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Human Genome and Diseases: Review

Dyskeratosis congenita

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Abstract. Dyskeratosis congenita is an inherited skin and bone marrow failure syndrome. There are X-linked, autosomal dominant and autosomal recessive forms of the disease. The X-linked form is due to mutations in the *DKC1* gene at Xq28. The encoded protein, dyskerin, is a component of both small nucleolar ribonuclear protein particles and the telomerase complex. Mutations in *DKC1* mainly lead to amino acid substitutions. The autosomal dominant form of the disease is due to mutations in hTR, the RNA component of telomerase, making it likely that the disease is due to defective telomerase activity. Mutations in hTR are predicted to either disrupt secondary structure or alter the template region. The gene or genes involved in the recessive forms of the disease remain elusive, though genes whose products are required for telomere maintenance are strong candidates.

Key words. Ageing; bone marrow failure; dyskerin; dyskeratosis congenita; DC registry; hTR; telomerase.

Introduction

Dyskeratosis congenita (DC, also known as Zinsser-Engman-Cole syndrome) is a rare inherited disorder that is characterised by its triad of abnormal cutaneous pigmentation, nail dystrophy and mucosal leucoplakia [1–3]. Although these features are accompanied with a variety of noncutaneous abnormalities (dental, gastrointestinal, genitourinary, neurological, ophthalmic, pulmonary and skeletal) [4–7], premature mortality is primarily caused by bone marrow (BM) failure (~67% of cases), a predisposition to malignancy (~6% of deaths mainly from epithelial carcinomas of the gastrointestinal tract and skin) and fatal pulmonary complications [~18% of deaths, half of which are post BM transplant (BMT)]. The remaining 9% were due to unrelated causes [8, 9]. The international dyskeratosis registry (DCR) was established at Hammersmith Hospital in 1995 to coordinate research and treatment; to date 165 families are enrolled in the registry.

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Clinical features of DC

DC is a very heterogeneous disorder with respect to clinical and haematological features and pattern of inheritance [9, 10]. No specific ethnic group is affected, and the disease affects all three germ layers [10]. X-linked, autosomal dominant and autosomal recessive forms of DC exist. A classic DC patient will usually develop the characteristic triad of skin pigmentation, nail dystrophy and mucosal leucoplakia by the age of 10 years with the mean age of death being in the third decade. Generally, cutaneous and noncutaneous abnormalities develop progressively. The severity of the triad and the additional noncutaneous features depends to some extent on the mode of inheritance (X-linked DC is generally more severe than autosomal recessive DC; autosomal dominant DC is less severe, but exceptions to the rule do exist). Heterogeneity has been observed within affected families and is more pronounced in female cases. Thus X-linked cases vary in severity from those diagnosed in their late twenties or thirties to those who die with BM failure within their first

year of life [Hoyeraal-Hreidarsson (HH) syndrome, see below]. Autosomal dominant cases vary from those who remain completely asymptomatic up to their 5th decade to those who develop cutaneous lesions and BM failure in their teens. Although no genetic loci have been associated with the recessive form of DC, the families showing clear autosomal recessive inheritance patterns again show wide variation in their clinical phenotype, from mild cutaneous abnormalities to severe BM failure in the first decade. Variation in clinical expression between members of the same family suggests that the DC phenotype can be modulated by other genetic and/or environmental factors [9].

Diagnosis of DC and treatment

Correct diagnosis of DC can be difficult due to the clinical and genetic heterogeneity of this disease. Depending on the age of onset and the severity of the cutaneous and noncutaneous features, it is possible for DC to be mistaken for other BM failure syndromes such as aplastic anaemia (AA) or Fanconi's anaemia (FA) (e.g. [10]). In some severe cases DC is not recognised because the patient dies before any of the cutaneous features develop. Most of the tissues affected in DC patients (e.g. skin and BM) tend to be tissues derived from highly regenerative and rapidly dividing cells, and therefore DC appears to share features of a premature ageing syndrome, as well as being associated with BM failure syndromes [11].
In cases where diagnosis is inconclusive, the use of cellular sensitivity to clastogens can be used to distinguish between DC and FA patients [12]. Diagnosis of X-linked DC can also be aided by the ability to detect skewed X-chromosome inactivation patterns (XCIPs) in X-linked female carriers (see below).
Current treatments for DC are symptomatic, palliative and remain unsatisfactory, resulting in poor prognosis for the patient. The anabolic steroid oxymetholone can improve haematopoietic function, though only for a variable period of time [13]. Allogeneic stem cell transplantation (SCT) is the preferred form of treatment using either sib or unrelated donors, but due to the increased risk of pulmonary complications and malignancies in older DC patients, this method has proved to be less successful than in FA patients [9].

