

Case presentation and review

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Herfstsymposium
15 October 2011

case presentation

SL 4 year old boy

Presents to the pediatrician for: evaluation developmental delay

Anamnesis:

In school noticed: clear delay and even regression in his development

Uneasy walking, less active, frequent falls; hardly speaks

No further physical complaints

Antecedents:

Pregnancy and birth: normal

On the age of 3 years admitted for Mexican fever (A.Z. Middelaars in

Deurne), otherwise healthy

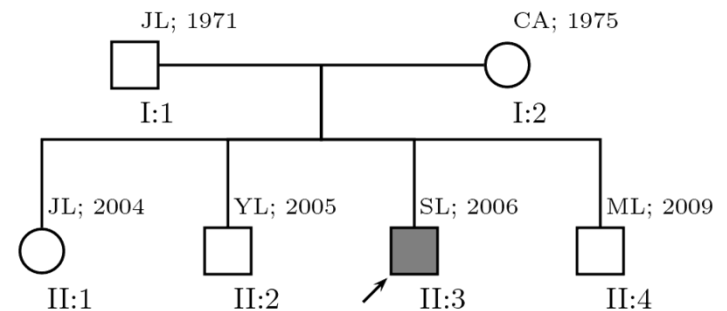
Family:

Parents from Morocco, consanguine (cousins)

Two healthy brothers and one healthy sister

No diseases in the family

(mother received RBC transfusion post partum 1x)



case presentation



Physical examination:

G 18,2 kg

L 102,5 cm

HO 55,5 cm (>p97)

Not sick, looks pale.

Pronounced large forehead,
broadened face.

Hart: systolic murmur 2/6.

Abdomen: liver 2 cm,
spleen 2-3 cm.

Normal male genitalia.

Neurological examination:

broad gang path,
mild hypotonia,
coordination normal,
normal cranial nerves.

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Problems

pallor

hepatosplenomegaly

enlarged head circumference

psychomotor delay

case presentation

Work-out:

CBC

Hb electroforesis

ferritin

liver- and renal function

MRI brain and RX lumbal spine

metablolic investigation (urine and blood)

genetic investigation (consultation and blood)

US abdomen

case presentation

Work-out:

CBC

Hb electrophoresis

ferritin **194**

liver- and renal function **normal**

MRI brain and RX lumbal spine **besides clearly thickened skull bone normal**

metabolic investigation (urine and blood) **normal**

genetic investigation (consultation and blood) **normal**

echo abdomen **hepatosplenomegaly**

case presentation

Work-out:

CBC

Hb electrophoresis

ferritin 194

liver- and renal function normal

MRI brain and RX lumbal spine besides clearly thickened
skull bone normal

metabolic investigation (urine and blood) normal

genetic investigation (consultation and blood) normal

echo abdomen hepatosplenomegaly

case presentation

Leukocyten	12,200 +	10E9/l	4,8 - 11,5
Hematocriet	0,169 -	l/l	0,324 - 0,396
Erythrocyten	2,51 -	10E12/l	3,93 - 4,99
Hemoglobine	5,70 --	g/dl	11,0 - 13,6
Mean cell volume	67,3 --	fl	76,1 - 86,7
Mean cell HB	22,7 -	pg	25,5 - 29,5
Mean cell HBconc.	33,7	g/dl	33,1 - 35,5
RDW	29,0 +	units	12,5 - 14,3
Trombocyten	215,00	10E9/l	205 - 450
Reticulocyten	30,000 +	/1000 RBC	8 - 20
Reticulo abs	76,000	10E9/l	29 - 80

Red Blood Cell Indices in Beta-Thalassemia

Red Blood Cell Index	Normal ¹		Affected	Carrier ¹
	Male	Female	β-Thal Maj	β-Thal Minor
Mean corpuscular volume (MCV fl)	89.1±5.01	87.6±5.5	50-70	<79
Mean corpuscular hemoglobin (MCH pg)	30.9±1.9	30.2±2.1	12-20	<27
Hemoglobin (Hb g/dL)	15.9±1.0	14.0±0.9	<7	Males: 11.5-15.3 Females: 9.1-14

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Work-out:

CBC

Hb electroforesis

ferritin 194

liver- and renal function normal

MRI brain and RX lumbal spine besides clearly thickened
skull bone normal

metablolic investigation (urine and blood) normal

genetic investigation (consultation and blood) normal

echo abdomen hepatosplenomegaly

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Hemoglobine A2	1,6	%	1,5 - 3,5	
Hemoglobine F	98,4 +	%	< 2,0	
Hb Electrof.(alcal)				HbF + HbA2
				Geen HbA aantoonbaar. Geen Hb-variant.
				Diagnose: beta-thalassemie maior.
				Gezien het lage HbA2 is er waarschijnlijk een
				beta-delta deletie en/of een bijkomende hereditaire persisterende HbF (HPHF).

Hemoglobin Patterns in Beta-Thalassemia (Age >12 Months)

Hemoglobin Type	Normal	Affected		Carrier
		β° -Thal Homozygotes	β^{+} -Thal Homozygotes or β^{+}/β° Compound Heterozygotes	β -Thal Minor
HbA	96%-98%	0	10%-30%	92%-95%
HbF	<1%	95%-98%	70%-90%	0.5%-4%
HbA ₂	2%-3%	2%-5%	2%-5%	>3.5%

case presentation



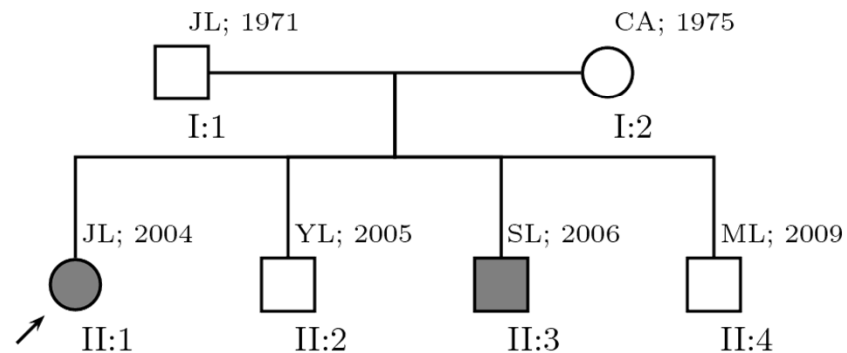
JL 6 year old sister came to visit her younger brother **SL**:

