

# New insights into the genetics of congenital neutropenia

*Konjenital nütropenilere genetik bakış*

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## **Abstract**

Several congenital disorders may cause neutropenia. The recent advances in the field of molecular biology have facilitated our knowledge concerning pathophysiological mechanisms leading to these disorders. The molecular basis of disorders with defective myelopoiesis (severe congenital neutropenia and cyclic neutropenia) and disorders due to defective RNA processing (Shwachman Diamond syndrome and cartilage hair hypoplasia) are the two congenital neutropenia syndromes that are addressed in this review. Molecular defects defined by means of these disorders are very important for our understanding of the cause of the disorders as well as some unknown molecular mechanisms.

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**Key words:** Congenital neutropenia, molecular basis, myelopoiesis, RNA processing

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## **Özet**

Bir çok konjenital hastalık nütropeni nedeni olabilir. Moleküler biyoloji alanındaki son gelişmeler bu hastalıklara yol açan patofizyolojik mekanizmalar hakkındaki bilgilerimize katkıda bulunmuştur. Bu makalede iki grup konjenital nütropeni sendromunun moleküler temelleri üzerinde duruldu; bozuk miyelopoezle gidenler (ağır konjenital nütropeni ve döngüsel nütropeni) ve RNA işlenmesindeki bozukluğa bağlı olanlar (Shwachman Diamond sendromu ve kıkırdak saç hipoplazisi). Bu hastalıklarda tanımlanan moleküler bozukluklar hem hastalıkların nedenlerinin, hem de moleküler biyoloji alanında bilinmeyen bazı mekanizmaların anlaşılmasında önem taşımaktadır. *(Turk J Hematol 2009; 26: 1-8)*

**Anahtar kelimeler:** Konjenital nütropeni, moleküler temeller, miyelopoezis, RNA işlenmesi

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Neutropenia is an important condition that brings about a tendency to severe, sometimes life-threatening infections. The cause of neutropenia can be congenital or acquired. Recently, major advances have occurred in the field of molecular genetics concerning congenital neutropenia (CN) that will be addressed in this review. Common causes of CN and related mutations are shown in Tables 1 and 2, respectively [1]. Most of these disorders are inherited in an autosomal recessive trait that increases the number of such patients in countries like Turkey, where consanguineous marriages are frequent.

## Congenital neutropenia with defective myelopoiesis

### Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) was identified by Kostmann, a Swedish pediatrician, in 1956 as “a new recessive lethal disease in man” (Kostmann syndrome) [2]. However, it has been understood that only one-third of patients inherited the disease as a recessive trait. Therefore, SCN was proposed as a general name of a group of disorders with different inheritance patterns caused by mutations in different genetic loci. The most characteristic clinical finding is recurrent, severe bacterial infections beginning from birth, such as abscesses of perirectal skin or liver, omphalitis, otitis media, and upper and lower respiratory tract infections, caused by common or uncommon bacterial agents [3,4]. Orodental problems, such

as gingival hyperplasia, which may lead to premature loss of permanent teeth, and aphthous stomatitis are frequently seen among patients with SCN. Splenomegaly has been observed in 20% of patients at first admission or after treatment with granulocyte-colony stimulating factor (G-CSF). Most patients have absolute neutrophil counts (ANC) of less than 200/mm<sup>3</sup>. Other features in the peripheral blood include mild anemia, thrombocytosis, monocytosis and eosinophilia. Increased serum immunoglobulin levels, especially Ig G, were reported in a group of patients from Iran [5]. Bone marrow of patients with SCN characteristically reveals a “maturation arrest” at the promyelocyte/myelocyte stage. As in the peripheral blood, monocytosis and eosinophilia may also be present in the bone marrow. Molecular studies concerning the etiology of SCN revealed mutations in a few genetic loci (Table 2).

