TEHRAN UNIVERSITY OF MEDICAL SCIENCES

Evaluation of Patients with Congenital Neutropenia

From Diagnostic Approach to Treatment Opportunities

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Neutropenia

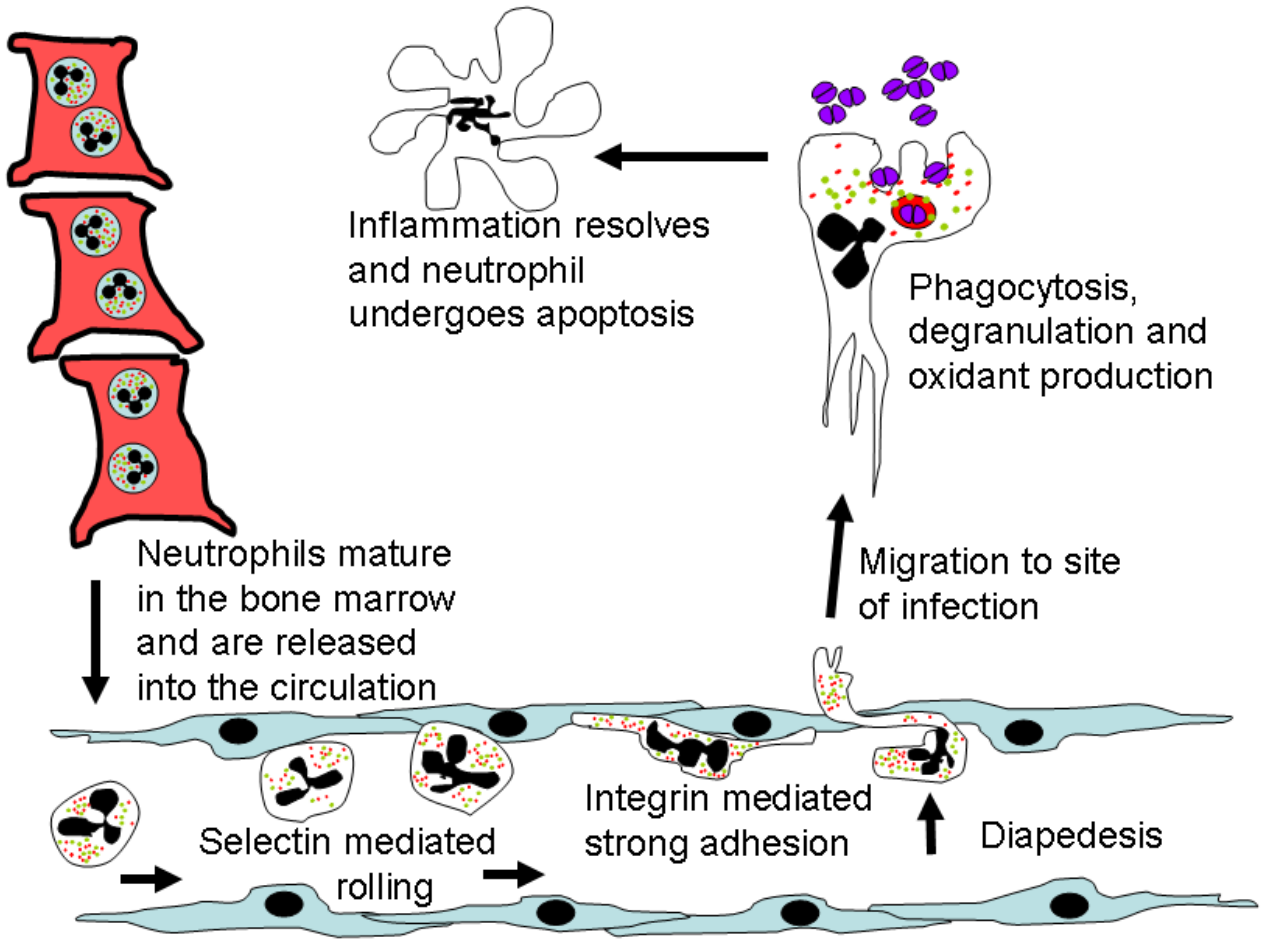
- The phagocytic system is an essential part of the host immune defense and is the main component of the innate immune system.

- The most commonly encountered phagocytic defect is a decrease in the absolute number of circulating neutrophils.

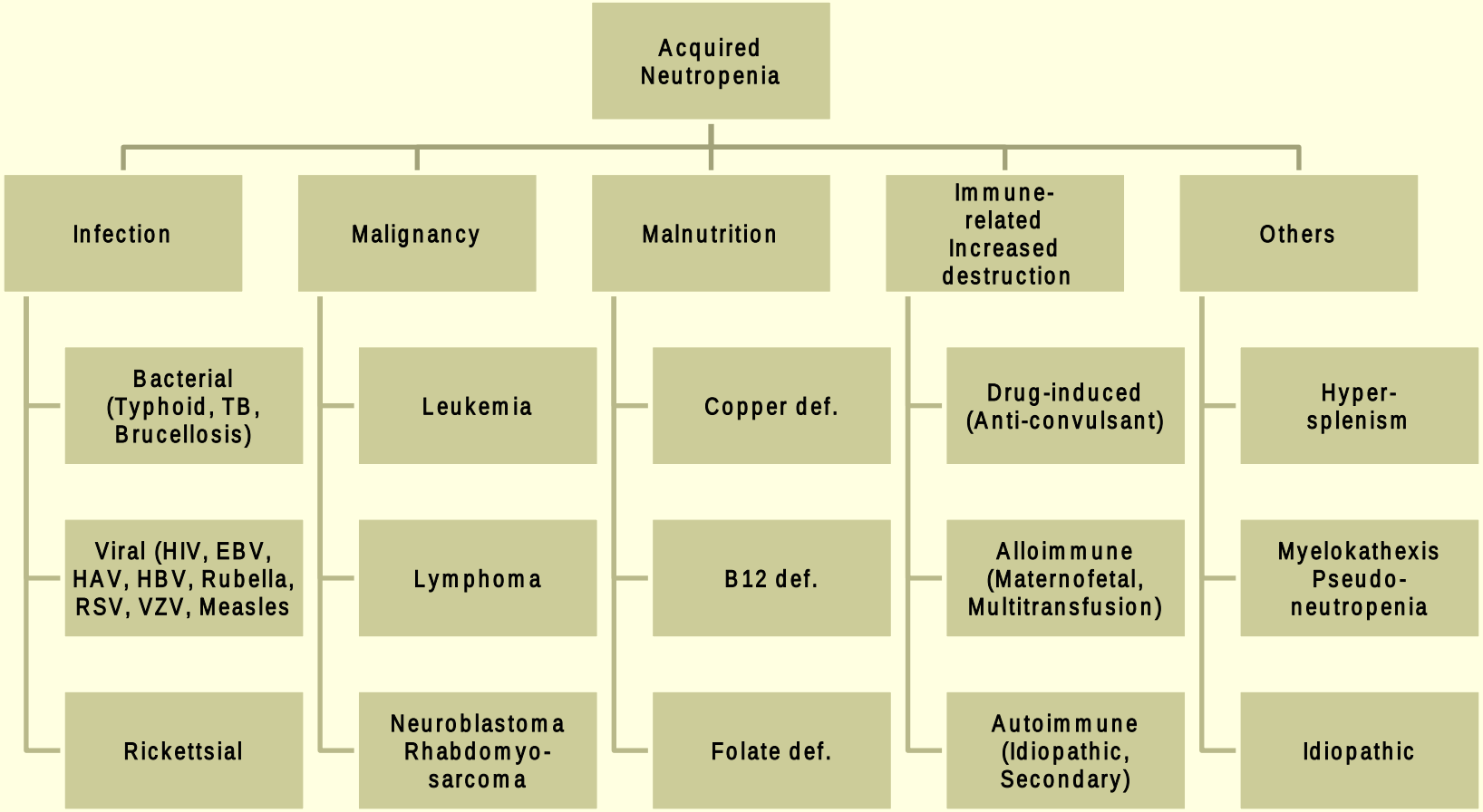
- Neutropenia (or granulocytopenia) is a reduction in the absolute neutrophil count to less than 1,500/mm^3.

