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Dyskeratosis congenita as a disorder of telomere maintenance

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Abstract

Since 1998, there have been great advances in our understanding of the pathogenesis of dyskeratosis congenita (DC), a rare inherited bone marrow failure and cancer predisposition syndrome with prominent mucocutaneous abnormalities and features of premature aging. DC is now characterized molecularly by the presence of short age-adjusted telomeres. Mutations in seven genes have been unequivocally associated with DC, each with a role in telomere length maintenance. These observations, combined with knowledge that progressive telomere shortening can impose a proliferative barrier on dividing cells and contribute to chromosome instability, have led to the understanding that extreme telomere shortening drives the clinical features of DC. However, some of the genes implicated in DC encode proteins that are also components of H/ACA-ribonucleoprotein enzymes, which are responsible for the posttranslational modification of ribosomal and spliceosomal RNAs, raising the question whether alterations in these activities play a role in the pathogenesis of DC. In addition, recent reports suggest that some cases of DC may not be characterized by short age-adjusted telomeres. This review will highlight our current knowledge of the telomere length defects in DC and the factors involved in its development.

Keywords

dyskeratosis congenita; telomerase; telomere

1. Clinical features of dyskeratosis congenita and its variants

The first descriptions of dyskeratosis congenita (DC) date back to the early 1900s [1]. These initial reports focused on a mucocutaneous triad of abnormal reticulated skin pigmentation, nail dystrophy and mucosal leukoplakia. Over the ensuing years, however, it became increasingly apparent that individuals with DC have a markedly high risk of bone marrow failure such that, today, DC is best known as an inherited bone marrow failure syndrome [2]. Bone marrow failure develops in 80 to 90 percent of patients with DC by age 30 years and is the leading cause of death [3]. There are no targeted therapies for patients with DC.

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Treatment with androgens may lead to improvement in anemia and, at times, platelet counts, but does not result in sustained responses. Historically, hematopoietic stem cell transplantation was associated with significant morbidity and mortality for patients with DC, however, the utilization of transplant regimens tailored for these patients is resulting in improved outcomes [4]. Thus, mortality due to bone marrow failure may decrease in the years to come.

Reports of clinical data from large cohorts with DC highlight the wide range of additional manifestations that may be present in this disorder [5, 6]. In addition to bone marrow failure, individuals with DC have increased risk of malignancy, with a 40 to 50 percent cumulative incidence by age 50 years [7]. For example, the risks of squamous cell carcinoma of the tongue, acute myeloid leukemia, and myelodysplastic syndrome, which may evolve into acute myeloid leukemia, are markedly increased. As outcomes following hematopoietic stem cell transplantation improve and patients survive their bone marrow failure, the incidence of malignancy in individuals with DC is expected to increase. Pulmonary fibrosis is an additional life-threatening manifestation of DC. In fact, pulmonary fibrosis alone, in the absence of other major findings, is the most common manifestation of the cellular defect underlying DC (discussed in detail in the article by M. Armanios in this issue) [8–11].

Additional “minor” features of DC include intrauterine growth retardation, developmental delay, microcephaly, abnormalities involving the eyes, teeth, and hair, including premature graying, excessive sweating, short stature, hypogonadism, enteropathy, liver disease, esophageal and urethral stenosis, osteoporosis and avascular necrosis of the hips or shoulders [5, 6]. Importantly, the recognition of DC as a multisystem disorder has allowed for broadening of the diagnostic criteria to beyond that of the classic triad of abnormal reticulated skin pigmentation, nail dystrophy and mucosal leukoplakia (Table 1) [12, 13].

Revesz syndrome (MIM #268130) and Hoyeraal Hreidarsson syndrome (MIM #300240) are two Mendelian conditions with significant clinical overlap with DC (Table 1). As discussed below, molecular genetics have clinched the link with these disorders. Each is considered a severe variant of DC, not only because of the clinical findings, but also the early childhood onset and apparently markedly shortened lifespan observed, with no reported cases older than 20 years of age [6]. The defining feature of Revesz syndrome is bilateral exudative retinopathy occurring in association with intracranial calcification (in most cases) and features of DC such as bone marrow failure, mucocutaneous abnormalities, intrauterine growth retardation, cerebellar hypoplasia, and developmental delay [14, 15]. Hoyeraal-Hreidarsson syndrome is characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, moderate to severe mental retardation, and progressive immunodeficiency and pancytopenia [16, 17]. Individuals may be diagnosed with Hoyeraal-Hreidarsson syndrome if four or more of these findings are present or if they have cerebellar hypoplasia and additional features of DC [12, 13]. Like Revesz syndrome, the mucocutaneous features of DC and cerebral calcifications may be present [18, 19].

2. Short telomeres underlie DC

Telomeric DNA consists of tandem repeats of TTAGGG sequence, and the length of telomeres is maintained only when sufficient levels of telomerase, the telomere replication enzyme, are expressed. A pivotal advance in our understanding of the underpinnings of DC came with the 1999 discoveries that dyskerin, the protein encoded by the gene mutated in X-linked recessive DC (*DKC1*) [20], is associated with telomerase, and that cells from *DKC1*-mutant DC patients have reduced levels of hTR, the RNA subunit of telomerase to which dyskerin binds, reduced telomerase activity, and short telomeres [21]. With these findings, many years of basic telomere biology research and translational and clinical DC studies

converged. Subsequent genome-wide linkage analysis and candidate gene sequencing led to the identification of six additional DC-associated genes, five of which impact telomerase [*TERT, hTR* (formally known as *TERC*), *TCAB1* (also known as *WRAP53* and *WDR79*), *NOP10*, and *NHP2*], and a sixth (*TINF2*), which encodes a central component of the telomere binding complex shelterin (TIN2) (Fig. 1) [22–28]. Furthermore, short age-adjusted telomeres are observed, not only in DC patients with mutations in these telomere biology genes, but also in the approximately 40 to 50 percent of those with genetically uncharacterized disease [29, 30]. Thus, DC has been referred to as a syndrome of telomere shortening [23].