- pallor
- hepatosplenomegaly
- pronounced forehead

Permission of the parents acquired for public presentation

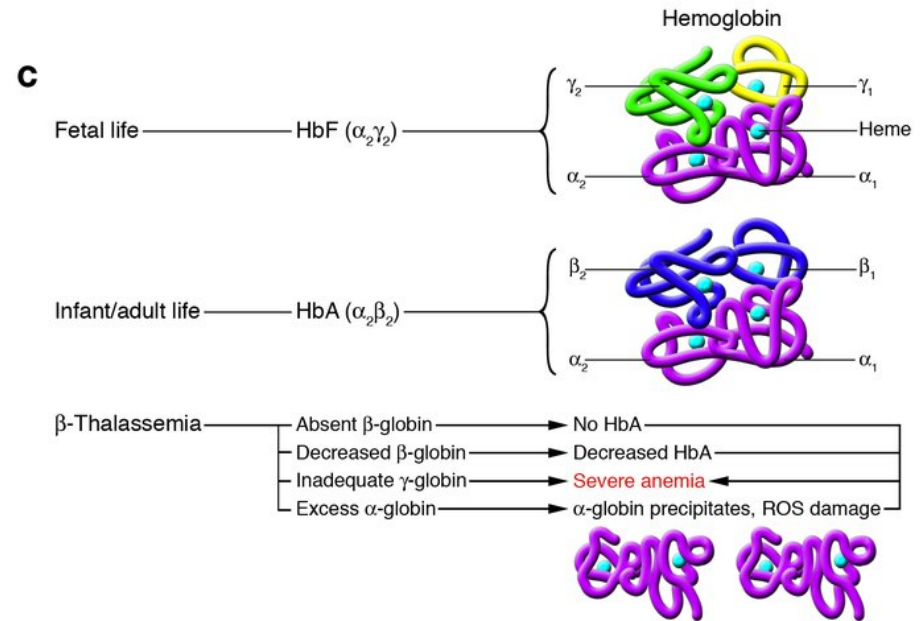
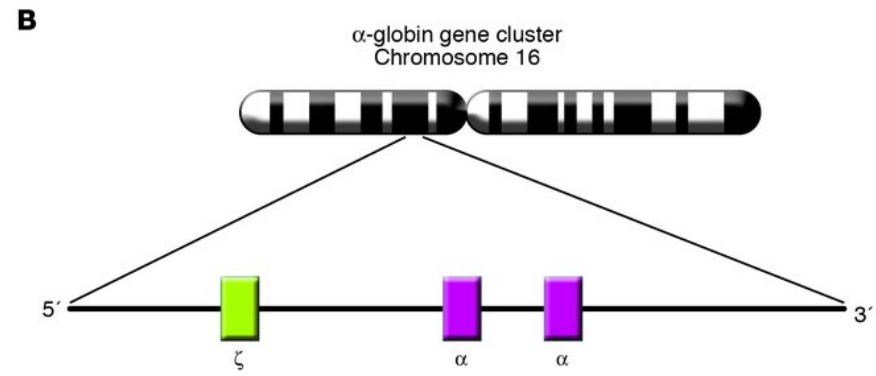
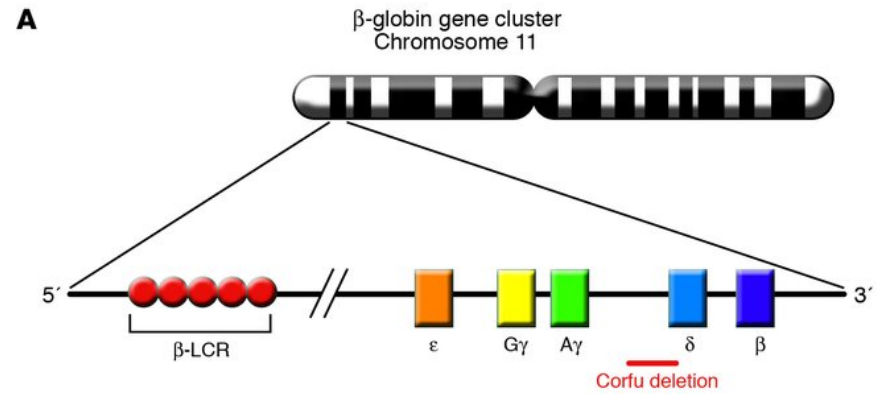
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	Hb	MCV	RBC	HbA2	HbF
father	13	65	5,7	5,2	6,2
mother	8,8	61	4,38	5,2	0,8
brother 5y	9,8	56	5,3	5,7	3,6
brother 15mo	9,8	56	5,2	5,1	14,4
SL 4y	5,7	67	2,5	1,6	98,4
JL 6y	6,1	73	2,5	2	98



Beta-Thalassemia

from the Greek,
thalassa (sea)
and *haima* (blood)



Definition

-group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis, resulting in reduced Hb, decreased RBC production and anemia

-most thalassemias are inherited as recessive traits

-Beta-thalassemias can be classified into:

1. Beta-thalassemia

- **Thalassemia major**
- **Thalassemia intermedia**
- **Thalassemia minor**

2. Beta-thalassemia with associated Hb anomalies

- HbC/Beta-thalassemia
- HbE/Beta-thalassemia
- HbS/Beta-thalassemia

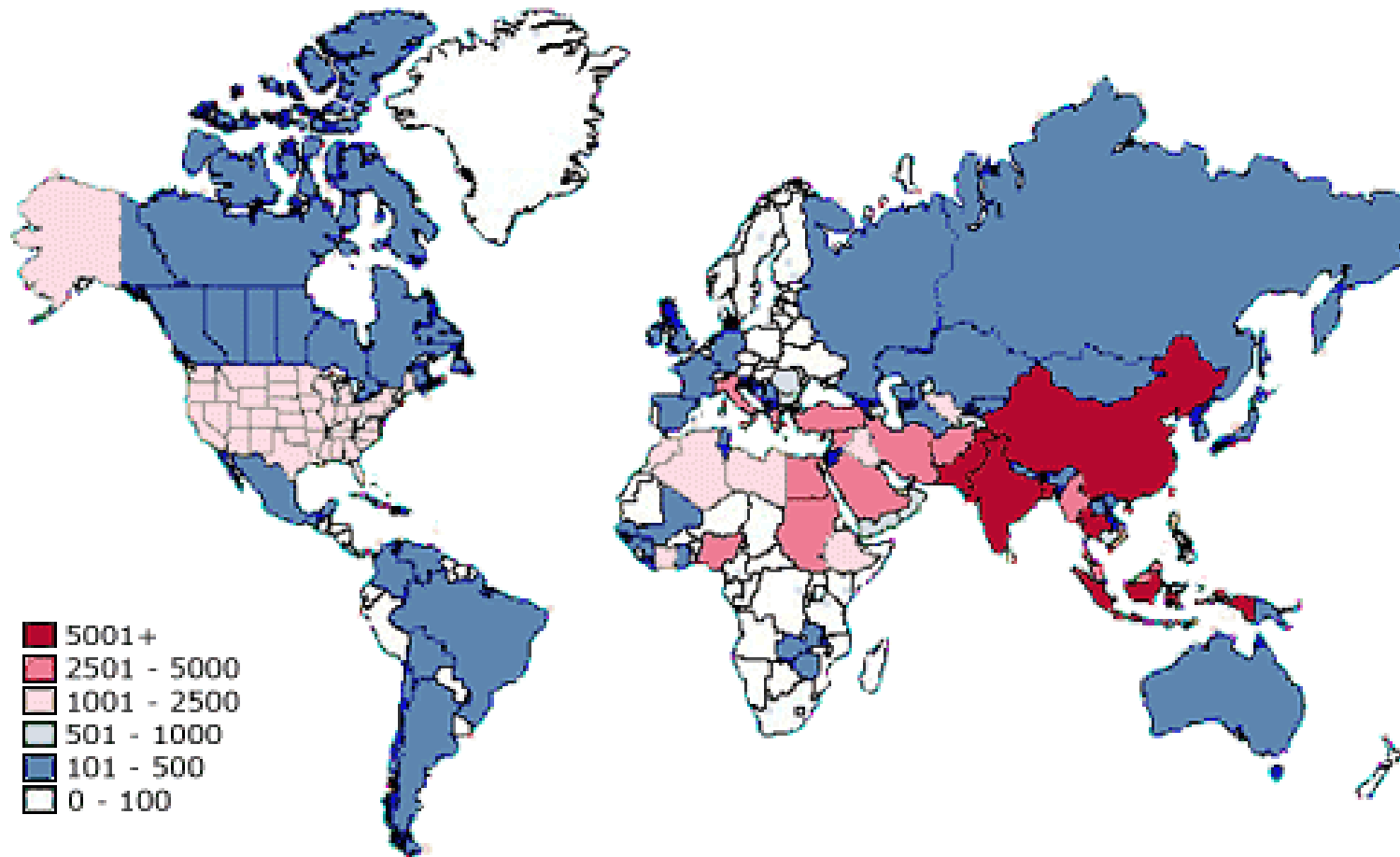
3. Hereditary persistence of fetal Hb and beta-thalassemia

