Dyskeratosis congenita

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Abstract

Classical dyskeratosis congenita (DC) is a rare multisystem disorder with a prevalence estimated to 1 in 1,000,000. DC is characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia. A variety of other abnormalities have been reported. Bone marrow (BM) failure is the principal cause of early mortality with an additional predisposition to malignancy and fatal pulmonary complications. DC exhibits considerable clinical and genetic heterogeneity. X-linked recessive, autosomal dominant and autosomal recessive forms are recognised. The genes (DKC1 and TERC) mutated in two subtypes of DC both encode components of the telomerase complex and DC is now believed to be principally due to defective telomerase function. The identification of DKC1 mutations in patients with Hoyeraal-Hreidarsson (HH) and of TERC mutations in some patients with aplastic anaemia (AA) and myelodysplasia (MDS) have extended the range of patients who can be regarded as having DC. From a clinical perspective the link between DC and AA and in turn to defective telomerase suggests that treatments directed at correction of telomerase activity might benefit DC/AA patients who do not respond to conventional therapy. At present haemopoietic stem cell transplantation (SCT) using low intensity protocols represents the only curative option for DC patients developing BM failure.

Key-words

Aplastic anaemia (AA), dyskeratosis congenita (DC), dyskerin, Hoyeraal-Hreidarsson syndrome (HH), telomerase

Name of the disease/Included diseases

Dyskeratosis congenital is also known as Zinsser-Engman-Cole syndrome. The Hoyeraal-Hreidarsson syndrome is a severe variant of DC. Mild forms of DC can present with aplastic anaemia.

Definition

DC is an inherited disorder, which in its classical form is characterized by a triad of abnormal skin pigmentation, nail dystrophy and leucoplakia. A range of other abnormalities can be seen in a given patient. DC patients have a high predisposition to developing bone marrow (BM)
failure and malignancy. The genes (DKC1 and TERC) mutated in two subtypes of DC both encode components of the telomerase complex and DC is now believed to be principally due to defective telomerase function. The identification of DKC1 mutations in patients with HH and of TERC mutations in some patients with aplastic anaemia (AA)/myelodysplasia (MDS) have highlighted its considerable heterogeneity and extended the range of patients who can be regarded as having DC.

Differential diagnosis

Patients with DC have features that overlap with Fanconi anaemia (FA) and idiopathic AA. The most challenging presentation is with AA. All such patients should have chromosomal breakage analysis with mitomycin-C (MMC) or diepoxybutane (DEB) to exclude FA. If the chromosomal breakage study is normal then it is logical to proceed to TERC analysis. Depending on the result of this and whether the patient has somatic features of DC it is then reasonable to study the DKC1 gene. Diagnosis of DC is relatively easy in patients who have the classical mucocutaneous features. At present diagnosis can be substantiated at the genetic level in ~40% (DKC1 and TERC) of DC patients.

Aetiology/pathophysiology

DC is a genetically heterogeneous disorder. X-linked recessive (MIM 305000), autosomal dominant (MIM 127550) and autosomal recessive (MIM 224230) genetic subtypes are recognised. Analysis of the Dyskeratosis Congenita Registry (DCR – at Hammersmith Hospital, London) families suggests that each of the autosomal forms of DC may be genetically heterogeneous. Recent studies have shown that the genes mutated in the X-linked recessive and autosomal dominant subtypes of DC encode core components of the telomerase complex.