Several methods have been used to assess telomere length in DC (see article by P. Lansdorp in this issue). With the telomere flow FISH method [31], which combines fluorescence in situ hybridization with flow cytometry, an absolute telomere length less than the first percentile for age in multiple white blood cell subsets (such as total lymphocytes or granulocytes, naïve T cells and B cells) is both sensitive and specific for DC, correctly identifying over 90% of the DC patients and excluding over 90% of the non-DC patients or unaffected DC relatives tested [29]. Thus, telomere length assessment has become an important aid in the clinics for screening for DC in patients, particularly those in whom the classical mucocutaneous findings of DC are not apparent. Importantly, abnormally short telomere lengths do not appear to be restricted to the hematopoietic compartment in DC, although normal distribution curves have not been established for cells other than hematopoietic. For example, relative telomere length in early passage primary skin fibroblasts was shorter in patients with DC as compared to normal (but not age-matched) controls [32]. In another study, strong correlation of relative telomere length was observed in blood and buccal cells, and buccal cells and fibroblasts isolated from individuals with DC [33]. Thus, leukocyte telomere length appears to be a good surrogate for so-called ‘constitutional’ telomere length.

The extent of telomere shortening correlates with the clinical manifestations of DC, such that patients with the shortest telomeres often present earlier and with more severe findings [23, 34, 35]. This relationship is further exemplified in autosomal dominant DC in which there is a progressive decrease in the average telomere length and an accumulation of the shortest telomeres in mutation carriers across generations [23, 34]. Importantly, disease anticipation, defined as a decrease in age of onset and increase in disease severity across successive generations, correlates with the progressive telomere length changes. This disease anticipation due to changes in telomere length rather than the presence of a mutation *per se* mimics the progressively severe phenotypes first reported with successive generations of the telomerase knockout mouse and telomerase knockout yeast [36–39] (see the articles by M. Armanios and K. Rudolph in this issue for further discussions on anticipation and mouse models, respectively).

This evidence makes it clear that accumulation of critically short telomeres underlies the pathophysiology of dyskeratosis congenita, and each of the seven genes mutated in DC, either the core telomerase components, factors required for telomerase biogenesis, or a telomere bound factor, results in defective telomere length maintenance. It has been well established that telomere length imposes a barrier on the proliferative capacity as demonstrated for primary human cells in culture and in mouse models [37, 40, 41]. Once telomeres have reached a critically short length, the cell cycle is arrested and cells then undergo either senescence or apoptosis [42–44]. Animal models indicate that, in DC, the failure of tissues such as bone marrow, skin, the gastrointestinal epithelium, liver and lung, is the result of restraint placed on the proliferative capacity of stem cells in these tissues by the critically short telomeres [37, 45].

3. X-linked recessive DC: *DKC1*

The seven genes implicated in DC result in three modes of transmission, X-linked recessive, autosomal dominant, and autosomal recessive. Mutations in *DKC1* can be identified in almost all cases where X-linked recessive inheritance is most clearly evident (i.e., there are multiple affected males, maternal transmission and no affected females). Overall, *DKC1* mutations are also the most common in DC, occurring in 30% of all probands [13, 35, 46]. Interestingly, even in one of the few exceptional cases of X-linked inheritance in which a deleterious *DKC1* variant could not be found, linkage analysis on the X-chromosome identified a single peak, which encompassed the *DKC1* locus, and dyskerin levels were found to be reduced in affected males [46]. Mothers heterozygous for *DKC1* mutations exhibit a skewed pattern of X-inactivation [47] and are generally asymptomatic, although limited mucocutaneous findings have been reported in some female carriers of X-linked disease [46, 48, 49]. Most often, males with *DKC1* mutations exhibit a severe disease phenotype and present in early childhood, including some with Hoyeraal Hreidarsson syndrome (Fig. 2) [50, 51].

Dyskerin is a component of the telomerase holoenyzme [52], which binds via the H/ACA domain of the telomerase RNA subunit, hTR [21]. Dyskerin is required for hTR stability and telomerase biogenesis [21, 53, 54]. Consequently, hTR levels are limiting in *DKC1* mutant cells, which harbor less than half the normal amount of telomerase [21, 54]. The importance of the hTR deficiency on the growth of *DKC1* mutant cells was demonstrated by the observation that telomere length maintenance and the premature senescence phenotype of human *DKC1*- mutant primary cells required the co-expression of *hTR* with *TERT*, in contrast to control cells, which could maintain telomere length and growth with forced expression of *TERT* alone [55].

Dyskerin is also a highly conserved pseudouridine synthase, which, when in complex with NOP10, NHP2, GAR1, and H/ACA-containing scaRNAs or snoRNAs, catalyses the pseudouridinylation of specific uridine residues in spliceosomal and ribosomal RNAs, respectively [56]. Therefore, it has been questioned whether loss of this activity may contribute to the often-severe clinical phenotype manifested by patients with *DKC1* mutations [55, 57–59]. Notably, however, most *DKC1* mutations map to the N and C terminal domains, and spare the TruB central pseudouridine synthase catalytic domain (Fig. 2) [60]. Additionally, the vast majority of DC-associated *DKC1* mutations, including the DKC1p.A353V mutation present in 40% of probands, result in single amino acid substitutions, and definitive N terminal truncating mutations (e.g., nonsense or frameshift) have not been reported (Fig. 2) [60]. This suggests that uridine synthase catalytic activity is essential for cell viability or, albeit much less likely, results in a viable phenotype other than DC when severely reduced or absent.

