



REVIEW ARTICLE

Molecular insight of dyskeratosis congenita: Defects in telomere length homeostasis

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ABSTRACT

Background: Dyskeratosis congenita (DC) is a rare disease and is a heterogenous disorder, with its inheritance patterns as autosomal dominant, autosomal recessive, and X-linked recessive. This disorder occurs due to faulty maintenance of telomeres in stem cells. This congenital condition is diagnosed with three symptoms: oral leukoplakia, nail dystrophy, and abnormal skin pigmentation. However, because it has a wide range of symptoms, it may have phenotypes similar to other diseases. For this reason, it is necessary to use methods of measuring the Telomere Length (TL) and determining the shortness of the telomere in these patients so that it can be distinguished from other diseases. Today, the Next Generation Sequencing technique accurately detects mutations in the target genes.

Aim: This work aims to review and summarize how each of the DC genes is involved in TL, and how to diagnose and differentiate the disease using clinical signs and methods to measure TL. It also offers treatments for DC patients, such as Hematopoietic Stem Cell Transplantation and Androgen therapy.

Relevance for Patients: In DC patients, the genes involved in telomere homeostasis are mutated. Because these patients may have an overlapping phenotype with other diseases, it is best to perform whole-exome sequencing after genetics counseling to find the relevant mutation. As DC is a multi-systemic disease, we need to monitor patients frequently through annual lung function tests, ultrasounds, gynecological examinations, and skin examinations.

1. Introduction

Vertebrate telomeres are a hexanucleotide complex of TTAGGG repeats, with the range between 10 to 15 kb in humans and 25 to 50 kb at the ends of mouse chromosomes [1]. Telomeres are specialist nucleoprotein structures at the extremities of chromosomes that protect the natural chromosomal end against the signaling and DNA repair activities produced at the end created by the DNA cleavage [2]. Telomeres are due to incomplete amplification of linear DNA molecules by DNA polymerases shrink with each cell division, which is called the telomere replication problem [3]. The longest telomere length (TL) is at the beginning of birth that gradually diminishes with age. Because of the end proliferation problem, telomeres are shortened at each cell division, with the human cells proliferation repeating regions of telomeres with each cell division at the 3' chromosome end and their telomeres gradually shortened [4]. The mechanism to counteract the problem of ultimate replication is reduced to 200–50 bp. Eventually, telomeres reach an important threshold, leading to an irreversible stop in growth, a phenomenon known as cell senescence. Short telomeres activate a control point depending on p53 leading to apoptosis or senescence. Telomere shortening as a major molecular factor plays an important function in the aging phenomenon. Thus, telomeres act as “clocks” that determine the lifetime of the cell

surface. The emergence of telomeres can cause short-lived telomeres that bring about cell aging or cellular crisis, including apoptosis, genomic instability, and shortening cell lifespan [5]. The question remains as to how the ends of the conserved chromosomes can lead to the discovery of telomeres and telomerase. Research in this area has led to the identification of new age-related diseases during the past few years with the identification of a group of telomeric mediated syndromes [6]. Shortened telomeres can be extended by telomerase, a ribonucleoprotein complex, consisting of telomerase RNA component (TERC) and telomerase reverse transcription (TERT) and shelterin [7].

1.1. Telomerase

The telomere maintenance requires the aggregation of the protein components, RNA from telomerase, and telomerase maturation and stability factors. Telomerase compensates for telomere aberration by adding TTAGGG repeats to the end of chromosomes in normally expressed cells, such as pluripotent stem cells. Telomerase is composed of a TERT and a TERC, which serve as a model for the de novo addition of telomere repetitions. Telomerase, which can produce new telomeres, solves the problems of ultimate proliferation [8]. Telomerase biogenesis requires the assembling of *TERT* and *TERC* in a stable complex that can function in telomeres. *TERT* after synthesis into the cytoplasm with HSP 90 and P23, which penetrates the nucleus by its nuclear location signal. On the other hand, telomerase RNA (TR) synthesis in the nucleus binds to the *NOPI10*, *NHP2*, *GARI*, and dyskerin complexes (*TERC* complex). *TERT* and *TERC* are then assembled to form the final telomerase. The telomerase enters the Cajal Body (CB) through the CAB box in TR. In the S phase of the cellular cycle, the telomerase is connected to the *TPP1* in the Shelterin complex through the TEN domain in *TERT* and enters the telomere. In other cell cycle phases, it is separated from the telomere [9]. A specific reverse transcriptase can help to extend the 3' end of the chromosomes from the telomerase complex. Disruptions in telomerase activity can damage the chromosome and cause the cell to become cancerous. Telomere dysfunction is also associated with other conditions including dyskeratosis congenita (DC) (Figure 1), Hoyeraal-Hreidarsson (HH) syndrome, and Idiopathic Pulmonary Fibrosis (IPF) [10].