- Impaired production, peripheral destruction, and abnormal distribution of neutrophils may lead to low numbers of circulating granulocytes.

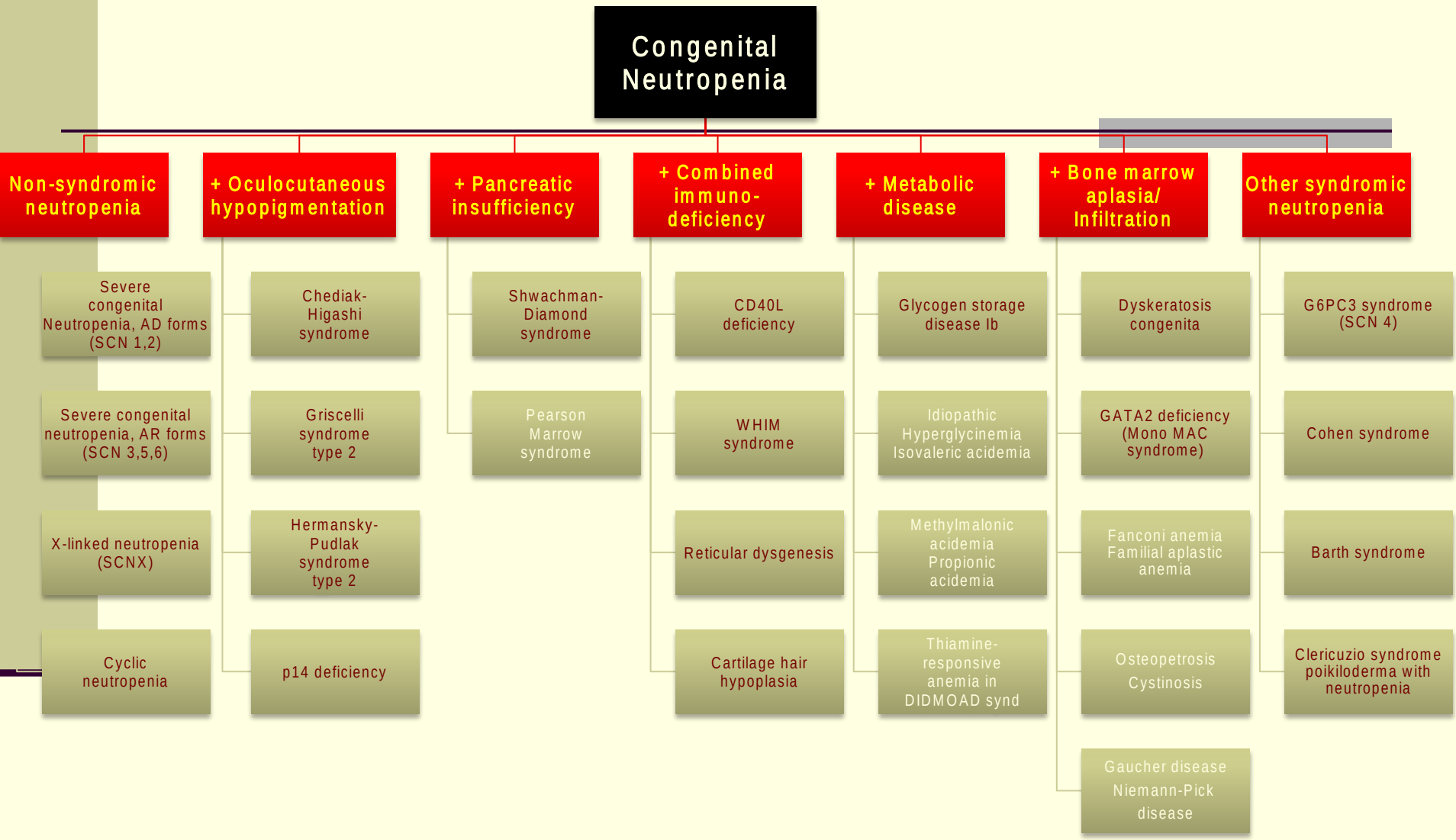
The life cycle of the neutrophil



Approach to acquired neutropenia



Approach to congenital neutropenia



Congenital Neutropenia and Primary Immunodeficiency Diseases

- Severe congenital neutropenia (SCN 1,2,3,5,6)
- Cyclic neutropenia
- X-linked neutropenia
- G6PC3 deficiency (SCN4)

- Chediak-Higashi syndrome
- Griscelli syndrome, type 2
- Hermansky-Pudlak syndrome, type 2
- p14 deficiency

- Shwachman-Diamond syndrome

- CD40L deficiency
- WHIM syndrome
- Cartilage hair hypoplasia
- Reticular dysgenesis

- Glycogen storage disease Ib
- Barth syndrome
- Cohen syndromes
- Poikiloderma with neutropenia

- Dyskeratosis congenita
- GATA2 deficiency

** Rezaei N, Moazzami K, Aghamohammadi A, Klein C. Neutropenia and primary immunodeficiency diseases. Int Rev Immunol. 2009; 28(5):335-66.*

*** Wintergerst U, Rosenzweig SD, Abinun M, Malech HL, Holland SM, Rezaei N. Phagocytes Defects. In: Rezaei N, Aghamohammadi A, Notarangelo LD (eds). Primary immunodeficiency diseases: definition, diagnosis and management. Springer-Verlag Berlin Heidelberg 2008, pp. 131-166.*

Severe Congenital Neutropenia

- Also, known as Kostmann syndrome
- Persistent severe neutropenia (ANC< 500/μL)
- Increased susceptibility to severe infections
- Early onset of recurrent bacterial infections
- Early-stage (promyelocyte-myelocyte) maturation arrest of myeloid differentiation in the bone marrow

Severe Congenital Neutropenia

✓Pathophysiology and all genetic defects associated with SCN has not been completely understood!