### Cyclic Neutropenia

Cyclic neutropenia (CyN) refers to an autosomal dominant disorder with characteristic oscillations in neutrophils in approximately 21 days [1]. The disease was defined in 1910 in an infant who had recurrent fever, furunculosis, and severe neutropenia [6]. During the nadir of ANC (<500/mm<sup>3</sup>) for 3-5 days, aphthous stomatitis, periodontitis and other pyogenic infections may take place. There are also detectable oscillations in reticulocytes and platelets and sometimes of eosinophils and lymphocytes from normal to high levels during neutropenia. In contrast to SCN, no tendency to malignancy has been defined in patients with CyN.

**Table 1. Common causes of congenital neutropenia**

1. Severe congenital neutropenia
2. Cyclic neutropenia
3. Familial benign (ethnic) neutropenia
4. Cohen syndrome
5. Neutropenia associated with immune deficiency <ul style="list-style-type: none"> <li>a. Shwachman Diamond syndrome</li> <li>b. Cartilage hair hypoplasia (chondrometaphyseal dysplasia)</li> <li>c. Myelokathexis/WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) syndrome</li> <li>d. X-linked agammaglobulinemia (Bruton's disease)</li> <li>e. Hyper IgM syndrome</li> <li>f. Reticular dysgenesis</li> <li>g. Dubowitz syndrome</li> <li>h. Cellular immune deficiency</li> </ul>
6. Neutropenia associated with oculocutaneous albinism or lysosomal defects <ul style="list-style-type: none"> <li>a. Hermansky Pudlak syndrome Type 2</li> <li>b. Chediak Higashi syndrome</li> <li>c. Griscelli syndrome</li> </ul>
7. Neutropenia associated with metabolic disorders <ul style="list-style-type: none"> <li>a. Glycogen storage disease Type 1b</li> <li>b. Idiopathic hyperglycinemia</li> <li>c. Organic (isovaleric, methyl malonic, propionic) acidemia</li> <li>d. Barth syndrome</li> </ul>

**Table 2. Genetic mutations related to congenital neutropenia**

Disorder	MIM#	Inheritance	Chromosomal locus	Gene	Diagnostic Features and Mechanism of Disease
SEVERE CONGENITAL NEUTROPENIA	202700	AD	19p13.3	ELA2	Increased apoptosis of early granulocytes caused by defective transport of mutant elastase (see text)
	608233	AR	5q14.1	AP3B1	Hermansky-Pudlak syndrome Type 2; Increased apoptosis of early granulocytes caused by defective transport of elastase due to APB3 mutations (see text)
	600871	AD	1q22	Gfi1	Increased apoptosis of early granulocytes caused by increased elastase levels due to defect in transcriptional repression of ELA2 gene by Gfi1 (see text)
	605998	AR	1q21.3	HAX1	Increased apoptosis due to impaired mitochondrial membrane stability (see text)
	138971	AR	1p35-p34.3	CSF3R	Acquired mutation; Activation of STAT5 due to truncation of G-CSFR may cause leukemic transformation (see text)
	300299	X-R	Xp11.23	WAS	Neutropenic variant of Wiskott-Aldrich syndrome (WAS) [55]; Decreases the stability of the autoinhibited structure of WAS protein, resulting in activation of actin; Increased numbers of CD3+/CD8+/CD57+ T lymphocytes may mediate myelosuppression
Cyclic neutropenia	162800	AD	19p13.3	ELA2	Excessive inhibition of myelopoiesis due to mutant elastase (see text)
Chediak-Higashi syndrome	214500	AR	1q42.1-q42.2	LYST	Partial albinism, giant lysosomes in peripheral blood cells, impaired platelet function; Caused by mutation in the lysosomal trafficking regulator gene <i>LYST</i> [56]; Tendency to malignancy
Cohen syndrome	216550	AR	8q22-q23	COH1	Mental retardation, microcephaly, characteristic facial features, childhood hypotonia and joint laxity, a cheerful disposition, and intermittent isolated neutropenia [57]; Disturbance in intracellular vesicle sorting and protein transport
Griselli syndrome Type 2	607624	AR	15q21	RAB27A	Partial albinism, frequent pyogenic infections, and acute episodes of fever, neutropenia, and thrombocytopenia [58]
Shwachman-Diamond syndrome	260400	AR	7q11	SBDS	Defective RNA processing (see text)
Cartilage hair hypoplasia	250250	AR	9p21-p12	RMRP	Defective RNA processing (see text)
Barth syndrome	302060	X-R	Xq28	TAZ	Dilated cardiomyopathy, skeletal myopathy, abnormal mitochondria, and neutropenia; Inhibition of acyl-specific remodelling of cardiolipin results in changes in mitochondrial architecture and function [59]
X-linked agammaglobulinemia	300300	X-R	Xq21.3-q22	BTK	Bruton disease; Decreased neutrophil numbers when rapid production is needed; Due to mutation in BTK (Bruton tyrosine kinase) gene, one of the genes responsible for myelopoiesis [60]
WHIM syndrome	193670	AD (?)	2q21	CXCR4	Warts, Hypogammaglobulinemia, tendency to Infections, and Myelokathexis that presents as neutrophils with cytoplasmic vacuoles and hypersegmented nuclei with dense, pyknotic lobes connected by long filaments; Caused by truncating mutations in the cytoplasmic tail domain of the gene encoding chemokine receptor-4 [61]
Hyper IgM Type 1	308230	X-R	Xq26	CD40Lgene	High IgM levels with absence of IgG, IgA, and IgE, resulting in a profound susceptibility to bacterial and opportunistic infections, increased frequency of autoimmune neutropenia; Caused by defects in either CD40 or CD40L (ligand) [62,63]
Hyper IgM Type 3	606843	AR	20q12-q13.2	CD40 gene	
Glycogen storage disease Type 1b	232220	AR	11q23	G6PT gene	Recurrent infections, neutropenia, chronic inflammatory bowel disease that resolves with G-CSF treatment; Neutrophils are also defective in both motility and respiratory burst functions [64]