Genetics of DC

Three distinct modes of inheritance of DC have been described. While a significant proportion of cases registered in the DCR appear to have an X-linked genetic inheritance pattern (MIM 305000), autosomal dominant (MIM 127550) and autosomal recessive (MIM 224230) DC cases have also been recognised (e.g. [9, 14, 15]). Figure 1 shows examples of pedigrees from the DCR illustrating these inheritance patterns.

X-linked DC

Genetic localisation of the X-linked gene, DKC1

Linkage analysis of one large X-linked DC family [16] mapped the disease locus to Xq28 [17], which Connor et al. confirmed with an additional three DC families [18]. Further linkage analysis eventually narrowed this region to a 3.5-Mb region between markers DXS1684 and DXS1108 [19, 20]. This region was then refined by identification of additional recombination events and the use of XCIP analysis to determine the carrier status and haplotype within eight X-linked DC families [20].
Random inactivation of one of the X chromosomes in each female cell occurs during early embryogenesis and results in the hypermethylation of genomic DNA on the inactive chromosome. Investigation of the methylation-sensitive *Hpa*II and *Hha*I sites within the highly polymorphic CAG repeat in exon1 of the androgen receptor locus at Xq11.2-Xq12 (HUMARA) [21] shows the proportion of active paternally and maternally inherited X chromosomes. These are usually seen in a 50:50 ratio, although this ratio is sometimes skewed, especially in older women [22]. In known DC carriers, however, the pattern is completely skewed, with all cells having the X chromosome that bears the mutant allele inactivated [23–25]. Since X inactivation is a random process, cells in which only the mutant gene is expressed must be completely outgrown by cells expressing the wild-type gene. Therefore, this completely skewed pattern of XCIP is useful in identifying female carriers of *DKC1* mutations. These findings also have some interesting implications. First, they fit in well with the BM failure phenotype seen in DC. Second, they imply that should a gene transfer approach be developed as a therapy for DC, cells receiving a wild-type copy of the gene can be expected to outgrow the patients cells – suggesting the transfer event would not need to be so efficient. Last, the skewed pattern of X inactivation could be used as a marker of the presence of the mutant gene in linkage studies to narrow down the important region [20], as illustrated in fig. 2.
Genetic linkage studies narrowed the DC region down to 1.4 Mb of Xq28. As this area had been extensively investigated, this resulted in reducing the number of positional gene candidates to a manageable 28 genes. Southern blot analysis using partial complementary DNA (cDNA) clones of these 28 candidate genes as probes found that one clone, *XAP101,* was partially deleted in a DC patient from pedigree DCR015 [26]. Subsequent cloning of the entire open reading frame (ORF) and screening of other DC patients identified an additional five missense mutations [27]. Characterisation of the gene responsible for X-linked DC, *DKC1*, revealed the gene consists of 15 exons

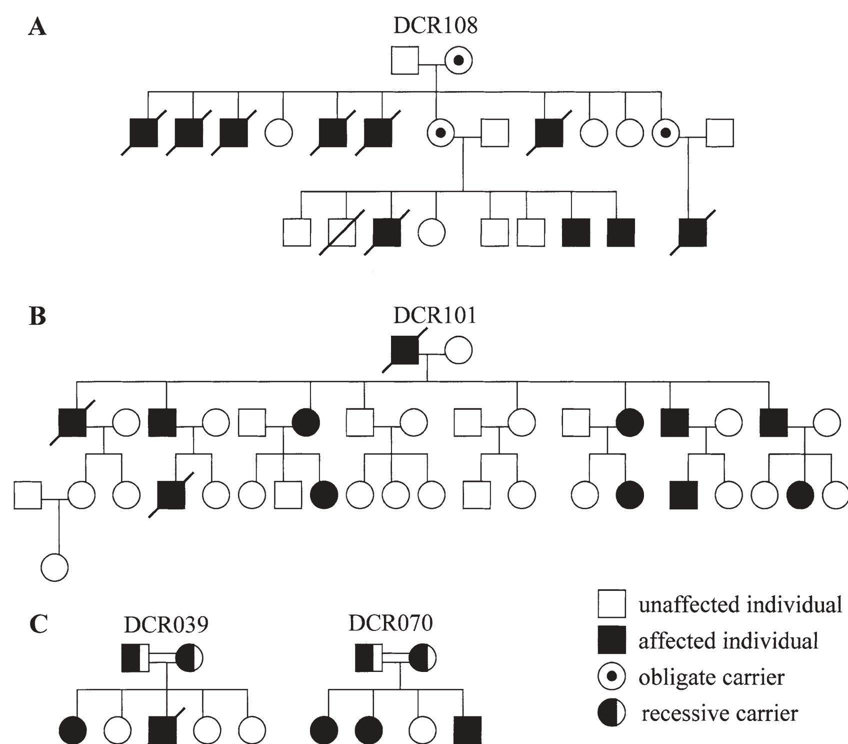

Figure 1. Examples of families within the DCR that have different genetic inheritance patterns. DCR108 (*A*) is a typical X-linked DC family pedigree showing only uncle and nephew affected cases. DCR101 (*B*) is a large autosomal dominant DC family tree showing affected males and females in consecutive generations, while DCR039 and DCR070 (*C*) are two typical autosomal recessive families where these parents are from consanguineous marriage, and both sexes in the following generation are affected.