1.2. Shelterin

The telomerase enzyme protects the TL of DNA. The shelterin complex accumulates along the telomere, holds the telomere, and regulates telomere protection and regulation of TL and formation of T-loop [11]. Shelterin comprises of six proteins that include Telomeric Repeat binding Factor1 (TRF1), Telomeric Repeat binding Factor2 (TRF2), TRF1-Interacting Nuclear protein2 (TIN2/TINF2), ACD/TPP1, TERF2 Interacting Protein (TERF2IP/RAP1), and Protection of Telomer1 (POT1), which prevent telomeres from binding to each other. Shelterin detects the TTAGGG repeat through TRAF1,2 (which connects to the double-strand part of the telomere) and POT1 (which connects to the single-strand part of the telomere D-loop). One of the functions of Shelterin is to regulate telomerase activity. TPP1 is an interaction with the telomerase. Other factors,

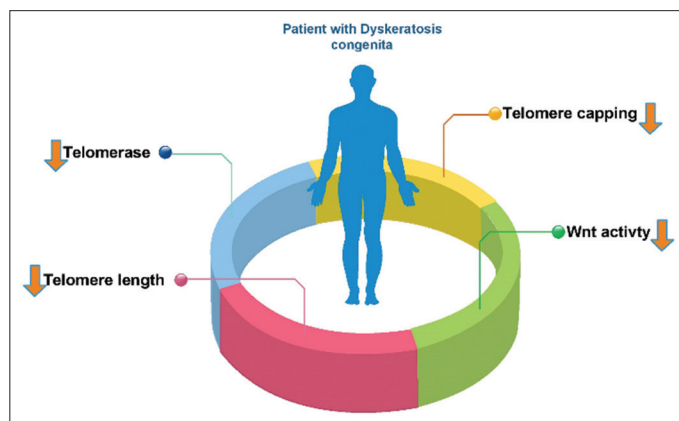


Figure 1. An overview of what happened to dyskeratosis congenita patients

such as CB and tankyrase1 and 2, also positively affect telomerase activity. By increasing the TTAGGG repeat and the length of ssDNA, POT1 binds to these repeats which leads to a negative regulator of telomerase (Figure 2). Another function of the shelterin is the prevention of the response of DNA damage response. These signals, generated when DNA is damaged, increase Non-Homologous End Joining (NHEJ) in the cell [12]. In Mouse models, it has been shown that the absence of TRF2 and RAD1 can cause more NHEJ. Therefore, shelterin plays a role in the inhibition of NHEJ [13].

1.3. DC

DC is a rare illness with a prevalence of one in one million. A total of 400 families worldwide are reported to have been affected by the DC [14], but this complex disorder accounts for about 1% of all telomere syndromes. This congenital condition can be inherited as autosomal recessive (AR), autosomal dominant (AD), and X-Link recessive (XLR). This is a premature aging syndrome with short telomeres. Telomere dysfunction can be caused by telomere loss, such as telomerase deficiency or loss, leading to inappropriate activation of DNA repair pathways [6]. The decrease in TL generally shows its symptoms in the skin, blood, and nails because these tissues are highly renewable. This renewability depends on the stem cells in these tissues. In stem cells, telomerase activity is high (TLs are almost constant). Therefore, any defect in telomerase or other factors that affect TL will damage stem cells and impair tissue renewability [15]. DC is a disorder of several systems in its classical form with triple skin abnormalities, nails and leukoplakia of the mouth (white spots on the inside of the mouth) or the presence of one of the features of the triplet in combination with BM failure and two other related findings [16] (Figure 3). The main reason for death among these patients is BMF, although PF, cancer, and hepatic cirrhosis are secondary causes of death [17]. In genetic terms, DC is almost equally heterogenous. Mutations directly involved in DC have been identified in the *DKC1*, *TERC*, *TERT*, *NOPI10*, *NHP2*, *PARN*, *RTEL1*, *TPP1*, *TCAB1*, *CTC1*, *POT1*, *STN1*, and *TINF2* genes [18] (Table 1).