**MIM:** Mendelian Inheritance in Man; **AR:** Autosomal recessive; **AD:** Autosomal dominant; **X-R:** X-linked recessive; **G6PT:** Glucose-6-phosphate translocase

### Mutations related to perturbation of intracellular trafficking of elastase

The first attributed locus was the elastase (ELA2) gene. After studies showing a relationship between ELA2 mutations and CyN, the same relationship with SCN was first shown in 2000 [7]. In contrast with the first description as a “recessive disease” by Kostmann, germ-line dominant inheritance of ELA2 mutations was shown for both SCN and CyN patients. In vivo evidence supporting germ-line dominant inheritance in a report revealed five SCN cases with the same ELA2 mutation born to four mothers whose ELA2 genes were all wild-type. The mothers were impregnated by same-donor sperm obtained from a sperm bank, suggesting inheritance of mutation from the father [8]. Studies concerning the role of elastase in the pathophysiology revealed that myeloid precursors having mutated elastase are damaged in the early stages of myeloid differentiation; therefore, no mature myeloid cells develop in the bone marrow and peripheral blood of these patients. The father of a SCN patient who was mosaic for ELA2 mutation is another in vivo evidence [9]. Peripheral blood analysis showed that he had mutated and wild type ELA2 genes in T lymphocytes; however, there was no neutrophil bearing mutated gene, indicating that the neutrophils with mutated gene were eliminated during myeloid maturation. The authors suggested this finding as the first in vivo confirmation of the pathogenic nature of ELA2 mutations in humans. Mutation of the ELA2 gene is the most frequent molecular marker in patients with SCN, accounting for nearly half of all patients [10]. However, this mutation was not present in patients with the autosomal recessive form of SCN (Kostmann syndrome), supporting the existence of different inheritance patterns.

Neutrophil elastase, a serine protease, is normally located within azurophil granules of neutrophils and degrades Gram-negative bacteria and some other proteins such as coagulation proteins, growth factors, extracellular matrix proteins, immunoglobulins, and complement proteins, as well as PML-RAR $\alpha$  fusion protein [11]. Although a relationship between ELA2 mutations and SCN has been shown, mice deficient in elastase had normal granulopoiesis, suggesting that these mutations cause a gain rather than a loss of function [12]. However, these mice were more susceptible to Gram-negative bacterial sepsis and death but not to Gram-positive bacteria.

