—Review—

Mutations of Telomerase Complex Genes Linked to Bone Marrow Failures

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Abstract

Dyskeratosis congenita (DKC) is a bone marrow failure (BMF) with characteristic physical anomalies, and is typically diagnosed in childhood. Some forms of DKC are known to be caused by mutations occurring in *DKC1*, telomerase RNA component (*TERC*), and telomerase reverse transcriptase (*TERT*). These genes are the main constituents of the telomerase complex that plays a role in replicating telomeres and stabilizing them against shortening. Mutations in these genes could shorten telomeres and impair the proliferative capacity of hematopoietic stem cells, eventually causing DKC. Recently, mutations in *TERC* and *TERT* have been reported in some cases of aplastic anemia (AA) and myelodysplastic syndrome (MDS). These cases are considered to be atypical forms of DKC that develop slowly in adulthood without characteristic physical anomalies. Genetic tests are essential in diagnosing this late-presenting DKC and determining the appropriate treatment. This article reviews mutations in the telomerase complex and their connections with DKC and bone marrow failures.
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Key words: bone marrow failure, dyskeratosis congenita, telomere, telomerase complex gene, mutation

Mutations of Telomerase Complex in Cases of Dyskeratosis Congenita

Dyskeratosis congenita (DKC) is a bone marrow failure (BMF) typically accompanied by reticulated skin pigmentation, nail dystrophy, mucosal leukoplakia, and other manifestations. In over 80% of cases of DKC, bone marrow failures develop by age 10 with the above-mentioned physical anomalies[1]. Various complications are also observed in 15 to 25% of cases, including retarded mental development, pulmonary diseases, short stature, dental anomalies, esophagostenosis, and loss or graying of scalp hair. Also, malignancies develop in 8% of cases, including squamous carcinoma and adenocarcinoma of the skin, the nasopharynx, and the gastrointestinal tract, as well as hematopoietic malignancies such as myelodysplastic syndrome (MDS), Hodgkin's disease, and acute myelocytic leukemia[1].

The inheritance patterns are X-linked recessive in 35% of cases, autosomal dominant in 5% of cases, and unknown in 60%[2]. The gene responsible for X-linked recessive inheritance is DKC1 located at

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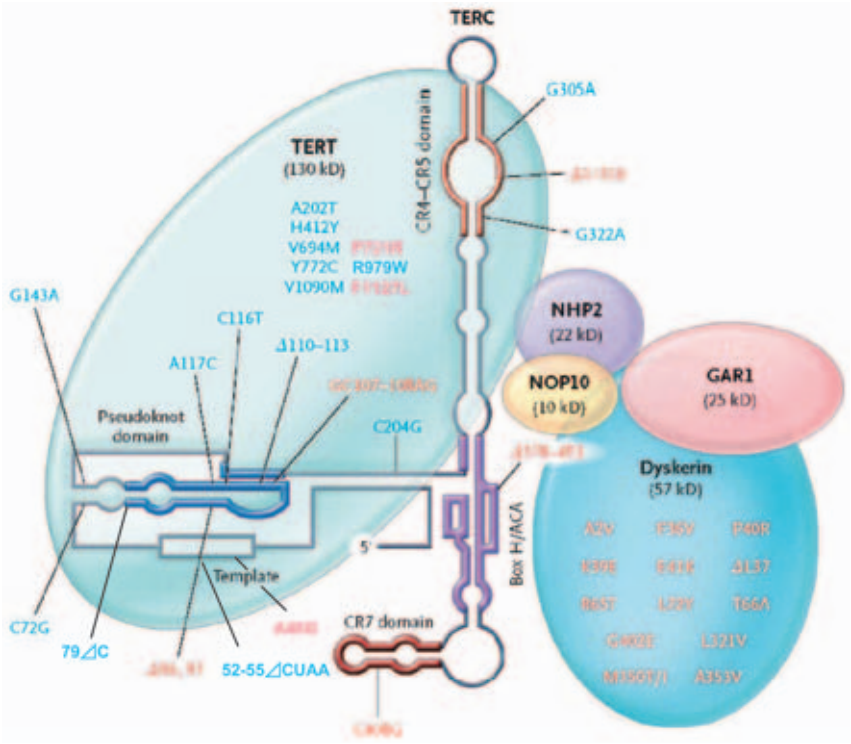


