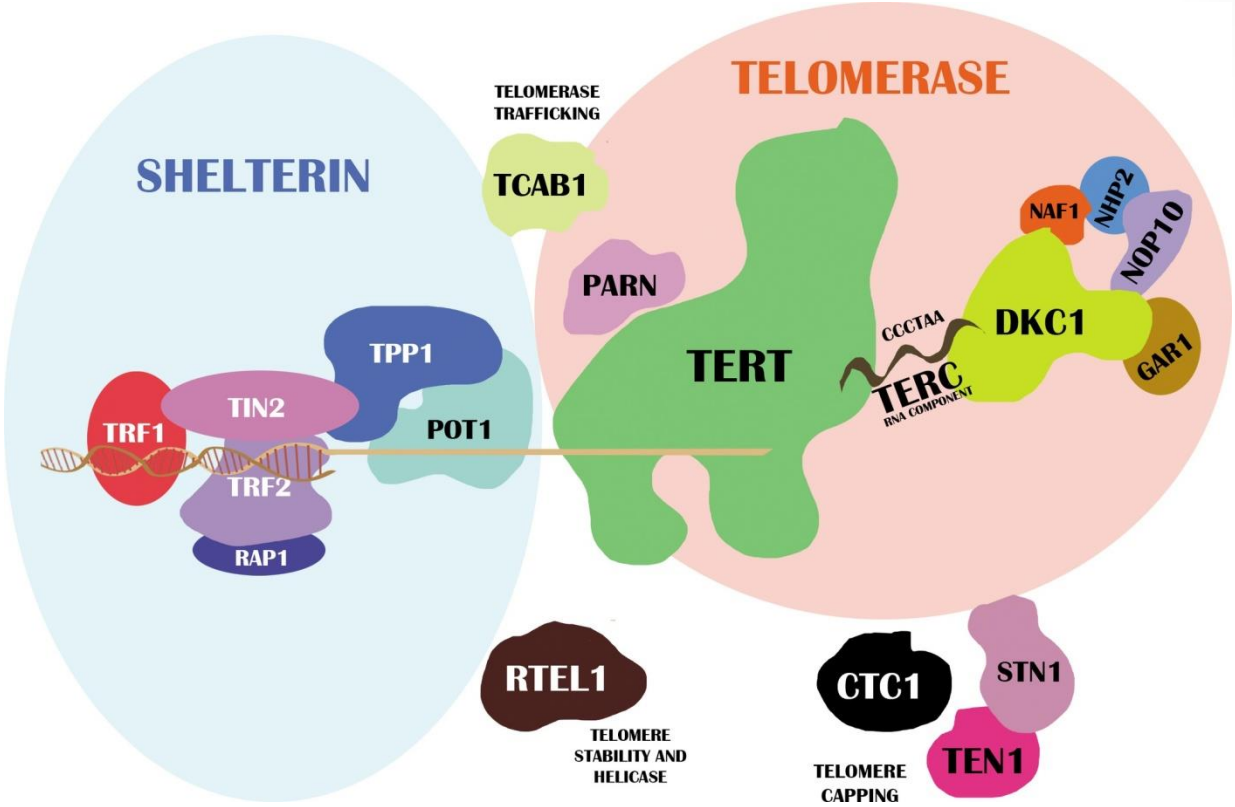


Telomerase

- Enzyme complex of ribonucleoproteins, responsible for maintaining the length of telomeres.
- ↑ expression in tissues with intense regenerative activity
- It consists of 5 components: TERC, TERT, Dyskerin, NOP10, NHP2



The genomics of inherited bone marrow failure: from mechanism to the clinic



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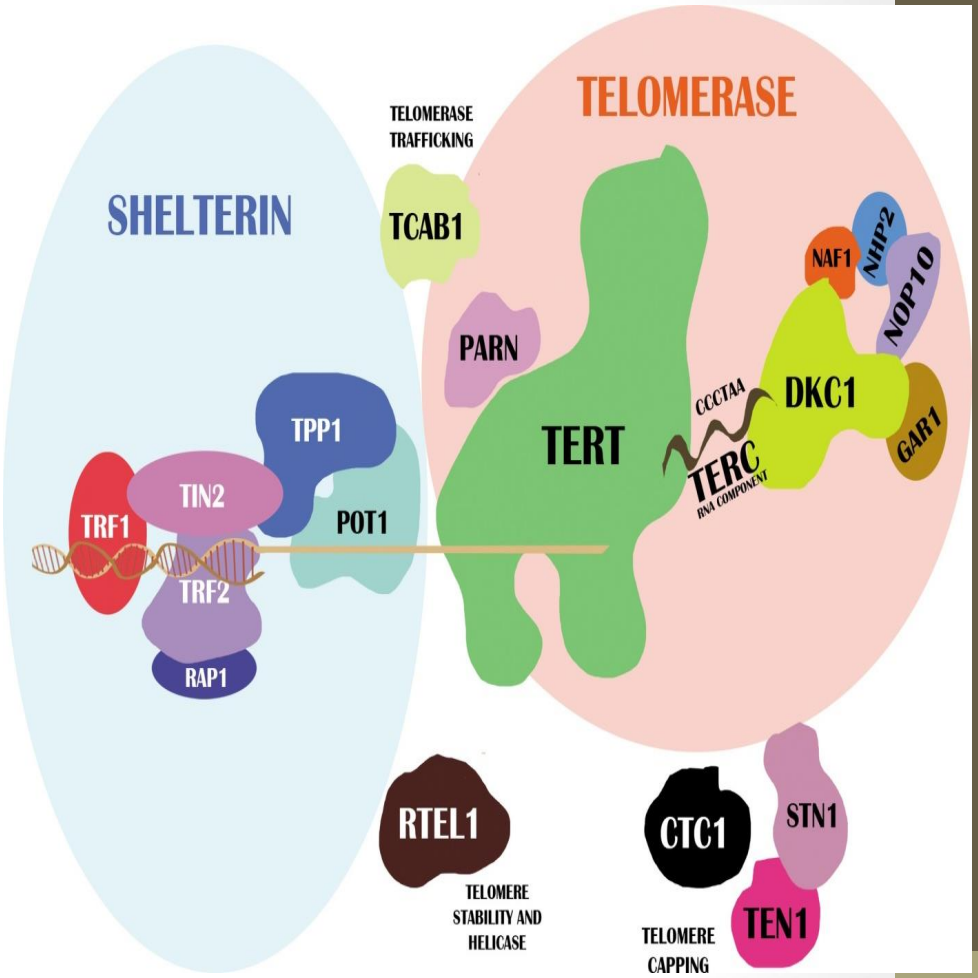
Inheritance

- 16 genes
- Types of inheritance
  - ✓ X-linked DKC1
  - ✓ Autosomal dominant TERC, TINF2
  - ✓ Autosomal recessive CTC1, NHP2, NOP10, PARN, WRAP53
    Autosomal recessive or dominant ACD, RTEL1, and TERT
  - ✓ De novo mutations
- Severe clinical picture: ***DKC1, TINF2, RTEL1***
- Delayed diagnosis: ***TERC, TERT*** (mucosal manifestations may be absent)