Mutations in the ELA2 gene result in disruption of elastase procession, preventing its transport to azurophil granules. The pathophysiological mechanism caused by ELA2 mutations has been explained in two ways. The first explanation [13] is based on evidence that the carboxy tail of elastase should be processed for its transport by adaptor protein 3 (AP3) to azurophil granules. Mutations in the ELA2 gene in SCN patients usually affect the carboxy terminal of elastase where it binds to AP3 [7]. Mutations in the binding site of AP3 also cause CN in patients with Hermansky-Pudlak syndrome type 2 (HPS2) [14]. There have been only four patients from three families diagnosed with HPS2. This syndrome, with typical findings including hypopigmentation and neutropenia, is equivalent to that found in three different species: gray collies, ruby drosophila and pearl mice [13]. In the case of SCN or HPS2, it is suggested that mutations disrupting the AP3 recognition signal cause overexpression of elastase erroneously localized in the plasma membrane. Another factor for increased overexpression of elastase is mutations in the transcriptional repressor of

that ELA2 gene, Gfi1, that lead to accumulation of elastase in the plasma membrane due to overproduction [15]. Gfi1 normally represses the expression of many other genes as well as of ELA2 [16]. Taken together, in this explanation, the authors suggest that overexpression of elastase in the plasma membrane is caused by “excessive routing of neutrophil elastase to the plasma membrane” due to mutations in ELA2, AP3 or Gfi1 genes. This overexpression occurring in myeloid precursors may cause loss of these cells, which results in the “maturation arrest” observed in the bone marrow of SCN patients.

The second explanation of the pathogenesis of SCN suggests that ELA2 mutations lead to accumulation of elastase within the cytoplasm, not in the membrane, of myeloid precursors [17]. The investigators used an inducible cell culture-based system to express a panel of ELA2 mutations and found that there was a disruption of intracellular elastase processing at different levels due to different mutations, as suggested in the above-mentioned studies. However, immunofluorescence studies revealed that the mutant elastase was localized within cytoplasm, and co-localized with calnexin, a marker of endoplasmic reticulum (ER). Furthermore, an identical pattern of elastase accumulation has also been shown through analysis of granulocytes from patients with SCN. Finally, the authors showed increased apoptosis in cells expressing mutant elastase associated with findings of “unfolded protein response” (UPR). In normal circumstances, neutrophil elastase is folded within ER like many other proteins. Accumulation of unfolded mutant elastase in ER triggers UPR, a coordinated adaptive response in order to relieve stress in ER [18]. The aim of this reaction is to reduce misfolded proteins within the ER. During this task, three transmembrane ER proteins, PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 (IRE1), regulate three main reactions to reduce misfolded proteins. Under normal circumstances, these three transmembrane ER proteins are associated with an ER chaperone protein, Bip/GRP78, that maintains them in an inactive state. During ER stress caused by misfolded proteins, Bip/GRP78 preferentially associates with misfolded proteins, a reaction that finally unbinds transmembrane ER proteins. If the ER stress is severe and these proteins are inadequate to reduce the stress, UPR triggers apoptosis [19]. Therefore, the authors suggested that mutant elastase within ER (in the cytoplasm) causes UPR and finally apoptosis of early myeloid cells.