X-linked DC: DKC1 and dyskerin

Initially, through linkage analysis in one large family with only affected males in more than one generation it was possible to map the gene for the X-linked form of the disease to Xq28 (Connor et al. 1986). The availability of genetic markers and additional X-linked families facilitated positional cloning of the gene (DKC1) that is mutated in X-linked DC (Knight et al. 1996; Knight et al. 1998; Heiss et al. 1998; Hassock et al. 1999). The DKC1 gene consists of 15 exons and spans ~15Kb within Xq28. It is transcribed into a ~2.5kb mRNA that is translated into a 514 aa protein. The identification of the DKC1 gene has made available a genetic test (Knight et al. 1999a; Vulliamy et al. 1999; Knight et al. 2001; Heiss et al. 2001; Salowsky et al. 2002; Hiramatsu et al. 2002; Lin et al. 2002; Cossu et al. 2002; Wong et al. 2004) that can be used to confirm diagnosis in suspected cases, identify carriers and provide antenatal diagnosis in X-linked families. The majority of mutations in DKC1 cause single acid substitutions, one of which (A353V) accounts for approximately 40% of X-linked DC cases. The phenotype in these patients with the same dyskerin mutation can vary considerably and suggests that other genetic/environmental factors influence the DC phenotype. Although the mutations are spread throughout the DKC1 gene there are two prominent clusters involving amino acids 31-72 and 314-420, encoded in exons 3-6 and exons 9-12. This suggests that different parts of the dyskerin molecule may have different functions and warrants further studies. Based on its homologues (see below) the following domains have been identified in dyskerin: TruB (tRNA pseudouridine synthase N terminal domain) at amino acids (aa) 107-24, PUA (putative RNA-binding domain in pseudouridine synthase and Archaeosine transglycosylase) at aa 296-371 and two nuclear localisation signals at aa 11-20 and aa 446-458. In future studies it will be interesting to determine whether dyskerin mutations have different molecular consequences depending on their precise physical location within the dyskerin molecule.

The DKC1 gene is expressed in all tissues of the body consistent with it having a “house keeping function” in the human cell. This correlates well with the multi-system phenotype of DC. The DKC1 gene and its encoded protein, dyskerin, a nucleolar protein (Youssoufian et al. 1999; Heiss et al. 1999) are highly conserved with many homologues including in yeast (Cbf5p), rat (NAP57), drosophila (mfl) and mouse (Dkc1) (Jiang et al 1993; Meier et al. 1994; Cadwell et al. 1997; Philips et al. 1998; Giordano et al. 1999; Ruggero et al. 2003). These dyskerin homologues associate with three core proteins (GAR1, NHP2 and NOP10) and form the core component of the H/ACA ribonucleoprotein particles (RNPs) (Henras et al. 1998). These H/ACA RNPs associate with H/ACA small nucleolar RNAs (H/ACA snRNAs) and guide the conversion of uridine to pseudouridine (pseudouridylation) at specific sites of ribosomal RNA (rRNA). Based on studies on its homologues, dyskerin has been therefore predicted to be important in pseudouridylation of specific residues of ribosomal RNA (Tollervey & kiss 1997; Lafontaine et al. 1998; Watkins et al. 1998; Zebrarjadian et al 1999; Filipowicz & Pogacic 2002; Wang and Meier 2004). This step is essential for ribosome biogenesis and it has therefore been suggested that part of the pathology in X-linked DC probably relates to
defective ribosome biogenesis (Luzzatto & Karadimitris 1998). Recent studies on the mouse dyskerin gene (Dkc1) (Ruggero et al. 2003; Mochizuki et al. 2004) provide additional support for this (Meier 2003). Further studies are necessary in human cells to clarify this issue. It has been shown that dyskerin and the other three proteins (GAR1, NH2P2 and NPO10) that form the core of the RNP's also associate with the RNA component of telomerase (TERC)(Pogacic et al. 2000; Dez et al. 2001), which also contains a H/ACA consensus sequence (Mitchell et al. 1999a; Mitchell et al. 1999b). Together with telomerase reverse transcriptase (TERT), TERC forms the core of the active telomerase complex, which is important in the maintenance of telomeres (McEachern et al. 2000; de Lange 2002; Chen and Greider 2004). Mitchell et al (1999b) found that in one fibroblast and four lymphoblast cell lines from patients with X-linked DC the level of TERC was reduced while no significant defect in rRNA processing or site-specific pseudouridylation was detected. Furthermore, telomere lengths in the lymphoblast cell lines were shorter than expected for age-matched normal individuals. Similar results have recently been obtained for the peripheral blood mononuclear cells of one X-linked DC patient (Wong et al. 2004). Telomerase activity, induced by overexpression of TERT in the X-linked DC fibroblast cell line, was shown to be reduced compared to similarly treated lines from DC carriers (Mitchell et al. 1999b). However, in the peripheral blood mononuclear cells from five X-linked DC patients telomerase activity seems to vary over a similar range to that seen in five normal controls (Vulliamy et al. 2001a). It has also been shown that telomeres are shorter in blood cells from patients with autosomal forms of DC (Vulliamy et al. 2001a). The combination of these findings suggested that DC might be principally a disease of telomere maintenance. Further clarification has come from the elucidation of the genetic basis of autosomal dominant DC (see below).