Fig. 1 Schematic structure of the telomerase complex and location of mutations associated with bone marrow failure syndromes
TERC, TERT, dyskerin, NOP10, NHP2, and GAR1 constitute the telomerase ribonucleoprotein complex. Mutations in patients with classic dyskeratosis congenita associated with physical anomalies are shown in red; mutations in patients with an unconventional marrow failure which develops later in adulthood without physical anomalies are shown in blue. Amino acids are represented in single-letter code.

Xq28[3], and the genes responsible for autosomal dominant inheritance are the telomerase RNA component (*TERC*) located at 3q21-28[4] and telomerase reverse transcriptase (*TERT*) located at 5p15[5–7] (**Fig. 1**). These genes are the main constituents of the telomerase complex that replicates telomeres and stabilizes their lengths[8]. Mutations of these genes are considered to cause telomere shortening[4,9,10], impair the proliferative capacity of cells including hematopoietic stem cells, and cause DKC with the above-mentioned symptoms[2]. Furthermore, correlations have been observed between the degree of telomere shortening and the onset age of DKC, associated symptoms, and the presence of hematopoietic damage[10]. With Hoyeraal-Hreidarsson syndrome (HHS), which is considered to be a severe form of DKC as described below, telomere shortening is reported to be more pronounced than with typical DKC[10].

HHS is a genetic disease that typically causes bone marrow failures in boys in early childhood. Besides bone marrow failures, HHS may also lead to such complications as microcephaly, hypoplastic cerebellum, growth retardation, unusual facies, reductions in B and NK cell counts, and cellular immunodeficiency. In most cases, patients die around the age of 10[1]. While the inheritance pattern had been assumed to be X-linked recessive in most cases, it has recently been reported that HHS develops in girls as well[1]. According to a recent report, one third of the HHS patients were girls, suggesting an autosomal recessive inheritance pattern[7]. While HHS was originally thought to have been a separate disease from DKC, mutations including T49M and S121G on *DKC1* have since been discovered in HHS patients, giving support to the view that HHS is a severe form of DKC[1,11].

In recent years, the existence of unconventional

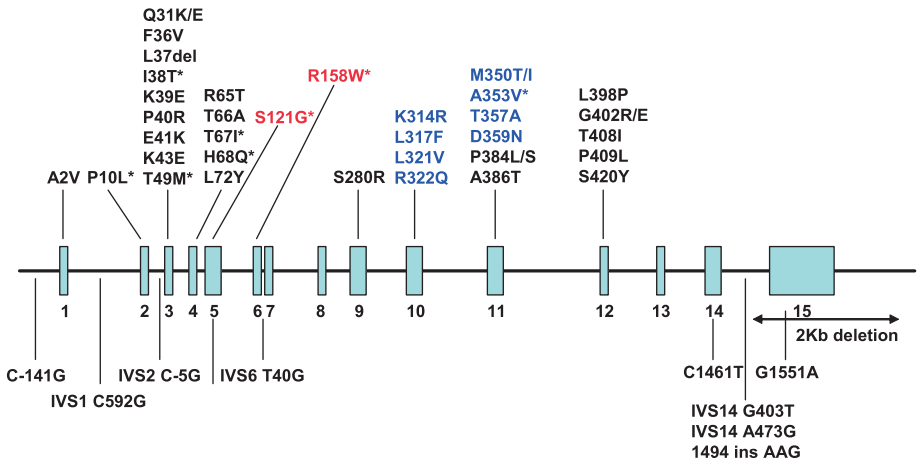


Fig. 2 *DKC1* mutations in dyskeratosis congenita
The mutations located within the TruB domain are in red. The mutations located within the PUA domain are in blue. The mutations marked with asterisks are seen in patients with HHS.