[28, 29], that it is transcribed into an ~2.5 kb messenger RNA that is translated into a 514 aa/~57 kDa protein, dyskerin, which is ubiquitously expressed in adult and foetal tissues [27] (fig. 3).

The *DKC1* gene and dyskerin

Dyskerin is a nucleolar protein [27, 30, 31] that is highly conserved among eukaryotes and appears to be the human homologue of the characterised yeast protein Cbf5p (centromere/microtubule binding protein), rat NAP57 (Nopp140-associated protein) nucleolar protein and *Drosophila* Nop60B/*mfl* gene [32–36]. This high level of conservation suggests this protein has an important function. Functional studies of Cbf5p and NAP57 suggest that dyskerin is a multifunctional protein involved in ribosomal RNA (rRNA) biosynthesis, ribosomal subunit assembly and/or centromere/microtubule binding. Identification of a protein motif present within Cbf5p, NAP57, and the yeast and bacterial class of TruB pseudouridine (ψ) synthases suggest that dyskerin and its homologues have pseudouridine synthase activity. This has been confirmed in *Saccharomyces cerevisiae* TruB homologues PUS4 and Cbf5p [37, 38]. Dyskerin has two TruB ψ synthase motifs and a carboxy-terminal lysine-rich repeat domain (fig. 3B) [27, 31, 39, 40].

Yeast studies have shown that Cbf5p is a constituent of a class of small nucleolar ribonucleoprotein particles called H/ACA snoRNPs [34, 38]. These complexes are involved in modifying particular residues during rRNA processing [41]. While the C/D box class of snoRNPs methylate residues in nascent rRNA chains, the H/ACA box class of snoRNPs pseudouridylate specific residues. In both cases the snoRNAs act as guides to direct the complexes to the appropriate residues [42]. The yeast H/ACA class of snoRNAs form a stable complex with the essential nucleolar proteins Gar1p, Nhp2p, Nop10p as well as Cbf5p (reviewed in [43, 44]). Reduced expression of Cbf5p (yeast) and *mfl* (*Drosophila*) results in reduced rRNA pseudouridylation, reduced H/ACA box snoRNP stability, increased accumulation of partially processed rRNA precursors and decreased numbers of mature ribosomes [34, 36, 45].

Following the observation that human telomerase RNA (hTR) contained a 3′ H/ACA domain [46], it was shown that dyskerin was also a component of telomerase [47], a nucleoprotein complex responsible for synthesising the telomere repeats at the ends of chromosomes. Immunoprecipitation experiments showed that dyskerin associated with both the H/ACA class of snoRNPs and the human telomerase RNPs [47] and that the other conserved