4. Autosomal dominant forms

5. Beta-thalassemia associated with other manifestations

- Beta-thalassemia-trichothiodystrophy
- X-linked thrombocytopenia with thalassemia

Epidemiology



Epidemiology

- highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia
- the high gene frequency in these regions is most likely related to the selective pressure from *Plasmodium falciparum* malaria
- about 1.5% of the global population (80 to 90 million people) are carriers of betathalassemia
- according to Thalassemia International Federation, only about 200,000 patients with Thalassemia Major (=TM) are alive and registered as receiving regular treatment
- most common combination of beta-thalassemia with abnormal Hb or structural Hb variant with thalassemic properties is HbE/betathalassemia which is most prevalent in Southeast Asia where the carrier frequency is around 50%

Clinical description

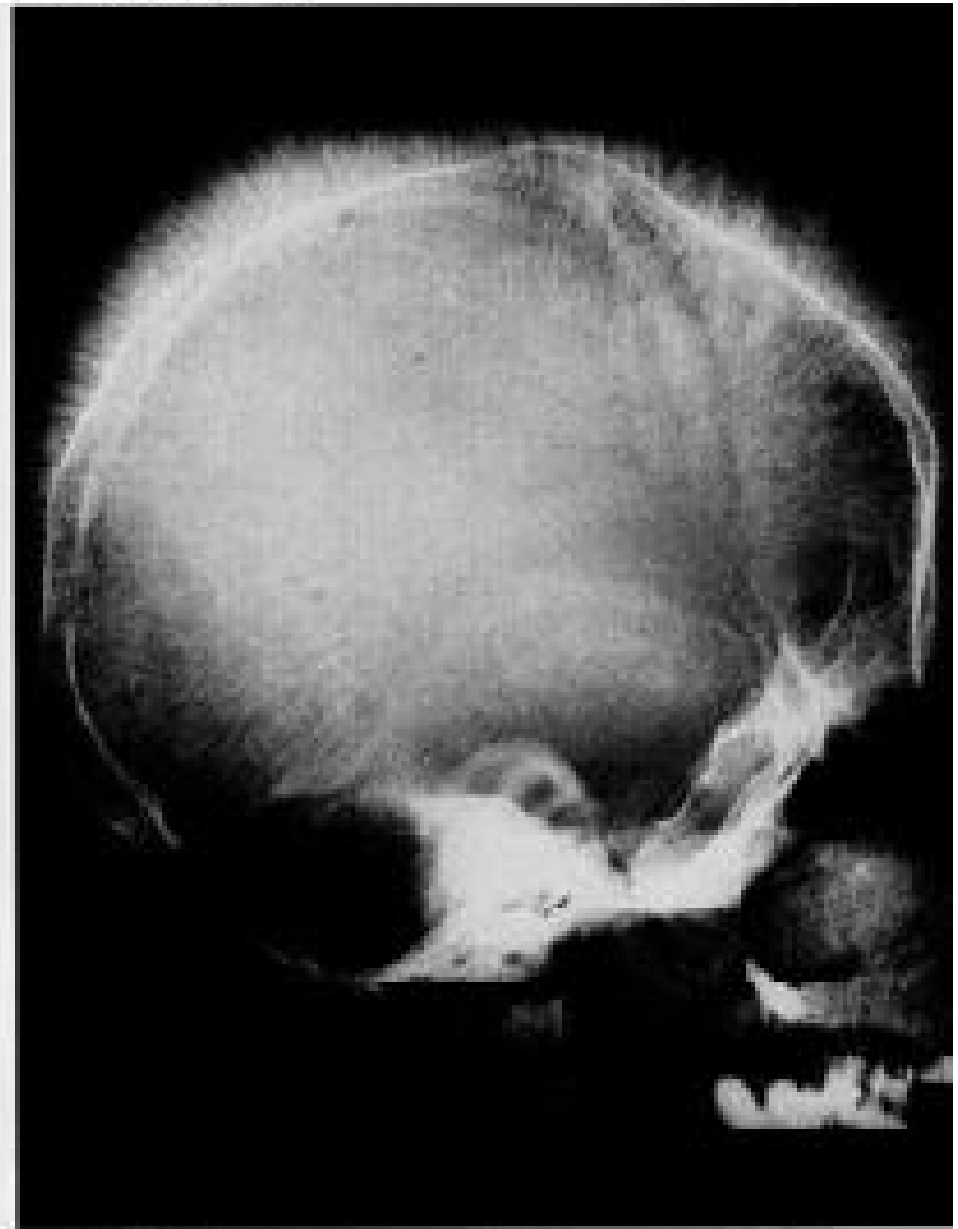
- children with TM usually come to medical attention within the first two years of life and require regular RBC transfusions to survive
- *Thalassemia intermedia* (=TI) includes patients who present later and do not require regular transfusion
- except in the rare dominant forms, heterozygous betathalassemia results in the clinically silent carrier state
- HbE/beta-thalassemia and HbC/beta-thalassemia exhibit a great range in terms of diversity of phenotypes and spectrum of severity

Clinical description

Thalassemia Major (TM)

- clinical presentation between 6 and 24 months
- failure to thrive, progressively pallor, feeding problems, diarrhea, irritability, fever, and progressive enlargement of the abdomen
- in some developing countries, the clinical picture of TM is characterized by:
 - growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, masses from extramedullary hematopoiesis, and skeletal changes from expansion of the bone marrow

Beta Thalassemia Major – bone changes



Clinical description

Thalassemia Major

- regular transfusion program (minimum Hb 9.5-10.5 g/dL)
gives normal growth and development
- transfused patients may develop complications
 1. related to iron overload
 - growth retardation and failure or delay of sexual maturation
 - heart (dilated cardiomyopathy or rarely arrhythmias)
 - liver (fibrosis and cirrhosis)
 - endocrine glands (DM, hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands)
 2. hypersplenism, chronic hepatitis (hepatitis B and/or C), HIV infection, venous thrombosis, and osteoporosis
 3. hepatocellular carcinoma

Clinical description

Thalassemia Major

- survival of individuals who have been regularly transfused and treated with appropriate chelation extends beyond age of 40 years
- myocardial siderosis is the most important life-limiting complication of iron overload in beta-thalassemia
(cardiac complications are the cause of the deaths in 71% of the patients with beta-thalassemia major)

Clinical description

Beta-thalassemia intermedia (TI)

- present later than TM, have milder anemia and do not require or only occasionally require transfusion
- wide clinical spectrum
- due to ineffective erythropoiesis and peripheral hemolysis more often than in TM:
 - gallstones
 - leg ulcers
 - thrombosis
- at risk of iron overload secondary to increased intestinal iron absorption, hypogonadism, hypothyroidism and diabetes are *not* common
- successful spontaneous pregnancies possible
- cardiac involvement mainly from a high-output state and pulmonary hypertension, while systolic left ventricle function is usually preserved

Beta-thalassemia minor

- usually clinically asymptomatic but sometimes have a mild anemia

Etiology

- more than 200 mutations have been so far reported
- majority are point mutations in functionally important regions of the beta globin gene, causing a reduced or absent production of beta globin chains
- deletions of the beta globin gene are *uncommon*

Table 1: Common types of beta-thalassemia: severity and ethnic distribution.