- Early onset recurrent bacterial infections

- Presenting features

Superficial abscesses	Oral ulcers
Cutaneous infections	Omphalitis
Pneumonia	Otitis media

- During the course of disease
  - Abscesses in different sites
  - Mucocutaneous manifestations
  - Respiratory infections
  - Diarrhea

Severe Congenital Neutropenia

✓Pathophysiology and underlying genetic defect of SCN is not completely understood
✓Multigene disorder with a common hematological and clinical phenotype

✓SCN1: *ELA2* (AD): either SCN or cyclic neutropenia
✓SCN2: *GFI1* (AD): B/T lymphopenia

✓SCN3: *HAX1* (AR): Cognitive and neurological defects
✓SCN5: *VPS45* (AR): BM fibrosis, Nephromegaly
✓SCN6: *JAGN1* (AR)

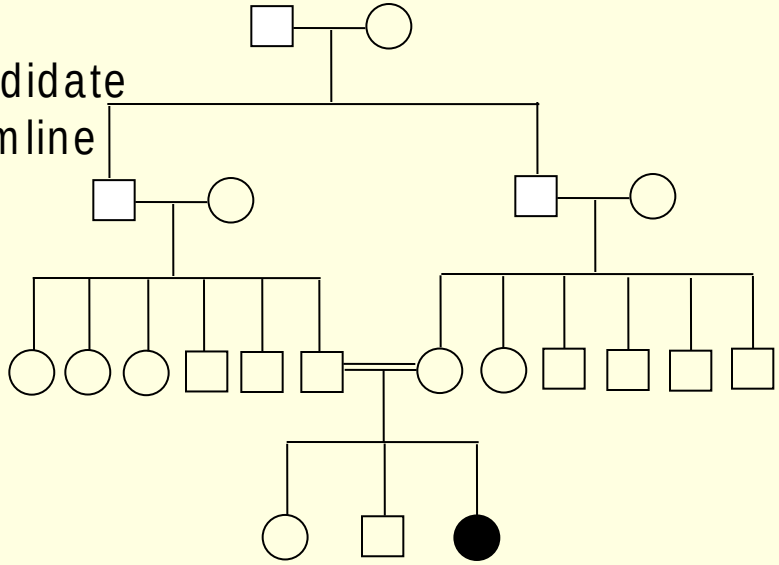
✓SCN4: *G6PC3* (AR): Structural heart defects, Urogenital abnormalities, Deafness, Venous Angiectasias

✓X-SCN: *WASP* (XL): Monocytopenia

HAX1 deficiency

✓HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease)

✓Using a positional cloning approach and candidate gene evaluation, a recurrent homozygous germline mutation in *HAX1* gene was found

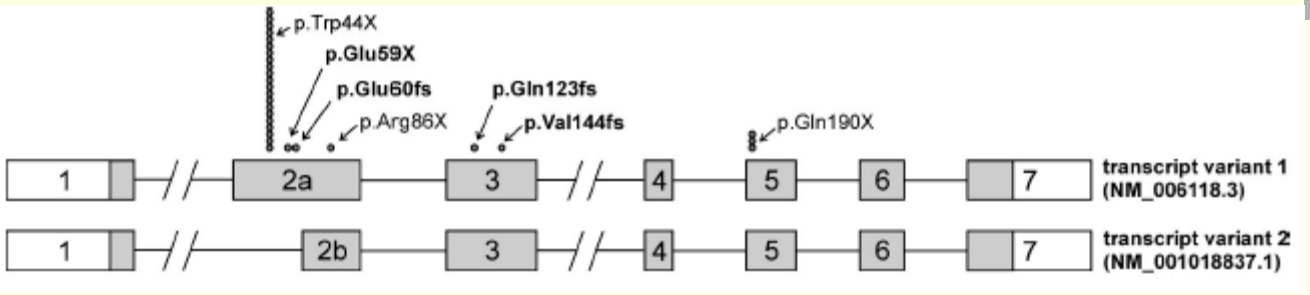


✓*HAX1* has a role in controlling the apoptosis

✓Mutant *HAX1* and also *ELA2* could accelerate apoptosis in myeloid progenitor cells of the patients

Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schaffer AA, Rathinam C, Boztug K, Schwinzer B, Rezaei N, Bohn G, Melin M, Carlsson G, Fadeel B, Dahl N, Palmblad J, Henter JI, Zeidler C, Grimbacher B, Welte K . HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet 2007; 39:86-92.

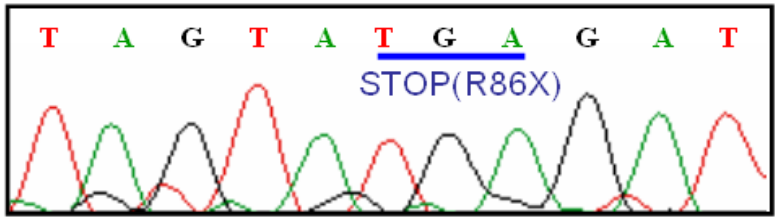
Genotype-Phenotype associations in HAX1 deficiency



- Mutations affecting transcript variant 1 only were associated with SCN, whereas mutations affecting both transcript variants caused CN and neurologic symptoms
- Transcript variant 2 was markedly expressed in human brain tissue
- The clinical phenotype of SCN appears to depend on the localization of the mutation and their influence on the transcript variants

** Germeshausen M, Grudzien M, Zeidler C, Abdollahpour H, Yetgin S, Rezaei N, Ballmaier M, Grimbacher B, Welte K, Klein C. Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. Blood 2008; 111(10): 4954-4957.*

- Neurological disorders
- R86X mutation in *HAX1* gene



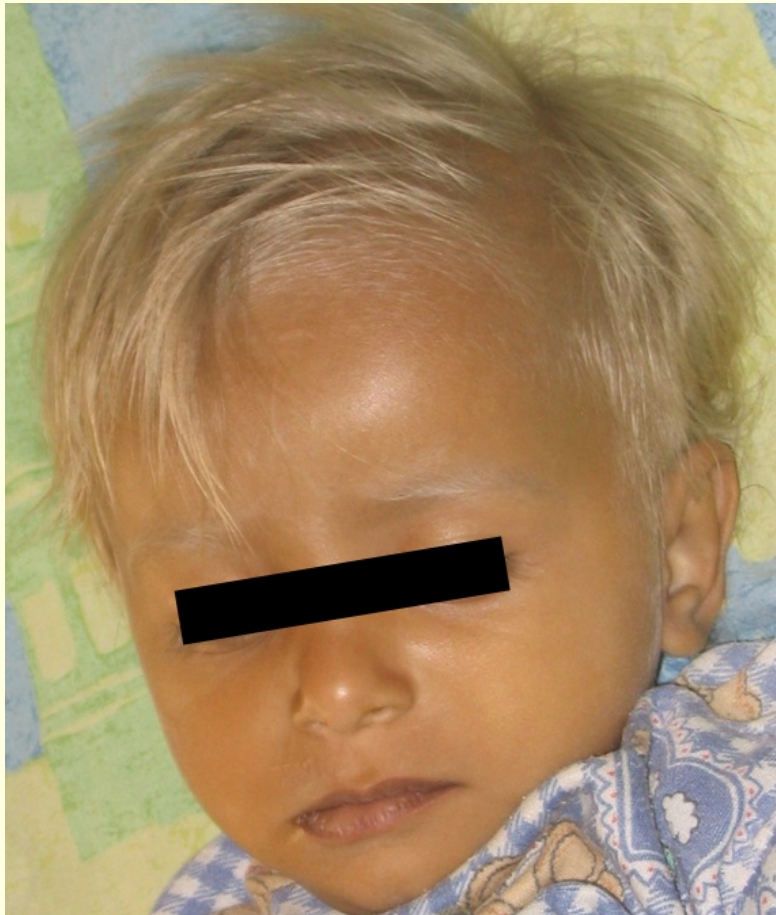
** Rezaei N, Chavoshzadeh Z, Alaei OR, Sandrock I, Klein C. Association of HAX1 deficiency with neurological disorder. Neuropediatrics 2007 Oct;38(5):261-263.*