forms of DKC that develop slowly in adulthood without characteristic physical anomalies has been reported[12]. In addition, many patients with aplastic anemia (AA) show significant reductions in telomere length[13–15], which may have correlations with the severity of symptoms and refractoriness to immunosuppressive therapy. With regards to AA, MDS, and other conditions that are considered to be variants of BMF, the responsible genes have not yet been identified. It is possible that AA patients with significant telomere shortening, as well as AA and MDS patients who do not respond to immunosuppressive drugs, may include those with late-presenting DKC.

DKC1 Gene Mutations

Dyskerin, a nuclear protein coded by *DKC1*, is a small nucleolar RNA (snoRNA) with a boxH/ACA domain. It forms a complex with other snoRNAs such as NOP10, NHP2 and GAR1, and is believed to be associated with ribosomal RNA (rRNA) processing and pseudouridylation of pre-rRNA transcripts[16]. Furthermore, the snoRNA complex including Dyskerin combines with the boxH/ACA domain of *TERC* to secure the processing and stability of the telomerase complex[16].

Although X-linked DKC is believed to be caused by mutations in *DKC1*, large deletions and splicing mutations are rare since most of the mutations are point mutations (**Fig. 2**). These mutations are concentrated in exons 3, 4, 10, 11 and 12, and mutations are especially numerous on the PUA pseudouridine synthase motif in exon 11. In particular, A353V is found in about 30% of cases and is considered to be a hot spot. Furthermore, it should be noted that S121G and R158W, which are mutations of the domain where the TruB pseudouridine synthase motif resides, exhibit a phenotype of HHS that is considered to be a severe form of DKC. In addition, the T49M mutation is observed only in HHS, suggesting that an unknown functional domain exists in this region[10]. However, the correlation between the site of mutation and the severity of DKC and HHS is still largely unknown. For example, A353V (considered to be a hot spot for DKC) is also found in 8% of HHS patients, and the P10L and I38T mutations linked to HHS are also found in cases that exhibit phenotypes of DKC. Further study is needed to explain these facts[1,2,10]. Furthermore, there are three GC-rich *cis*-elements in the promoter domain of *DKC1* where Sp1 and Sp3 control the expression of *DKC1*. The −141C/G mutation in the Sp1 binding site is known to reduce the expression level of *DKC1* and cause DKC, suggesting that both qualitative and quantitative abnormalities of Dyskerin mutations could lead to DKC[17].

The occurrence of fetal lethality in *DKC1* knockout mice has been reported, indicating that Dyskerin has such important functions as pseudouridylating pre-rRNA within a cell and repairing telomeres[18]. As a model for DKC, an analysis was performed on Dyskerin hypomorphic mutant mice with deletions in exons 12～15 or just in exon 15 of *DKC1*[19]. While the expression of *DKC1* was significantly reduced in these mice, the phenotype of DKC reappeared by the second generation[19,20]. Also, reductions in m*TERC* expression and telomerase activity were observed. At the 4～6th generation, a shortening of telomere length was observed[19,20]. More recently, an in vitro functional analysis was performed by introducing the hot spot A353V into mouse ES cells, proving that A353V can influence the pathological expression by altering the levels of *TERC* and snoRNA accumulation, telomerase activity, and rRNA processing and pseudouridylation[21].

However, *DKC1* mutations have not yet been reported in such types of BMF as AA and MDS[22]. *DKC1* mutations may cause severe disorders in such functions as telomere repair in stem cells and pseudouridylation, which in turn could lead to serious impairment of hematopoietic as well as dermal and gastrointestinal mucosal stem cells, resulting in the pathological expression of DKC and HHS.