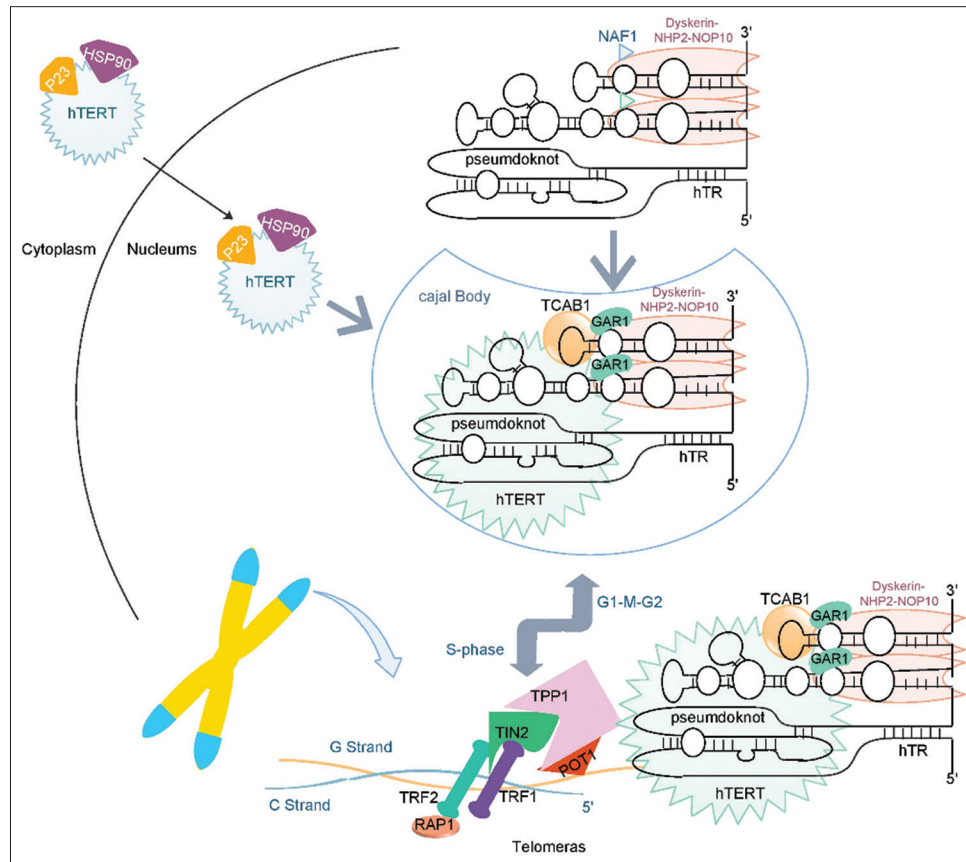


Figure 2. Telomere assembly and recruitment. TERT, after being synthesized in the cytoplasm enters the nucleus with the chaperones. TR is made in the nucleus but is not expressed because it is a non-coding RNA. TR has different domains to which NOP10, NHP2, Dyskerin, and GAR1 proteins bind and form the TERC complex. TERT and TERC are then entered into CB by TCAB1. In CB, telomerase is formed and matures. In the S phase of the cell cycle for telomere lengthening, telomerase is recruited to telomere by TPP1 Shelterin complex (the shelterin complex consists of TPP1, POT1, TRF1, TRF2, TIN2, Rap1 proteins). Telomerase, along with the shelterin, adds TTAGGG repetitions to the telomere. Once the TL is sufficient, the negative feedback generated by the POT1 disables the telomerase and re-enters the CB.

2. DC Genes

2.1. TERT

TERT (also called *TP2*, *hTERT*, and *DKCA2*) is an abbreviated form of TERT. It is situated at 5p15.33 with 16 exons. *TERT* is expressed in Testis, lymph node, bone marrow, brain, skin, and 22 other tissues [19]. *TERT* forms the catalytic part of the telomerase, which in replication, adds TTAGGG repeats to the telomere end of each chromosome [20]. *TERT* has four functional areas: 1- telomerase N-terminal (TEN) domain. 2- TR-binding domain (TRBD). 3- Reverse transcriptase (RT) domain. 4- C-terminal extension (CTE) [21]. RT plays a major role in *TERT*. *TERT* binds to TR through TRBD and RT. These two areas are bound to the CR4/CR5 and pseudoknot domains in TR, respectively [22]. The TEN domain is involved in telomerase recruitment to telomeres and CTE is part of the reverse transcriptase complex [9]. In cancerous cells and stem cells, telomerase is highly active. These cells increase telomerase activity by augmenting *TERT* expression [23]. Higher *TERT* activity in cancer cells can be due to epigenetic changes, mutations in the promoter, and the

impact on signaling pathways in *TERT* [24]. However, a decrease in telomerase activity that shortens TL due to a mutation in *TERT* causes DC disease. The AD mutation in *TERT* causes DC classic [25], but the AR mutation causes a more severe form called HH syndrome. In addition to the three symptoms of DC, intrauterine growth delay, cerebellar hypoplasia, gradual failure of the bone marrow in microcephaly, and immunodeficiency are seen in HH syndrome [26]. More severe symptoms of HH syndrome surface because the TL in these patients is much shorter than that in DC patients [27].

2.2. TERC

TERC (also called *hTR*, *TRC3*, and *DKCA1*) is an abbreviated form of TERC. It is located in 3q26.2 with one exon. Telomerase is a ribonucleoprotein polymerase that retains the ends of the telomere by adding TTAGGG to the telomere. The enzyme is composed of a reverse transcriptase protein component and an RNA component coded by this gene, which serves as a telomere repetition model. Deregulation of expression telomerase in somatic cells may be involved in oncogenesis [28]. TR has five important areas: 1- A template area for telomere synthesis.