Cyclic Neutropenia

- Oscillations of circulating neutrophil counts
- Neutropenia for 3-6 days with an average cycle lasting 21-days
- Severe infections during the neutropenic phases
- Cyclic anemia and monocytopenia

Oculocutaneous hypopigmentation and immunodeficiency

- Chédiak-Higashi syndrome
- Griscelli syndrome, type 2
- Hermansky-Pudlak syndrome, type 2
- p14 deficiency
- Pallidin deficiency
- Vici syndrome

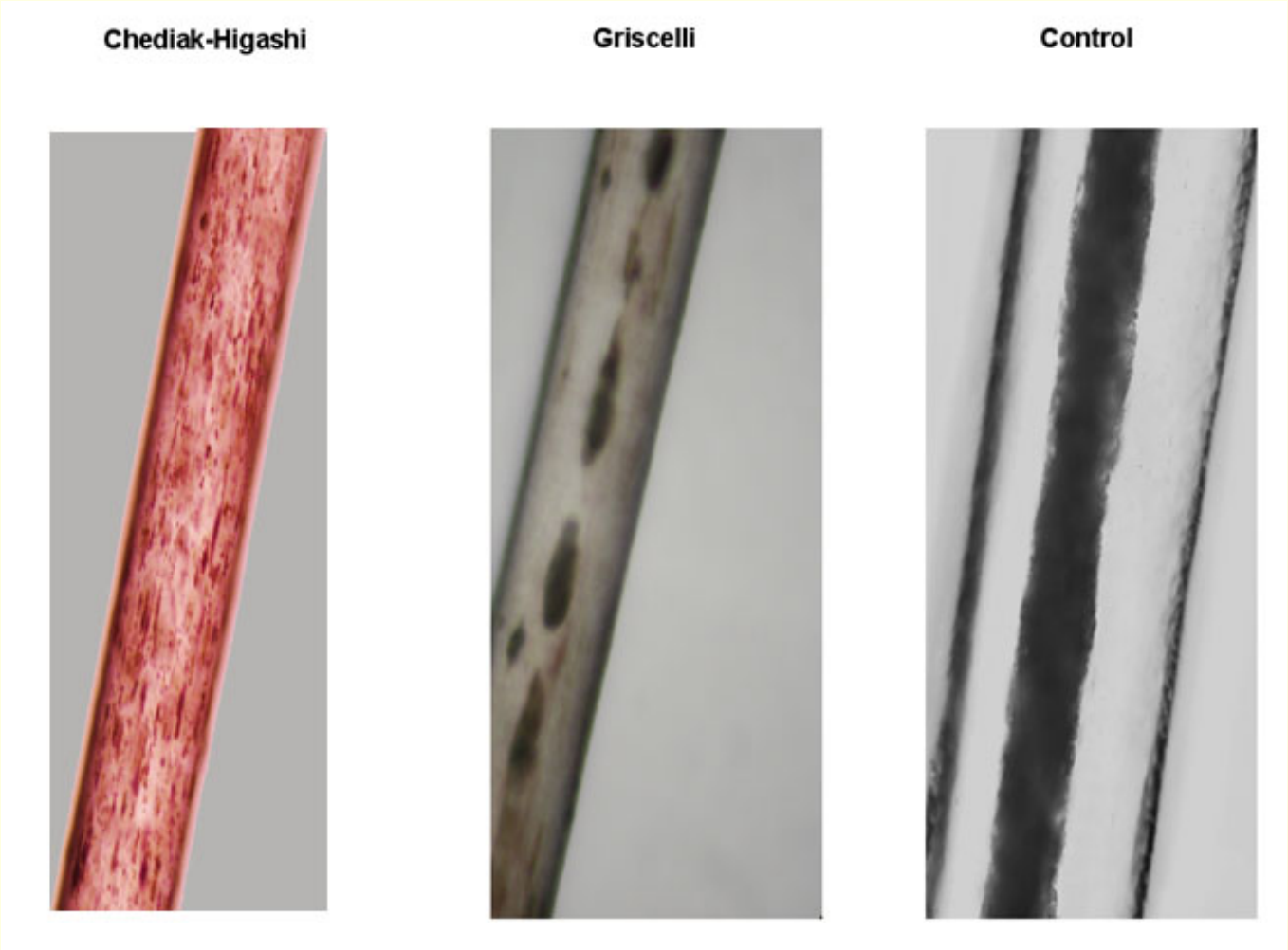


Oculocutaneous hypopigmentation in a patient with Griscelli syndrome, type 2 .

Characteristics of the immunodeficiency syndromes with hypopigmentation

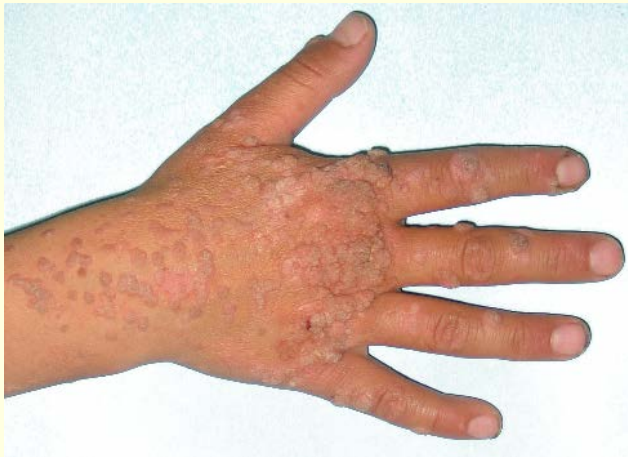
	Chédiak-Higashi syndrome	*Griscelli syndrome, type 2*	*Hermansky-Pudlak syndrome, type 2*	*p14 deficiency*
Hypopigmentation	*Variable*	*Variable*	*Prominent*	*Prominent*
Hair shaft findings	*Distributed regular melanin granules*	*Large irregular melanin granules*	*Normal or distributed small clumps of pigment*	-
Prominent facial features	-	-	+	+
Neutropenia	+	+/-	+	+
Bleeding disorder	+	-	+	-
Giant intracellular granules	+	-	-	-
Hemophagocytic lymphohistiocytosis	+	+	+/-	-
Neurological disorder	+	-	-	-
Pulmonary fibrosis	-	-	+/-	-
Developmental delay	+/-	-	+/-	-
Short stature	-	-	-	+

Light-microscopic hair shaft analysis of CHS and GS2 *vs.* control



WHIM syndrome

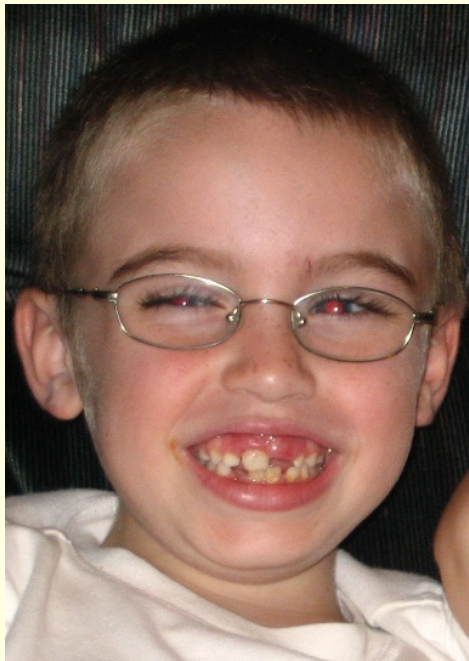
- *W*arts
- *H*ypogammaglobulinemia
- *I*nfections
- *M*yelokathexis : Neutrophils are retained in the bone marrow and not released into the peripheral blood stream



Wart lesions in a patient with WHIM syndrome.