TERC Gene Mutations

TERC bonds with *TERT* (telomere reverse transcriptase) as well as the above-mentioned snoRNA protein, TEP1, p23, hsp90, etc. to form the telomerase complex. *TERC* resides within the complex as an RNA at 451bp without being translated into protein, and serves as a mold for telomere lengthening. *TERC* forms a secondary structure by itself. The pseudoknot domain and the CR4-CR5 domain in the 5' end bond with *TERT* and participate in telomerase activity. In the 3' end, the boxH/ACA domain is bonded to a snoRNA protein such as Dyskerin, and the CR7 domain bonds with the small Cajalbody RNAs protein (scaRNAs) via the CAB box (**Fig. 1**), participating in the processing and stabilization of the telomerase complex[8,23]. The ScaRNAs protein resides in the Cajalbody in the nucleus, and is thought to pseudouridylate, methylate, or otherwise modify the rRNA in a way similar to the snoRNA[23].

Autosomal dominant DKC is considered to develop due to *TERC* mutations (**Fig. 1**). In DKC families where *TERC* mutations are found, genetic anticipation has been observed in which the onset age of the disease becomes earlier and telomere shortening becomes more pronounced as the disease is passed on to the next generation[24]. Furthermore, in recent years, telomere shortening and *TERC* mutations have been reported in some cases of AA and MDS[2,12,23,25,26]. In Japan, too, a case of MDS with *TERC* mutation has been reported[27]. In these cases of AA and MDS, no characteristic physical anomalies of DKC were observed, and the disease developed slowly in adulthood. This suggests that some of the patients diagnosed with a conventional BMF may in fact have abnormalities in the telomerase complex. In addition, mutations were found at Sp1 binding sites in the promoter domain of *TERC* in patients with paroxysmal nocturnal hemoglobinuria (PNH)[28]. This suggests that BMF may develop through qualitative as well as quantitative abnormalities caused by *TERC* mutations, as was the case with *DKC1*.

Regarding *TERC* mutations, the results of prior analyses performed with knockout mice are available[19]. According to this study, no abnormal phenotypes were found in the first to third generations, although average telomere shortening of 4.3kb per generation was observed. Later generations presented chromosome aneuploidy and various phenotypes, including aspermatogenesis, testicular atrophy, impaired proliferative capacity of hematopoietic cells, premature aging in organs with active cellular outgrowth, and reduction in stress responses such as wound healing. The findings are interesting as they show similarities with the above-mentioned genetic anticipation in the DKC family with *TERC* mutations. Furthermore, telomere shortening of $terc^{+/-}$ mice was about 50% that of $terc^{-/-}$ mice, suggesting that the haploinsufficiency effect impedes the process of repairing telomeres to

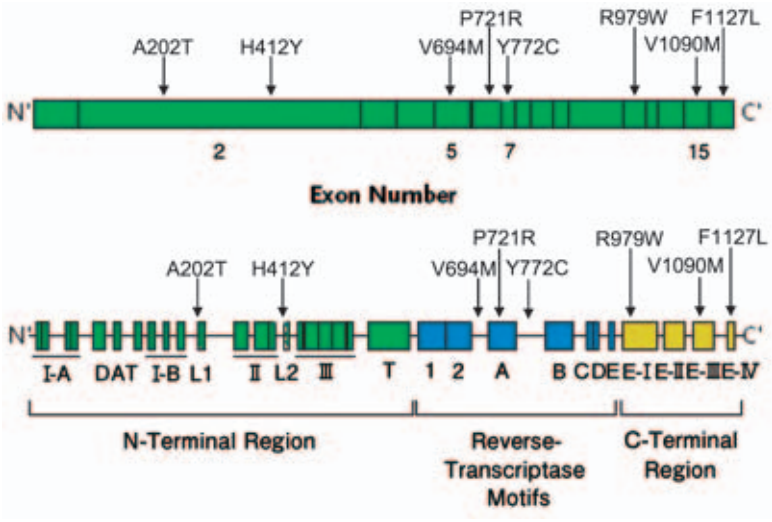


Fig. 3 The linear structure of *TERT* gene and location of mutations
P721R is located within motif A of the reverse transcriptase domain. R979W is located within essential region I of the nuclear export signal (NES) domain. F1127L is located within essential region IV of the C-terminal domain that dissociates the activities of telomerase (C-DAT).