**Autosomal Dominant DC: TERC and DC pathophysiology**

Linkage analysis in one large DC family showed that the gene for autosomal dominant DC is on chromosome 3q, in the same area where the gene for TERC had been previously mapped. This led to TERC mutation analysis in this and other DC families and the demonstration that autosomal dominant DC is due to mutations in the TERC gene (Vulliamy et al. 2001b). TERC is a 451 nucleotide RNA. TERC consists of four structural domains: the pseudoknot domain, CR4-CR5 domain, the H/ACA domain and the CR7 domain. Structure function analysis reveals that the pseudoknot and CR4-CR5 domains (together with TERT) are required for the catalytic function while the H/ACA and CR7 domains for TERC RNA accumulation.

Mutations in TERC have been found in several of the TERC domains. The functional consequences of these mutations and their effect on telomerase activity (either in a cell free system or after introduction of mutant TERC into W138VA13 cells not expressing telomerase) and/or on the structure of telomerase RNA (e.g. using NMR spectroscopy) have been studied. Collectively, these functional studies (Comolli et al. 2002; Theimer et al. 2003a; Theimer et al. 2003b; Fu et al. 2003; Ly et al. 2003, Ren et al. 2003; Marrone et al. 2004) have demonstrated that TERC mutations seen in AD-DC patients result in reduced telomerase activity either through impaired RNA accumulation/stability (found to be the case for the following mutations: deletion from nucleotide 378 and 408 C->G) or a catalytic defect (found to be the case for the following mutations: 72C->G, 96-97 del CT, 107/108 GC->AG, and 110-113 del GACT). Furthermore, experiments reconstituting telomerase with both normal and mutant TERC molecules showed no evidence for a dominant negative effect. Instead the data suggests that the TERC mutations act via haplo-insufficiency (Fu & Collins 2003; Marrone et al. 2004). Additionally, it has been established for some of these mutations that are located within the conserved stem cell structure (e.g. 72C->G; 107-108 GC->AG, 408 C->G), that it is their effect on the secondary structure rather than the primary sequence that determines the functional consequences (Comolli et al. 2002; Ly et al. 2003; Marrone et al. 2004). Analysis of these disease causing TERC mutations has thus provided important confirmatory data on the different functional TERC domains.

Since the DKC1 encoded protein dyskerin and TERC are both components of the telomerase complex and all DC patients have very short telomeres, it is currently believed that DC arises principally from an abnormality in telomerase activity (Marciniak & Guarente 2001). This telomerase deficiency results in accelerated telomere shortening in DC cells (Mitchell et al. 1999; Vulliamy et al. 2001; Wong et al. 2004) and is associated with increased loss of cells (Montanaro et al. 2002) particularly from tissues which need constant renewal, such as the haemopoietic and dermatological systems that bear the brunt of DC. Evidence for a haemopoietic stem/progenitor cell defect in DC has been established in several studies (Colvin et al 1984; Friedland et al 1985; Alter et al 1992; Marsh et al 1992; Marley et al. 1999) and is
The demonstration of \textit{D KC1} and \textit{TERC} mutations in DC families provides an accurate diagnostic test, including antenatal diagnosis, in approximately 40\% of DC cases (data based on the DCR). It also provides the basis for designing the much needed new treatments. Since in any given patient DC is a single gene disorder and the cells that need to be targeted (haemopoietic stem cells) are accessible, DC is a good candidate for haemopoietic gene therapy. Furthermore there is evidence from fibroblast culture studies and from the skewed patterns of X-chromosome inactivation seen in carriers of X-linked DC (Devriendt \textit{et al}. 1997; Vulliamy \textit{et al}. 1997) that cells transfected with the normal gene would have growth/survival advantage compared to the uncorrected cells. Such an advantage would also be predicted from the role of dyskerin and \textit{TERC} in telomere maintenance.