Why mutations at the same genetic locus cause two different clinical pictures, SCN and CyN, is not yet clear. One explanation is that the mutations in the ELA2 gene occupy different sites in patients with CyN and SCN. It has been reported that in CyN, mutations usually affect one of three different positions in intron 4 [20]. They disrupt the normal splice donor site at the end of the 4th exon, causing use of a cryptic, upstream splice acceptor site. That results in the internal inframe deletion causing synthesis of a stumpy protein. However, in patients with SCN, chain-terminating mutations are prevalent and intron 4 mutations are very rare. The molecular pathophysiology of CyN is not well understood. The defect is in the stem cell, as it was observed in an in vivo example. A report of a patient with leukemia in relapse who was transplanted from her HLA-identical sibling suffering from CyN resulted in the same oscillations in neutrophils in the recipient [21]. It was hypothesized that if the normal inhibitory feedback from neutrophils to regulate myelopoiesis is in extreme inhibition, myeloid maturation stops

and neutropenia occurs. But this suppression is a temporary event that leads to subsequent myelopoietic activity [22]. A neutrophil membrane extract, CAMAL (common antigen of myelogenous leukemia), has been shown to have this inhibitory property [23]. Elastase is an active component of CAMAL, supporting a role for mutant elastase in the extreme inhibition of myelopoiesis observed in CyN [24].

#### Mutations related to mitochondrial membrane stability

Since ELA2 mutations are responsible for approximately half of the patients with SCN and at least two forms of inheritance have been described by clinical studies, recent research has been focused on finding new mutations that cause this phenotype. In the search of a gene responsible for the recessive inheritance observed in 20 Middle Eastern children mostly from Turkey and three Swedish children from the original Kostmann family, a new genetic locus at chromosome 1q21.3, HAX1, was linked to SCN [25]. The HAX1 gene protein product is involved in stabilizing the mitochondrial membrane potential [26]. HAX1-deficient neutrophils have revealed increased spontaneous and tumor necrosis factor (TNF) $\alpha$ - or H<sub>2</sub>O<sub>2</sub>-induced apoptosis [25]. Reconstitution of the cellular phenotype of HAX1-deficient cells by retroviral wild-type gene transfer resulted in a decrease in the rate of apoptosis in cell cultures. Since HAX1 is a ubiquitously expressed gene, the authors searched non-hematopoietic cells for apoptosis in HAX1-deficient patients. Interestingly, fibroblasts from patients showed a more rapid loss of their membrane potential after exposure to valinomycin compared to those from healthy donors. Their conclusion on the basis of myeloid-specific apoptosis was that this effect might be due to intrinsic differences in the molecular control of apoptosis in neutrophils compared with other cell types. HAX1 mutation is responsible for approximately 30% of SCN cases. Molecular analysis showed that the same mutation in the HAX1 gene was present in patients from Turkey, Iran and Lebanon (W44X). Splenomegaly was a prominent clinical feature in these patients. However, the Kostmann family members had a different mutation (Q190X) in the HAX1 gene. None of these patients had ELA2 mutation.

#### Mutations related to transcriptional activation

Recently, lymphoid enhancer-binding factor (LEF-1) has been shown to play a key leading role in the regulation of proliferation and differentiation of myeloid cells [27]. It acts as an architectural transcription factor that regulates expression of many genes, including ELA2, by bending helical phasing of transcription binding sites [28]. Compared to healthy controls, LEF-1 mRNA expression was low in promyelocytes derived from SCN patients. It has been shown that LEF-1 is a transcriptional activator of both the ELA2 gene and the C/EBP $\alpha$  gene, which is also a transcriptional activator of the ELA2 gene. Analysis of CD33<sup>+</sup> bone marrow cells from patients with SCN showed significant decrease in mRNA levels of LEF-1 and its target genes cyclin D1, c-myc, survivin C/EBP $\alpha$ , and ELA2, those involved in survival, proliferation and differentiation (Figure 1). Importantly, reconstitution of LEF-1 in early hematopoietic progenitors corrected the defective myelopoiesis in two individuals with SCN. It has been suggested that LEF-1 is a decisive factor for the regulation of myelopoiesis and its absence plays a critical role in the defective myeloid maturation in individuals with CN.