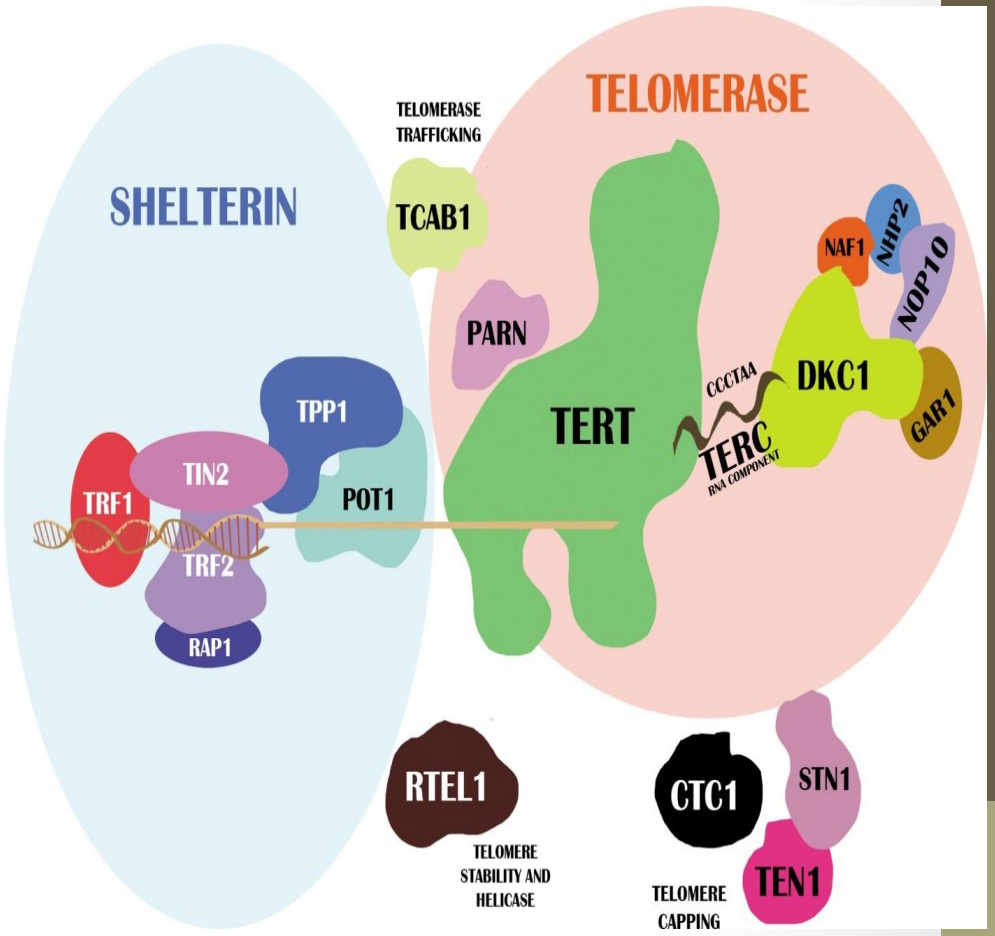
X-linked recessive

- The most severe form with early clinical manifestations
- *DKC1* gene (Xq28)→Dyskerin
- It is expressed in all tissues and along with snoRNAs: uracil→pseudouracil conversion of rRNA
- Process important for the synthesis of ribosomes
- It interacts with the RNA component of telomerase (TERC)



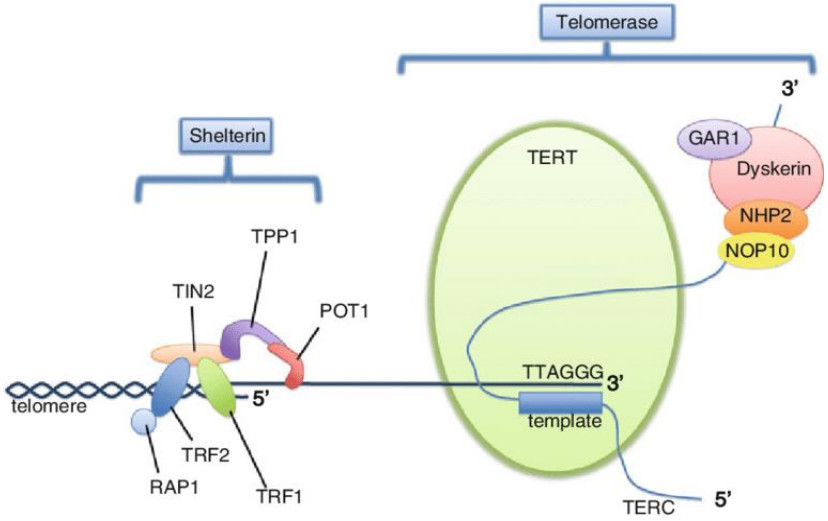
Autosomal recessive inheritance

- Homozygous mutation in ***TERT*** (S.Hoyeraal-Hreidarsson)
  - growth disorders
  - neurological disorders
  - bone marrow failure
  - Immunodeficiency



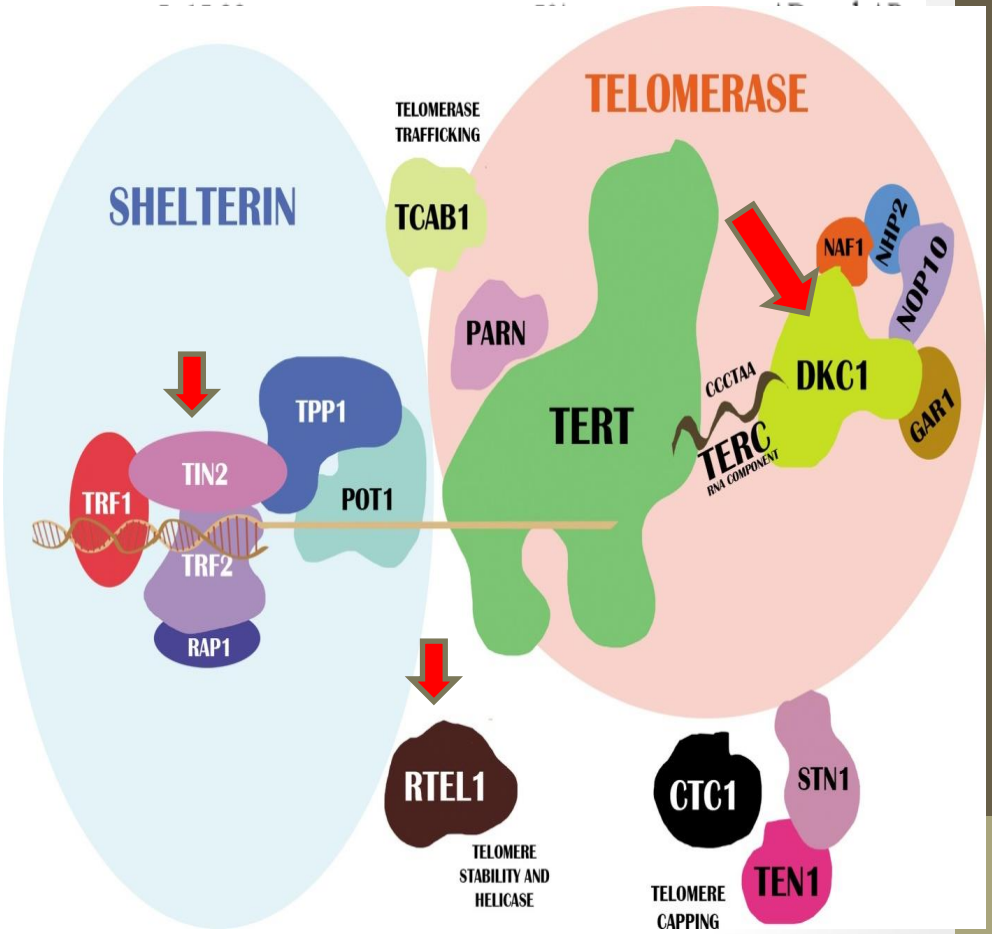
Sporadic cases- Shelterin

- Complex of 6 proteins
- Protection of telomeres from unnecessary action of the DNA-repair mechanism
- A mutation in a protein of the Shelterin complex (***TINF2***) has been found in DC patients.



- This particular mutation leads to the creation of extremely short telomeres and is accompanied by a severe clinical picture:
- **Hoyeraal-Hreidarsson s**
  - ✓ Growth disorders
  - ✓ Neurological disorders bone
  - ✓ Bone marrow failure
  - ✓ Immunodeficiency
- **Revesz. S**
  - ✓ Retinopathy (exudations)
  - ✓ IUGR
  - ✓ Bone marrow failure
  - ✓ CNS calcifications

Dyskeratosis congzenita	*DKC1*	Xq28	20-25%	XLR
	TINF2	1q11.2	11–20%	AD
	TERT			
	TERC			
	RTEL1			
	ACD			
	CTC1			
	NOP10			
	NHP2			
	PARN			
	WRAP53			
	*STN1**			
	NAF1†			



Diagnosis – clinical presentation

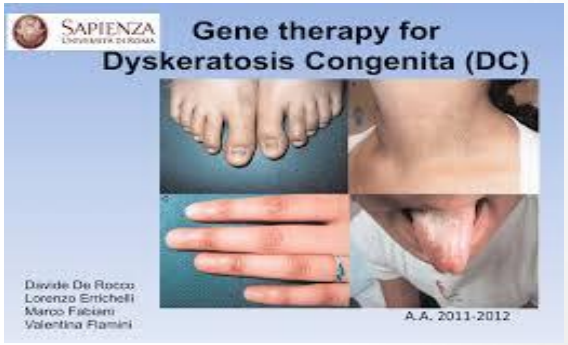
- **Classic presentation**:
  - ✓ Triad +/- hematological manifestations
  - ✓ 1 stigma from the triad + two of the remaining morphological stigmata + hypocellular BM
- **'Silent carriers'**: people with no stigmata or hematological abnormalities who carry the same mutation as a patient in the family – need follow-up
- **Adults with "AA"** with mutations of the *TERC, TERT* genes
- Patients with a **family history of pulmonary fibrosis** *(TERT)*

Diagnostic tests

- Determination of telomere length (FISH-flow cytometry) in peripheral blood lymphocytes (screening test)
- BM aspiration /biopsy/ bone marrow cytogenetic test: cannot distinguish DC from other BFMs or MDS (not a specific diagnostic test but necessary)
- Definite diagnosis: disease-related genes