\textbf{Clinical description}

\textbf{Classical DC}

Classical DC is an inherited BM failure syndrome characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy and mucosal leucoplaikia (Zinsser 1906; Engman 1926; Cole 1930). A variety of other (dental, gastrointestinal, genitourinary, hair greying/loss, immunological, neurological, ophthalmic, pulmonary and skeletal) somatic abnormalities have also been reported (Table 1; Sirinavin and Trowbridge 1975; Drachtman and Alter 1995; Knight \textit{et al}. 1998; Solder \textit{et al}. 1998; Dokal 2000). BM failure is the principal cause of early mortality with an additional predisposition to malignancy and fatal pulmonary complications (Dokal 2000). Clinical manifestations in DC often appear during childhood although there is a wide age range. The skin pigmentation and nail changes typically appear first, usually by the age of 10 years. BM failure usually develops below the age of 20 years; 80-90\% of patients will have developed BM abnormalities by the age of 30 years (Knight \textit{et al}. 1998a; Dokal 2000). In some patients the BM abnormalities may appear before the mucocutaneous manifestations and can lead to an initial diagnosis of "idiopathic aplastic anaemia" (Do Boeck \textit{et al}. 1981; Philips \textit{et al}. 1992; Forni \textit{et al}. 1993; Dokal 2000; Fogarty \textit{et al}. 2003). There is considerable clinical variability between patients, sometimes even within the same family. Although it is difficult to make generalisations, the X-linked recessive form appears to be associated with a more severe phenotype (more abnormalities and a younger age of onset) than the autosomal dominant form; patients with autosomal dominant DC tend to have less abnormalities and a later age of onset. The mucocutaneous abnormalities in autosomal dominant DC can also be "mild" and can make diagnosis difficult. The autosomal recessive families also show considerable heterogeneity, with some patients having severe bone marrow failure by the age of 10 years, yet others having no haematological abnormalities even by the age of 40 years. The main causes of death are BM failure/immunodeficiency (~60-70\%), pulmonary
complications (~10-15%) and malignancy (~5-10%) (Knight et al. 1998a; Dokal 2000).

Table 1. Somatic abnormalities in patients with classical DC

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical presentation</td>
<td></td>
</tr>
<tr>
<td>Abnormal skin pigmentation</td>
<td>89</td>
</tr>
<tr>
<td>Nail dystrophy</td>
<td>88</td>
</tr>
<tr>
<td>Bone marrow failure</td>
<td>85.5</td>
</tr>
<tr>
<td>Leucoplakia</td>
<td>78</td>
</tr>
<tr>
<td>Other abnormalities</td>
<td></td>
</tr>
<tr>
<td>Epiphora</td>
<td>30.5</td>
</tr>
<tr>
<td>Learning difficulties/developmental delay/mental retardation</td>
<td>25.4</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>20.3</td>
</tr>
<tr>
<td>Short stature</td>
<td>19.5</td>
</tr>
<tr>
<td>Extensive dental caries/loss</td>
<td>16.9</td>
</tr>
<tr>
<td>Oesophageal stricture</td>
<td>16.9</td>
</tr>
<tr>
<td>Premature hair loss/greying/sparse eyelashes</td>
<td>16.1</td>
</tr>
<tr>
<td>Hyperhidrosis</td>
<td>15.3</td>
</tr>
<tr>
<td>Malignancy</td>
<td>9.8</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>7.6</td>
</tr>
<tr>
<td>Liver disease/peptic ulceration/enteropathy</td>
<td>7.3</td>
</tr>
<tr>
<td>Ataxia/cerebellar hypoplasia</td>
<td>6.8</td>
</tr>
<tr>
<td>Hypogonadism/undescended testes</td>
<td>5.9</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>5.9</td>
</tr>
<tr>
<td>Urethral stricture/phimosis</td>
<td>5.1</td>
</tr>
<tr>
<td>Osteoporosis/aseptic necrosis/scoliosis</td>
<td>5.1</td>
</tr>
<tr>
<td>Deafness</td>
<td>0.8</td>
</tr>
</tbody>
</table>

NB – In the future as patients identified to have DC on the basis of mutation analysis are included, these percentages are likely to change.