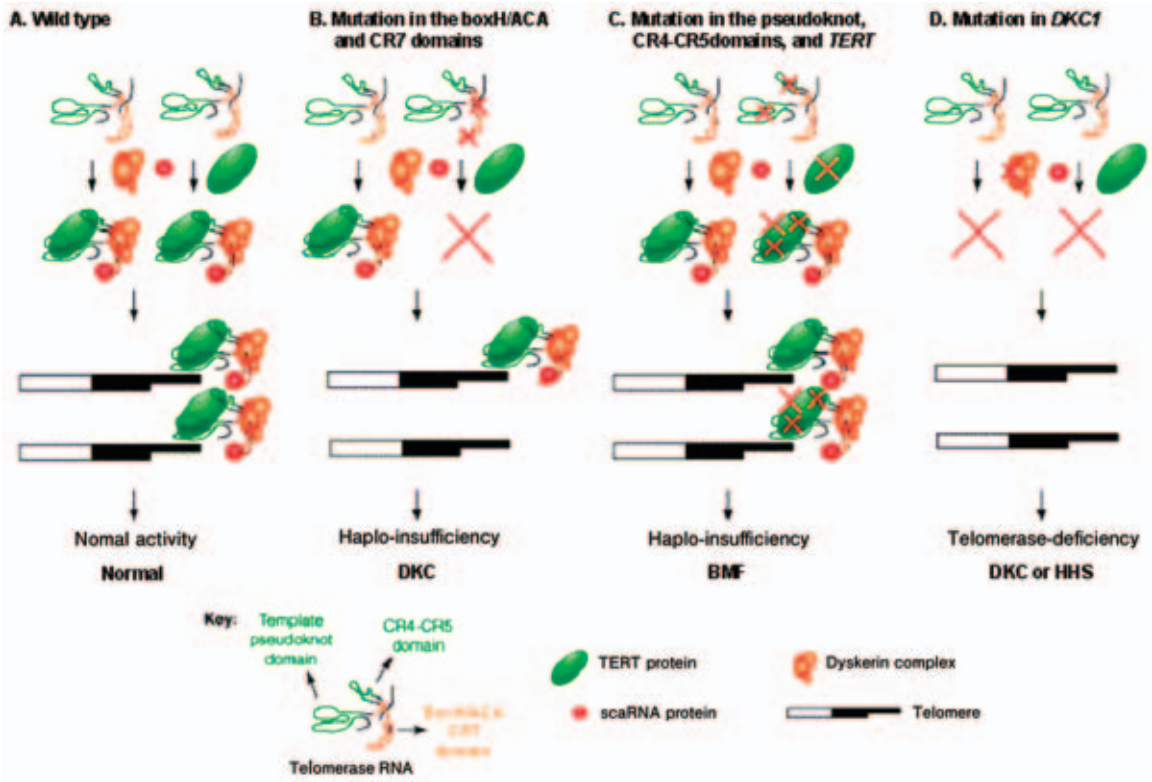
proper lengths[29]. In addition, the functional analysis of *TERC* mutations in vitro showed that mutations of the pseudoknot and CR4-CR5 domains in the 5' end directly impair the telomerase activity, while mutations of the boxH/ACA and CR7 domains in the 3' end indirectly impair the telomerase activity by impeding the assembly and accumulation of the telomerase complex[30,31]. Recently, two *TERC* variants with sequence changes located within the template region have been shown to exert dominant-negative effects[32]. However, other mutations are considered to impair the telomerase activity through haploinsufficiency rather than through dominant-negative effects[30,31].