Population	β -gene mutation	Severity
Indian	-619 del	β^0
Mediterranean	-101 CTT	β^{++}
Black	-88 CTT	β^{++}
Mediterranean; African	-87 CTG	β^{++}
Japanese	-31 ATG	β^{++}
African	-29 ATG	β^{++}
Southeast Asian	-28 ATC	β^{++}
Mediterranean; Asian Indian	IVS1-nt1 GTA	β^0
East Asian; Asian Indian	IVS1-nt5 GTC	β^0
Mediterranean	IVS1-nt6 TTC	$\beta^{+}/++$
Mediterranean	IVS1-nt110 GTA	β^+
Chinese	IVS2-nt654 CTT	β^+
Mediterranean	IVS2-nt745 CTG	β^+
Mediterranean	codon 39 CTT	β^0
Mediterranean	codon 5 -CT	β^0
Mediterranean; African-American	codon 6 -A	β^0
Southeast Asian	codon 41/42 -TTCT	β^0
African-American	AATAAA to AACAAA	β^{++}
Mediterranean	AATAAA to AATGAA	β^{++}
Mediterranean	codon 27 GIT Hb (Hb Knossos)	β^{++}
Southeast Asian	codon 79 G>A (Hb E)	β^{++}
Malaysia	Codon 19 G>A (Hb Malay)	

β^0 :complete absence of beta globin on the affected allele

β^+ :residual production of beta globin (around 10%)

β^{++} :very mild reduction in beta globin production

Etiology

Genetic modifiers

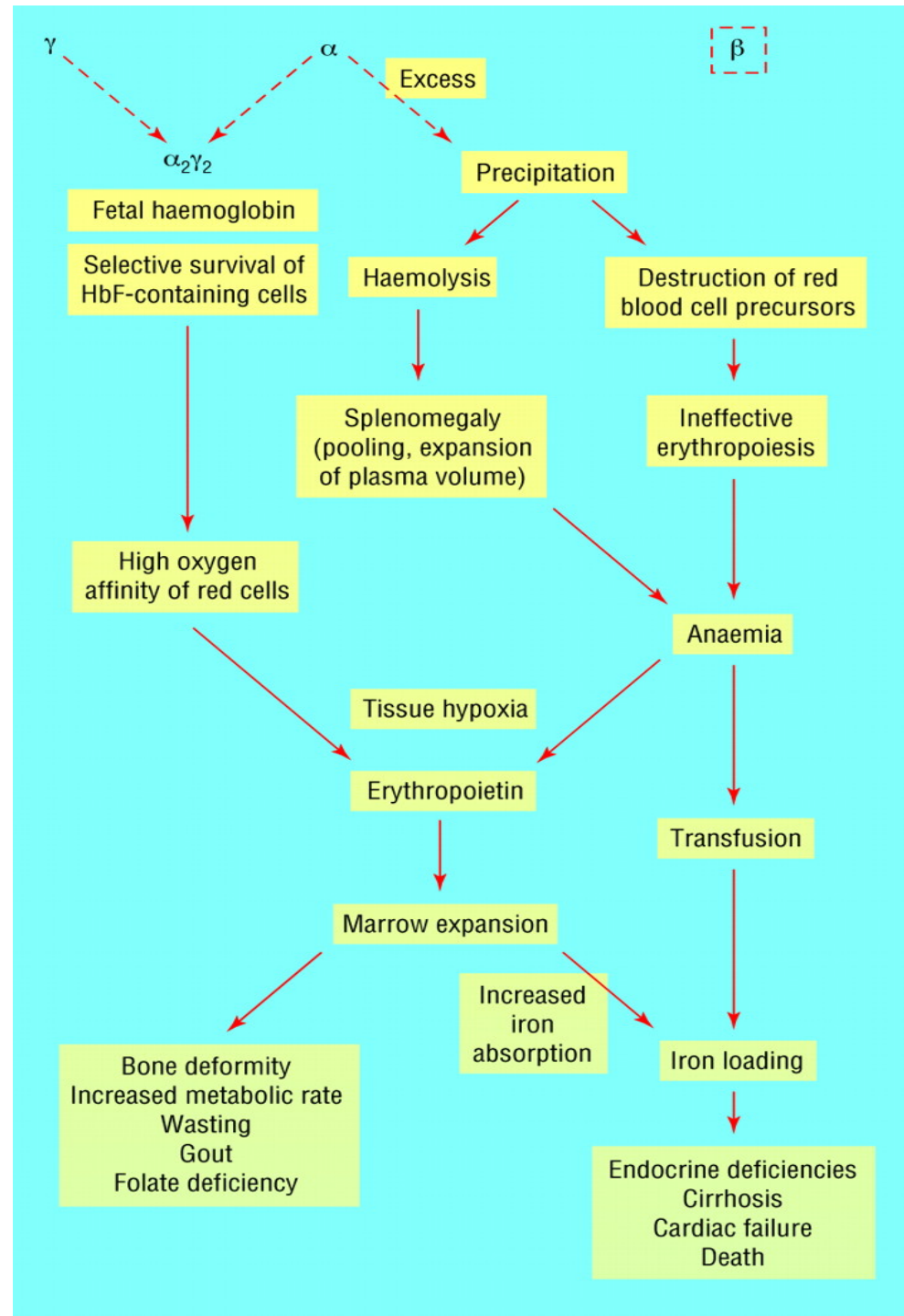
= genetic variants that lead to differences in disease phenotype, affecting the clinical severity of the disease

for example:

- mutations increasing HbF production, associated with deletional and non-deletional HPFH linked to the beta globin gene cluster
- presence of (TA)₇ polymorphism in the promoter region of the uridine diphosphate-glucuronosyltransferase gene, which in the homozygous state is associated with the Gilbert syndrome, is a risk factor for the development of cholelithiasis in TM and TI
- the apolipoprotein E ϵ 4 allele and some HLA haplotypes, which seem to be genetic risk factors for left ventricular failure in homozygous beta-thalassemia
- polymorphism in glutathione-S-transferase M1 gene has been associated with an increased risk of heart iron overload in TM

Etiology

Pathophysiology



Etiology

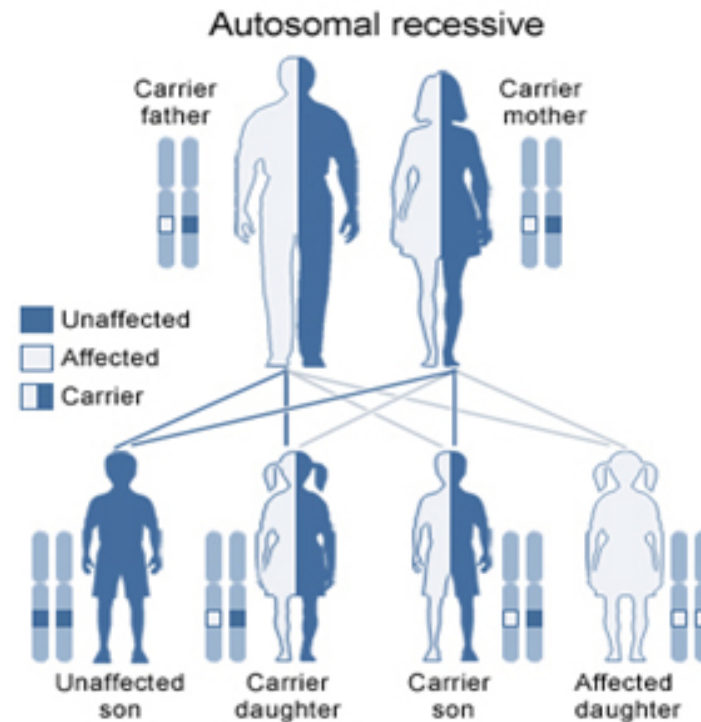
Pathophysiology

- degree of globin chain reduction is determined by the nature of the mutation at the beta globin gene located on chromosome 11
- peripheral hemolysis contributing to anemia is less prominent in TM than in TI, and occurs when insoluble alpha globin chains induce membrane damage to the peripheral erythrocytes
- anemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the bone marrow (up 25 to 30x), causing typical bone deformities
- prolonged and severe anemia and increased erythropoietic drive result in hepatosplenomegaly and extramedullary erythropoiesis