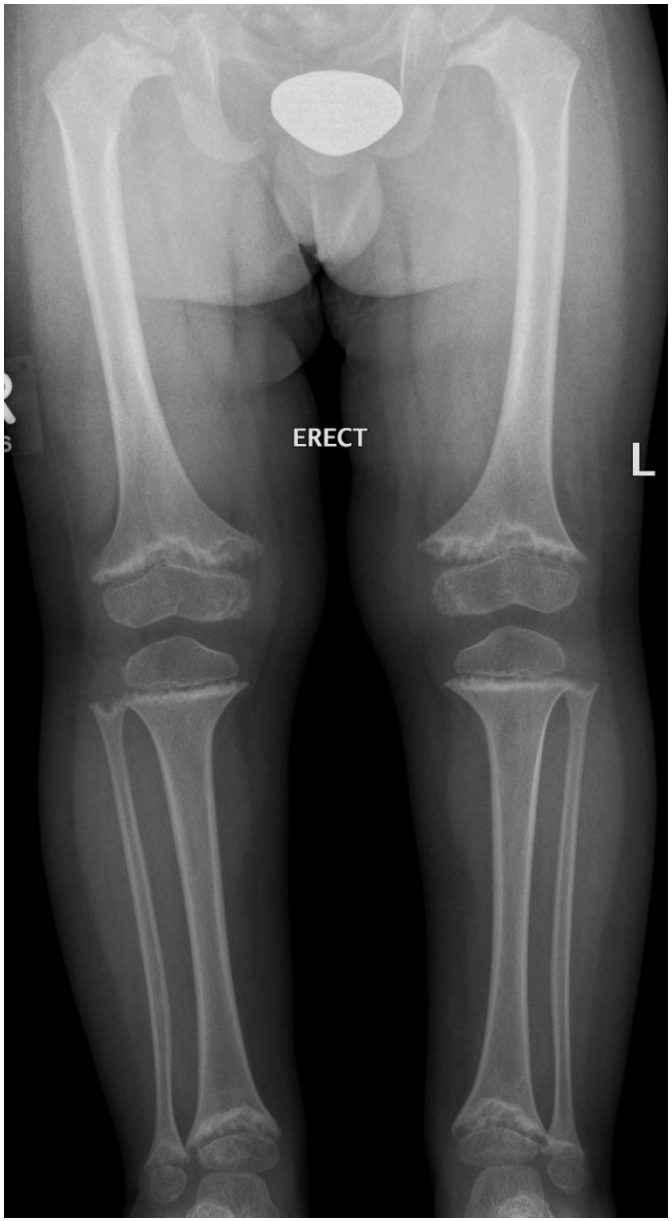
Treatment-follow up

- **Oxymetholone** (0.5-1.0 mg/kg/day)
- **EPO, G-CSF** (without oxymetholone – splenic rupture)
  - FBC, imaging and biochemical evaluation of liver function every 3 months
  - Annual check-up with BM aspirate/biopsy/cytogenetics
  - Screening for epithelial cancers
- **Allogeneic HSCT**
  - serious respiratory complications, avoid use of busulphan and radiation (annual check-up of pulmonary functions)
  - Ideal candidate: no pulmonary involvement, available compatible relative donor
- Gene therapy ???



Shwachman – Diamond s.

- 1964 : Children with malabsorption and neutropenia
- Autosomal recessive inheritance
- M/F 1.48:1
- Incidence: 1/350000 births
- It is characterized by:
  - ***Pancreatic exocrine insufficiency***
  - ***Neutropenia***
  - ***Bone marrow failure*** (20% pancytopenia), MDS, AML (25%, M:F=3:1)
  - ***Physical abnormalities***
    - short stature (50%)
    - metaphyseal chondrodysplasia (25%)


ERECT
L

Clinical presentation

- Delayed growth from infancy
- Short stature
- Hepatomegaly
- Abnormalities of the ribs
- Syndactyly
- Abnormalities of the palate
- Tooth dysplasia
- Neurodevelopmental disorders

Diagnosis

- Neutropenia: persistent stable in 1/3, intermittent 2/3 of patients (+/- other cytopenias, ↑HbF, ↑MCV)
- 20% pancytopenia
- Exocrine pancreatic function
  - Serum trypsinogen ↓(<3 years)
  - Serum Isoamylase ↓ (>3 years)
- Fatty infiltration of the pancreas
- Molecular testing

Inheritance - genes

Autosomal recessive

(2003) ***SBDS*** gene (7q11)

>90% of patients carry SBDS mutations

DNAJC21, EFL1

Autosomal dominant

SRP54

All genes are related to ribosomal function

SBDS gene

Production of a protein involved in the maturation of the 60s ribosomal subunit

↓

Increased apoptosis and short telomeres

↓

Ribosome dysfunction

GENE	FREQUENCY
SBDS	**92%**
EFL1	**<1%**
DNAJC21	**<1%**
SRP54	**<1%**
Unknown mutation	**<10%**

Nelson A, *GeneReviews*, 2018

Progression of the disease

- Average life expectancy: 36 yr
- Causes of death
  - ✓ AML / MDS
  - ✓ AA
  - ✓ Infections

Treatment

- Malabsorption → administration of pancreatic enzymes (remission in 50% of patients after 5 years of age)
- Neutropenia → G-CSF
- Treatment of infections
- Oxymetholone
- Allogeneic HSCT (58% 2-year survival)
- Malignancies: Hematological only (MDS/AML)

Diamond – Blackfan anemia

- First described in 1938 (congenital aplasia of the red cell line)
- Ribosomopathy
- Annual incidence: ≈ $5/10^6$ live births
- It manifests during early infancy with characteristic: selective reduction of red cell precursors, orthochromic macrocytic anemia, reduced %rets

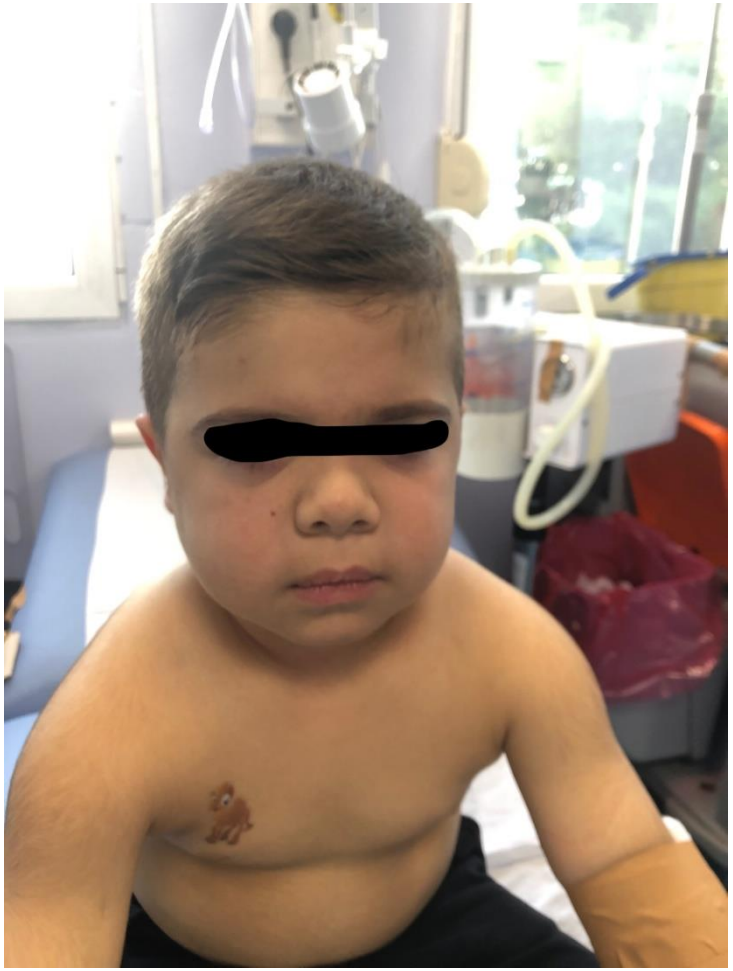
Diamond
Blackfan
Anemia

Clinical picture
(50% congenital anomalies)

- Craniofacial malformations
- Thumb malformations (*RPL5*, *RPL11*)
- Cardiac abnormalities
- Abnormalities of the genitourinary system
- Short stature
- Hydrops fetalis – mild anemia adult life 20% premature birth
- 28 %IUGR

Genotype-phenotype correlation:

- ***RPS19***: fewer stigmata
- ***RPL5***: more stigmata



Diagnostic criteria

- Orthochromic, macrocytic anemia
- ↓ Rets
- Normal bone marrow cellularity with red cell line reduction only (EB<5%)
- WBC: Normal or ↗
- Platelets Normal or ↗
- ↑ eADA activity (75-90% of non-transfused patients)
- ↑Hb F
- ↑ EPO