#### Malignant transformation and G-CSF receptor mutations

In SCN patients, cumulative incidence of leukemia has been reported as 21% after 10 years [29]. Incidence of leuke-

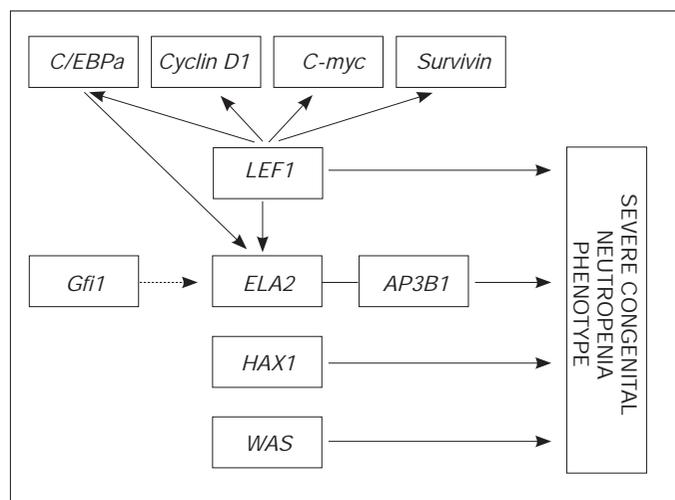
mia is elevated in patients who need higher (more than 8  $\mu$ g/kg) doses of G-CSF (40%) compared to those who need lower doses (11%). Leukemic transformation occurred in patients with both ELA2 and HAX1 mutations [30,31]. Patients with RAS and G-CSF receptor (G-CSFR) gene mutations, monosomy 7, and Down syndrome are at high risk of developing leukemia. Recently, abnormal sensitivity and clonal expansion of cells with monosomy 7 in response to G-CSF has been reported [32].

Mutations in the G-CSFR gene are deemed to be acquired since they have not been detected at birth in any patient with SCN. This mutation has been found only in patients with SCN, and not in any other form of CN. Incidence of G-CSFR mutations is remarkably high in SCN patients who developed leukemia (80%) compared to those without leukemia (30%), which suggests a role for this gene in leukemogenesis [33]. Interestingly, the loss of mutated clone after cessation and regrowth after reinduction of G-CSF treatment has been reported. Mutations mostly result in a truncated G-CSFR protein that activates STAT5, a signaling protein known to be involved in leukemogenesis [34].

### Congenital neutropenia due to defective RNA processing

#### Shwachman Diamond syndrome

Shwachman Diamond syndrome (SDS) is an autosomal recessive disorder that mainly affects two organs, the bone marrow and pancreas, with a strong propensity to malignancy. It was described by Shwachman and Diamond [35] in 1964 and subsequently by Bodian et al. [36]; thus, Shwachman Bodian Diamond syndrome (SBDS) is a synonym for this syndrome. Clinical manifestations begin from early infancy, including failure to thrive and steatorrhea due to exocrine pancreatic insufficiency; however, steatorrhea may resolve in half of the patients with ageing [37]. Serum isoamylase level is low at all ages and trypsinogen level is low below the age of three years. Pathologic and imaging studies show fatty replacement of pancreatic tissue. Elevated liver enzymes may accompany the clinical picture frequently during the first two years, after which, as with steatorrhea, they normalize with age. Neutropenia



**Figure 1.** Schematic representation of genetic interactions in SCN. Except for Gfi1, all the arrows represent activating effect on target genes.

(ANC <1500/mm<sup>3</sup>) is found in the majority of patients, being intermittent in two-thirds and chronic in the remainder. Furthermore, neutrophil chemotaxis was found to be decreased [38]. Anemia, thrombocytopenia, pancytopenia, and elevated hemoglobin F levels may also be associated with neutropenia. Bone marrow cytology is usually not pathognomonic. On the other hand, baseline dysplasia in all three hematopoietic lineages, which should be distinguished from myelodysplastic syndrome, can be present. Although the clinical significance is not clear, clonal cytogenetic abnormalities, mostly in chromosome 7, are common in patients with SDS [39]. Acute myeloblastic leukemia/myelodysplastic syndrome with complex clonal cytogenetics and poor prognosis frequently develops in these patients. Median age at diagnosis of malignancy is 19 years [40]. Growth retardation, metaphyseal dysostosis most commonly affecting the femoral head, and some endocrine disorders such as type 2 diabetes mellitus, growth hormone deficiency, and hypothyroidism are some other features of SDS.