Etiology

Hereditary transmission

- beta-thalassemias are inherited in an AR recessive manner
- parents of an affected child are obligate heterozygotes and carry a single copy of a disease-causing beta globin gene mutation



Diagnosis

Clinical Diagnosis

- suspect when: infant younger than two years of age
with severe microcytic anemia,
mild jaundice and
hepatosplenomegaly
- TI presents at a later age with milder clinical findings
- carriers are usually asymptomatic, but sometimes may have mild anemia

Diagnosis

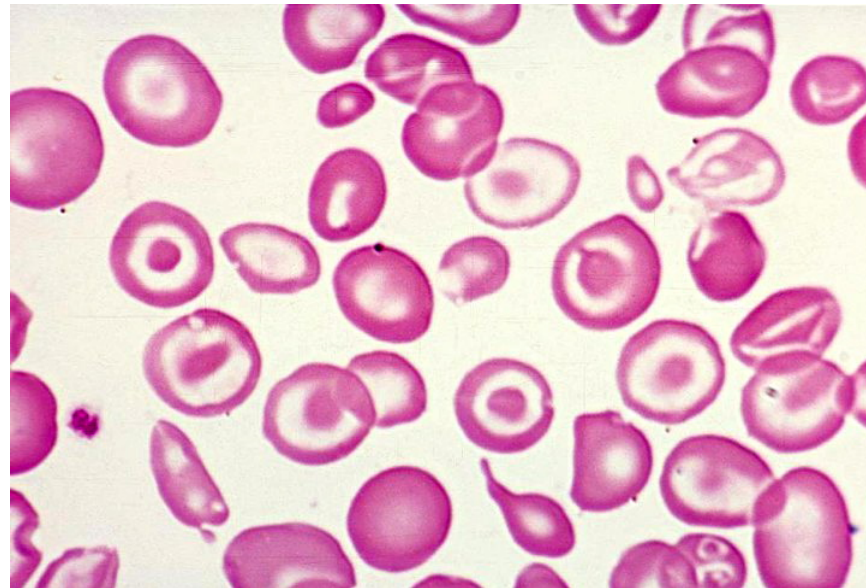
Hematologic Diagnosis

RBC indices show microcytic anemia

- TM → Hb <7 g/dl, MCV 50-70 fl, MCH 12-20 pg
- TI → Hb 7-10 g/dl, MCV 50-80 fl, MCH 16-24 pg
- Thalassemia minor → reduced MCV and MCH, increased Hb A2

Peripheral blood smear

- RBC → microcytosis, hypochromia, anisocytosis, poikilocytosis and nucleated RBC
- Carriers have less severe RBC morphologic changes



Diagnosis

Hematologic Diagnosis

Qualitative and quantitative Hb analysis (by cellulose acetate electrophoresis and DE-52 microchromatography or HPLC) identifies the amount and type of Hb present:

- beta⁰ thalassemia, homozygotes → HbA absent and HbF 92-95%
- beta⁺ thalassemia homozygotes → HbA levels 10-30% and HbF between 70-90%

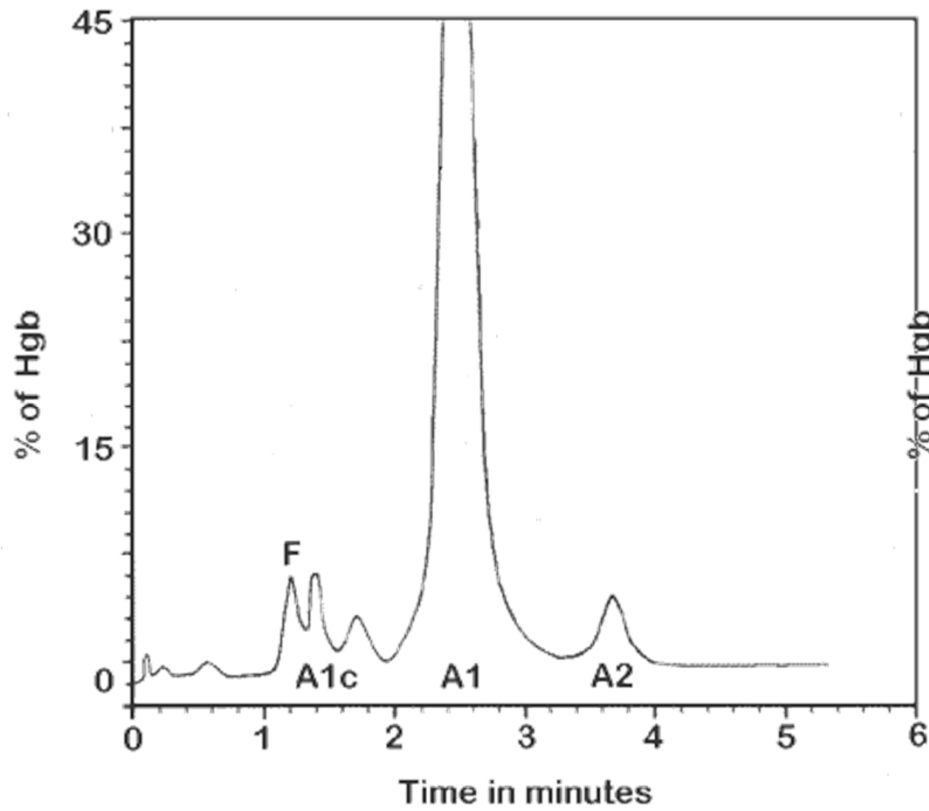
HbA₂ is variable in beta thalassemia homozygotes

- HbA₂ enhanced in beta thalassemia minor.

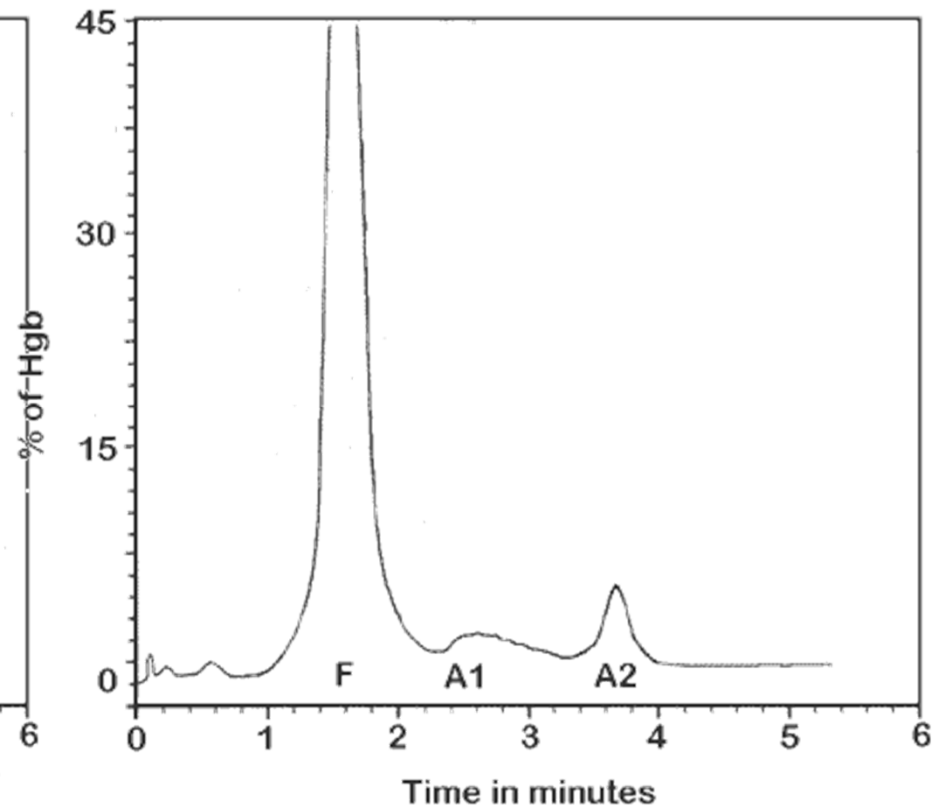
Hb electrophoresis and HPLC also detect other hemoglobinopathies (S, C, E, OArab, Lepore) that may interact with beta-thalassemia