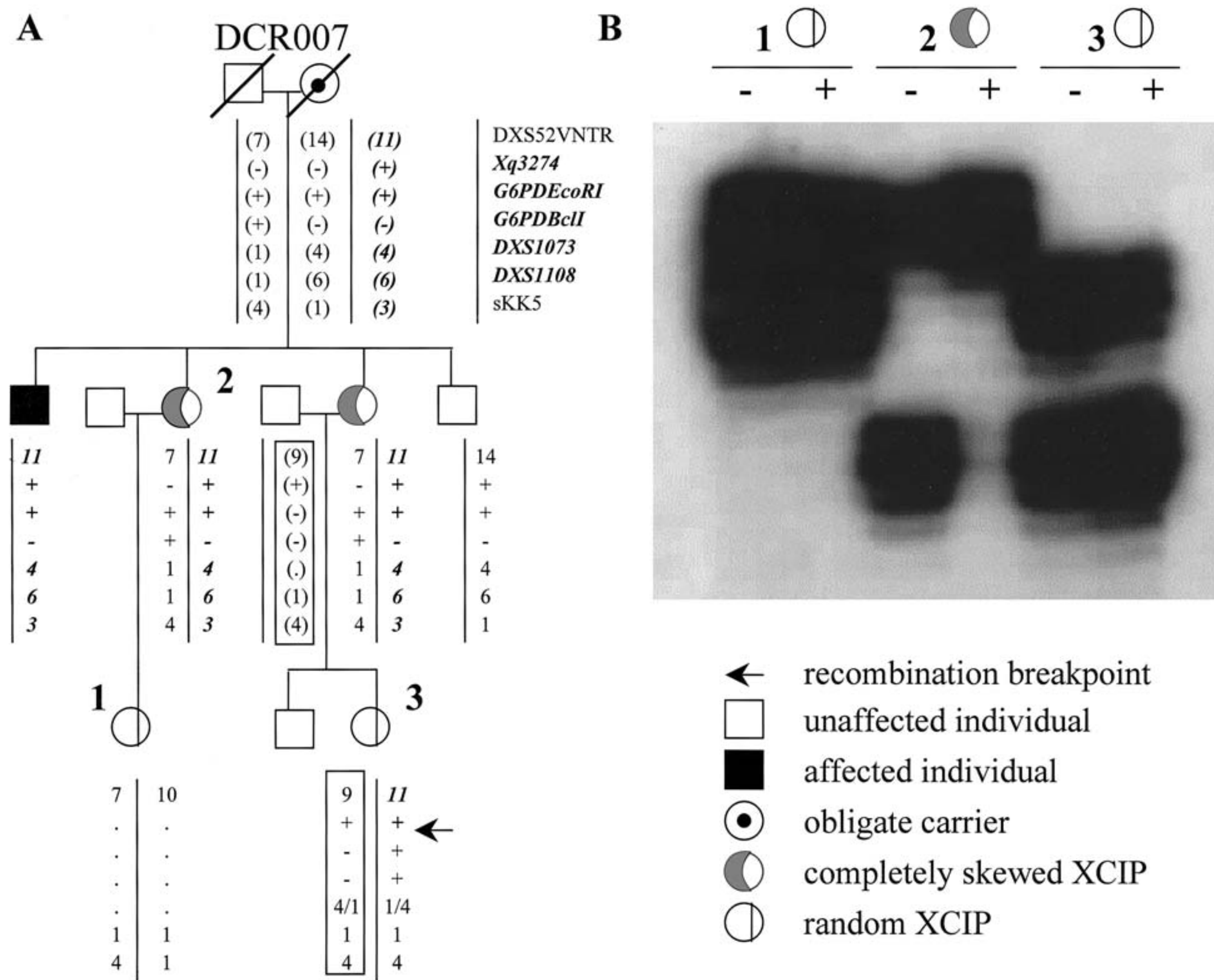

Figure 2. The X-linked DC candidate gene was fine mapped on the X chromosome through a combination of linkage analysis (*A*) and XCIP analysis (*B*). Inferred haplotypes are denoted by brackets, and those boxed are inferred from the predicted father's haplotype. The disease-associated haplotypes and their corresponding markers are shown in italics. The haplotypes for the pedigrees were deduced by assuming the minimum number of recombination events. XCIP analysis by amplification of the HUMARA polymorphism without (–) and with (+) previous digestion with *Hpa*II on 3 females from DCR007 (*B*) showed that while individual 2 was completely skewed and therefore a carrier of the mutant DC locus, individuals 1 and 3 were not skewed and therefore were assumed not to carry the mutant DC locus. This together with the intervals gained from pedigrees DCR006 and DCR025 (not shown) narrows the region of interest to a 1.4-Mb interval between Xq3274 and DXS1108.

proteins GAR1, NOP10 and NHP2 were also present in the telomerase complex [48]. The role of these proteins in telomerase is not known, but they may be necessary for efficient assembly of telomerase RNP in the nucleolus [49–51]. Male X-linked DC fibroblasts showed lower telomerase RNA accumulation and activity with reduced telomere lengths when compared with normal controls [47, 52], yet they did not show a detectable reduction in other H/ACA snoRNA accumulation or function [47]. Therefore, the biological consequences of mutations in dyskerin may be in defective rRNA pseudouridylation and/or defective telomere maintenance, but the available evidence favours defects in telomerase [53].

An intriguing aspect of *DKC1* gene structure is the presence of a H/ACA snoRNA gene within intron 8 (fig. 3 A) [54]. H/ACA snoRNA genes are encoded in the introns of ubiquitously expressed genes, and the mature RNAs are produced from the liberated introns by nucleolytic processing. Most H/ACA snoRNAs contain antisense elements with recognisable homology to the sites of known pseudouridines in ribosomal RNAs. The H/ACA snoRNA in intron 8 of the *DKC1* gene does not contain antisense elements that would recognise any rRNA, suggesting it may target some other kind of RNA molecule such as mRNA. Curiously the *mfl* gene in *Drosophila* also encodes a H/ACA box snoRNA in an intron [36], although here there is some antisense homology with a specific sequence in 18S rRNA.