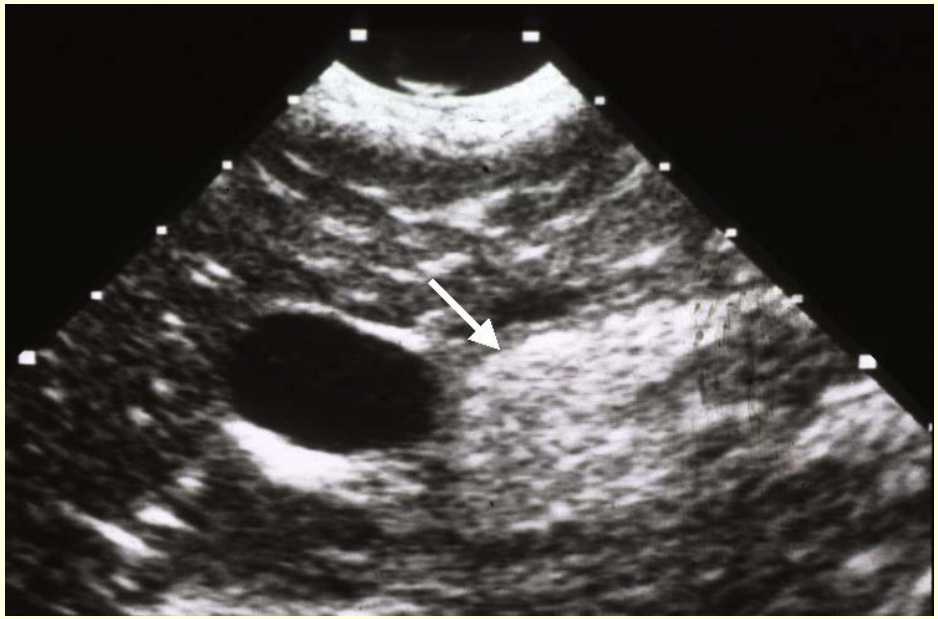
Shwachman-Diamond syndrome

- Congenital bone marrow failure syndrome
- Varying degrees of cytopenia
- A marked propensity to develop myelodysplastic syndrome and acute myelogenous leukemia
  - Exocrine pancreatic insufficiency
  - Broad spectrum of skeletal abnormalities
  - Growth retardation
  - Dental caries
  - Neurodevelopmental delay
  - Hepatic dysfunction

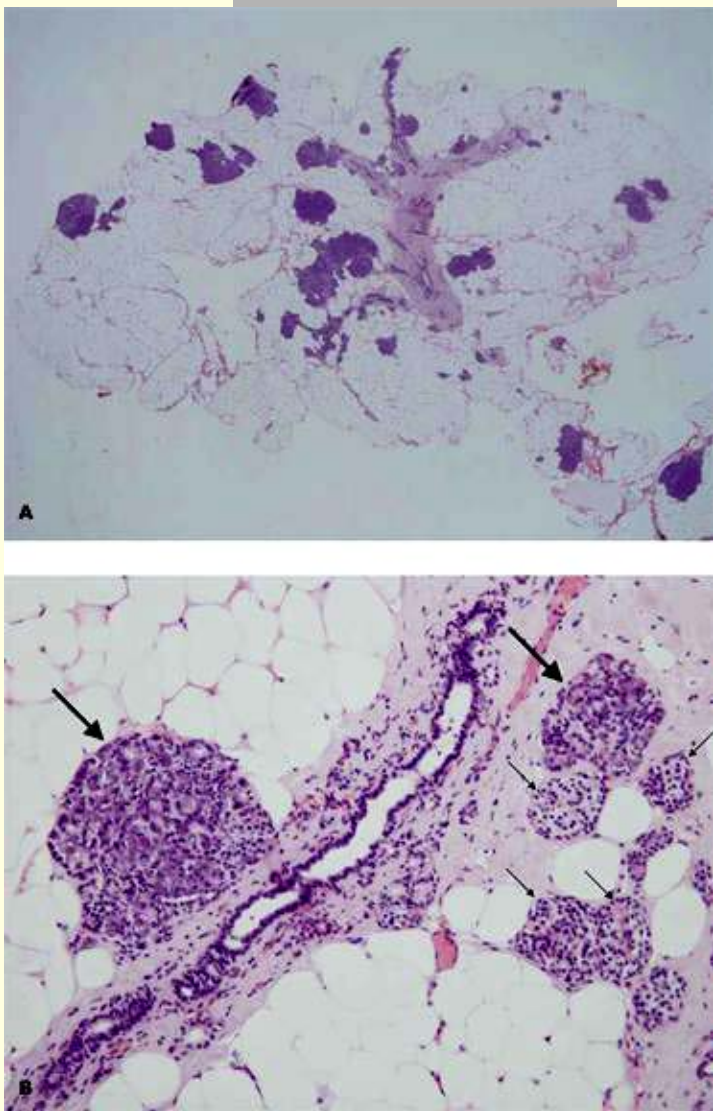


http://www.shwachmandiamondamerica.org

Shwachman-Diamond syndrome



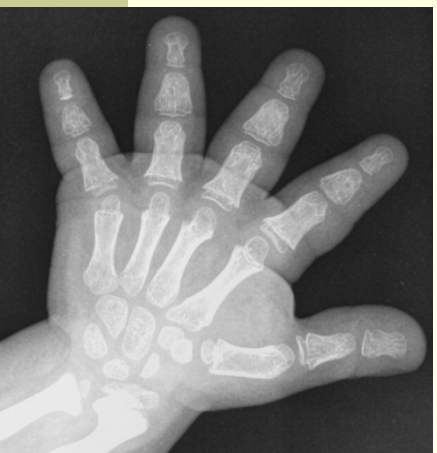
Abdominal sonography of a patient with SDS and typical "white" pancreas (arrows) due to lipomatosis

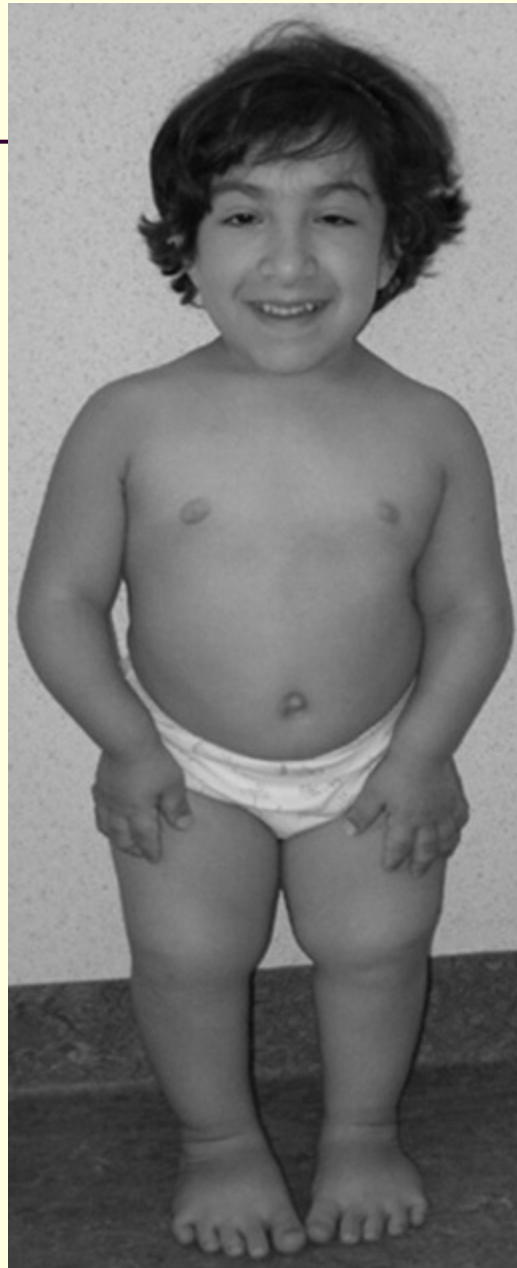


Typical histology of the pancreas of a patient with SDS. Note the extensive replacement of the exocrine pancreas by adipose tissue surrounding acini (large arrows) with remaining small islands of parenchyma (small arrows).