Table 1. Dyskeratosis congenita genes and related disorders (OMIM)

GENE/OMIM NUMBER	Protein function	Disorders/Inheritance
TERT (187270)	Reverse transcriptase in telomerase	DC (AD, AR) Leukemia-acute myeloid (AD) Pulmonary fibrosis and/or bone marrow failure (AD)
TERC (602322)	RNA template in telomerase for addition telomeric repeat	Aplastic anemia (AD) Pulmonary fibrosis idiopathic (AD) DC (AD)
DKC1 (300126)	Component of telomerase	DC (X-linked)
NOP10/NOLA3 (606471)	Component of telomerase	DC (AR)
NHP2/NOLA2 (606470)	Component of telomerase	DC (AR)
TINF2/TIN2 (604319)	Component of shelterin	DC (AD) Revesz syndrome (AD)
TPP1/ACD (609377)	Component of shelterin	DC (AD, AR)
TCAB1 (612661)	Recruitment telomerase to Cajal body	DC (AR)
RTEL1 (608833)	Prevent the formation of the second structure in G-overhang and T-loop unwinding	DC (AD, AR) Pulmonary fibrosis and/or bone marrow failure (AD)
PARN (604212)	Degrades poly (A) tail in 3' end TR	DC (AR) Pulmonary fibrosis and/or bone marrow failure (AD)
STN1 (613128)	Component of the CST complex	DC (AR) Cerebrotinal microangiopathy with calcifications and cysts 2 (AR)
CTC1 (613129)	Component of the CST complex	DC (AR) Cerebrotinal microangiopathy with calcifications and cysts (AR)
POT1 (606478)	Component of shelterin	DC

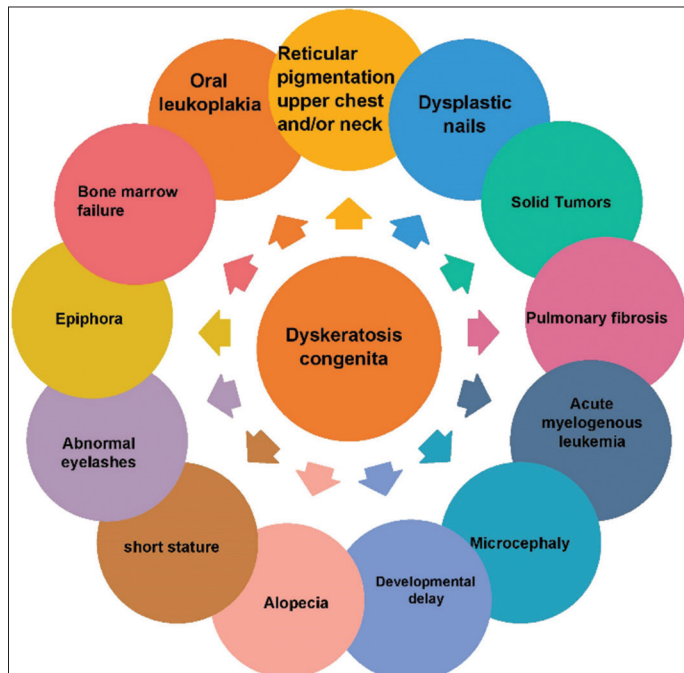


Figure 3. DC is diagnosed by triad significant symptoms of oral leukoplakia, dysplastic nail, reticular pigmentation. Having two major signs or one major sign and two or more minor signs (written in the figure) can diagnose DC. Because DC causes a wide range of symptoms, it needs careful diagnosis because it overlaps with the symptoms of other diseases.

2- pseudoknot domain. 3- CR4/CR5 domain. 4- H/ACA domain. 5- CR7 domain [29]. H/ACA interacts with *NOP10*, *GARI*, *NHP2*, and dyskerin. H/ACA has two hairpin areas bound by the H box and ACA areas. In the H/ACA domain, there is an area called the CAB Box, which is bound to the *TCAB1* and assists with telomerase localization in the CB [9]. For two reasons, the accumulation of TR in CB is implicated in the telomere lengthening, first in CB, telomerase maturation, and second in telomerase recruitment to telomeres [30].

2.3. *DKC1*

DKC1 (also called *CBF5* and *DKCX*) is a shortened form of dyskerin pseudouridine synthase 1. It is located in Xq28, and it has 14 exons. *DKC1* is expressed in bone marrow, lymph node, testis, skin, and 23 other tissues [31]. *DKC* plays a role in TR stability [9]. By binding to the H/ACA Domain, *DKC* prevents excessive cleavage at the TR 3' end. Mutations in *DKC* reduce *TERC* [32]. One of the post-translational changes that occur in *DKC* is sumoylation, which occurs in specific *DKC* lysines. Reportedly, sumoylation plays a role in regulating *DKC* stability. Defects in sumoylation reduce *DKC* stability and decrease TR, shortening telomere and causing the DC [33]. Defects in *DKC* also disrupt the rRNA and biogenesis of the ribosome because *DKC* interacts with the H/ACA domain in snoRNA to form a complex that plays a role in altering certain uridines in the rRNA. *DKC* in this complex activates the enzyme pseudouridine synthase [34].

2.4. *NOP10*

NOP10 (also called *DKCB1* and *NOLA3*) is located in 15q14 and has two exons. *NOP10* is expressed in bone marrow, spleen, appendix, skin, and 23 other tissues. This gene is part of the family of H/ACA snoRNPs (small nuclear ribonucleoprotein). SnoRNPs contribute to various aspects of the treatment and modify the rRNA [35]. *NOP10* plays a role in telomerase stability. Mutations in *NOP10* are associated with a decrease in *TERC* [36].