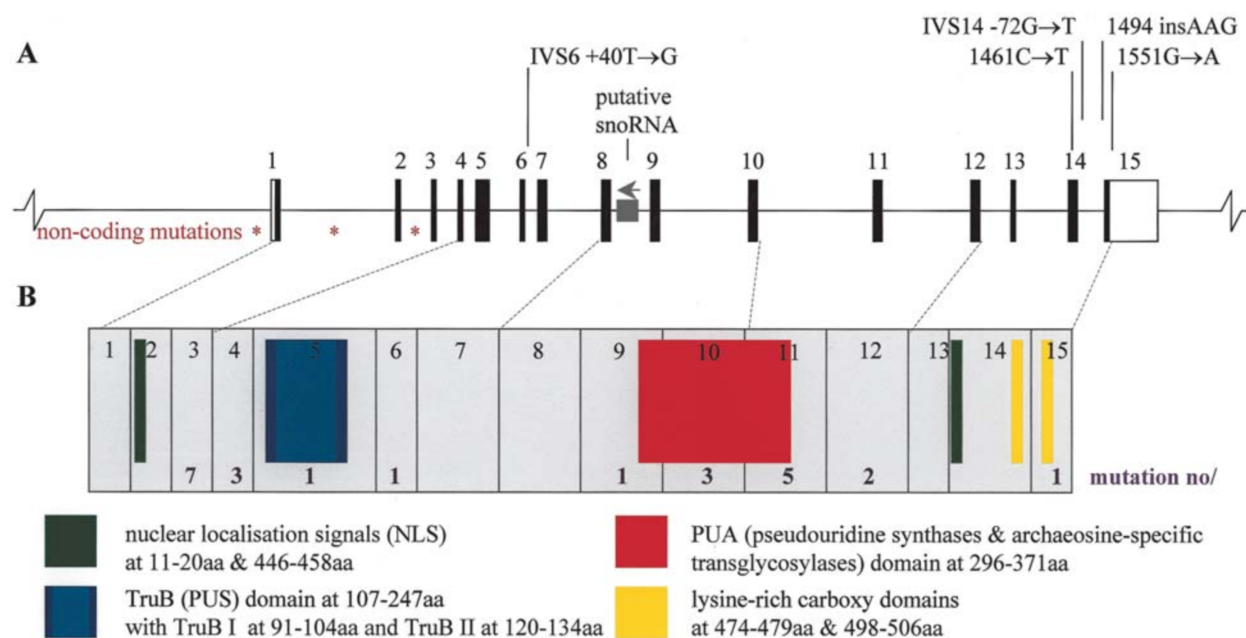

Figure 3. The genomic and protein structure of *DKC1*. *DKC1* (*A*) contains 15 exons which are translated into a ~57-kDa protein, dyskerin (*B*). To date three noncoding mutations (*) are known to occur in *DKC1,* while the rest (purple) appear to form two clusters spanning exon 2→6 and exon 9→12. Known polymorphisms are indicated within the genomic structure (*A*) along with the putative snoRNA located in intron 8 (grey box).

Mutations found in *DKC1*

The majority of mutations found in *DKC1* to date cause a single amino acid substitution in dyskerin (table 1) [26, 27, 29, 55, 56]. One mutation, causing the A353V substitution, accounts for 30% of X-linked DC cases and in many cases has been shown to be a de novo event [29]. We suspect the high frequency of this mutation is related to the fact that it is a CpG-to-TpG mutation, which is relatively frequent in mammalian DNA. Like the other mutations, it must also have the rather special properties of affecting the function of dyskerin mildly enough to not perturb embryonic development but severely enough to cause DC. The distribution of mutations with respect to the amino acid sequence shows there are two clusters of mutations involving amino acids encoded in exons 2→6 and 9→12. There is little information regarding the function of these parts of the protein, and the role of these mutations in the molecular pathology of DC awaits further experiments. The only mutation that affects a residue in a conserved TruB domain is the 121 Ser→Gly, which is close to the highly conserved catalytic aspartic acid residue (125) [40].

Only one large deletion (exon15) has been found [26]. This deletion removes only the last 22 amino acids of the protein, which are relatively less well conserved and is compatible with the idea that some function is retained. Two intron mutations and a promoter mutation have been found to be the only mutations in the *DKC1* gene in three families. The promoter mutation –141C→G destroys a consensus transcription factor (Sp1) binding site. One of the intron mutations IVS1 592 C→G has been shown to affect splicing, while the other IVS2 473 C→G alters a conserved motif. These three noncoding changes may indicate that decreased production of dyskerin as well as structurally altered dyskerin is capable of causing DC [57].

Mutations in *DKC1* have also been found to result in HH syndrome (MIM 600545), a BM failure syndrome with cerebelar hypoplasia and immunodeficiency problems (table 1) [58, 59]. This syndrome can therefore be regarded as a severe form of DC where death from BM failure occurs before the appearance of the classical diagnostic features of DC. The severe phenotype is not obviously related to the sites of the mutations since the A353V mutation has been found in an HH case and in milder, more typical DC. Rather, the severe expression represents an extreme case of heterogeneity in DC that must be due to other genetic and possibly environmental factors.