More directly, although effects on pseudouridinylation of ribosomal RNAs and ribosome biogenesis have been observed in *DKC1* mutant mouse models [57, 61], no discernible effects have been observed in patient-derived *DKC1* mutant primary fibroblasts or lymphoblastoid cell lines [21, 55]. Additionally, no defects in ribosome biogenesis were detected *DKC1* mutant primary human cells revitalized with forced expression of *hTR* and *TERT* [55]. Lastly, modeling of N and C terminal DC-associated dyskerin mutations onto the structure of the archaeal H/ACA ribonucleoprotein complex suggested that the mutations cluster in a single domain formed by the N and C termini that does not overlap with NOP10, NHP2, or Gar1 binding sites [62]. Consistent with this, *DKC1* mutations do not significantly impact dyskerin/NOP10/NHP2/NAF1 complex formation in vitro [63]. Notably, the dyskerin mutations also do not overlap with RNA binding sites nor alter the association of the dyskerin/NOP10/NHP2/NAF1 complex with the H/ACA domain of hTR [62, 63].

Instead, they have been found to modulate the interaction of dyskerin with the pre-H/ACA ribonucleoprotein assembly factor SHQ1 [64]. How altering dyskerin – SHQ1 interactions might differentially affect the telomerase RNP remains to be determined. Allosteric effects on hTR binding may be at play, as a set of assayed mutants, including the A353V mutant, showed impaired interaction with hTR in vitro [65].

4. Sporadic (de novo) autosomal dominant DC: *TINF2*

The second most commonly mutated gene in DC is *TINF2*, which bears mutations in approximately 15% of all probands [13, 27, 66]. All reported mutations are heterozygous, resulting in autosomal dominant disease. Although a family with autosomal inheritance of a *TINF2* mutation has been reported [27], these mutations are most often de novo, and result in a dramatic telomere shortening within the first generation [27, 66]. Moreover, compared to DC patients with other mutations, those with *TINF2* mutations have shorter telomere lengths, earlier onset of disease, and are more likely to present with bone marrow failure prior to manifesting any signs of the classic triad [27, 66]. Not surprisingly, *TINF2* mutations have been identified in some patients with the severe DC variants Revesz and Hoyeraal Hreidarsson syndromes and, thus far, are only gene mutations associated with Revesz syndrome.

Unlike the other factors implicated in DC, TIN2 is a component of the shelterin complex, where it occupies a central position, binding the double-stranded telomeric DNA binding factors, TRF1 and TRF2, and, thereby recruiting another binding partner, TPP1 (and secondarily its binding partner POT1), to regions of double-stranded telomeric DNA (Fig. 1) [67]. Knockdown of TIN2 in human cell lines results in telomere elongation indicating it functions as a negative regulator of telomere length [68]. TIN2's positive modulation of the levels of TRF1, another known negative telomere length regulator, has been proposed to underlie this regulation. Conversely, TIN2 depletion has also been shown to impair the recruitment of telomerase to telomeres, likely via its crucial role in anchoring TPP1 at telomeres [69]. Thus, TIN2 may have both positive and negative roles in telomere length regulation. However, because TIN2 depletion is accompanied by reduction in both TRF1 and TPP1 levels [69, 70], TIN2-independent contributions to length regulation have not been established. TIN2 also functions in sister telomere cohesion, and secondarily in telomere break repair, via its interaction with the cohesin subunit SA1 [70, 71]. Lastly, two isoforms of TIN2 are expressed in human cells, including a longer isoform, known as TIN2L, which is formed by the inclusion of three C-terminal exons through alternative splicing [72]. While both isoforms appear to bind similarly to TRF1, TRF2, and TPP1, TIN2L has been shown to a have greater association with the nuclear matrix and telomeres. Therefore, it is unlikely to perform functions identical to those of the shorter isoform, analyzed in the prior functional studies.

Understanding the mechanism(s) responsible for the rapid and severe shortening observed in patients with germline *TINF2* mutations has been of great interest, but to date remains unclear. In contrast to the reduction in hTR levels that is observed with certain telomerase associated mutations (e.g., in *DKC1*), hTR levels are unaffected in TIN2 mutant cells [66]. Additionally, ectopically overexpressed TIN2 bearing the most common DC-associated mutations does not differentially impact telomerase activity levels (as measured in a semiquantitative assay); hTR, TRF1, TPP1, or TRF2 levels; the ability of TIN2 to interact with TRF1, TRF2 or TPP; or the subcellular and telomere localization of TIN2 [73]. Instead, the DC mutations lead to a reduction in TIN2's association with telomerase, consistent with a role for TIN2 in modulating telomerase recruitment. The extent to which this accounts for the mechanism by which TIN2 mutations result in telomere shortening in patients, however, is questioned given only a modest effect is observed in the *in vitro* telomerase-association

assay (at most a 40% reduction), whereas dramatic shortening is observed in patient-derived cells, which still also carry a wild type allele.

Strikingly, nearly all of the pathogenic TIN2 mutations reported to date cluster within a highly conserved 18 amino acid region from K280 to Q298 (Fig. 3) [27, 60, 66]. The function of this segment of TIN2 remains to be elucidated, but prior interaction mapping indicates it lies C-terminal to its TRF1, TRF2, TPP1, and SA1 binding domains and, therefore, is unlikely to impact significantly on TIN2's interaction with any of these proteins [70, 74–76]. Recently, two additional nonsense mutations at amino acids 269 and 271 have been reported in patients with DC, extending the mutated region of TIN2 to include amino acids directly adjacent to the TRF1 binding domain [77]. These truncation proteins are expressed, and the most N terminal truncation protein, encompassing the first 269 amino acids of TIN2, was found to have markedly reduced interactions with TRF1, while the most common missense mutation, TIN2p.R282H, had little effect on this interaction. This suggests that while some mutations may affect TRF1 binding to TIN2, it is unlikely that this is the underlying mechanism driving pathogenesis in all DC patients with *TINF2* mutations.