### SBDS gene mutations

Molecular analysis showed that SDS is caused by mutations in the SBDS gene on chromosome 7q11. A pseudogene, SBDSP, occupies a locally duplicated genomic segment at the distal part of chromosome 7 and shares 97% nucleotide identity with SBDS [41]. Conversion mutations due to recombination of SBDS with SBDSP resulting in unidirectional gene conversion from the pseudogene to SBDS have been shown to be responsible for disease (Figure 2). It was revealed that converted segments were restricted to a short fragment extending approximately 240 bp in exon 2 and consistently included at least one of two pseudogene-like sequence changes that result in protein truncation. However, a Japanese study showed that conversion mutations, either homozygous or compound heterozygous, may take place at exons 1 and 3 as well as exon 2 [42].

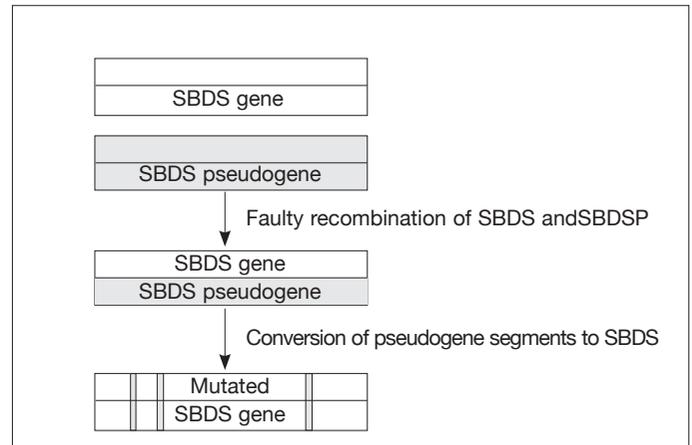
SBDS protein is a member of a highly conserved protein family of unknown function with putative orthologs in dissimilar species such as plants, yeast, and vertebrate animals, located within highly conserved operons [43]. These are homologs of RNA-processing genes, suggesting that SDS may be caused by a deficiency in an aspect of RNA metabolism [44]. An elegant study done in SBDS gene knockdown zebra fish showed a morphogenetic defect in the pancreas that alters the spatial relationship between exocrine and endocrine components, and also defective granulopoiesis, resembling patients with SDS [45]. This study also showed that SDS protein is expressed throughout embryogenesis.

### Cartilage hair hypoplasia/metaphyseal chondrodysplasia

Cartilage hair hypoplasia (CHH) or metaphyseal chondrodysplasia was first described among Old Order Amish by McKusick et al. [46] in 1965 as an autosomal recessive disease. Characteristic features include short-limbed short stature, metaphyseal chondrodysplasia of the tubular bones, hypoplastic hair, cellular immune deficiency, macrocytic anemia that spontaneously recovers before adulthood, and moderate to severe neutropenia (ANC: 100-2000/mm<sup>3</sup>) [47]. The patients suffer from recurrent severe infections, particularly varicella zoster due to neutropenia and cellular immune deficiency [48].

### RMRP mutations in CHH and related disorders

In eukaryotes, approximately 80% of the RNA is in the form of ribosomal RNA (rRNA). There are four kinds of rRNA (5S, 5.8S, 18S, and 28S), and each represents as one copy per