Autosomal dominant DC

Genetics of autosomal dominant DC

Within the DCR, relatively few families show this form of genetic inheritance (6/165 families). Linkage analysis of a single large family, DCR101, gave a LOD score of 1.8 for marker D3S3725 using six affected and two unaffected related individuals. This mapped to an interval of ~30 cM on chromosome 3 [60]. As dyskerin has been

Table 1. Mutations found in DKC1 within DC and HH (**bold**) families.

Mutation	AA substitution	Exon	Family	Reference
–141 C→G	N/A	5′ flanking	DCR035	57
5 C→T	A2V	1	DCR041	29
IVS1 592 C→G	N/A	IVS1	DCR025	57
IVS2 473 C→G	N/A	IVS2	DCR050	29
106 T→G	F36V	3	DCR026	27
109-111 ΔCTT	ΔL37	3	DCR013	27
115 A→G	K39E	3	DCR008	29
119 C→G	P40R	3	DCR007	27
121 G→A	E41K	3	DCR038	29
127 A→G	K43E	3	NH1	56
146 C→T	T49M	3	**DCR052, DCR117, DCR152**	58
194 G→C	R65T	4	DCR033	29
196 A→G	T66A	4	DCR012	29
214,5 CT→TA	L72Y	4	DCR002	27
361 A→G	S121G	5	**DCR064**	58
472 C→T	R158W	6	DCR091	57
838 A→C	S280R	9	DCR017	57
949 C→T	L317F	10	KR2	55
961 C→G	L321V	10	DCR009	29
965 G→A	R322Q	10	KR1	55
1049 T→C	M350T	11	DCR031, DCR071	29
1050 G→A	M350I	11	DCR021	29
1058 C→T	A353V	11	DCR004, DCR005, DCR006, DCR020, DCR029, DCR030, DCR044, DCR046, DCR051, DCR055, DCR056, DCR059, DCR062, **DCR065**, DCR066, DCR100, DCR115, DCR140, DCR141, DCR142, DCR154, DCR155	29
1150 C→T	P384S	11	KR3	55
1151 C→T	P384L	11	DCR075	57
1204 G→A	G402R	12	DCR027	29
1205 G→A	G402E	12	DCR001	27
ΔExon 15	Δ493-514	15	DCR015	26

Δ, deletion.

shown to be a component of the telomerase complex [47] and *hTR* has been cytogenetically mapped to chromosome 3q [61], *hTR* was a likely candidate for autosomal dominant DC. Mutation screening of the gene encoding this 451 bp RNA detected an 821 bp deletion within affected members of DCR101, which removed 74 bp from the 3′ end of one of the copies of the *hTR* coding sequence [60]. Additional single/double point mutations were also found in two other, but not all, autosomal dominant DC families within the DCR: DCR063 and DCR082, as well as several unrelated AA patients (table 2; fig. 4A) [62].

hTR

Telomerase is responsible for maintaining the simple telomere (TTAGGG)n repeats at the ends of chromosomes [63, 64]. It consists of two major components, the reverse transcriptase (hTERT) and the RNA component (hTR), which acts as a template [65, 66]. Several other proteins are associated with hTR and hTERT, including dyskerin and the other conserved H/ACA snoRNA-associated proteins (see above). Most somatic cells do not express hTERT and so have no telomerase activity. Telomeres in these cells shorten with each cell division until they reach a critical length [67] where the cell enters senescence and cell death via apoptosis takes place. Germ cells and most cancer cells express hTERT and do not undergo telomere shortening [68–71]. Therefore, study of telomerase activity has become important in cancer and ageing research. hTR was shown to contain a H/ACA domain within the 3′ end of the RNA that is essential for in vivo hTR accumulation, hTR 3′ end processing and telomerase activity [46, 72]. Although all species investigated appear to have telomerase RNA (TR) molecules and H/ACA box domain snoRNAs, only vertebrates have been shown to possess a H/ACA domain within the telomerase RNA [46].

The finding that both X-linked and autosomal dominant DC are caused by disruption of elements of the telomerase complex suggests strongly that DC is a disease of defective telomere maintenance. The difference in telomere lengths between DC patients and normals is evident from an early age, implying that considerable telomere shortening takes place before birth. AA is another BM failure condition that often occurs with no known cause

Table 2. Mutations found in *hTR* within DC and AA (*italics*) patients.

Mutation	RE site	Disease	Family	Reference	Figure 4A
58 G→A	N/A	*AA*	(*597* and *598*), *652*, *.205*	62	1
72 C→G	loss of *Nar*I	*AA*	*4518N*	62	2
107,8 GC→AG	gain of *Dde*I	DC	DCR082	60	3
110-3 ΔGACT	N/A	*AA*	(*812* and *813*)	62	4
Δ378-3′ end	N/A	DC	DCR101	60	5
408 C→G	loss of *Tfi*I	DC	DCR063	60	6

Δ, deletion.