High Pressure Liquid Chromatography (HPLC)

Beta thalassemia minor



Beta thalassemia major



Diagnosis

Molecular Genetic Analysis

- commonly occurring mutations of the beta globin gene are detected by PCR-based procedures
- If targeted mutation analysis fails to detect the mutation, beta globin gene sequence analysis can be used to detect mutations in the beta globin gene

Diagnosis

Genetic counseling and prenatal diagnosis

- prevention of beta-thalassemia is based on:

1. carrier identification

2. genetic counseling → mode of inheritance, the genetic risk of having affected children and the natural history of the disease including the available treatment and therapies under investigation

3. prenatal diagnosis → by analysis of DNA extracted from fetal cells obtained by amniocentesis (5-18 weeks' gestation or chorionic villi sampling (11 weeks' gestation)

→ analysis of fetal cells in maternal blood and analysis of fetal DNA in maternal plasma for the presence of the father's mutation are currently under investigation

→ preimplantation genetic diagnosis may be available for families in which the disease-causing mutations have been identified (Brussel, Bonduelle)



Management

Transfusions

- goals: correction of anemia
 - suppression of erythropoiesis
 - inhibition of gastrointestinal iron absorption
- indications with confirmed diagnosis of thalassemia:
 - Hb < 7 g/dl
 - Hb > 7 g/dl with other factors (like facial changes, poor growth, evidence of bony expansion and increasing splenomegaly). When possible,
- pretransfusional Hb level of 9 to 10 g/dl
- prevents growth impairment, organ damage and bone deformities
- usually every two to four weeks



Management

Transfusion-dependent complications

Iron overload

Infections

Known

- *Viral (HIV, HCV, HBV, HTLV1, West Nile virus)*

- *Bacterial*

- *Parasitic*

Rare

- *Creutzfeld-Jacob disease*

- *Emerging and new pathogens*

Hemolytic
reactions

Acute hemolytic reactions

Delayed hemolytic reactions

Autoimmune hemolytic anemia

Non-Hemolytic
reactions

Allergic and anaphylactic reactions

Febrile non-hemolytic reactions

Transfusion-related acute lung injury (TRALI)

Transfusion-associated graft-versus-host disease

Circulatory overload

Post-transfusion purpura

Management

Assessment and treatment of Iron overload

- clinical manifestations:

hypogonadism (35-55% of the patients)

hypothyroidism (9-11%)

hypoparathyroidism (4%)

diabetes (6-10%)

liver fibrosis

heart dysfunction (33%)

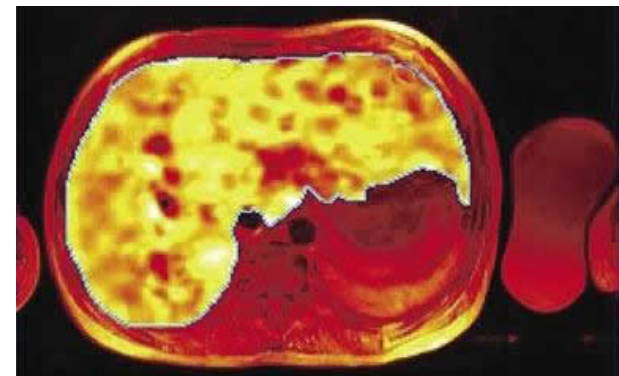
- iron status assessment:

1. serum ferritin

2. liver iron concentration (liver biopsy)

3. MRI techniques: R2 and parameters
cardiac T2*

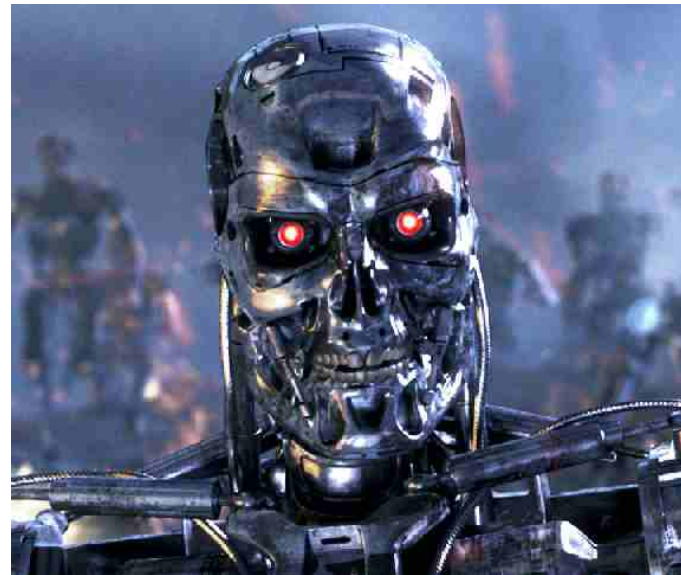
4. magnetic biosusceptometry (SQUID)



FerriScan (MRI- based new technology for the non-invasive measurement of liver iron concentrations)

Management

- the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool
- start iron chelation treatment:
 - 10-20 transfusions or
 - ferritin >1000 ng/ml



Management

- *deferoxamine* (DFO)

usually SC (or IV) 8- to 12-hour nightly infusion, 5-7 nights a week
dosage is 20-40 mg/kg body weight for children

in high risk cases: continuous administration of DFO via Port-acath or sc
50 and 60 mg/kg per day

iron is excreted both in faeces (about 40%) and in urine

most frequent adverse effects of DFO:

local reactions at the site of infusion

occasionally accompanied by fever, chills and malaise

other complications (mainly high doses in young patients and low ferritin values):

- sensorineural hypoacusia, particularly at high frequencies
- ocular toxicity (night-blindness, blurred vision, decreased visual acuity, impairment of colour vision, cataract and other disturbances of the eye)
- retarded growth and skeletal changes with a disproportionately short trunk and dysplasia of the long bones
- infections by *Yersinia Enterocolitica*, and other pathogens (*Kl. Pneumoniae*).