The identification of *TINF2* mutations in patients with DC raises the question as to whether mutations in genes encoding other shelterin components might be present in among the 40 to 50 percent genetically uncharacterized cases. To address this question, Savage et al, analyzed the sequences of the other five shelterin genes (*ACD, POT1, TERF1, TERF2* and *TERF2IP*, which encode TPP1, POT1, TRF1, TRF2 and hRAP1, respectively) in a cohort of 16 patients with DC and 7 patients with very short telomeres in some or all leukocyte subsets, bone marrow failure, and features suggestive of DC, but who did meet diagnostic criteria [78]. Although rare sequence variants were identified, none were predicted to alter coding sequence or affect splicing. Therefore, mutations in the other shelterin components, at the very least, do not appear to be a common cause of DC.

5. Inherited autosomal dominant DC: *hTR* and *TERT*

A third mechanism leading to telomere shortening in DC patients is via mutations in the genes encoding the essential telomerase components hTR and TERT (Fig. 1) [22–24]. Indeed, the identification of germline mutations *hTR* and *TERT* point directly to a role for critically short telomeres in the pathogenesis of this disorder. *TERT* and *hTR* are mutated in 5–10% of DC cases each [13]. In nearly all cases, except for a few reported *TERT* cases [35, 79], the mutations are heterozygous, and, hence, cause autosomal dominant disease [22, 80]. In families carrying these mutations, disease anticipation is often seen [23]. In contrast to *DKC1* and *TINF2* mutations, *hTR* and *TERT* mutations have been found not only in patients with DC, but also in individuals suffering from a variety of disorders including idiopathic pulmonary fibrosis (discussed in detail by M. Armanios in this issue), aplastic anemia (sometimes referred to as acquired), myelodysplastic syndrome, and familial and sporadic liver disease [8, 81–83].

Since the original description of *hTR* mutations in three DC families [22], approximately 40 additional mutations, either nucleotide substitutions or deletions, have been reported in the 451 nucleotide hTR RNA (Fig. 4) [60]. Although disease-associated mutations are distributed throughout the RNA, including those that manifest as DC, the majority map to the pseudoknot/core domain, which contains sequences required for catalysis, a TERT binding site, and the template, which specifies the telomeric repeat sequence. Some mutations result in decreased hTR levels, whereas others have no discernible effect on hTR accumulation [22, 84]. Functional analyses of a number of these indicate that they are predominantly loss of function, rather than dominant negative mutations [84]. Mechanisms of loss of function include reduced association with TERT, decreased catalytic activity of

the assembled RNP, and altered fidelity of telomeric repeat addition [85]. Taken together, these findings indicate that hTR is limiting within cells and *hTR* haploinsufficiency can lead to clinical features of DC [86], as shown in the heterozygous telomerase RNA knockout mouse model [87, 88].

Similar to *hTR*, close to 50 distinct disease-associated mutations have been reported in *TERT* [60]. These map to each of its various functional domains (Fig. 5). As with *hTR*, mutations in *TERT* have been identified not only in patients with DC, but also Hoyeraal Hreidarsson syndrome and other diseases associated with telomere shortening, most prominently and more frequently, idiopathic pulmonary fibrosis (see article by M. Armanios in this issue) [8, 79, 80, 83, 89, 90]. Notably, however, *TERT* mutations associated with the DC phenotype map predominantly to the C terminal portion of the protein including the reverse transcriptase domain and the C terminal extension, which promotes processivity [91–94]. Functional analysis of select mutations has revealed that the majority impact correspondingly on catalytic activity or repeat processivity [23, 35, 79, 85, 95, 96]. Dominant negative effects have not been observed. Thus, similar to *hTR* mutations, haploinsufficiency underlies the mechanism of *TERT* mutation-associated disease [23].

6. Autosomal recessive DC: *TCAB1, NOP10, NHP2*, and *TERT*

Some cases of DC are due to autosomal recessive mutations. Recently, the identification of germline compound missense mutations in the gene encoding TCAB1 (*TCAB1*; formally *WRAP53*) revealed defective telomerase trafficking as a cause of autosomal recessive DC (Fig. 1) [28]. TCAB1 mediates the localization of specific H/ACA class RNAs, including hTR, to Cajal bodies through recognition of a CAB box [97, 98]. TCAB1 associates with active telomerase and is required for its delivery to the telomeres in S phase [97]. Knockdown of TCAB1 results in loss of dyskerin and hTR from Cajal bodies, mislocalization of hTR to the nucleolus, and progressive telomere shortening over time [28, 97]. Thus, the directing of the telomerase complex to the Cajal body by TCAB1 appears to play a crucial role in telomere length maintenance.

In the first report of *TCAB1* mutations in humans, the two unrelated probands had classical DC and blood lymphocyte telomere lengths below the first percentile [28]. Parents and siblings of the probands were found to have heterozygous mutations in *TCAB1* and telomere lengths within normal range, consistent with an autosomal recessive inheritance. The mutated residues were highly conserved and significantly decreased the overall TCAB1 protein level, and its localization to Cajal bodies in patient-derived Epstein Barr virus transformed lymphoblasts. Similar to the effects of knockdown of TCAB1 in cancer cell lines, the biallelic *TCAB1* mutations also resulted in loss of dyskerin and hTR in Cajal bodies, and nucleolar hTR in the lymphoblastoid cells suggesting a loss-of-function mechanism. In contrast to dyskerin-mutant lymphoblasts, the *TCAB1*-mutant lymphoblasts had normal hTR levels, further underscoring the distinct mechanisms by which these hTR-associated proteins impact on telomerase. Precisely how the recruitment of the telomerase complex to the Cajal body impacts on telomerase function or regulation remains to be determined. Any additional factors identified would be prime candidates for screening genetically uncharacterized cases of DC.