Cartilage hair hypoplasia

- Disproportionate short limbed short stature
- Metaphyseal chondrodysplasia
- Hypoplastic hair
- Macrocytic anemia
- Neuronal dysplasia of intestine
- Limited elbow extension
- Ligamentous laxity
- Predisposition to cancer





American Journal of Human Genetics

Other phagocyte defects associated with neutropenia

- Glycogen storage disease Ib (*G6PT1*)
  - * fasting hypogycemia, lactic acidosis, hyperlipidemia, osteopenia
  - * hepatomegaly
  - * growth retardation
- Barth syndrome (*TAZ*)
  - * heart failure (cardiomyopathy)
  - * skeletal myopathy
  - * growth retardation
  - * cognitive impairment
- Cohen syndrome (*COH1*)
  - * facial dysmorphisms, microcephaly, mental retardation, short stature
  - * hypotonia, obesity
  - * retinopathy
- Clericuzio syndrome poikiloderma with neutropenia (*C16ORF57*)
  - * progressive erythematous rash
  - * telangiectasia

Other PIDs associated with neutropenia

- CD40 ligand deficiency (X-linked HIGM)
  - * low levels of IgG, IgA and IgE
  - * normal or elevated IgM level
  - * respiratory and GI infections
  - * opportunistic microorganisms
- Reticular dysgenesis
  - * rare form of severe combined immunodeficiency
  - * absent numbers of lymphocytes and hypoplasia of lymphoid tissues
- Dyskeratosis congenita
  - * abnormal pigmentations
  - * hyperhidrosis
  - * nail dystrophy
  - * oral leukoplakia

Other PIDs associated with neutropenia

- Glycogen storage disease Ib
  - * metabolic symptoms
  - * hepatomegaly
  - * growth retardation
  - * osteopenia
- Barth syndrome
  - * heart failure
  - * growth retardation
  - * skeletal myopathy
  - * cognitive impairment
- Dyskeratosis congenita
  - * abnormal pigmentations
  - * nail dystrophy
  - * hyperhidrosis
  - * oral leukoplakia
- Cohen syndrome
  - * hypotonia
  - * mental retardation
  - * obesity
  - * microcephaly
  - * short stature
  - * characteristic facial features
- Poikiloderma with neutropenia
  - * early onset poikiloderma
  - * palmo-plantar hyperkeratosis
  - * pachyonychia
  - * skeletal defects
- Reticular dysgenesis
  - * rare form of severe combined immunodeficiency

Suspicious to congenital neutropenia

- Presence of neutropenia in association with early onset severe and recurrent infections

- Timely referral to a hematologist and/or clinical immunologist:
  * Early diagnosis
  * Appropriate treatment

Diagnosis

- Review of the clinical history:
  To rule out drug exposure and underlying illness such as autoimmune diseases

- Serial complete blood cell count (CBC):
  To determine the chronicity and severity
  To exclude other causes of secondary neutropenia

Further Steps

- Bone marrow examinations
- Immunological studies considering all immunodeficiency diseases associated with neutropenia
- Molecular studies to make definite diagnosis

Management

- In the absence of appropriate treatment, affected children suffer from life-threatening infections
- Bone marrow transplantation (BMT) used to be the only treatment option
- Since CSF therapy became available, it has became possible to manage patients without a requirement for BMT
- Recombinant G-CSF is the first choice of treatment for neutropenia
- G-CSF increases the number of neutrophils and consequently reduce the number of infections and days of hospitalization
- Prophylactic antibiotics should be prescribed
- Associate complications should be treated separately

Hematopoietic stem cell transplantation

- In SCN, HSCT is recommended in following cases with SCN:
* Those who do not respond to G-CSF treatment
* Those with continuing severe bacterial infections
* Those who complicated with development of myelodysplasia

- In SDS, HSCT should be offered to patients with
* Pancytopenia
* MDS
* Overt leukemia in remission