2.5. *NHP2*

NHP2 (also called *DKCB2* and *NOLA2*) is located in 5q35.3 and has four exons. It is expressed in Esophagus, brain, bone marrow, skin, and 23 other tissues. This gene belongs to the H/ACA snoRNPs (small nuclear ribonucleoprotein) gene family [37]. Mutations in *NHP2* reduce TR and subsequently lower telomerase activity. *NHP2*, together with *NOP10*, *GARI*, and *DKC* interact with snoRNA and form the snoRNP complex. This complex is involved in pseudouridylation and cleavage rRNA. Therefore, *NHP2* deficiency is associated with pre-rRNA maturation defects and ribosome biogenesis. In 2020, Benyelles and *et al* reported the first case of HH syndrome with mutations in both *NHP2* alleles [38].

2.6. *TINF2*

TINF2 (also called include *TIN2* and *DKCA3*) is an abbreviated form of *TERF1* interacting nuclear factor 2. It is located in 14q12 and has six exons. It is expressed in the Lymph node, adrenal, bone marrow, skin, and 23 other tissues [39]. *TINF2* is one of the six proteins that make up the shelterin [34]. It also binds from C-terminal to *TRF1* and from N-terminal to *TRF2* and *TPP1* (binding to *TPP1* causes the *TIN2*, with indirect binding to *POT1*). These binds establish communication between the double-strand and single-strand telomere areas, which has a pivotal role in the stability of the Shelterin complex [40,41]. Furthermore, *TINF2* binds to *TRF1* in the cytoplasm to prevent ubiquitination by E3-ligase Fbx4 and its degradation. *TINF2* is ubiquitinated and destroyed by E3-ligase siah2 after entering the nucleus. In general, *TINF2* affects the dynamics of the Shelterin and Telomere complexes to match the telomere with the cell cycle [42]. The mutation in *TINF2* disrupts the stability of the shelterin and recruitment telomerase complex [43]. Mutations in *TINF2* have been seen in patients with Revesz syndrome, a severe form of DC. In addition to the triad symptoms of DC, pancytopenia and cerebellar hypoplasia can be seen in Revesz syndrome [44]. *TINF2* is also involved in metabolism. In this way, in its N-terminal, it has targeting sequences for mitochondria. In mitochondria, it undergoes post-translational changes. Studies have shown that when *TINF2* expression decreases with RNAi, oxidative phosphorylation is reduced and glycolysis is weakened in cancer cells [45].

2.7. *TPP1*

TPP1 (Also called *ACD*, *PTOP*, and *TINT1*) is located in 16q22.1 and has 12 exons. *TPP1* is expressed in Testis, ovary,

skin, bone marrow, and 23 other tissues. This gene codes for a protein associated with telomere function. This protein assembles and stabilizes this complex and facilitates telomerase access [46]. *TPP1* binds from the C-terminal to the *TIN2*, from its central domain to the *POT1* [47], and from the OB (oligosaccharide/oligonucleotide -binding) domain to the *TERT* in the N-terminal. In the OB domain, there is an area called TELpatch (TPP1 glutamate(E)- leucine(L)- rich patch) which is involved in telomerase activity [48]. Heterozygous mutations in TELpatch have been seen in people with HH syndrome [49]. Growth signals, which the cell responds to changes in the environment, play a role in protecting the telomere. One of the pathways involved in signal response is the PI3-K/AKT. This pathway protects telomere and prevents cellular senescence. This pathway adjusts the telomere by the impact on *TPP1*. Through the OB domain, *TPP1* forms a homodimer. By binding to the *TPP1*, AKT increases homodimerization. This dimerization is essential for telomerase performance and increasing TL as regulated by AKT. During nutritional stress, AKT activity is inhibited, which disrupts the formation of the homodimer, *TPP1-POT1* heterodimer, and the shelterin complex. In general, disturbance in the AKT pathway disrupts the function of the *TPP1* and causes damage to the telomere [50]. Heterodimer *TPP1-POT1* is involved in binding the primer (the same 3' end) to the template strand in the *TERT*, which reduces the primer detachment and increases the RAP (Repeat addition processivity). On the other hand, *TPP1-POT1* causes slipped DNA strand on the TR template strand, which causes repetitions to be made on DNA in a row [51]. In general, *TPP1* recruits telomerase into the telomere, telomerase activity, and the negative feedback of the TL through the *POT1* [52].

2.8. *TCAB1*

TCAB1 (also called *DKCB3* and *WRAP53*) is an abbreviated form of Telomerase CB protein 1. It is located in 17p13.1 and has 13 exons. *TCAB1* is expressed in the Lymph node, testis, bone marrow, skin, and 23 other tissues [53]. Telomerase is found in most cellular cycles in CB. Its placement in CB is determined by the interaction between CAB Box in hTR and *TCAB1* in CB [54]. In the CB, telomerase matures. Thus, *TCAB1* is involved in telomerase maturation and telomerase recruitment to telomere [55]. *TACB1* is involved in repairing HR and NHEJ. In response to DNA damage, ATM phosphorylates *TCAB1* in S64. *TCAB1* forms a complex before being phosphorylated by MDC1 and RNF8. After *TCAB1* is phosphorylated, the complex binds to H2AX through *TCAB1* and phosphorylates it. Phosphoryl *TCAB1* also causes 53BP1 to be applied to the lesion site. In general, *TCAB1* initiates repair pathways in response to DNA damage [56].