NOP10 and *NHP2* have also been implicated in autosomal recessive DC (Fig. 1). NOP10 and NHP2 associate with dyskerin, and are likewise required for the pseudouridinylation of specific uridine residues in spliceosomal and ribosomal RNAs [56]. Also similar to dyskerin, they associate with hTR and are constituents of the active telomerase complex [52, 56]. Homozygosity mapping of affected individuals from consanguineous families with genetically uncharacterized DC led to the identification of a single *NOP10* mutation

(homozygous R34W) in one of 16 families [25]. Directed sequencing of an additional 117 uncharacterized probands revealed no additional pathogenic changes. In a subsequent study, *NHP2* sequencing revealed deleterious mutations in two (Y139H/Y139H and V126M/X154RextX*52) of the 117 probands [26]. Thus, *NHP2* and *NOP10* mutations are very rare.

As with *DKC1* mutations, the individuals with *NOP10* or *NHP2* mutations had severe telomere shortening and reduced hTR levels. In contrast to *DKC1* mutations, DC-associated *NOP10* and *NHP2* mutations were found to impact pre-RNP assembly in vitro, with NHP2p.Y139H and V126M affecting dyskerin-NOP10-NHP2-GAR1 tetramer formation, and NOP10p.R34W affecting the association of the tetramer with hTR and a subset of H/ACA RNAs [63]. Because of the rarity of the naturally occurring mutations, genotype-phenotype correlations await additional characterized patients.

Lastly, some cases of autosomal recessive DC, including the variant Hoyeraal Hreidarsson syndrome, have been attributed to mutations in *TERT* [35, 79]. These mutations are located in either the reverse transcriptase or C terminal extension domains of TERT, and have been found in consanguineous families. In these autosomal recessive cases, the unaffected heterozygous parents have telomere lengths ranging from very short to normal [25, 26, 79, 96], indicating parents of some autosomal recessive cases may themselves manifest DC-related pathologies later in life.

7. DC with normal telomere length?

Recently, six families in the London-based Dyskeratosis Congenita Registry were found to have homozygous mutations in *C16orf57* [30], a gene implicated previously in Poikiloderma with Neutropenia (MIM #604173). Poikiloderma with Neutropenia is another rare Mendelian syndrome, which has overlapping clinical features with DC, including abnormal skin pigmentation, nail dystrophy, neutropenia, and pulmonary disease [99]. *C16orf57* encodes a protein of unknown function. Notably, the individuals in the Dyskeratosis Congenita Registry with *C16orf57* mutations were found to have normal leukocyte telomere length in stark contrast to the very short leukocyte telomere length observed in the vast majority individuals with, not only genetically characterized, but also genetically uncharacterized DC [30]. Could it be then that defective telomere maintenance is not the sole basis of DC? An untested possibility is that *C16orf57* mutations result in a length-independent telomere defect (e.g., loss of the 3’ terminal single strand extension, known as the G-overhang) that leads to telomere dysfunction, leading to similar cellular and tissue pathology as occurs in the context of DC with critically short telomeres. Alternatively, it may be that clinical features alone, such as the appearance, distribution and timing of skin changes can distinguish individuals who have *C16orf57* mutations and normal telomeres from those with classical DC and very short telomeres. Clearly, further research is needed to better elucidate the role C16orf57 plays within normal cells, and, if any, at the telomere and in DC. Importantly, for those cases in which DC is suspected, but telomere lengths are normal, *C16orf57* sequence analysis should be considered.

Two additional reports support the notion that some cases of DC or its variants may be due to telomere length-independent telomere dysfunction. Both reports describe children with the DC-variant Hoyeraal Hreidarsson syndrome. In the first report, the patient expressed a unique splice variant of Apollo, a 5’ to 3’ exonuclease implicated in both intrastrand crosslink repair and telomere protection [100]. Primary dermal patient fibroblasts exhibited increased telomere dysfunction induced foci, despite normal bulk telomere length. Whether patient leukocyte subsets exhibited normal telomere length as well was not ascertained. Additionally, SV40-transformed patient fibroblasts exhibited an increased frequency of telomeric doublets, possibly reflecting impaired telomere replication. Consistent with these

findings, mammalian cell culture studies have revealed roles for Apollo in relieving telomere replicative stress and in the maintenance of telomere end structure [101–103]. The identification of additional patients with Apollo defects will be needed to determine the mechanism and role of Apollo-mediated telomere dysfunction in the context of DC or, possibly specifically, Hoyeraal Hreidarsson syndrome. This case suggests that the development of additional clinical assays to assess telomere function may be helpful in the future.

Similarly intriguing results have been reported in another patient with Hoyeraal Hreidarsson syndrome [104]. As in the patient described above, the primary dermal fibroblasts from this patient had increased telomere dysfunction induced foci yet normal telomere length. In this second study, however, leukocyte telomere length was measured and found to be severely short in the proband and affected siblings. One proposed explanation for the differences in telomere lengths is that it relates to differences in the replicative demand on dermal fibroblasts versus peripheral blood leukocytes. Interestingly, the length of the 3’ telomeric overhang was similarly diminished in the leukocytes and dermal fibroblasts, pointing to loss of telomere end structure as the primary mechanism of disease. The gene encoding Apollo was not sequenced in this patient, although the genes known to be mutated in DC were found to be normal as was the telomerase activity level. Further study of patients such as this will broaden our understanding of the mechanisms driving telomere dysfunction in human disease.