TERT Gene Mutations

TERT, a reverse transcription enzyme in the telomerase complex coded at 5p15, comprises the following 3 regions: the N-terminus with the *TERC* binding function, the reverse transcriptase (RT) region with 7 conserved motifs that exhibits reverse transcription activity, and the C-terminus for multimerization of telomerase **(Fig. 3)**[8].

We recently performed mutation analyses of *TERT*, *DKC1*, *Nop10* and *NHP2* on three groups of AA patients: those who presented telomere shortening but no *TERC* abnormality; those with a family history; and those who did not respond to the immunosuppressive therapy. We found heterozygous mutations of *TERT* **(Fig. 3)** in these patients, and their telomere shortening was much more pronounced than in the age-match control group[22]. Vulliamy T. et al. also performed a *TERT* mutation analysis on patients with DKC or familial AA without abnormalities in *DKC1* and *TERC*, and found heterozygous mutations of *TERT*[5,10]. In Japan, *TERT* mutations have been reported in 2 cases of infantile AA[33]. In the cases we reported, the onset of disease was late (30 years or older) and no physical disorders distinctive to DKC were observed, while in the cases reported by Vulliamy, T. et al., the onset of disease was early (10 years or younger) and some of the patients exhibited phenotypes of HHS. We believe, therefore, that the clinical features of BMF with *TERT* mutations require further study.

Previous analyses of *TERT* knockout mice did not show any severe phenotypic disorders, although telomere shortening progressed with each new generation[19]. As was the case with $terc^{+/-}$ mice, however, the attenuation of telomerase activity and telomere shortening in $tert^{+/-}$ mice were about 50% of those in $tert^{-/-}$ mice, indicating that *TERC* and *TERT* in the telomerase complex control the



Reference 23 modified

Fig. 4 Models for the mechanisms underlying bone marrow failures due to telomerase complex gene mutations

A: The wild-type telomerase RNA is assembled with small nucleolar RNAs (snoRNA) and small Cajal-body RNA (scaRNA) proteins [including dyskerin complex (orange) and the putative CAB box-binding protein (red)] and telomerase reverse transcriptase (*TERT*; green) to form a functional telomerase complex that will later be recruited to the chromosome ends and maintain telomere length.

B: Mutations in the box H/ACA domain and the CR7 domain generate unstable *TERT* and cause reductions in RNA level and telomerase activity. This results in a haploinsufficiency phenotype.

C: Mutations in the pseudoknot domain (excluding the template region) and those in the CR4-CR5 domain generate stable but inactive RNA that can assemble with *TERT* to produce inactive enzyme. These inactive complexes might also lead to a haploinsufficiency phenotype and fail to maintain telomere length.

D: Mutations in the dyskerin affect the processing and stability of telomerase RNA. This then results in a global reduction in telomerase RNA level and telomerase activity, and telomere shortening occurs.

augmentation of telomere lengths with considerable precision[34]. An *in vitro* functional analysis of *TERT* mutations also showed that haploinsufficiency attenuates the telomerase activity and affects the repair and lengthening of telomeres[22]. These functional analyses indicate that *TERT* mutations impede the repair mechanism of telomeres and contribute to the development of BMF. However, the mechanism by which *TERT* mutations lead to the onset of BMF is still largely unclear. In a family with the A202T mutation (a genetic condition similar to that of *TERT* knockout mice), for example, telomere shortening is observed in family members other than the patient, too, but they have no indications of hematologic disorders[22]. Patients with cri du chat syndrome, which is caused by the deletion of chromosome 5p where *TERT* is coded, exhibit heterozygous deletions of *TERT*, which attenuate telomerase activity through haploinsufficiency[35]. Few hematological complications have been reported with this syndrome, although it should be taken into account that there are few cases of long-term survival. Considering the above, we believe *TERT* mutations lead to the onset of BMF

by impeding the telomere repair mechanism and through other factors such as aging.