Pathophysiology

Disorder of ribosomal subunit assembly and function

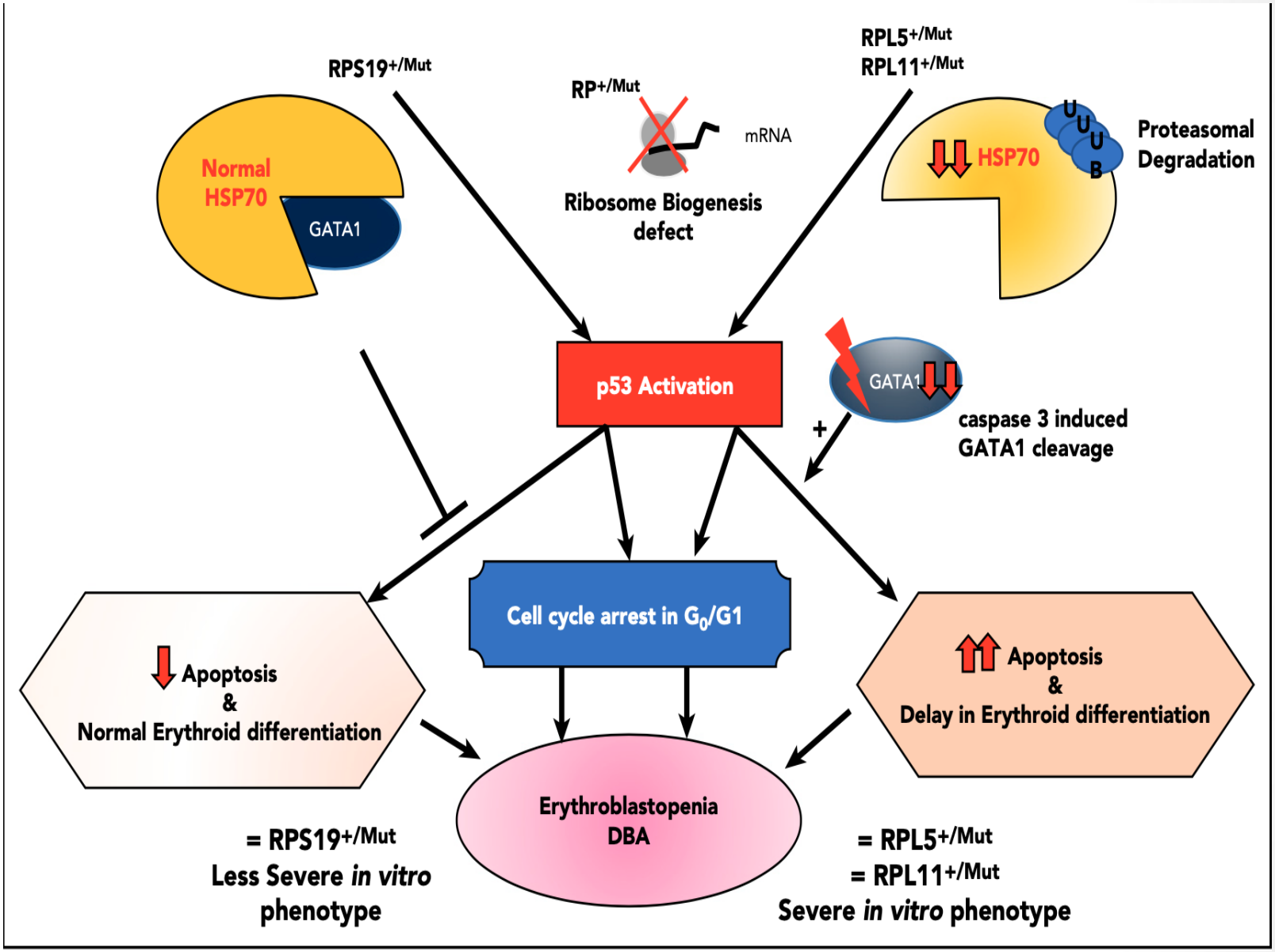
- ✓ **40S** - *RPS19, RPS17, RPS24*
- ✓ **60S** -*RPL35A, RPL5, RPL11*

p53 gene activation and cell destruction

Unbalanced globulin/heme ratio

↓

EARLY APOPTOSIS



Da Costa L, et al. Blood 2020

Inheritance

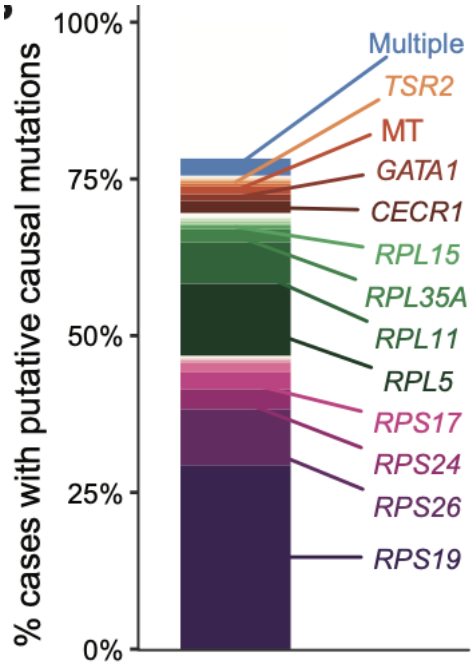
- Autosomal dominant trait (20 gen.)
  - ✓ *RPS19* (DBA 1) - 25% of patients
  - ✓ *RPL5, RPL11, RPS10, RPL35A, RPS26, RPS 24, RPS 17, RPS 7, RPL 19, RPL 26*
  - ✓ 70% of patients carry mutations in **6 RP** genes (*RPS19, PRL5, RPS26, RPL11, RPL35a, RPS24*)
- X-linked Inheritance:
  - ✓ *GATA 1*: Transcriptional hemopoietic factor that regulates the maturation of the red and MGC series. Only hematologic manifestations without morphological stigmata (a few cases)
- De novo mutations
- 25-40% of patients: unknown mutations

Mutated gene	RP	Incidence in DBA population
Genes involved in DBA*		
RPS19	eS19	25%-30%
Large deletions		10%-20%
RPL5	uL18	7%-12%
RPS26	eS26	6.6%-9%
RPL11	uL5	5%-7%
RPL35a	eL33	2%-3%
RPS10	eS10	1%-3%
RPS24	eS24	2.4%-3%
RPS17	eS17	1%-3%
RPL15	eL15	1 case 6 cases
RPS28	eS28	2 families
RPS29	uS14	2 families
RPS7	eS7	1 case
RPS15	uS19	1 case
RPS27a	eS31	1 case
RPS27	eS27	1 case
RPL9	uL6	1 case
RPL18	eL18	1 family
RPL26	uL24	1 case
RPL27	eL27	1 case
RPL31	eL31	1 case
TSR2 (X linked)†		1 family
Genes involved in DBA-like diseases		
GATA1 (X linked)‡		5 families
EPO		1 case
ADA2§		9 individuals

Da Costa L, et al. Blood 2020

Genetics

- 19q13.2 →***RPS19*** (1999)
- 25% of cases in the West
- Encodes the RPS19 protein (145 aa) component of the 40s ribosomal subunit
- Heterozygotes for this protein can be:
  - Patients
  - Relatives of patients without clinical manifestations who show isolated ↑e ADA



Ulirsch JC, et al. The American J of Human Genetics, 2018

Complications

- Cumulative risk of developing malignancies: 20%
  - Development of MDS, AML
  - Development of non-haematological malignancies (osteosarcoma)
- Aplastic anemia (Studies in cell cultures showed damage to all three cell lines and not just the erythrocyte)