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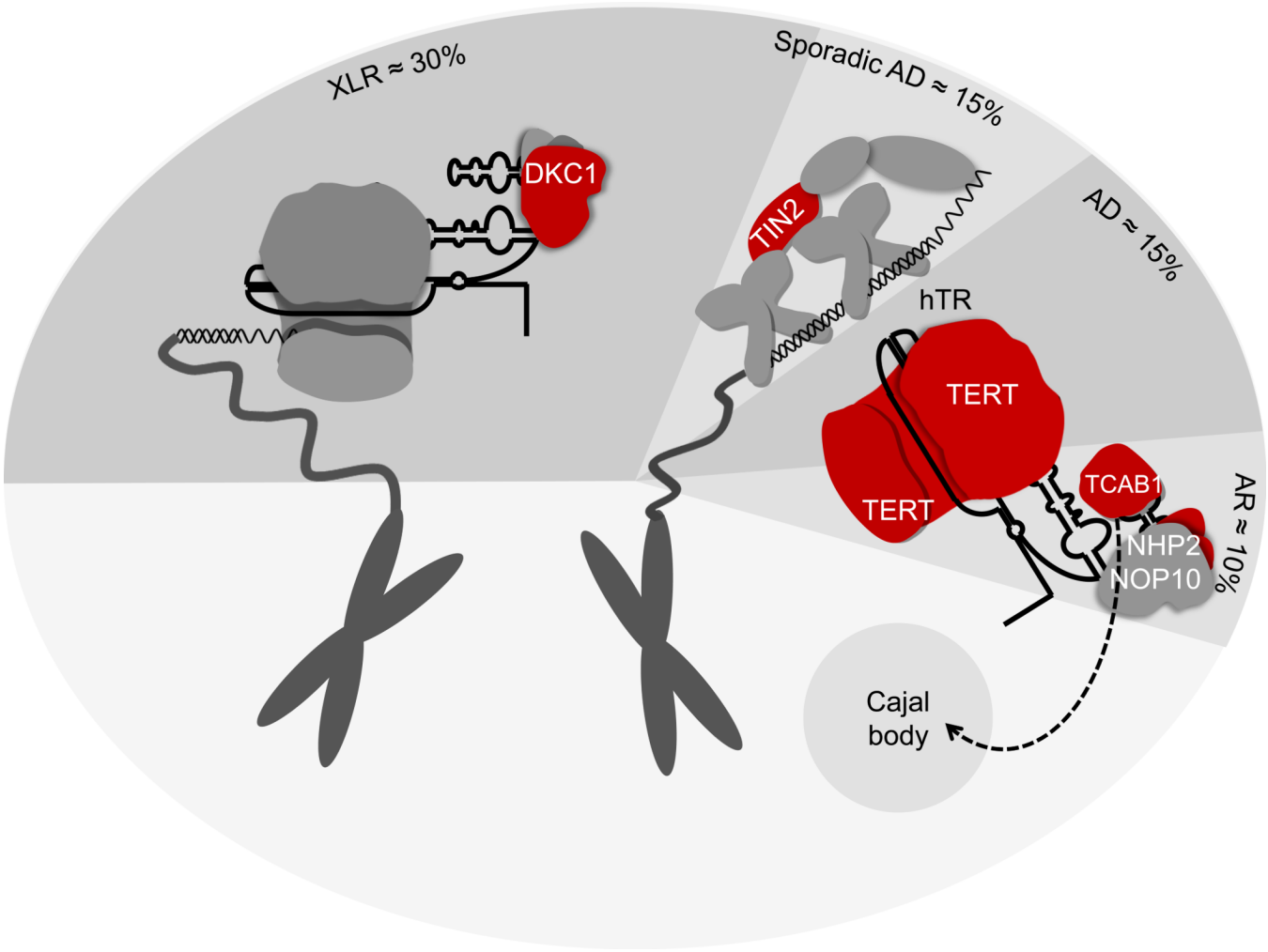

Figure 1.
The affected proteins and/or RNA in DC, the associated modes of inheritance and relative frequencies. The implicated proteins in each form of inheritance are indicated in red, while the remainder of the complex is in grey. The secondary structure of hTR is diagrammed in black. XLR, X-linked recessive; AD, autosomal dominant; AR, autosomal recessive.

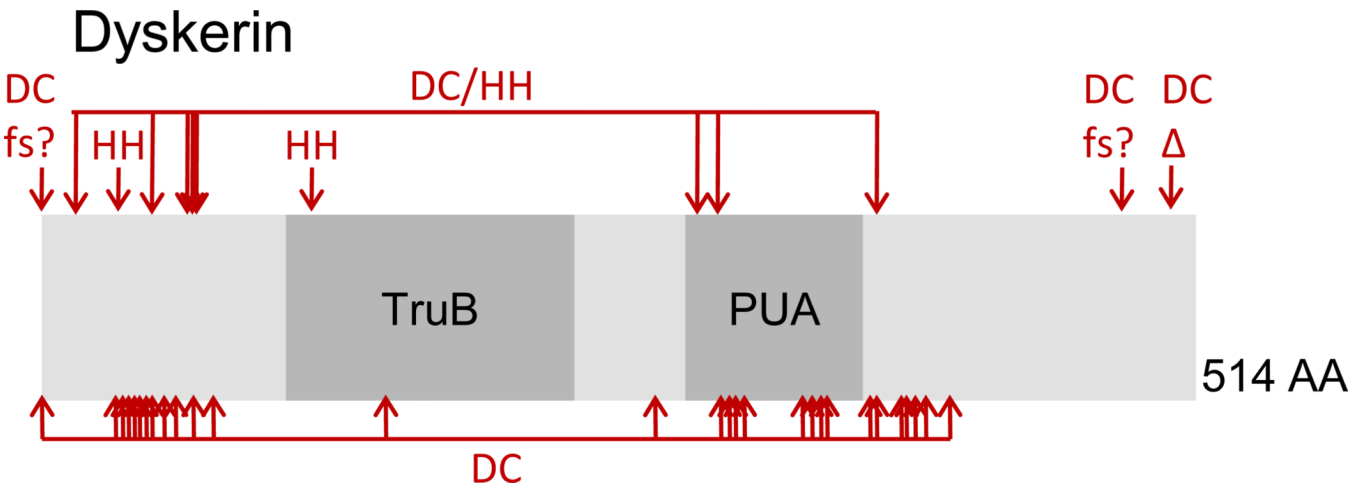

Figure 2.