2.9. *RTEL1*

RTEL1 (also called *NHL* and *DKCA4*) is an abbreviated regulator of telomere elongation helicase 1. It is located in 20q13.33 and has 35 exons. *RTEL1* is expressed in Testis, appendix, skin, bone marrow, and 23 other tissues [57]. *RTEL1*, with its helicase activity, prevents the formation of the second structure in the

G-overhang so that *POT1* can bind to G-overhang. Since *POT1* is involved in telomerase activity, then disruption of *RTEL1* reduces *POT1* binding and subsequently reduces telomerase activity and telomere shortening [58]. *RTEL1* is involved in T-loop unwinding. *TRF2* is involved in the recruitment of *RTEL1* to telomere by binding to the C4C4 motif in *RTEL1* in the S phase of the cell cycle. After the *RTEL1* is placed in the telomere, the *RTEL1*-PCNA causes the T-loop to disassemble so that there is no barrier at the end of the telomere for the replication fork. In the event of an *RTEL1* dysfunction, the replication fork stops near the T-loop. Cleavage by nucleases shortens the telomere. Therefore, *RTEL1* is involved in replication, telomerase activity, and telomere protection [59].

2.10. *PARN*

PARN (also called *DAN* and *DKCB6*) is an abbreviated poly(A)-specific ribonuclease. It is located in 16p13.12 and has 27 exons. *PARN* is expressed in the thyroid, testis, bone marrow, skin, and 23 other tissues [60]. Poly A tail mRNA affects stability. In any case, the stability of mRNAs decreases over time and decomposes, which is essential. Several factors are involved in the breakdown of mRNA, one of which is *PARN*, a deadenylase. *PARN* has a domain with nuclease activity and two domains bound to RNA called R3H and RRM [61]. TR is an RNA that needs to be processed. It is necessary for TR to deadenylation in the 3' end by *PARN*. If the *PARN* is disrupted and lacks deadenylation, several adenyls attach to the 3' end of the TR, making the TR susceptible to destruction by exonucleases such as *Exosc10*. In general, mutations in *PARN* reduce the amount of TR and impair telomerase and telomere [62].

2.11. *STNI*

STNI is located in 10q24.33 and has 10 exons. *STNI* is expressed in the testis, lung, esophagus, skin, and 23 other tissues [63]. The CST complex (*CTCI*-*STNI*-*TEN1*) binds single-stranded DNA with high affinity in a sequence-independent manner, while isolated subunits bind DNA with low affinity by themselves [64]. Initially, the CST complex has been proposed to protect telomeres from DNA degradation. However, the CST complex is involved in several aspects of telomere replication [65]. The CST complex inhibits telomerase and is involved in TL homeostasis; it is proposed to bind to newly telomerase-synthesized 3' overhangs and terminates telomerase action implicating the association with the ACD: *POT1* complex, thus interfering with its telomerase stimulation activity [66]. The CST complex is also proposed to be involved in fill-in synthesis of the telomeric C-strand, probably implicating recruitment and activation of DNA polymerase alpha [67].

2.12. *POT1*

POT1 is an abbreviation of protection of telomeres 1. It is located in 7q31.33 and has 23 exons. *POT1* is a member of the telomere family and encodes a nuclear protein involved in telomere maintenance [68]. Specifically, this protein functions as a member of a multi-protein complex that binds to the TTAGGG

repeats of telomeres, regulating TL and protecting chromosome ends from illegitimate recombination, catastrophic chromosome instability, and abnormal chromosome segregation [69].

2.13. *CTCI*

CTCI gene is an abbreviated form of CST telomere replication complex component. It is located in 17p13.1 and has 23 exons. This gene is expressed in the spleen, lymph node, and 25 other tissues [70]. *CTCI* encodes a component of the CST complex. Essentially, it protects telomeres from degradation [71]. The component of the CST complex acts as a specialized replication factor promoting DNA replication under conditions of replications stress or natural replication barriers such as telomere duplex. The complex is involved in TL homeostasis [72].