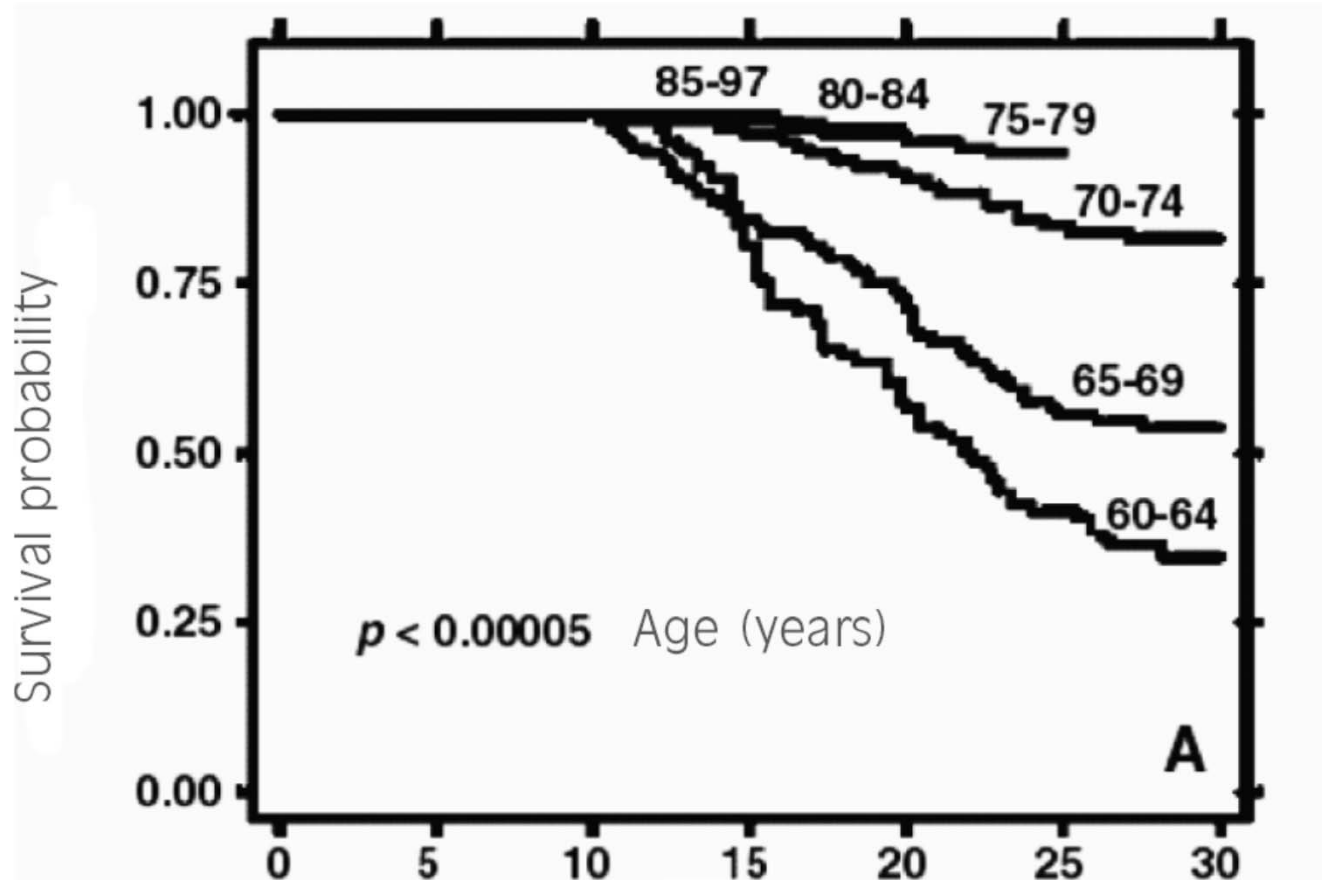
monitor: audiometric and ophthalmologic tests

growth and bone changes

→ use of DFO decreases morbidity and mortality



Management



however, because of the side effects and parenteral administration often non-compliance, limiting the usefulness of this chelator

Management



- *deferiprone* (DFP)

orally active iron chelator

75-100 mg/kg/day (3x per day)

DFP monotherapy more effective than DFO in decreasing myocardial siderosis in TM

side effects:

agranulocytosis (1% of the patients)

more common but less severe:

gastrointestinal symptoms

arthralgia

zinc deficiency

fluctuating liver enzymes

DFO and DFP can be used in combination to achieve levels of iron excretion that cannot be achieved by either drug alone without increasing toxicity, reversal of severe iron-related heart failure reported (myocardial T2*)

- combination therapy should be considered as an alternative to continuous intravenous DFO monotherapy when an intensive chelation is required

Management

Deferasirox (DFX)

once-daily, oral iron chelator

10-30 mg/kg/day

adverse events: - transient, mild-to-moderate gastrointestinal disturbances

- skin rash

- mild increase serum creatinine (a third of patients)

(S)-3'-(OH)-desazadesferrithiocin-polyether

magnesium salt, once-daily, oral iron chelator

excrete iron mainly in the stools

granted in the USA and Europe



Management

Treatment of iron overload-related complications

Delayed puberty, hypogonadism and assisted reproduction

- **delayed puberty in girls:** ethinyl estradiol
- **delayed puberty in males:** IM depot-testosterone esters
- **azoospermia or asthenospermia:** combination therapy with hCG and hMG IM or SC
- **primary or secondary amenorrhea:** ovarian function is preserved in most patients

Growth deficiency

- GH to those patients proven to have GH deficiency

Hypothyroidism

- L-thyroxine

Hypoparathyroidism

- severe hypocalcemia → IV calcium under careful ecg monitoring, followed by oral vit.D
- milder → calcitriol 0.25-1 µg twice daily

Diabetes and impaired glucose tolerance

- acarbose 100 mg/daily SC insulin

Osteoporosis

- prevention is the basis of the management

Splenectomy

- consider when annual red cell requirement exceeds 180-200 ml/Kg of RBC
- other indications for splenectomy: symptoms of splenic enlargement
leukopenia and/or thrombocytopenia and
increasing iron overload despite good chelation

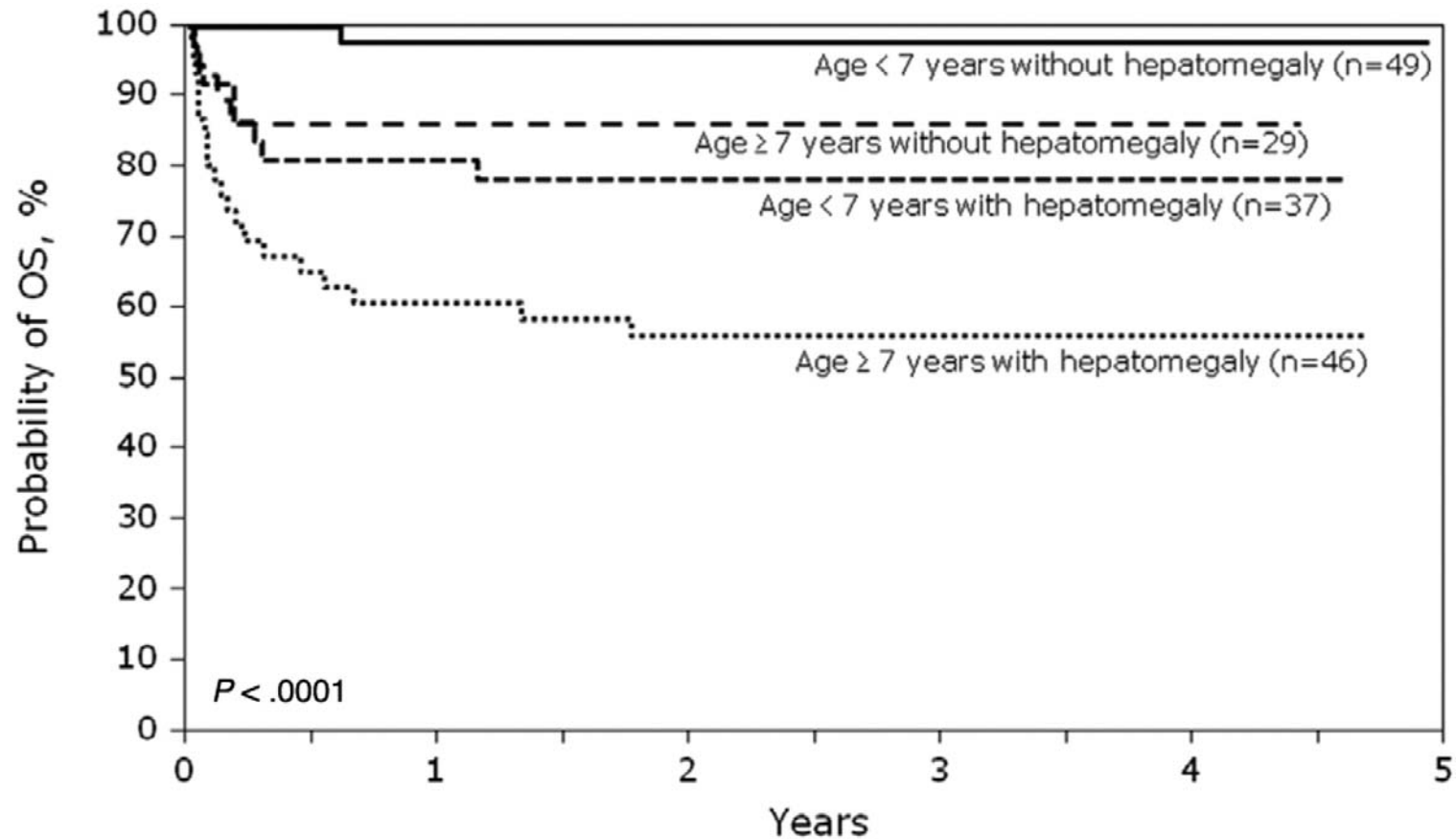
Management

Bone marrow and cord blood transplantation

BMT only definitive cure currently available

- outcome of BMT related to the pretransplantation clinical conditions:
 - presence of hepatomegaly
 - extent of liver fibrosis
 - history of regular chelation
 - severity of iron accumulation
- without the above risk factors, SCT HLA identical sibling diseasefree survival rate over 90%