Structural organization of dyskerin with localization of disease-associated mutations. See the Telomerase Database for a periodically updated compilation of disease-associated *DKC1* mutations [60, 105]. Abbreviations: Δ, deletion; DC, dyskeratosis congenita; fs?, putative frameshift; HH, Hoyeraal Hreidarsson syndrome.

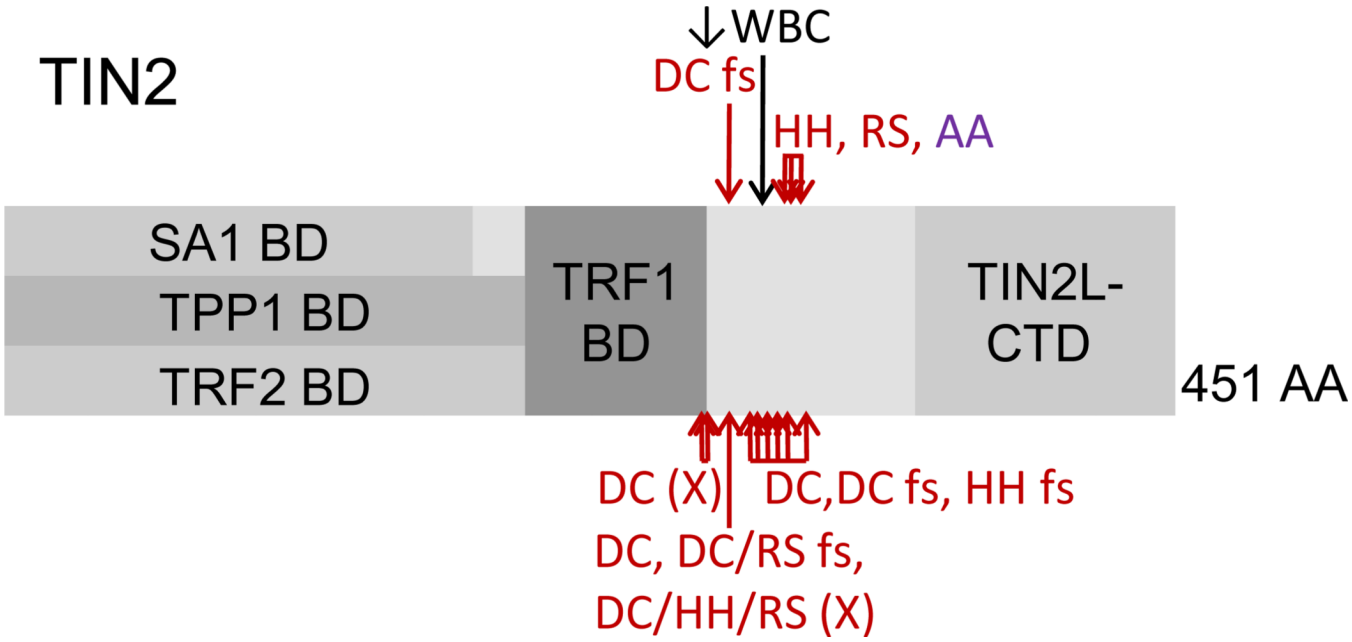

Figure 3.
Structural organization of TIN2 with localization of disease-associated mutations. See the Telomerase Database for a periodically updated compilation of disease-associated *TINF2* mutations [60, 105]. Abbreviations: AA, aplastic anemia; BD, binding domain; BMF, bone marrow failure; DC, dyskeratosis congenita; fs, frameshift mutation; HH, Hoyeraal Hreidarsson syndrome; RS, Revesz syndrome; TIN2L-CTD, TIN2L C terminal domain; ↓WBC, decreased WBC; (X), nonsense mutation.