3. Diagnosis

3.1. Overlapping phenotype and whole-exome sequencing

To diagnose DC patients, the three main symptoms, including nail dystrophy, oral leukoplakia, and abnormal skin pigmentation, or the association of one or two of the main symptoms with other symptoms are used as demonstrated in the figure. Some people have been shown to have delayed symptoms in adulthood, a condition known as cryptic DC [6]. Diagnosing cryptic DC patients is a challenging issue because they have different symptoms. As physicians expect early symptoms for hereditary diseases, diagnosing cryptic DC may not be easily performed, but its symptoms appear in adulthood [73]. Furthermore, DC is hard to accurately diagnose because it includes a wide range of symptoms and overlapping phenotypes with other diseases. For example, in a survey of 12 families of patients with DC-like symptoms, they were initially diagnosed with DC. However, by Whole Exome Sequencing (WES), mutant genes, none of which were associated with DC but related to different syndromes. Error in diagnosis due to nail dystrophy, abnormal skin pigmentation, bone marrow failure in 60% of these patients has been reported. The genes in which the mutation was found were related to the following syndromes: 1- Poikiloderma with Neutropenia, in which patients have symptoms of short stature, nail abnormalities, and suspected infection; 2- Ectodermal dysplasia/short stature syndrome, in which patients have symptoms of short stature, nail dystrophy, and abnormal pigmentation; and 3- Dubowitz syndrome, where patients have symptoms of microcephaly, eczema, short stature, and mental retardation. The phenotype of these syndromes overlaps with the DC phenotype, which might cause misdiagnosis [74]. Besides, the mucocutaneous symptoms appear late, so the presence of cryptic DC and overlapping phenotype make the diagnosis even more difficult. Also, early treatment for DC patients is vital. Given the above, we need a method to quickly and accurately detect mutations. Today, the WES technique detects Telomere Biology Disorders (TBD) such as DC [75]. WES helps diagnose heterogeneous diseases and diseases that are difficult to diagnose on phenotypic symptoms. WES also helps physicians diagnose the disease quickly to provide the best treatment for the patient and provide more accurate genetic counseling [74].

3.2. TL measurement methods

In DC, various methods are used to measure TL such as quantitative -PCR, Terminal Restriction Fragment (TRF) analysis, Q -FISH, Single TL Analysis, Telomere Shortest Length Assay, Monochrome Multiplex -qPCR (MM-qPCR), and flow -FISH [76,77]. Peripheral Blood Leukocytes (PBL) are used to measure TL. All of the above methods have advantages and disadvantages. In most studies, two or more methods have been compared with each other in the case of DC patients. For example, two flow-FISH and MM-qPCR were used to measure TL in 105 samples in one study. People with very short TL performed the WES to identify the mutation accurately. In general, with the results of this study, flow-FISH is more accurate to measure TL, but MM-qPCR is more suitable for study on a large cohort. Flow-FISH is a method routinely used to measure TL. One of its advantages is high accuracy and sensitivity to TL changes, a low rate of false-positive results, and reproducibility. Yet, the disadvantages include the difficulty of analysis, the need for a suitable cell population, and the need for a fresh sample [76,78]. High-throughput Q -FISH is an accurate method of checking short telomeres (<3 kbp). After taking a blood sample, the PBL is purified and stored in liquid nitrogen in this method. To perform the test after thawing, the cells are counted and fixed on a plate. Fluorescent probes (CCCTAA) are then bound to the telomere repeats with high affinity. Photographs are taken using digital microscopes and evaluated by the photo analysis software. Flow-FISH, like Q-FISH, uses fluorescent probes analyzed by flow cytometry [78,79]. Q -PCR is less expensive than other methods, has higher sensitivity, and can be performed with a small amount of DNA sample in this technique. Furthermore, the size of the TL estimating is based on the Threshold Cycle (CT). The sample CT depends on the number of repetitions of the telomeres. The q -PCR result is expressed as a Telomeric repeats/Single copy gene (T/S) ratio. In fact, it is difficult to optimize this method, so standards are set for all stages to make the test more reliable [76]. TRF measures the average TL in a cell population. This method is the oldest method for measuring TL and is also the gold standard [79]. TRF has six steps:

- 1- DNA extraction from the sample, which can be used by phenol-chloroform or salt precipitation
- 2- Examining DNA integrity to ensure a good DNA sample. Run the sample together in the Gel electrophoresis. The sample that forms a long smear in the gel is not a good sample and its DNA has been decomposed.
- 3- DNA cleavages by restriction endonuclease enzymes, usually *HinfI* and *RsaI*. The area of repeated telomere repetitions does not require cleavage.
- 4- The cleavage DNA to be run on the Agarose gel.
- 5- Probes that have CCCTAA sequence and complement the repetitive telomere sequence to be hybridized with the telomere sequence after being labeled with digoxigenin (DIG). These probes are identified by chemiluminescence and Anti-DIG -AP.
- 6- Data analysis to be performed after exposure to the X-ray.

One of the disadvantages of this method is that because the short

telomeres have low signal intensity in hybridization, it might not accurately detect short telomeres [80].

4. Treatment

To diagnose and manage the complications in DC patients, we need to monitor patients frequently through annual lung function tests, ultrasounds, gynecological examinations, and skin examinations [81]. As DC is a multi-systemic disease, parts of the body need to be under constant supervision [82]. Since no specific treatment can be assigned to DC, the most commonly used treatment is supportive therapy with blood transfusion. Diagnosis of genetic causes of DC has an important role in managing blood problems [83].

4.1. Immunosuppressive Therapy (IST)

IST with cyclosporine and anti-thymocyte globulin was used to treat severe Aplastic Anemia. DC patients treated with IST developed a transient response [83]. Researches have shown that BMF and immunodeficiency cause between 60% and 70% of premature deaths in DC patients. DC patients are not treated with IST [84]. Therefore, it cannot be considered a treatment for DC, but some evidence suggests an improvement in adult patients with mutations in *TERT* or *TERC* [85].