**Atypical forms of DC**

**Hoyeraal-Hreidarsson syndrome**

The Hoyeraal-Hreidarsson (HH) syndrome (MIM 600545) is a severe multi-system disorder that can present in the neonatal period and infancy. It is characterized by severe growth retardation, bone marrow failure, immunodeficiency and neurological abnormalities (Hoyeraal et al. 1970; Hreidarsson et al. 1988; Berthet et al. 1994; Aalfs et al. 1995; Ohga et al. 1995; Nespoli et al. 1997). The overlap of these HH features with some DC patients led to analysis of the DKC1 gene in HH patients. These studies demonstrated that some male HH cases are a severe variant of DC where death from BM failure/immunodeficiency occurs before the appearance of the diagnostic features of DC (Knight et al. 1999b). It also highlighted the immunological defects that can be seen in DC, ranging from the severe “T+B-NK- severe immunodeficiency” in HH patients (Knight et al. 1999b; Cosu et al. 2002) to the more variable immunological abnormalities observed in other DC patients (Wiedemann et al. 1984; Rose et al. 1992; Solder et al. 1998; Dokal 2000; Knudson et al. 2004). Several mutations in dyskerin have now been identified in HH patients (Knight et al. 1999b; Yagahmai et al. 2000; Cosu et al. 2002; Sznajer et al. 2003). Female cases of HH are also recognised (Mahmood et al. 1998; Akaboshi et al. 2000; DCR families) and it is likely that they represent a severe variant of the autosomal recessive form(s) of DC, the genetic basis of which presently remains unknown.

**Idiopathic aplastic anaemia and myelodysplasia**

In some of the DCR families, affected members have died of severe AA before the age of 10 years and a diagnosis of DC was made subsequently, only when other members of the family survived long enough to develop the classical mucocutaneous features. If it were not for the presence of subsequent members, these would have been characterized as idiopathic AA. In addition, it has been shown that patients with AA have short telomeres compared to age matched controls (Ball et al. 1998; Brummendorf et al. 2001). These observations led to analysis of the DKC1 and TERC genes in AA patients. Although the DKC1 gene screen was found to be normal (unpublished observations), mutations in TERC have been found in some cases of AA (including paroxysmal nocturnal haemoglobinuria) and MDS (Vulliamy et al. 2002; Yamaguchi et al. 2003; Vulliamy et al. 2004; Keith et al. 2004). It is noteworthy that MDS patients also have short telomeres (Boulwood et al. 1997). Patients with TERC mutations who present predominantly with features of AA or MDS can be regarded as having “mild/cryptic” DC.

These findings show that in some cases of AA/MDS the primary defect is in the maintenance of telomeres and this has
implications for the management of patients failing conventional therapies such as immunosuppressive therapy. They also highlight the clinical and genetic heterogeneity of DC and a possible rational for screening the DC genes in uncharacterised patients (e.g. unexplained pulmonary or liver disease) who have clinical features that overlap with DC.

Diagnostic methods
The diagnosis of DC is relatively easy when all the classical mucocutaneous features are present. However the age at which these features develop is very variable and some patients may initially present with non-cutaneous features of DC, which makes diagnosis based on clinical criteria very difficult. For example, some patients may present with aplastic anemia as their first presentation as highlighted above. Since the genes mutated in the X-linked recessive (DKC1) and autosomal dominant (TERC) DC sub-types are now known it is possible to substantiate the diagnosis in a significant proportion of DC patients. It is appropriate to screen for the DKC1 gene if patients are male and have 2 out the following: abnormal skin pigmentation, nail dystrophy, leucoplakia, BM failure. The situation regarding the TERC screen is different for two reasons. Firstly we already know that a sub-group of patients with AA have mutations in TERC. Secondly, screen for TERC is relatively easy. Therefore it is reasonable to undertake analysis of the TERC gene in all patients presenting with aplastic anemia. There is as yet no easy universal functional test for DC. In patients presenting with AA it is also important to undertake chromosomal breakage analysis for FA.