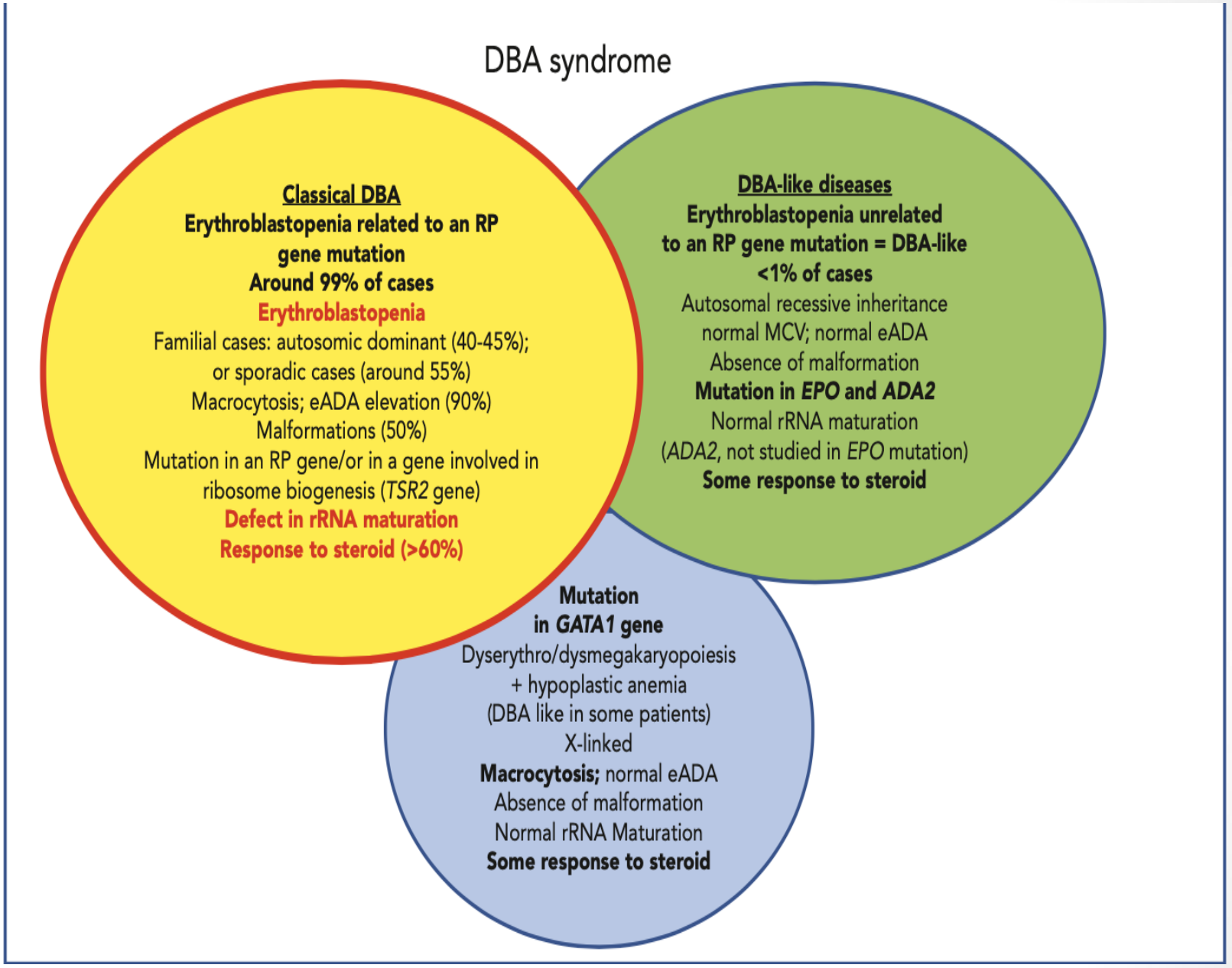
Diagnosis

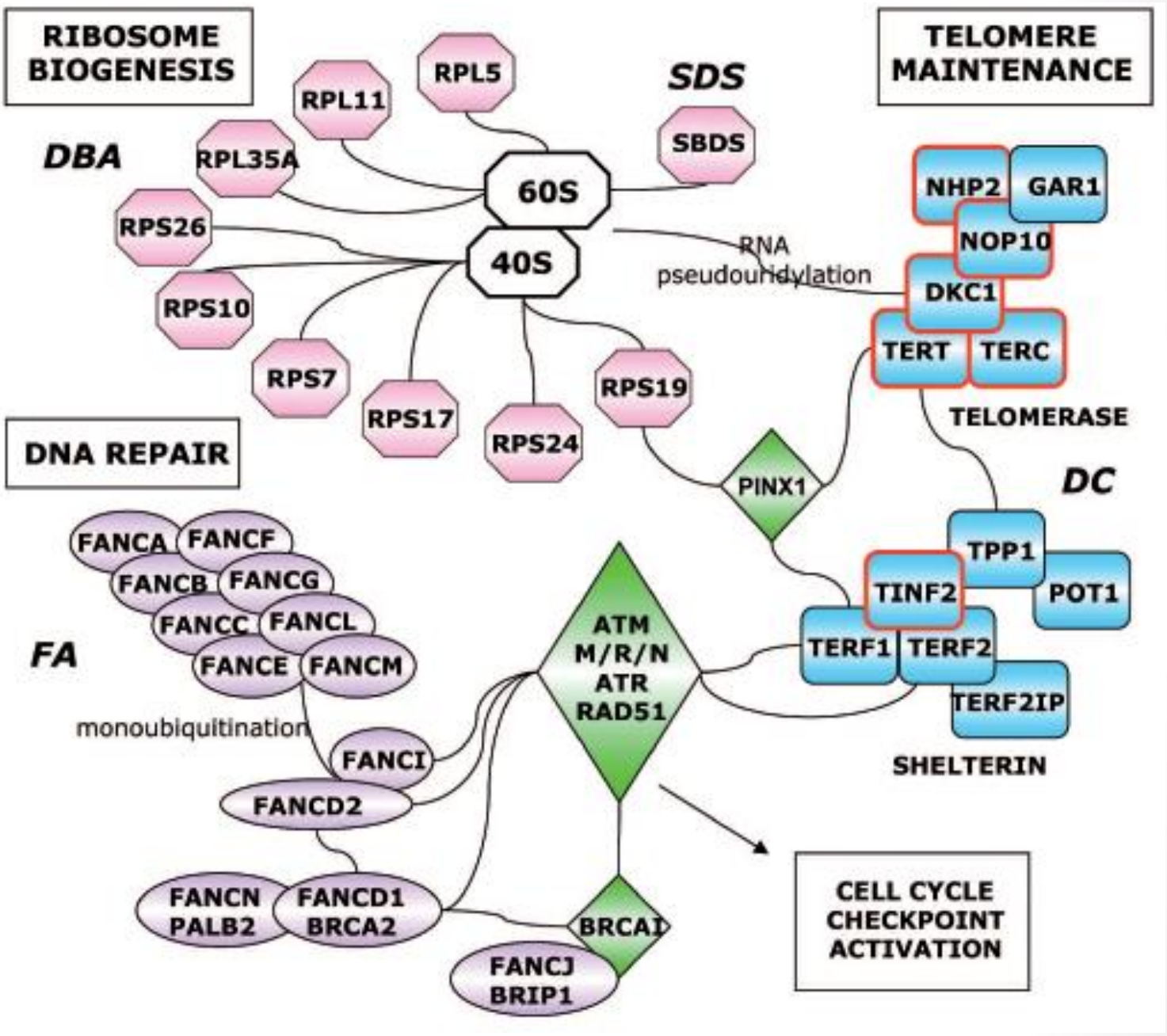
- Definitive diagnosis 50-70% of patients (known mutations)
- For the rest, the diagnosis is based on:
  - ✓ clinical picture
  - ✓ hematologic findings
- Family screening for **'silent carriers'**
  - ✓ FBC ↑ MCV
  - ✓ ↑ HbF
  - ✓ ↑ eADA
- Genetic analysis

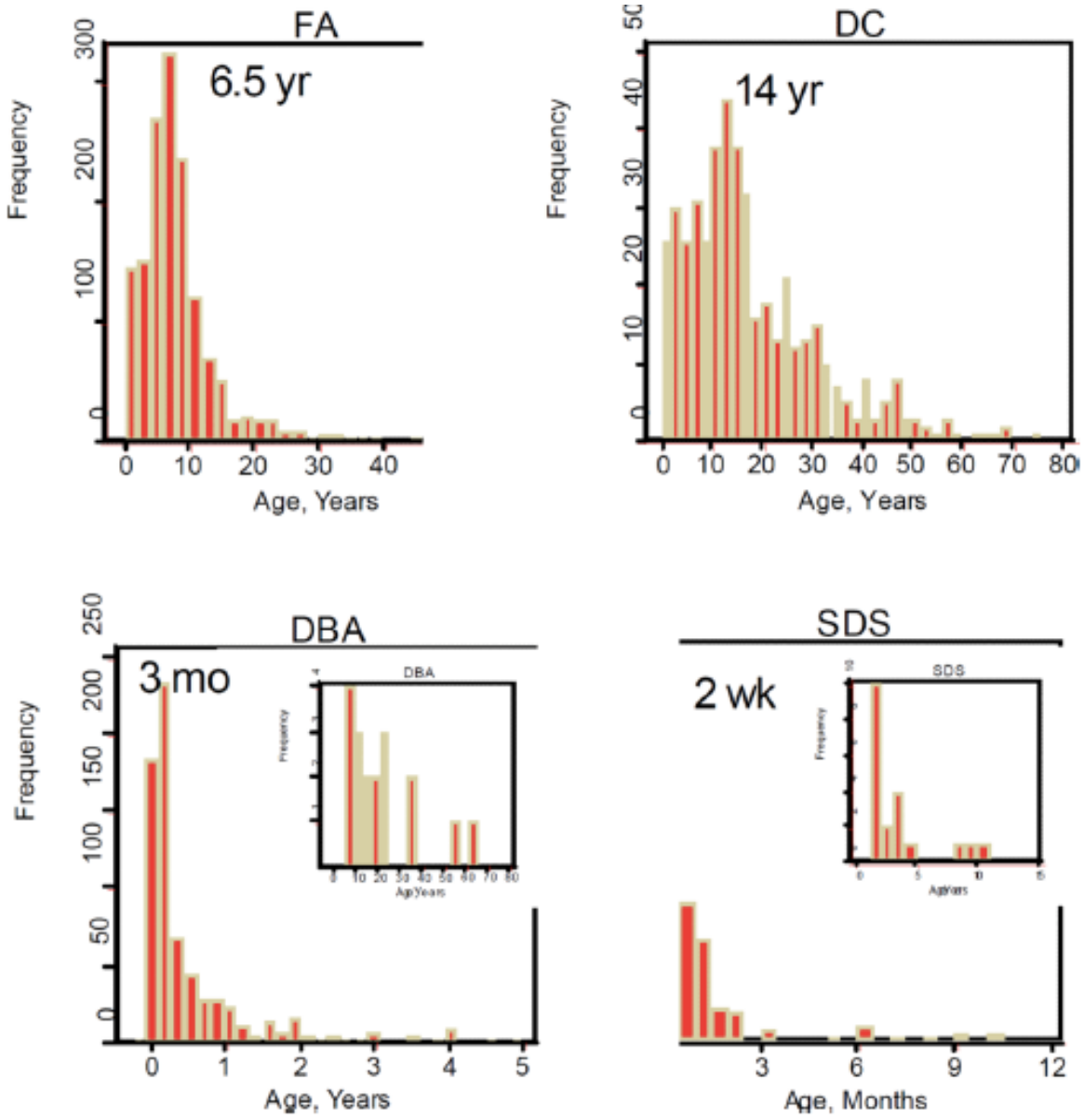
Treatment

- Corticosteroids (35% resistance)
- Transfusion program for corticosteroid-resistant patients
- Allogeneic HSCT
  - ✓ 5 years - survival: 70%
  - ✓ Best donor: compatible sibling
- 10-25% of patients: spontaneous remission
- Gene therapy ???