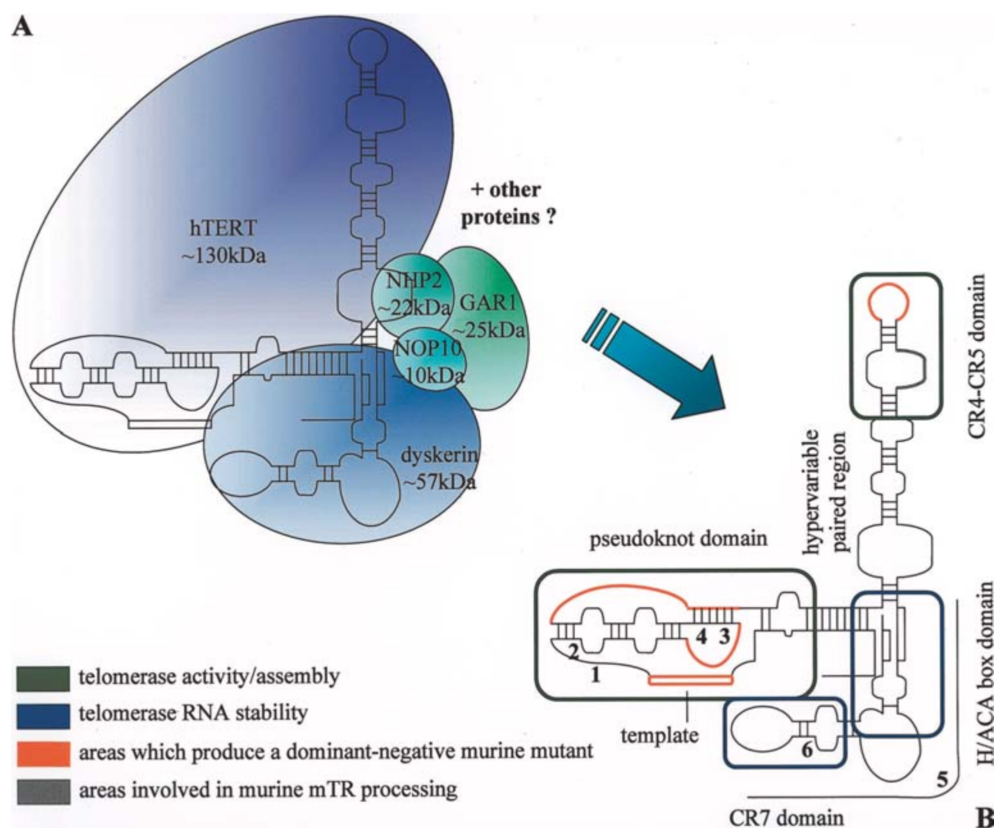

Figure 4. The putative protein associations of hTR (*A*) and its RNA structure (*B*). Mutagenesis analysis on mTR telomerase activity found that the areas in green were required for telomerase activity/assembly, the areas in blue were required for telomerase stability, and mutations in the areas coloured red or grey induced dominant-negative murine effects or altered mTR processing, respectively. The known hTR mutations listed in table 2 are also numbered. If the same holds true cross-species, any of the mutations located in the pseudoknot domain (1→4) would alter telomerase activity/assembly and may also have a dominant-negative effect. The loss of the H/ACA box and CR7 domains in 5 would reduce stability and hTERT accumulation, while 6 may only reduce stability while maintaining normal hTERT accumulation.

(idiopathic) or can sometimes be precipitated by exposure to certain chemicals or drugs and is in rare cases constitutional (familial or accompanied by other somatic abnormalities) [73]. In a recent study involving a small number of AA patients, a few (~10%) of both idiopathic and constitutional cases were found to have *hTR* mutations [62]. These patients had short telomeres, as do a significant number of AA patients [74]. Thus, mutations affecting telomerase activity may be involved in other BM failure conditions.

Mutations in *hTR* causing DC and AA

The mutations found in *hTR* from DC and AA patients are shown in table 2. Although the effect of the mutations on telomerase activity has not been characterised, the mutations seem to affect residues shown to be important in mammalian TR molecules. Thus, residue 58 is close to the template region, the large deletion removes all of the 3′H/ACA domain and the other mutations are part of the stem structures that form the secondary structure of the molecule [66]. The fact that these *hTR* mutations are

dominant implies either that 50% of the normal amount of *hTR* is insufficient for telomere maintenance or that the mutations exert a dominant-negative effect, resulting in less than 50% of the normal activity. In the case of the large deletion, no aberrant RNA could be detected in patients, cells by either Northern blotting or reverse-transcription polymerase chain reaction (RT-PCR) analysis showing that in this case at least haploinsufficiency of hTR is causing the problem [60]. We conclude that levels of hTR are finely regulated in human cells.