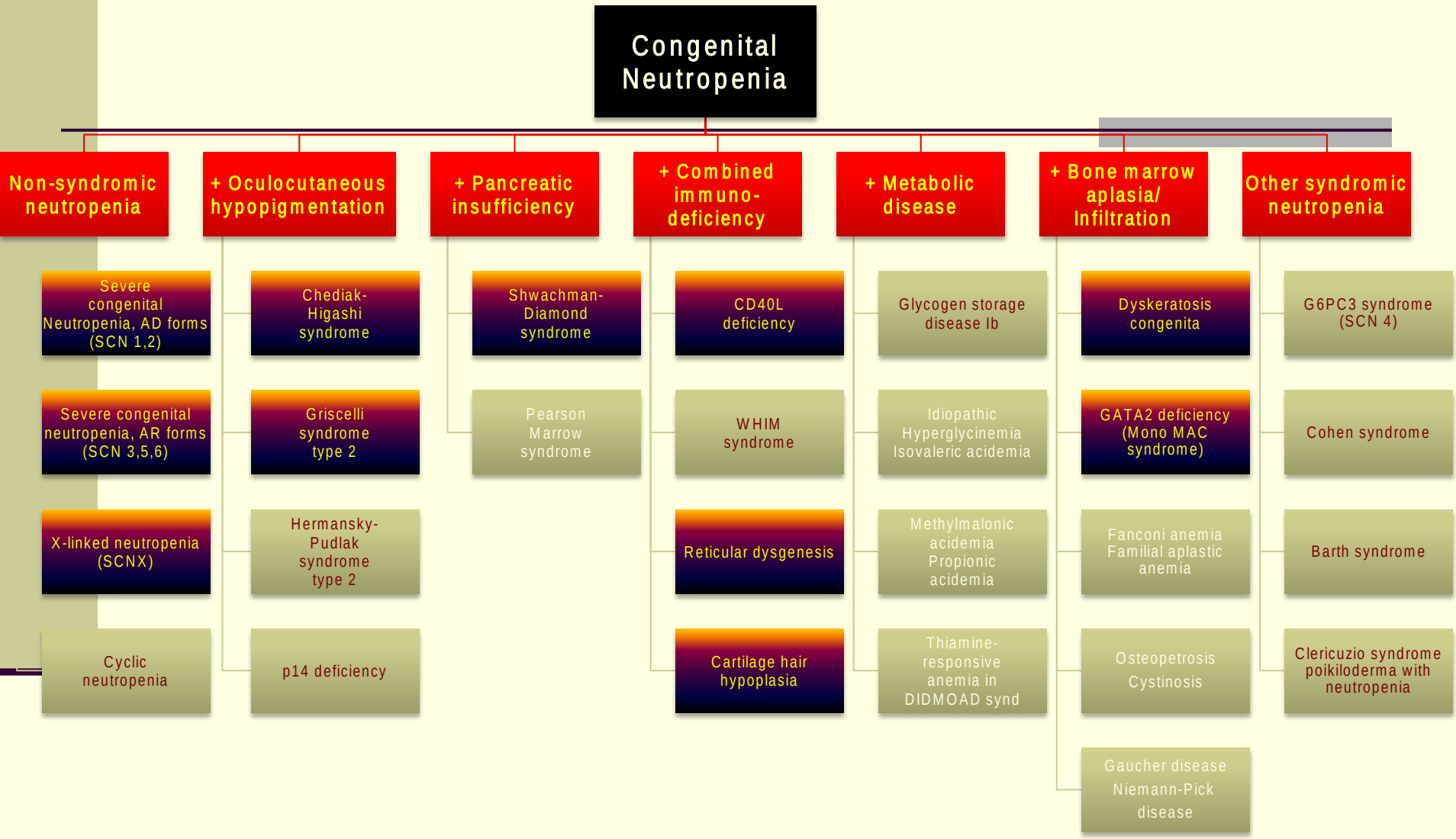
Follow-up

- Follow-up at least twice per year
- Dental follow-up and gingival care
- Complete blood cell counts at least every 3 months
- Repeat bone marrow examination (morphology plus cytology)
- G-CSF receptor analysis for SCN cases

Gene defects associated with various neutropenic phenotypes

CN variant	Congenital neutropenia	Osteopenia	Skeletal system (Growth delay/dysmorphic features)	Skin/Hair	Neurological system	Cardiovascular system	Urogenital system	Gastrointestinal system	Endocrine system	Adaptive immune system	Mutated gene
SCN-ELA2	•	•									*ELA2*
SCN-GFI1	•	•								•	*GFI1*
SCN-WAS	•									•	*WAS*
SCN-HAX1	•	•			•						*HAX1*
SCN-AK2	•				•					•	*AK2*
Glycogenosis Ib	•	•	•			•		•	•		*SLC37A4*
G6PC3 deficiency	•		•	•		•	•	•			*G6PC3*
Barth syndrome	•					•					*TAZ*
SBDS	•	•	•		•	•		•		•	*SBDS*
CHH	•	•	•	•	•			•		•	*RBDS*
CHS	•		•	•	•					•	*LYST*
GS type II	•			•						•	*RAB27A*
HPS II	•		•	•						•	*AP3B1*
P14-deficiency	•		•	•						•	*ROBL3*
Cohen syndrome	•		•	•							*COH1*
Poikiloderma with neutropenia	•		•	•							*C16orf57*
Neutropenia-CMT-II	•				•						*DNM2*
Pearson syndrome	•				•	•	•	•	•		*Mitochondrial DNA*

HSCT in congenital neutropenia



Approach to Congenital defects of phagocytes

Recurrent respiratory and cutaneous infections, ulcers and abscesses

- **Normal ANC**
  - **Defective NBT/DHR**
    - X-CGD (CYBB)
    - AR-CGD (CYBA, NCF1, NCF2, NCF4)
  - **Defective Chemotaxis**
    - **Impaired wound healing, Leukocytosis**
      - **Low CD18:** LAD 1
      - **Mental retardation, hh blood group:** LAD 2
      - **Bleeding tendency:** LAD 3
      - RAC2 deficiency
    - **Mainly periodontitis**
      - Localized juvenile periodontitis
      - **Palmoplantar Hyperkeratosis:** Papillon-Lefèvre syndrome
    - **Other motility defects**
      - **Mental retardation, Short stature:** β actin deficiency
      - Specific granule deficiency
- **Severe Persistent Neutropenia**
  - **Non-syndromic neutropenia**
    - AD-SCN (ELANE, GFI1)
    - AR-SCN (HAX1, VPS45, JAGN1)
    - X-linked neutropenia (WASP)
    - Cyclic neutropenia
  - **Syndromic neutropenia**
    - **Albinism, Hypogammaglobulinemia:** p14 (LAMTOR2) deficiency
    - **Pancreatic Insufficiency:** Shwachman-Diamond syndrome
    - **Metabolic symptoms, Hepatomegaly:** Glycogen storage disease Ib
    - **Mycobacteria, Alveolar proteinosis:** GATA2 deficiency (Mono MAC syndrome)
    - **Structural heart defects, Urogenital abnormalities, Venous Angiectasias:** G6PC3 syndrome (SCN 4)
    - **Facial dysmorphisms, Hypotonia, Retinopathy:** Cohen syndrome
    - **Cardiomyopathy, Skeletal myopathy:** Barth syndrome
    - **Progressive erythematous rash, Telangiectasia:** Clericuzio syndrome poikiloderma with neutropenia