Epidemiology
The precise incidence/prevalence of DC is unknown. It has been observed in many racial subtypes and it is estimated that the prevalence of DC is approximately 1 per 1000000 persons. The DCR at the Hammersmith Hospital (London, UK) has information on 340 patients of all races and distinguishes DC into X-linked recessive (MIM 305000), autosomal dominant (MIM 127550) and autosomal recessive (MIM 224230) subtypes.

Genetic counselling
Identification of a mutation in either the DKC1 or TERC gene facilitates accurate genetic counselling. Counselling should adhere to standards established for all genetic disorders.

Antenatal diagnosis
In families where mutation in the DKC1 or TERC gene has been characterized antenatal diagnosis is possible.

Management including treatment
DC is a multi-system disorder and may require the input of many sub-specialists. It is advisable that DC patients should avoid exposure to sunlight (use barrier creams). They should also avoid smoking and alcohol if possible as the livers and lungs of DC patients are more susceptible to damage. Use of moisturising creams to prevent damage to skin and good oral hygiene are also important. Bone marrow failure/immunodeficiency is the principal cause of premature mortality in DC patients. Oxymetholone (an anabolic steroid) can produce an improvement in haemopoietic function in many patients (approximately 60%) for a variable period of time (Smith et al, 1979; Dokal unpublished data). Successful responses to hematopoietic growth factors such as GM-CSF, G-CSF and erythropoietin have also been reported (Russo et al. 1990; Alter et al. 1997; Erduran et al. 2003) although usually these are transient. The main current treatment for severe BM failure is allogeneic haemopoietic stem cell transplantation (SCT) and there is some experience using both sibling and alternative stem cell donors (Berthou et al. 1991; Dokal et al. 1992; Langston et al. 1996; Yabe et al. 1997; Rocha et al. 1998; Ghavamzadeh et al. 1999; Lau et al. 1999). Unfortunately because of early and late fatal pulmonary/vascular complications following SCT, the results of allogeneic SCT have been less successful. The presence of pulmonary disease in a significant proportion of DC patients (Table 1, Paul et al. 1992; Verra et al. 1992; Knight et al. 1998; Safa et al. 2001; Kilic et al. 2003) explains the high incidence of fatal pulmonary complications in the setting of SCT. It also highlights the need to avoid agents, which are associated with pulmonary toxicity (such as busulphan and radiotherapy). Since BM failure is the main cause of premature death in DC patients and SCT is currently the only curative option for the BM failure, SCT should continue to be performed on selected patients. The best candidates for SCT are patients with no pre-existing pulmonary disease and who have sibling donors. SCT using fludarabine-based reduced-intensity conditioning regimens, which avoid busulphan and radiotherapy, appears to be giving encouraging results (Cossu et al. 2002; Nobili et al. 2002; Dror et al. 2003; Gungor et al. 2003). For patients lacking compatible SCT donors there is a great need to develop new treatment strategies.
It is also important to monitor the pulmonary system and to screen for malignancies, as these are the other important causes of mortality.

**Unresolved questions**

Many aspects of DC remain unresolved. These include some of the following:

1. What are the functions of dyskerin?
   Does it have a role in pseudouridylation in addition to telomere maintenance?
2. What are the relative contributions of defective telomere maintenance and ribosomal biogenesis in the pathophysiology of DC, particularly X-linked DC?
3. What is the genetic/molecular basis of autosomal recessive DC?
4. What is the proportion of idiopathic AA that is due to defects in DC genes?
5. What is the best conditioning regimen for DC patients undergoing SCT?
6. Will it be possible to develop new treatment strategies based on correction of the telomerase defect?

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