Depnath S, et al. Mol Ther 2017


DBA syndrome
Classical DBA
Erythroblastopenia related to an RP gene mutation
Around 99% of cases
Erythroblastopenia
Familial cases: autosomic dominant (40-45%); or sporadic cases (around 55%)
Macrocytosis; eADA elevation (90%)
Malformations (50%)
Mutation in an RP gene/or in a gene involved in ribosome biogenesis (TSR2 gene)
Defect in rRNA maturation
Response to steroid (>60%)
DBA-like diseases
Erythroblastopenia unrelated to an RP gene mutation = DBA-like
<1% of cases
Autosomal recessive inheritance
normal MCV; normal eADA
Absence of malformation
Mutation in EPO and ADA2
Normal rRNA maturation
(ADA2, not studied in EPO mutation)
Some response to steroid
Mutation in GATA1 gene
Dyserythro/dysmegakaryopoiesis + hypoplastic anemia
(DBA like in some patients)
X-linked
Macrocytosis; normal eADA
Absence of malformation
Normal rRNA Maturation
Some response to steroid


RIBOSOME BIOGENESIS
TELOMERE MAINTENANCE
SDS
DBA
RPL5
RPL11
RPL35A
SBDS
60S
40S
RPS26
RPS10
RPS7
RPS17
RPS24
RPS19
RNA pseudouridylation
NHP2
GAR1
NOP10
DKC1
TERT
TERC
TELOMERASE
DC
PINX1
DNA REPAIR
FANCA
FANCF
FANCB
FANCG
FANCC
FANCL
FANCE
FANCM
FA
monoubiquitination
FANCI
FANCD2
ATM
M/R/N
ATR
RAD51
TPP1
TINF2
POT1
TERF1
TERF2
TERF2IP
SHELTERIN
FANCN
PALB2
FANCD1
BRCA2
BRCA1
FANCJ
BRIP1
CELL CYCLE
CHECKPOINT
ACTIVATION


FA
6.5 yr
Frequency
Age, Years
DC
14 yr
Frequency
Age, Years
DBA
3 mo
Frequency
Age, Years
SDS
2 wk
Age, Months

Congenital Amegakaryocytic thrombocytopenia (CAMT)

- Thrombocytopenia during the first year of life due to decreased production of PLTs by the bone marrow
- Physical appearance normal
- BM aspirate: absence of megakaryocytes
- 50% of the patients develop AA by the age 5 yrs
- Increased risk of MDS/AML
- Both sexes equally affected
- Germilne mutations of the 1p34 gene ***(c-MPL)*****,** encoding the thrombopoietin receptor (c-MPL)
- It is characterized by genetic heterogeneity
- Autosomal recessive inheritance in some patients
- Other cell lines may be affected, CNS, heart defects, mental retardation

Types of CAMT

- **I – CAMT** (Severe clinical picture)
  - ✓ Complete loss of function of the TPO receptor (nonsense, frameshift mutation of MPL)
  - ✓ Severe thrombocytopenia from birth
  - ✓ Early onset pancytopenia, MDS (2 years)
- **II – CAMT** (Moderate clinical picture)
  - ✓ Missense mutation of the MPL gene
  - ✓ Transient increase of PLTs in the first years of life and delayed onset of AA (5 years) or no occurrence
  - ✓ Probability of no progression to pancytopenia
- **III – CAMT**
  - ✓ Damage to a gene other than MPL (RUNX1 haplo)

DIAGNOSIS

- Thrombocytopenia
- Elevated serum TPO levels
- Normal MPV
- Absence of MGC in the marrow
- Detection of TPO receptor mutations

TREATMENT

- HSCT
- Antifibrinolytic agents
- PLTs transfusions

PROGNOSIS

- 30% death due to bleeding
- 20% death after HSCT

TAR (Thrombocytopenia – Absent Radius)

- Thrombocytopenia from birth
- Absence of radius (thumbs present dd of FA)
- Other skeletal abnormalities, heart, kidney abnormalities
- 50% of patients are allergic to cow's milk
- L/W: Increased plasma TPO with decreased MGC presence in the marrow
- 50-75% inherited with autosomal recessive / dominant inheritance
- 25% de novo mutations
- Thrombocytopenia improves after 1 year of life (rare ALL, AML)
- Genetic analysis: mutation in the *RBM8A* gene (protein synthesis – RNA-binding motif protein 8A)
- Treatment: PLT transfusions, HSCT

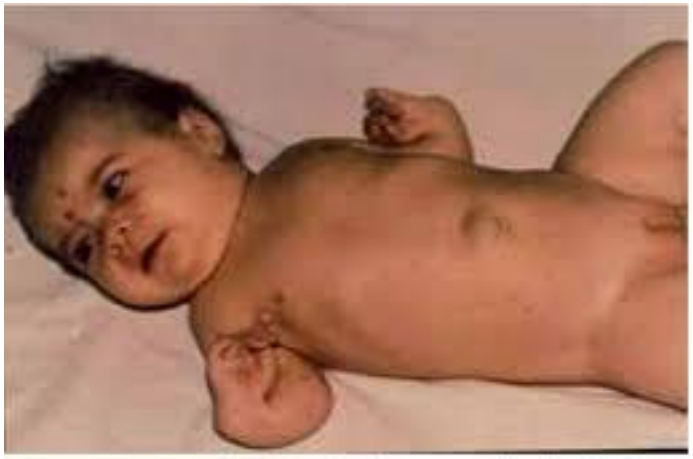
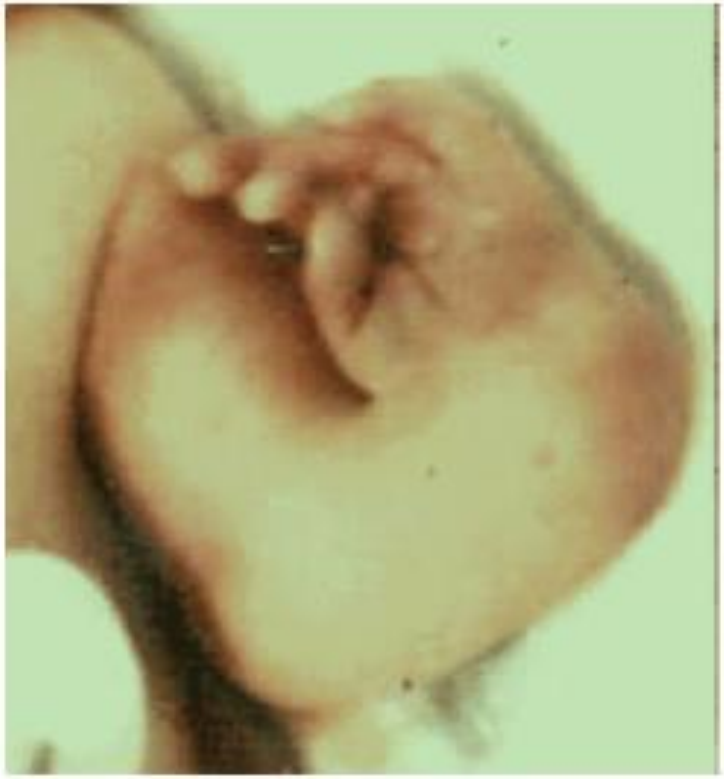
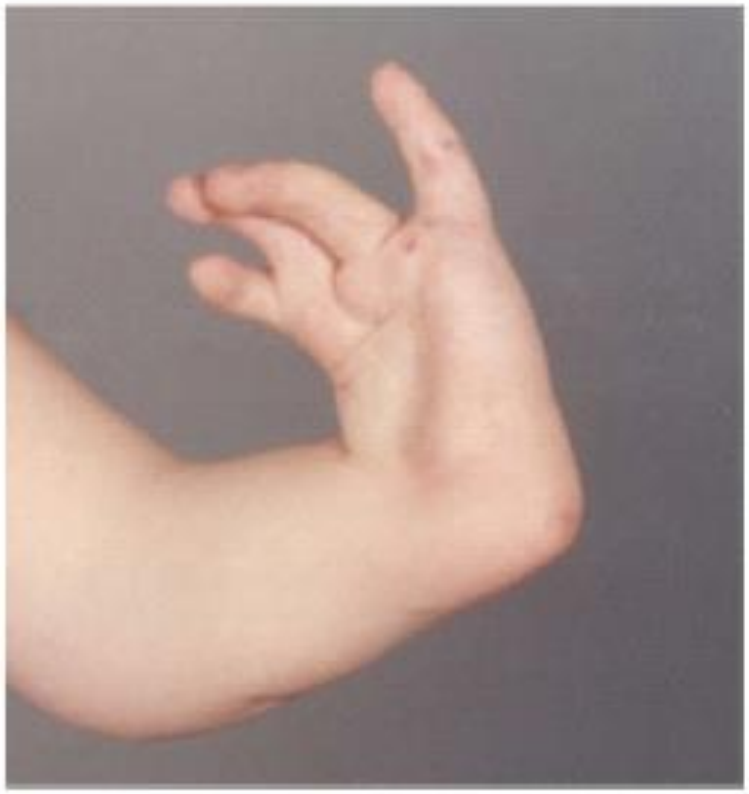