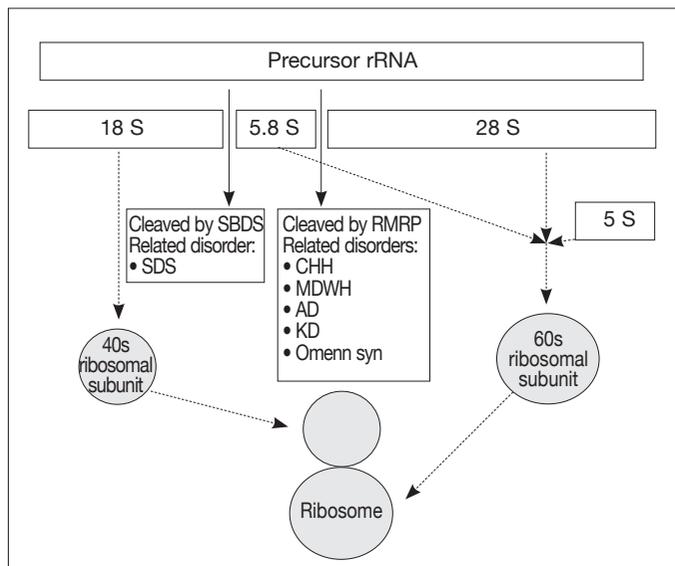


**Figure 2.** Mechanism of mutagenesis in the SBDS gene. The two adjacent boxes represent each strand of DNA of SBDS and SBDSP. After faulty recombination of SBDS and SBDSP strands, conversion of some nucleotides from pseudogene to SBDS may occur.

ribosome [49]. Three of them (5.8S, 18S, and 28S) are made by chemical modification and cleavage of a large precursor rRNA. Modification is made at specific positions guided by special RNAs termed as “guide RNA” or “SnoRNA” (small nucleolar RNA).

Recently, mutations in RNAse mitochondrial RNA processing RNA (RMRP), a noncoding SnoRNA that functions itself without being translated into a protein, have been connected to disorders mostly exhibiting skeletal dysplasia. These disorders include metaphyseal dysplasia without hypotrichosis (MDWH), anauxetic dysplasia (AD), kyphomelic dysplasia (KD) and Omenn syndrome, as well as CHH (Figure 3) [50]. Some other gene mutations in rRNA processing other than RMRP have been shown to result in disorders with bone marrow involvement such as dyskeratosis congenita caused by DKC1 mutations affecting rRNA modification, and Diamond-Blackfan anemia caused by RPS19 affecting 40S ribosomal subunit maturation. It is interesting that there is accumulating evidence showing a connection between rRNA processing defects and disorders affecting bone and bone marrow.

At least three functions have been attributed to RMRP: processing of mitochondrial RNA that functions as a primer for mitochondrial DNA replication in mitochondria, endonucleolytic cleavage of precursor 5.8S rRNA and processing of CLB2 mRNA that normally disappears rapidly as cells complete mitosis [51]. Despite being very similar to CHH, patients with MDWH (MIM#, 250460) do not have hair defect or immune deficiency. Patients with AD (MIM#, 607095) have extremely short stature, hypodontia, and mild mental retardation, but do not have tendency to cancer. Comparison of phenotype scores and rRNA and mRNA cleavage activities disclosed significant negative correlations between the degree of bone dysplasia and rRNA cleavage activity, between the degree of immunodeficiency or hematological abnormalities and mRNA cleavage activity, and between the incidence of hair hypoplasia and mRNA cleavage activity [52]. These findings may explain genotype/phenotype differences among patients with CHH, MDWH, and AD. Mutations that lead to CHH mostly originated from the transcribed region and the promoter region. The founder mutation in CHH is a 70A>G point mutation that affects both rRNA and mRNA processing, whereas mutations resulting in AD



**Figure 3.** Schematic representation of rRNA processing. In normal circumstances, a precursor rRNA is cleaved into three parts by means of SnoRNAs, such as SBDS protein and RMRP. Defect in this process due to mutations in SnoRNAs causes disorders frequently affecting bone and bone marrow.

affected ribosomal construction but not CLB2 mRNA levels [53,54]. In CHH, defect in processing CLB2 mRNA results in inability to exit from mitosis and therefore malignancy.

In conclusion, the recent advances in molecular medicine have led to new insights into the congenital neutropenias. Therefore, the invention of underlying molecular defects defined by means of these disorders is very important, not only for understanding the pathogenesis causing these disorders but also for understanding some unknown molecular mechanisms.

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