4.2. Growth factors

Granulocyte-Macrophage Colony- Stimulating Factor (GM-CSF) and erythropoietin did not show therapeutic success by affecting platelet count, but it has been suggested that combination therapy may be successful for neutropenia and anemia. Furthermore, it could be used as a Therapeutic approach for DC patients without a suitable donor [86]. Hematopoietic growth factors are sometimes used for BMF patients, but it is not advisable to use them with androgen [87].

4.3. Androgen therapy

According to studies, androgens can cause hematological responses in patients with BMF syndrome, such as DC and FA. In these, Danazol, Fluoxymesterone, and Oxymetholone can be mentioned as androgens [88,89], accounting for 70% response without transfusion dependence. The mechanism that stimulates hematopoiesis by androgens is unclear. Androgens can act by increasing telomerase activity [90] and stimulating erythropoietic stem cells, and to a lesser extent, myeloid progenitor cells stimulator in the bone marrow [91]. Recent studies have shown the effect of androgens on erythropoietin receptors (not erythropoietin itself) and receiving hematological responses [92]. Treatment with androgens has several side effects. For example, Oxymetholone can cause dyslipidemia and virilization [88]. Danazol is another synthetic androgen candidate for treating DC for its known side effects and good efficacy [83]. The estimated time for androgens can be between 1 and 3 months. Patients receiving androgens should avoid growth factors such as granulocyte-stimulating factor because of the risk of developing splenic peliosis and vascular Rupture [88].

4.4. Hematopoietic stem cell transplantation (HSCT)

Allogeneic HSCT is the only therapy that works for BMF in DC patients. In this regard, for a successful transplant, an HLA donor is needed. In the absence of a related donor, an unrelated donor can be used for transplantation. Therefore, donors should be evaluated for TL or mutations [82]. In a study for HCT, using the flow FISH method, a donor with a long telomere leukocyte was selected. They found that a donor with a long telomere increased the chances of survival and reduced the incidence of malignancies in recipients. It has also been shown that increasing each Kb of leukocyte telomere in the donor causes a reduction of 23% in the risk of death in the recipients [93]. HSCT can be associated with toxicity and high rates of premature mortality in patients. This toxicity can be due to the use of high doses of chemo-radiotherapy and chemotherapy [94]. Issues related to transplants include transplant failure, Graft versus host disease, organ toxicity such as liver cirrhosis, and pulmonary fibrosis [95], which probably causes the immunosuppressive condition, busulfan, high dose cyclophosphamide and increased use of Fludarabine [96]. Fludarabine is a type of reduced-intensity packaging diet used in HSCT with the safety suppressor effect of T-Cells in DC [97]. The use of non-myeloablative allogeneic HSCT is promising due to lower toxicity and greater success in transplantation [82]. Reduced-intensity conditioning (RIC) HSCT is a treatment to enhance the survival chance of patients with low side effects. Although there is no evidence of RIC support in DC patients, it requires less toxicity [98]. Recent reports have shown that RIC can reduce the likelihood of lung diseases without a significant effect on survival. However, evidence suggests better survival with complications in some cases [99]. A new study in patients with DC demonstrates that mesenchymal stem cells derived from the bone marrow can construct peripheral hematopoietic and may effectively manage DC patients with hematopoietic stem cell deficiency [100]. HSCTs cannot correct genetic defects such as *TERT* or *TERC* germ cell mutations related to aplastic anemia and lung fibrosis, so pulmonary complications after HSCT may be accelerated [82]. These patients are also highly vulnerable to malignancy after treatment, so contact with factors that trigger DNA damage should be minimized [101].

4.5. New treatment for mutation in *PARN*

TR plays a crucial role in maintaining telomeres. TR is affected by post-translational changes to maturity. One of the factors involved in the maturation of TR is *PARN*, which causes the deadenylation to the 3' end of the TR. On the other hand, *PAPD5* is poly (A) polymerase, and by adding adenine to the 3' end, it causes TR to be targeted and decomposed by RNA exome, which in turn shortens telomere in stem cells. It was found in one study that *PARN* gene mutated cells without deadenylation in TR can be used as *PAPD5* inhibitors such as BCCH001 and RG7834, which can inactivate *PAPD5* to prevent the addition of poly(A) to TR and its degradation, in order to enhance telomerase activity [102].

5. Conclusion

TL homeostasis is essential for cell development in highly renewable tissues. Defects in TL homeostasis are seen in cancer and

BMFs such as DC. TL is maintained by the two main components of telomerase and shelterin. Telomerase, which includes *TERC* and *TERT*, has a vital role in the TL. *TERC* as a template and *TERT* with their enzymatic activity add TTAGGG repetitions to the telomere. Mutations that increase and decrease telomerase activities are seen in cancer and DC, respectively. As DC has a wide range of phenotypes, caution needs to be practiced for an overlapping phenotype with other diseases. However, TL measurement methods can be used to distinguish it from other disorders. Today, the Next Generation Sequencing technique helps accurate diagnosis of the disease. This study addressed the role of each of the DC genes and the diagnosis of DC using clinical phenotype and TL measurement methods and treatments for DC patients.

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Conflict of Interests

The authors declare no conflict of interests.

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