Conclusion

While DKC is typically diagnosed in childhood and has characteristic physical anomalies, some forms of DKC have been found to present no such physical anomalies and develop slowly in adulthood. Considering the gene defects found in previous cases of DKC, we may reasonably assume that DKC is caused by abnormalities in the telomerase complex. However, the mutation screening of DKC patients with high-performance liquid chromatography (HPLC) conducted by Vulliamy T. et al. detected abnormalities of *DKC1*, *TERC* or *TERT* in only half of them[10]. Telomere shortening is also observed in various other cases of BMF, the responsible genes for which are yet to be identified, and it is possible that abnormalities in some other telomerase complex genes or telomere binding protein genes may prove to be responsible.

An outline of the mutations in the responsible genes is shown in **Figure 1**. In most cases, DKC that develops in childhood with characteristic physical anomalies is linked to a defect either in *TERC* at the 3' end or *DKC1* bonded to that site **(Fig. 1**, red). In contrast, DKC that develops slowly in adulthood without characteristic physical anomalies is linked to a defect in *TERC* at the 5' end or *TERT* **(Fig. 1**, blue). The telomerase complex regulates telomerase activity at the 5' end of *TERC* and controls RNA processing and stability at the 3' end[8,23]. Thus there is a possibility that dysfunctions of different mutation sites in the telomerase complex are responsible for different disease patterns **(Fig. 4)**.

DKC that develops slowly in adulthood with no physical anomalies has tended to be diagnosed as other forms of BMF such as AA or MDS. Given the fact that hematopoietic stem cell transplantation is a proven treatment option for DKC, applying genetic diagnosis to cases of BMF and correctly identifying late-presenting DKC will be essential in determining the appropriate course of treatment.

References

1. Dokal I: Dyskeratosis congenita in all its forms. Br J Haematol 2000; 110: 768–779.
2. Marrone A, Walne A, Dokal I: Dyskeratosis congenita: telomerase, telomeres and anticipation. Curr Opin Genet Dev 2005; 15: 249–257.
3. Heiss NS, Knight SW, Vulliamy TJ, et al.: X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. Nat Genet 1998; 19: 32–38.
4. Vulliamy T, Marrone A, Goldman F, et al.: The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 2001; 413: 432–435.
5. Vulliamy TJ, Walne A, Baskaradas A, et al.: Mutations in the reverse transcriptase component of telomerase (*TERT*) in patients with bone marrow failure. Blood Cells Mol Dis 2005; 34: 257–263.
6. Armanios M, Chen JL, Chang YP, et al.: Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A 2005; 102: 15960–15964.
7. Vulliamy TJ, Marrone A, Knight SW, et al.: Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation. Blood 2006; 107: 2680–2685.
8. Cong YS, Wright WE, Shay JW: Human telomerase and its regulation. Microbiol Mol Biol Rev 2002; 66: 407–425.
9. Vulliamy TJ, Knight SW, Mason PJ, et al.: Very short telomeres in the peripheral blood of patients with X-linked and autosomal dyskeratosis congenita. Blood Cells Mol Dis 2001; 27: 353–357.
10. Vulliamy TJ, Marrone A, Knight SW, et al.: Mutations in dyskeratosis congenita: their impact on telomere length and the diversity of clinical presentation. Blood 2006; 107: 2680–2685.
11. Marrone A, Dokal I: Dyskeratosis congenita: molecular insights into telomerase function, ageing and cancer. Expert Rev Mol Med 2004; 6: 1–23.
12. Fogarty PF, Yamaguchi H, Wiestner A, et al.: Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet 2003; 362: 1628–1630.
13. Ball SE, Gibson FM, Rizzo S, et al.: Progressive telomere shortening in aplastic anemia. Blood 1998; 91: 3582–3592.
14. Brummendorf TH, Maciejewski JP, Mak J, et al.: Telomere length in leukocyte subpopulations of patients with aplastic anemia. Blood 2001; 97: 895–900.
15. Lee JJ, Kook H, Chung IJ, et al.: Telomere length changes in patients with aplastic anaemia. Br J Haematol 2001; 112: 1025–1030.
16. Filipowicz W, Pogacic V: Biogenesis of small nucleolar ribonucleoproteins. Curr Opin Cell Biol 2002; 14: 319–327.
17. Salowsky R, Heiss NS, Benner A, et al.: Basal transcription activity of the dyskeratosis congenita gene is mediated by Sp1 and Sp3 and a patient mutation in a Sp1 binding site is associated with