FIGURE 1. Note petechial bleeding, brusing in the forehead

TAR



FA



Severe Congenital Neutropenia (SCN)

- Prevalence: 3-8.5/10^6 population
- It is characterized by:
  - ✓ Frequent bacterial infections from early infancy
  - ✓ ANC $< 0.5\text{-}0.2 \times 10^9$/L
  - ✓ Maturation arrest at the promyelocyte / myelocyte stage
- Considered a preleukemic condition (21% develop leukemia after 10 years)
- Inheritance:
  - Autosomal dominant:
    - ✓ Mutations in the ***ELANE*** gene (60% of cases).
    - ✓ Other cases are sporadic
  - Autosomal recessive (Kostmann):
    - ✓ Mutations in the ***HAX1*** gene (30% of cases)
  - X-linked
  - Sporadic cases

Pathophysiology

Mutations of *ELANE, HAX1*

↓

Decreased expression of transcriptional factors of granulocytes

The type of mutation is related to the severity of the manifestations

↓

Early apoptosis of progenitor granulocyte cells

Elastase 2 (*ELANE*)

- Serine protease produced at the promyelocyte stage
- It is stored in the primary granules of neutrophils
- >200 mutations have been described
- Specific mutations (p.C151Y, p.G214R)) are associated with poor prognosis

Hax 1 (Kostmann s.)

- Mitochondrial protein
- Antiapoptotic action
- Mutations of the *HAX1* gene lead to inactivation of the related protein, depolarization of mitochondrial membrane and release of pro-apoptotic proteins resulting in early apoptosis
- Neutropenia, neurological symptoms (epilepsy, mental retardation)

Growth factor-independent protein 1

- Transcription protein
- Its mutations lead to:
  - ✓ overexpression of *ELA 2* and early apoptosis
  - ✓ overexpression of CSF-1 and conversion of granulocytic progenitors to macrophages

G6PC3

- Increased apoptosis
- Severe neutropenia
- Heart and genitourinary system defects

WAS

- Mutations in the *WAS* gene lead to: disorders in mitosis, decreased proliferation & increased apoptosis of progenitor cells of the granulocytic cell line
- Inheritance:X-linked

Other proteins: *CD40 ligand, MAPBPIP, AP3B1, CHS1/LYST*

GATA 2 deficiency

- Hematopoietic transcription factor that affects the number and quality of primitive hematopoietic cells
- Autosomal dominant inheritance
  - ✓ Susceptibility to infections
  - ✓ Respiratory infection
  - ✓ Lymphedema
  - ✓ Autoimmune manifestations
- Predisposition to malignancy
- 7% of children with MDS have chronic unexplained neutropenia
- L/W: neutropenia and monocytopenia ↓B, NK
- BM: reduced cellularity with fibrosis, MGC line with atypias

Diagnosis of SCN

- Medical history
- Clinical picture
- Neutropenia on the context of a syndrome
  - Heart (G6PC3, TAZ)
  - Genitourinary (G6PC3)
  - Skeletal Abnormalities, Pancreatic Dysfunction (SBDS)
- Neutropenia without other abnormalities
  - ANC < 500/μL
- Typical image of bone marrow aspirate
- Search for specific mutations (ELANE, HAX1)
- Genetic panel

Therapy

- Treatment of infections
- Administration of G-CSF
- Increased risk of leukaemia:
  - *ELANE*, acquired mutations of the G-CSF receptor (CSF3R)
  - 80% of patients who develop AML carry these mutations
- HSCT: unresponsive to G-CSF, AML/MDS
- Gene therapy ???

Congenital dyserythropoietic anemias (CDA)

Anemia and ineffective erythropoiesis:

- Increased marrow cellularity and Fe deposition
- Dyserythopoietic EB with increased apoptosis of red lineage progenitor cells
- Reduced %Rets
- Degree of hemolysis (jaundice, LDH)
- Splenomegaly
- Types (I-VII) (often based on case reports)

CDA categorization

CDA I

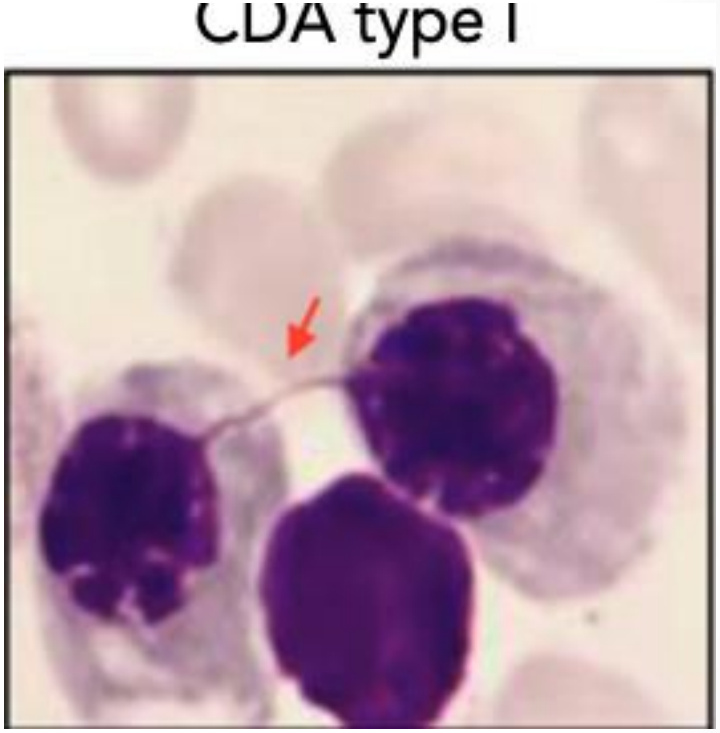
- *CDAN-1* protein
- *C15orf41*
  - DNA repair
  - Chromatin formation

Inheritance: autos. rec.

PB: anisopoikilocytosis (micro, macroovalocytes),

↑ MCV, NRBC, basophilic stippling

BM: >20% binucleated EBs, megablastic lesions and internuclear bridges

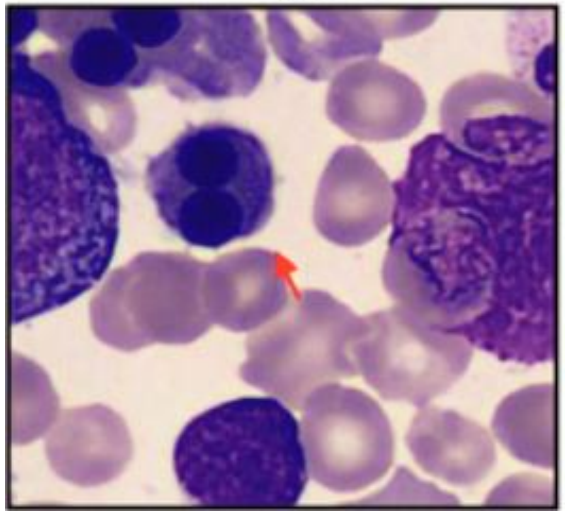


Splenomegaly and anemia
+/-Skeletal anomalies

CDA II (most common type)

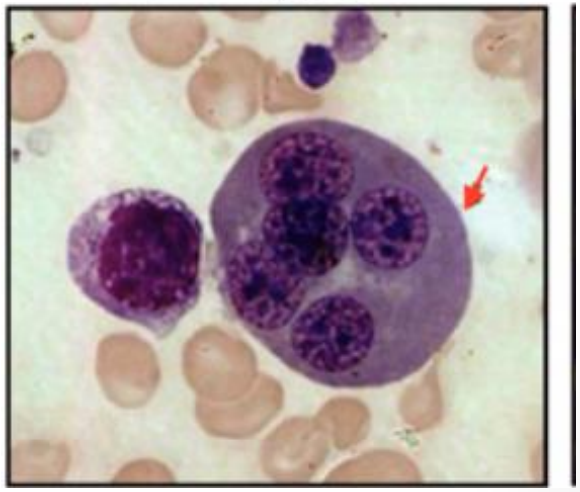
- *SEC23B* protein
- Appears in late childhood and adolescence
- Inheritance: AR.
- **PB:** normochromic anemia, Ret n/↑
- **BM**: >10% binucleated EB & >2% karyoblasts
- Jaundice, liver/splenomegaly Iron accumulation (20% liver cirrhosis)

CDA type II



- **CDA III** (rare),
- *KIF 23* protein (mitosis, intrac. trafficking)
- Ihneritance: AD
- **PB**: mild anemia, Ret↓
- **BM**: Multinucleated EB Jaundice, ↑LDH, ferritin n.