mTR$^{-/-}$ **mice**

The effect of mutation of one allele of human telomerase RNA in families with autosomal dominant DC is radically different from the effect on laboratory mice of knocking out both alleles of the gene coding for murine telomerase RNA (*mTR*) [75]. These mice showed no significant abnormalities in early generations, but the fourth and later generations of *mTR*$^{-/-}$ mice show poor wound healing, decreased proliferation of blood cells, gut abnormalities and increased incidence of end-to-end chromosome fusions and tumour formation [76, 77]. Though the clinical features of DC are highly variable, the similarity with late generation *mTR*$^{-/-}$ mice is evident [11]. The lag in appearance of the phenotype in *mTR*$^{-/-}$ mice is probably due to the fact that laboratory strains of mice have relatively long telomeres, and several generations are needed before a critical amount of shortening has occurred [78]. Logically, if telomeres in *mTR*$^{-/-}$ mice can shorten for several generations before phenotypic effects are observed, it follows that ageing in laboratory mice must be independent of telomere shortening [64, 77]. Nevertheless, *mTR*$^{-/-}$ mice may be useful in modelling DC and have already proved invaluable in studies of the role of telomeres in cancer.

Autosomal recessive DC

Genetics of autosomal recessive DC

No genetic mutations to date have been found to cause autosomal recessive DC. We currently have 14/165 families who show clear autosomal recessive inheritance patterns registered in the DCR. Since dyskerin and hTR have been shown to interact specifically with the H/ACA class of snoRNPs (fig. 4B), the associated proteins involved within this complex, rRNA biosynthesis and/or telomerase RNP assembly are likely autosomal recessive DC gene candidates. Yeast studies have shown that all H/ACA snoRNAs form a stable complex with the essential nucleolar proteins Gar1p, Nhp2, Nop10p as well as Cbf5p [79–82]. This snoRNP complex is required for global ψ formation on pre-rRNA [34, 81, 83], and Gar1p is also essential for 18S rRNA synthesis [83, 84]. Loss of Cbf5p, Nhp2p or Nop10p results in the depletion of the other H/ACA snoRNPs and snoRNA, suggesting that one or more of these core proteins directly binds to the H/ACA region of the snoRNA [34, 81]. Loss of these three proteins also effects Gar1p accumulation, which is not required for H/ACA snoRNA stability but is required for the stable association of snR36 (and possibly other H/ACA box snoRNAs) with the pre-rRNA [83]. Therefore it is plausible that the human homologues of Gar1p (GAR1), Nhp2p (NHP2) and Nop10p (NOP10) may cause autosomal recessive DC when mutated.

In a recent study, we analysed these three candidate genes in a selection of families showing autosomal recessive inheritance. No coding mutations were found in these three genes within 20 unrelated DC families, suggesting that *GAR1*, *NHP2* and *NOP10* are not commonly mutated in autosomal DC patients [A. Marrone, T. Vulliamy and B. Ng, unpublished data].

Although the core proteins within the H/ACA snoRNP are known, not all of the protein components of the telomerase complex have been identified. Active telomerase RNPs from HeLa cell nuclear extracts are approximately 1000 kDa in size [85], while those partially purified from rat S100 extracts appear to be larger [86]. Other protein components include hTERT [87], TEP1 [88], p23 and hsp90 [89]. Using the consanguineous families within the Hammersmith Hospital DCR, this laboratory is currently using linkage analysis to identify chromosomal areas of interest. This is being used in conjunction with SSCP analysis screening of candidate genes identified to be associated with the telomerase complex. As the human genome project continues, other candidate genes may become more apparent.

Future prospects

The discovery of the importance of the telomerase pathway in the pathogenesis of DC and AA raises many important questions. The unexpected convergence of H/ACA snoRNPs and telomerase RNPs is interesting from the functional and evolutionary viewpoints. When in evolution did a H/ACA domain become part of telomerase RNA, and what is the role of this domain and its associated proteins in telomere maintenance? Is it important for telomerase assembly in the nucleolus? Is the pseudouridylation activity of dyskerin important in telomere maintenance, and if so what is its template? Careful cell biology studies should reveal the answers to these questions. It is also possible that the identity of the other genetic loci that are responsible for the recessive forms of DC may provide important clues.

From the clinical point of view, the discovery that rapid shortening of telomeres is fundamental to the development of DC and possibly AA and other conditions may suggest avenues of research into therapies that aim to prevent or repair telomere shortening. The skewing of X in-

activation in female carriers of X-linked DC demonstrates that the normal cells have a growth advantage and that if a few stem cells in male patients could be corrected by gene transfer techniques, they could expand in vivo to populate the bone marrow.

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