decreased promoter activity. Gene 2002; 293: 9–19.
18. He J, Navarrete S, Jasinski M, et al.: Targeted disruption of Dkc1, the gene mutated in X-linked dyskeratosis congenita, causes embryonic lethality in mice. Oncogene 2002; 21: 7740–7744.
19. Cheong C, Hong KU, Lee HW: Mouse models for telomere and telomerase biology. Exp Mol Med 2003; 35: 141–153.
20. Ruggero D, Grisendi S, Piazza F, et al.: Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. Science 2003; 299: 259–262.
21. Mochizuki Y, He J, Kulkarni S, et al.: Mouse dyskerin mutations affect accumulation of telomerase RNA and small nucleolar RNA, telomerase activity, and ribosomal RNA processing. Proc Natl Acad Sci U S A 2004; 101: 10756–10761.
22. Yamaguchi H, Calado RT, Ly H, et al.: Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med 2005; 352: 1413–1424.
23. Chen JL, Greider CW: Telomerase RNA structure and function: implications for dyskeratosis congenita. Trends Biochem Sci 2004; 29: 183–192.
24. Vulliamy T, Marrone A, Szydlo R, et al.: Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. Nat Genet 2004; 36: 447–449.
25. Vulliamy T, Marrone A, Dokal I, et al.: Association between aplastic anaemia and mutations in telomerase RNA. Lancet 2002; 359: 2168–2170.
26. Yamaguchi H, Baerlocher GM, Lansdorp PM, et al.: Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood 2003; 102: 916–918.
27. Takeuchi J, Hinh L, Adam B, et al.: Identification and Functional Characterization of Telomerase RNA Variant Alleles in Japanese Patients with Aplastic Anemia or Myelodysplastic Syndrome. Blood 2006; 108: 292a #977.
28. Keith WN, Vulliamy T, Zhao J, et al.: A mutation in a functional Sp1 binding site of the telomerase RNA gene (hTERC) promoter in a patient with Paroxysmal Nocturnal Haemoglobinuria. BMC Blood Disord 2004; 4: 3.
29. Hathcock KS, Hemann MT, Opperman KK, et al.: Haploinsufficiency of mTR results in defects in telomere elongation. Proc Natl Acad Sci U S A 2002; 99: 3591–3596.
30. Marrone A, Stevens D, Vulliamy T, et al.: Heterozygous telomerase RNA mutations found in dyskeratosis congenita and aplastic anemia reduce telomerase activity via haploinsufficiency. Blood 2004; 104: 3936–3942.
31. Ly H, Calado RT, Allard P, et al.: Functional characterization of telomerase RNA variants found in patients with hematologic disorders. Blood 2005; 105: 2332–2339.
32. Xin ZT, Beauchamp AD, Calado RT, et al.: Functional characterization of natural telomerase mutations found in patients with hematologic disorders. Blood 2007; 109: 524–532.
33. Liang J, Yagasaki H, Kamachi Y, et al.: Mutations in telomerase catalytic protein in Japanese children with aplastic anemia. Haematologica 2006; 91: 656–658.
34. Liu Y, Snow BE, Hande MP, et al.: The telomerase reverse transcriptase is limiting and necessary for telomerase function in vivo. Curr Biol 2000; 10: 1459–1462. [Erratum, Curr Biol 2001; 11: 907.].
35. Zhang A, Zheng C, Hou M, et al.: Deletion of the telomerase reverse transcriptase gene and haploinsufficiency of telomere maintenance in Cri du chat syndrome. Am J Hum Genet 2003; 72: 940–948.

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