CDA type III



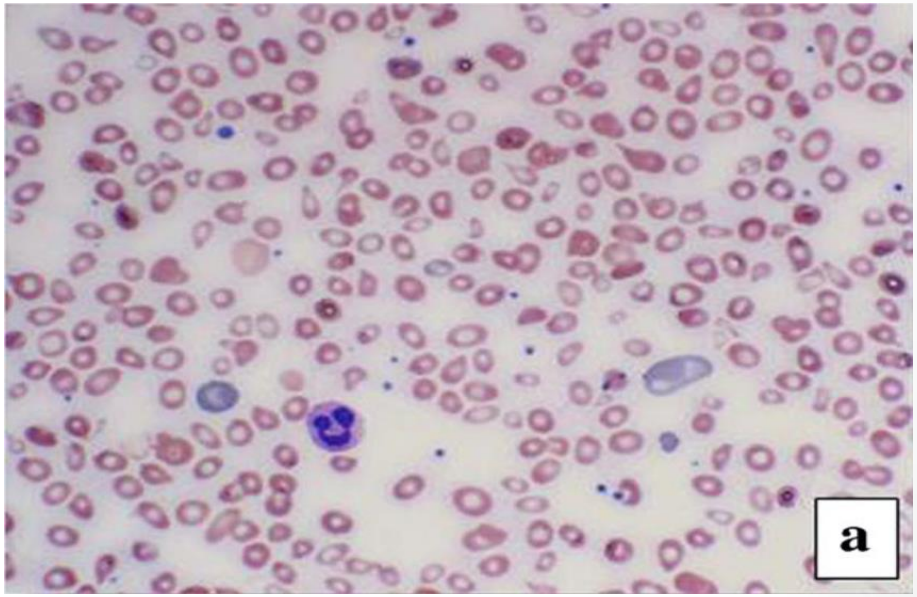
Other mutations:
GATA 1, KLF1

Diagnosis

- Compatible hematological findings
- Molecular testing

Treatment

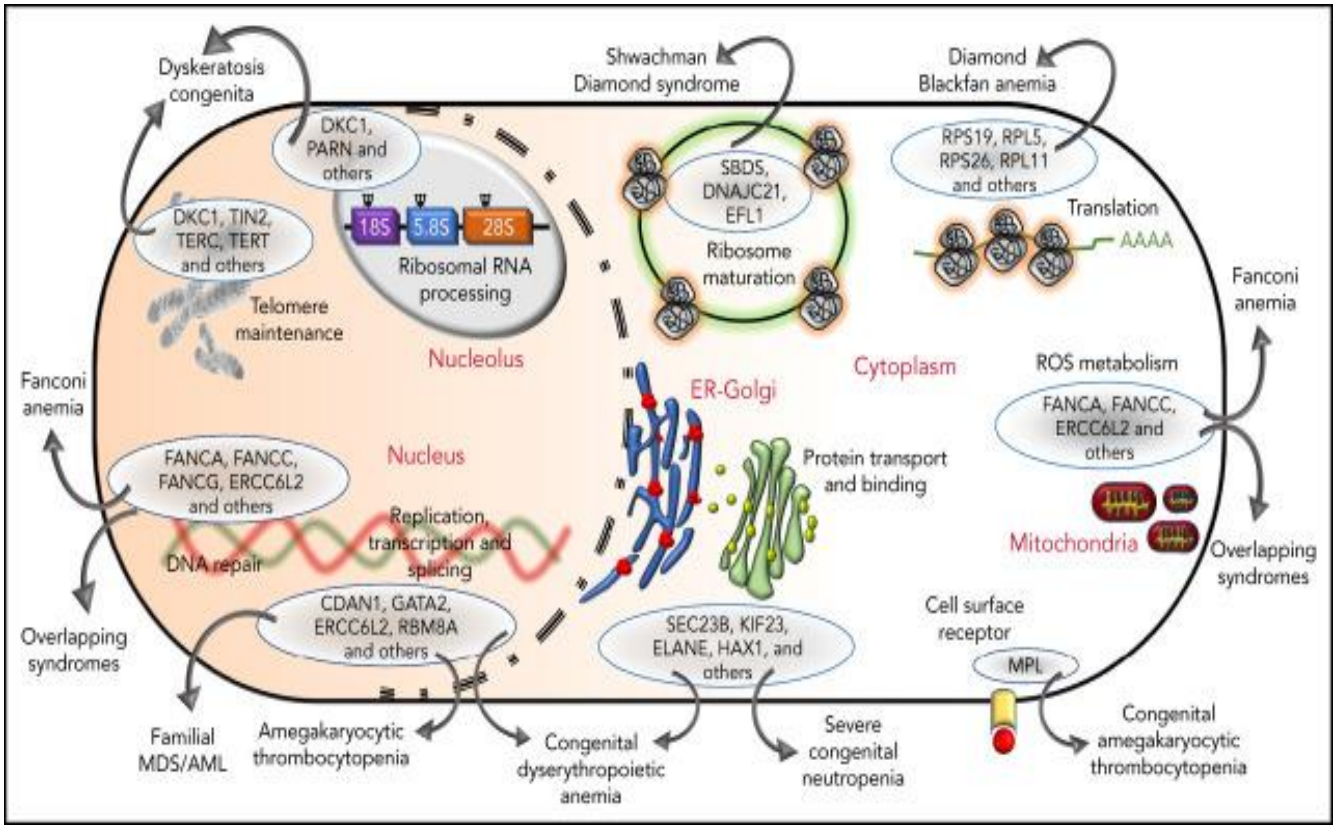
- Transfusions
- Splenectomy / cholecystectomy
- HSCT



IBMFS	Non-haematological clinical features	Laboratory findings	Associated cancers	Molecular mechanism
Fanconi anaemia	Radial ray anomalies, short stature, microcephaly, café	Pancytopenia, macrocytosis, elevated HbF, increased	MDS, AML, squamous cell cancers of head, neck, and anogenital region, other solid malignancies in *FANCD2*	DNA Repair: FA/BRCA pathway
Con am thr		reduced	Case report of ALL and one of MDS	Haematopoietic stem cell and megakaryocyte regulation
Dyskeratosis congenita	Skin pigmentation, nail dysplasia, oral leucoplakia, pulmonary fibrosis, stenosis of the oesophagus, liver disease	Pancytopenia, macrocytosis, elevated HbF, very short telomeres	MDS, AML, squamous cell cancers of skin, head, neck and anogenital region	Telomere biology
Diamond Blackfan anaemia	Short stature, malformation of craniofacilskeleton, eyes, heart, visceral, organs and limbs, bifid thumb	Anaemia, elevated red blood cell adenosine deaminase, macrocytosis, elevated HbF	MDS, AML, ALL, osteosarcoma, colon, possibly others	Ribosome biogenesis and processing
GATA2 deficiency			MDS, AML	
Severe congenital neutropenia	Severe infections	Neutropenia	MDS, AML	Myeloid lineage growth arrest
Shwachman Diamond syndrome	Exocrine pancreatic insufficiency, neurodevelopment and skeletal abnormalities	Neutropenia, low serum isoamylase, low serum trypsinogen	MDS, AML, ALL	Ribosome biogenesis and processing
Thrombocytopenia absent radii syndrome	Bilateral radial hypoplasia or aplasia with preservation of thumbs, other bony defects, congenital heart disease	Thrombocytopenia	Case reports of AML and ALL	mRNA maturation and processing

Every year:

- **FA**
- **SDS**
- **SCN**



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Who must be checked for IBMFs?

Personal history
- Cytopenias
- Short stature
- Congenital anomalies
- Other features of IBMFs
- Excessive treatment-related toxicity after cancer treatment

Family history
- Cytopenias
- Congenital anomalies
- Other features of IBMFs
- Cancers at young age
- Multiple 1st-2nd degree relatives with malignancy

Laboratory testing
- Cytopenias
- Elevated MCV
- Elevated HbF
- Low trypsinogen/pancr isoalmylase
- Elevated eADA activity
- Low Igs
- Abnormal lymphocyte subsets

Physical exam/imaging studies
- Short stature
- Failure to thrive
- Dysmorphologies
- Congenital anomalies

In conclusion...

- IBMFs are rare and fatal without treatment
- The clinical picture varies both in terms of severity and age of first manifestation
- Young adults with epithelial cancers may have IBMFs
- In recent years, with the help of genetics, important steps have been taken in understanding the molecular basis of IBMFs that help diagnose when the picture is not typical
- Successful HSCT solves the hematological problem but does not remove the risk of other malignancies...
- Gene therapy will be the definitive therapeutic solution ???

Thank you!!!