Management



Management

Bone marrow and cord blood transplantation

- major limitation is lack of an HLA-identical sibling donor for the majority of affected patients (appr. 25-30% have MRD)
- provided that selection of the donor is based on stringent criteria of HLA compatibility and that individuals have limited iron overload, results are comparable to those obtained when the donor is a compatible sib → however limited number of individuals
- GVHD in 5-8% of individuals

Cord blood transplantation from a related donor offers a good probability of successful cure and associated with a low risk of GVHD

Management

Management of thalassemia intermedia

- symptomatic

- splenectomy

 - indications: hypersplenism, retarded growth, mechanical disturbance

 - risks: infections mainly encapsulated bacteria (*Str. Pneumoniae*, *H. Influenzae* and *N. Meningitidis*)
thromboembolic events

 - prevention of post-splenectomy sepsis: immunization, AB prophylaxis, early ABTx
splenectomy combined with cholecystectomy

- extramedullary erythropoietic masses

 - therapy: radiotherapy, transfusions, or hydroxycarbamide

- leg ulcer

 - therapy: blood transfusions, zinc, pentoxifylline, oxygen chamber, hydrea, PDGF

- thrombosis

 - therapy: proper anticoagulation prior to procedures
platelet anti-aggregating agents (when Plt > 700.000)
LMW heparin with documented thrombosis

- iron overload

 - therapy: chelation therapy (when ferritin > 300 ng/ml)

- supplementary folic acid can be prescribed

Management



Lifestyle and diet in beta-thalassemia

- no specific dietary requirements
- glass of black tea with meals reduces iron absorption from food (TI)
- avoid foods very rich in iron
- adequate calcium (calcium supplements only with clear indication)
- folate supplements (1 mg/day)
- vitamin C (iron overload causes vitamin C to be oxidized; vitamin C may increase iron absorption from gut)
- discouraged from consuming alcohol (facilitates oxidative damage of iron and aggravates the effect of HBV and HCV on liver tissue)
- physical activity must always be encouraged
- vaccinations
- important psychological implications

Prognosis

thalassemia minor

- excellent
- increased risk for cholelithiasis, esp. with Gilbert mutation

thalassemia intermedia

- usually have severe hemosiderosis are less prone to cardiac problems, however, pulmonary hypertension, thromboembolic complications, overwhelming postsplenectomy sepsis, and the development of hepatocarcinoma may reduce survival

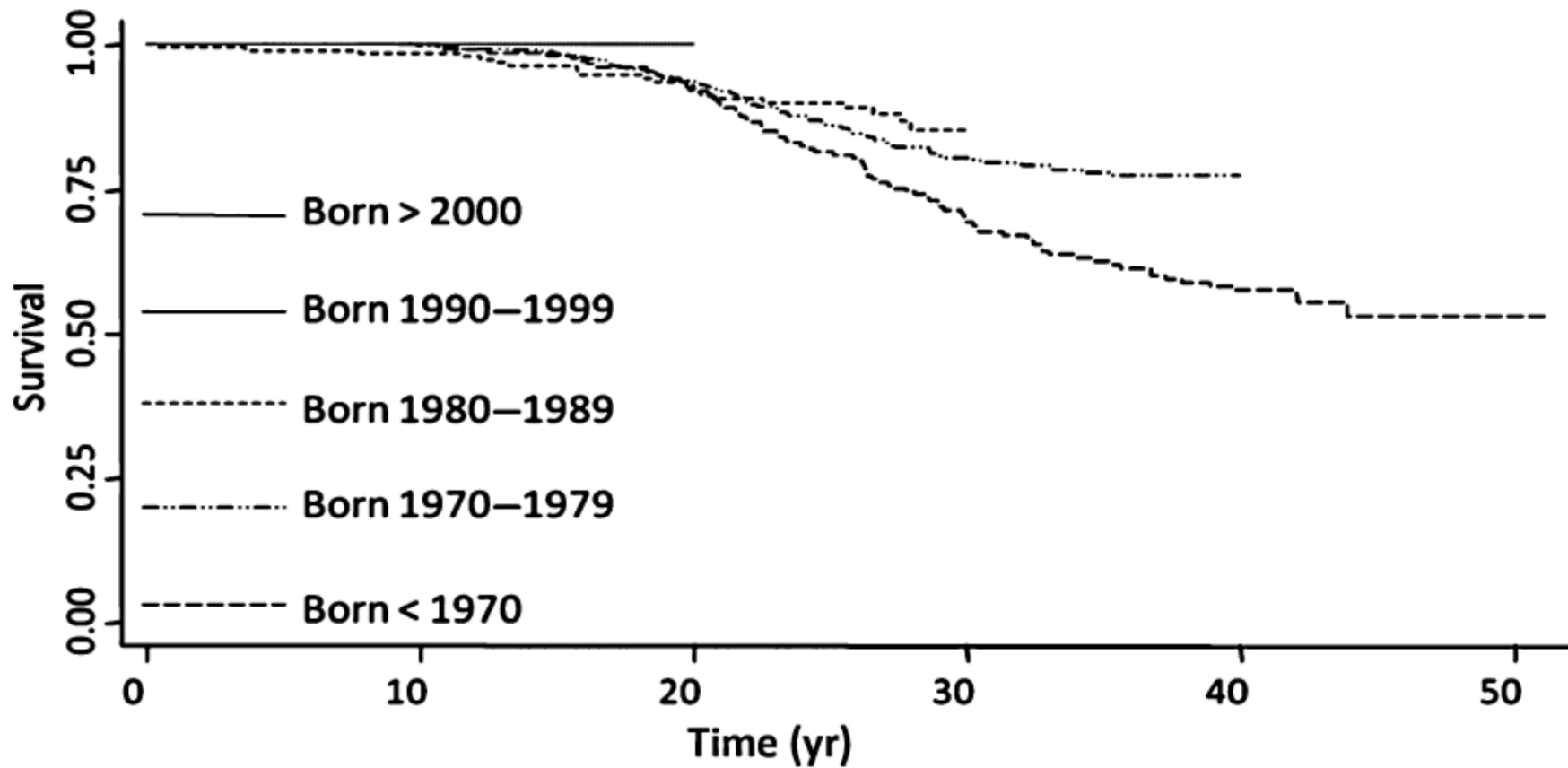
betathalassemia major

- no treatment death by age five (infections and cachexia)
- blood transfusions into 2nd decade
- DFO and good monitoring
- in recent years, children who have been chelated since a very young age have an excellent long-term prognosis

Prognosis

Kaplan–Meier curves* of overall survival according to decade of birth.

*Logrank test: P-value < 0.0001. *Test for trend: P-value < 0.0001



References

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<http://www.thalassemia.org.cy>

Thank you for your attention!

questions?