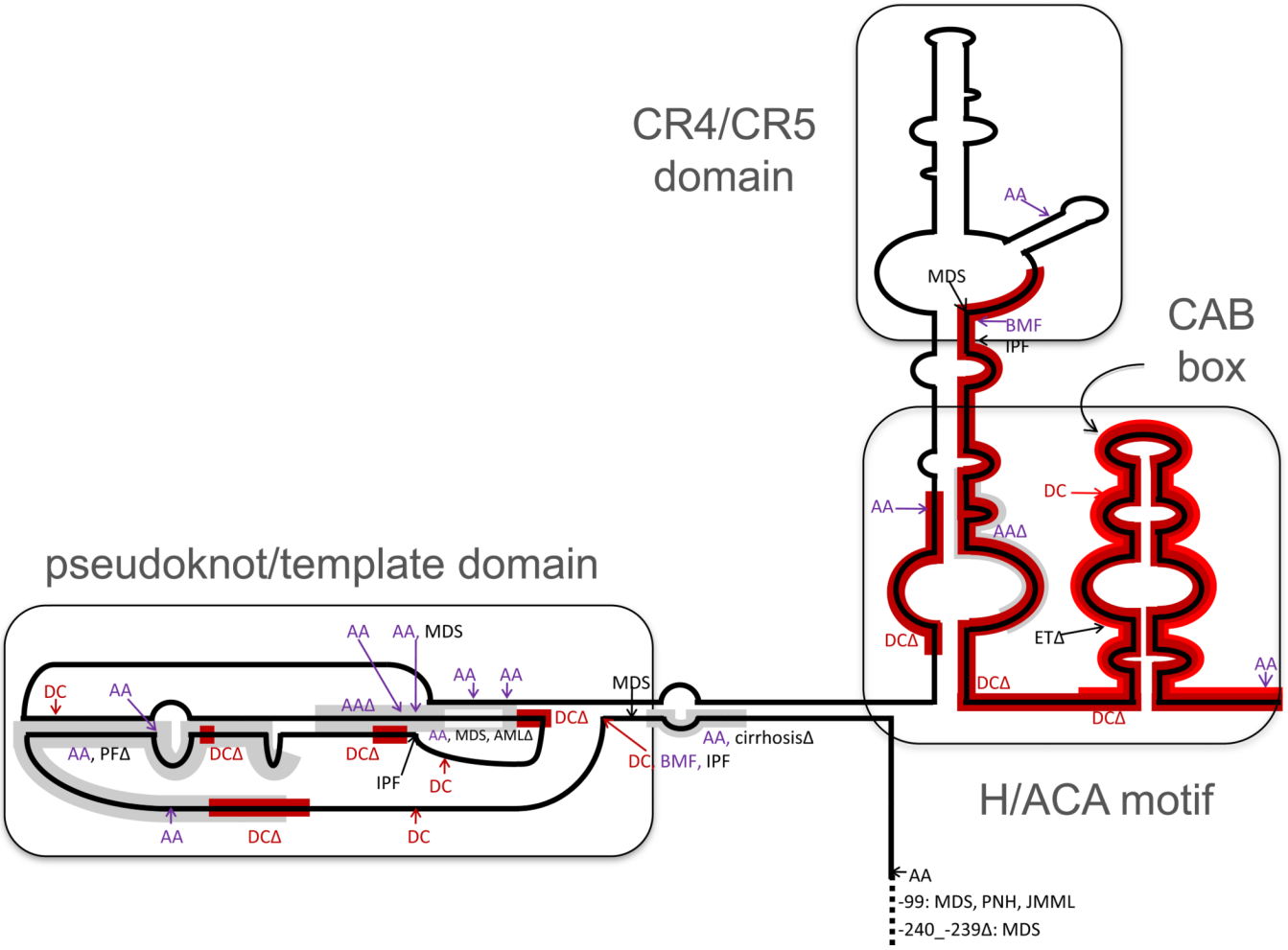

Figure 4.

Localization of disease associated mutations on hTR secondary structure. Thick red and gray lines indicate areas of deletion in DC and other disorders, respectively. Locations of point mutations are noted. Although the disease(s) in which the mutation has been reported are specified, this does not imply that the mutation is necessarily restricted to that phenotype. The reader is referred to the Telomerase Database for a periodically updated compilation of disease-associated *hTR* mutations [60, 105]. Abbreviations: Δ, deletion; AA, aplastic anemia; BMF, bone marrow failure; DC, dyskeratosis congenita; ET, essential thrombocythemia; IPF, idiopathic pulmonary fibrosis; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria; PF, pulmonary fibrosis.

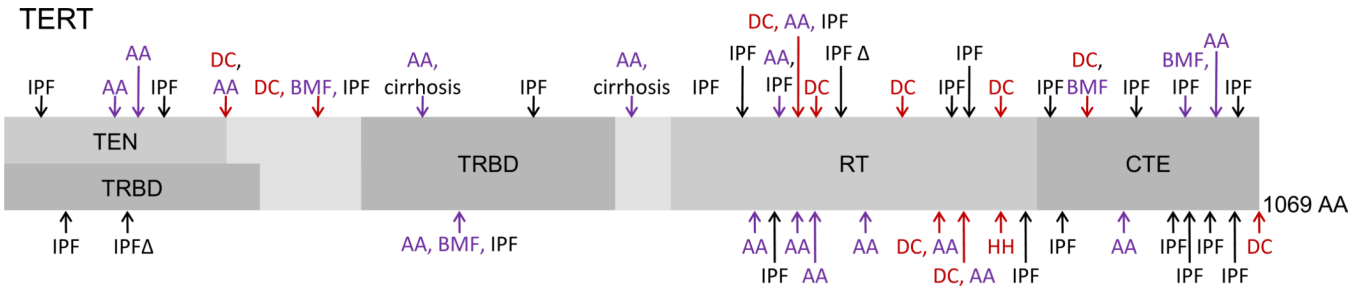

Figure 5.

Structural organization of TERT with localization of disease-associated mutations. See the Telomerase Database for a periodically updated compilation of disease-associated *TERT* mutations [60, 105]. As in Fig. 4, although the disease(s) in which the mutation has been reported are specified, this does not imply that the mutation is necessarily restricted to that phenotype. Abbreviations: Δ, deletion; AA, aplastic anemia; BMF, bone marrow failure; CTE, Cterminal extension; DC, dyskeratosis congenita; IPF, idiopathic pulmonary fibrosis; HH, Hoyeraal Hreidarsson syndrome; TEN, telomerase essential N terminal domain; TRBD, telomerase RNA binding domain; RT, reverse transcriptase domain.

Table 1

Diagnostic criteria of dyskeratosis congenita and its variants

A. Dyskeratosis congenita

1. Simultaneous presence of abnormal skin pigmentation, nail dystrophy and leukoplakia.
2. Presence of one of the mucocutaneous triad, bone marrow failure, and two of the other somatic features of DC.
3. Presence of two or more features seen in DC associated with very short telomeres (below the first percentile in multiple leukocyte subsets).

B. Hoyeraal Hreidarsson syndrome

1. Presence of four or more of the features growth retardation, developmental delay, microcephaly, bone-marrow failure, immunodeficiency and cerebellar hypoplasia.
2. Cerebellar hypoplasia and additional manifestations of DC.

C. Revesz syndrome

1. Bilateral exudative retinopathy and additional manifestations of DC.