Molecular Diagnosis for Neutropenia

http://chmc.tums.ac.ir
http://rcid.tums.ac.ir

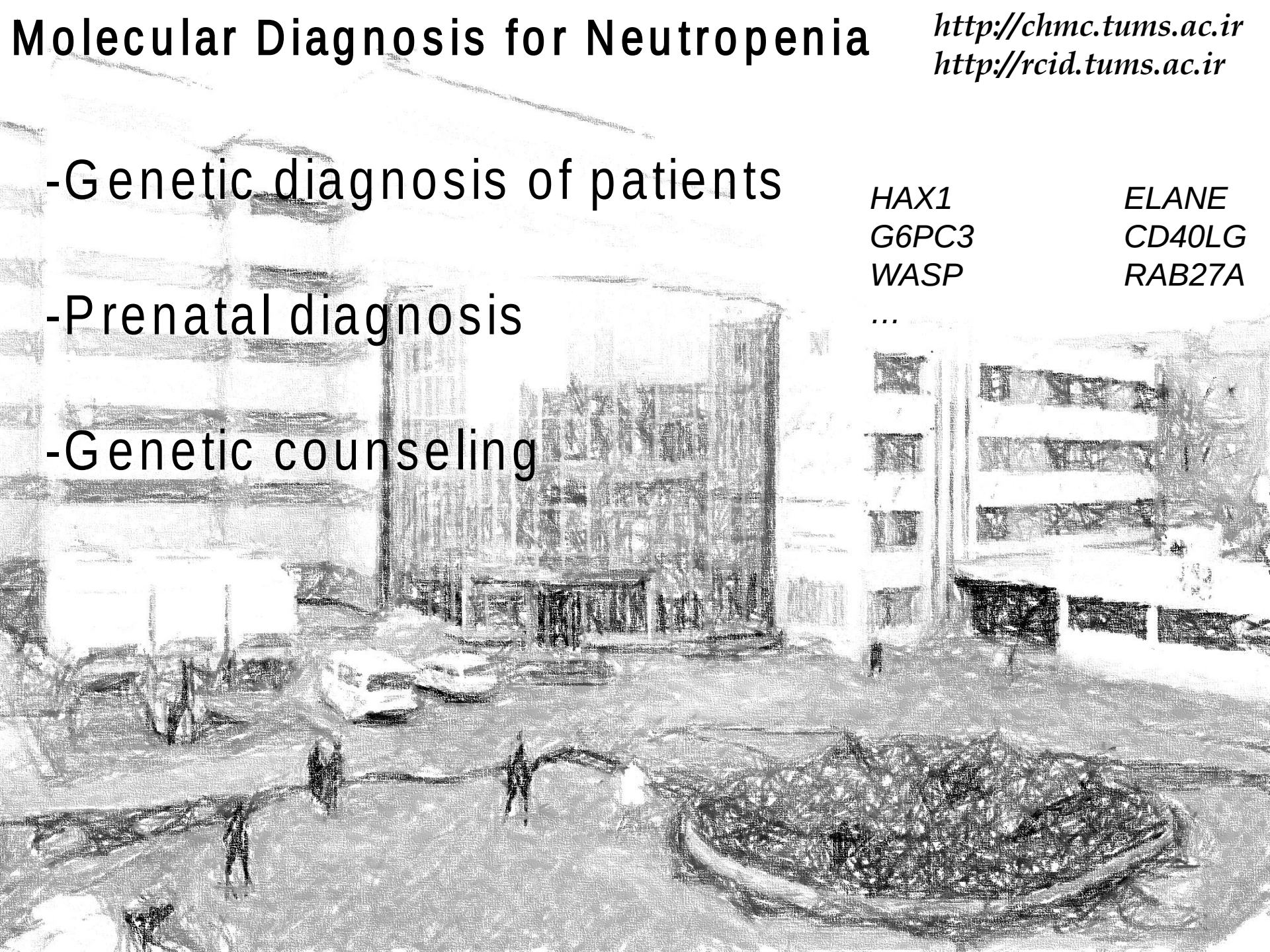
-Genetic diagnosis of patients

-Prenatal diagnosis

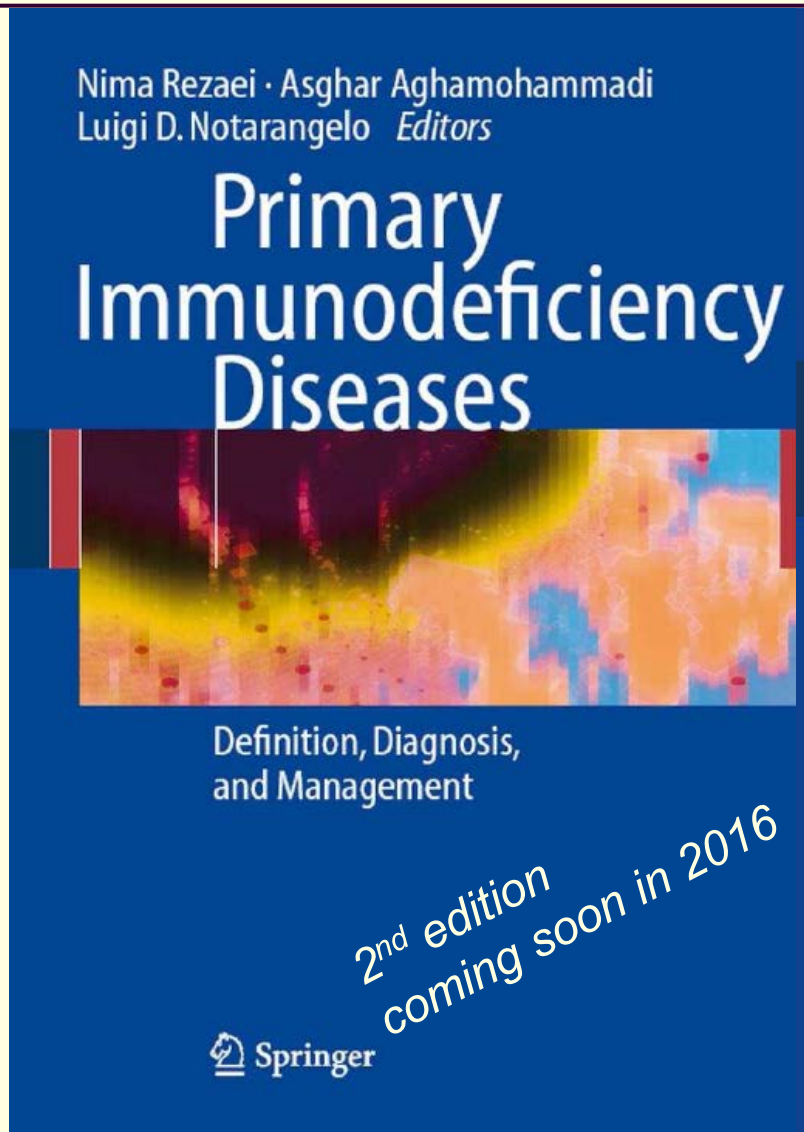
-Genetic counseling

HAX1
G6PC3
WASP
...

ELANE
CD40LG
RAB27A



References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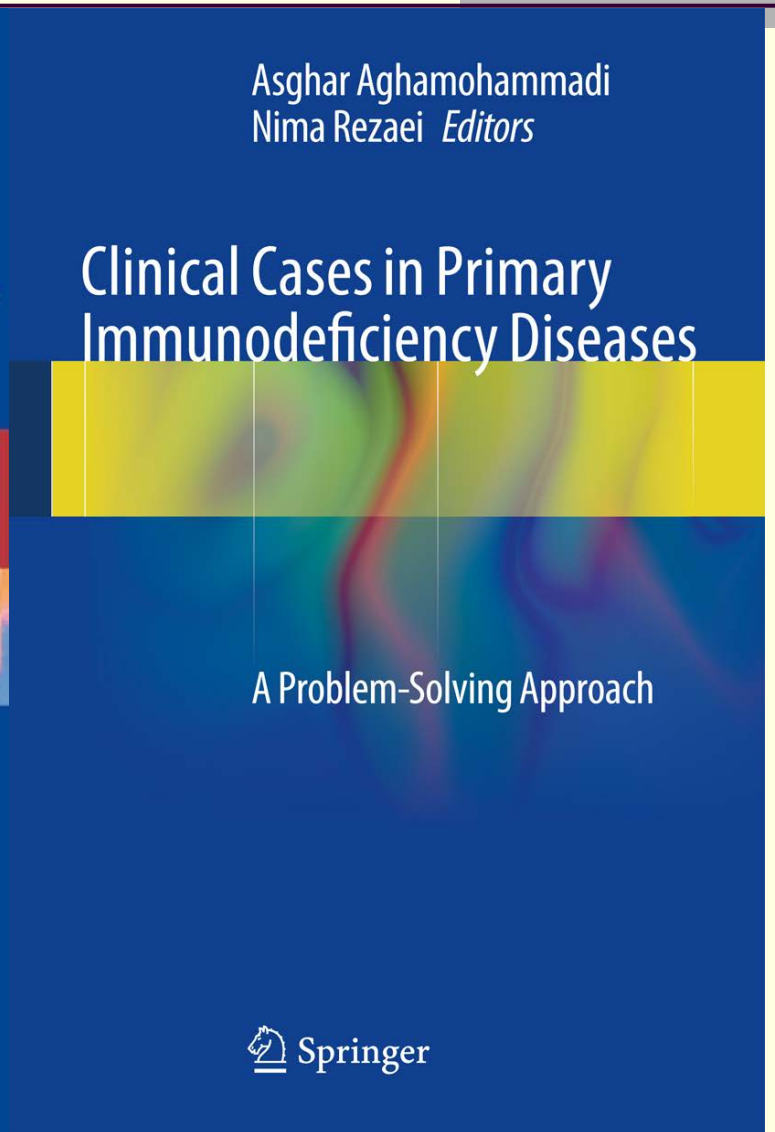
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