

## Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA

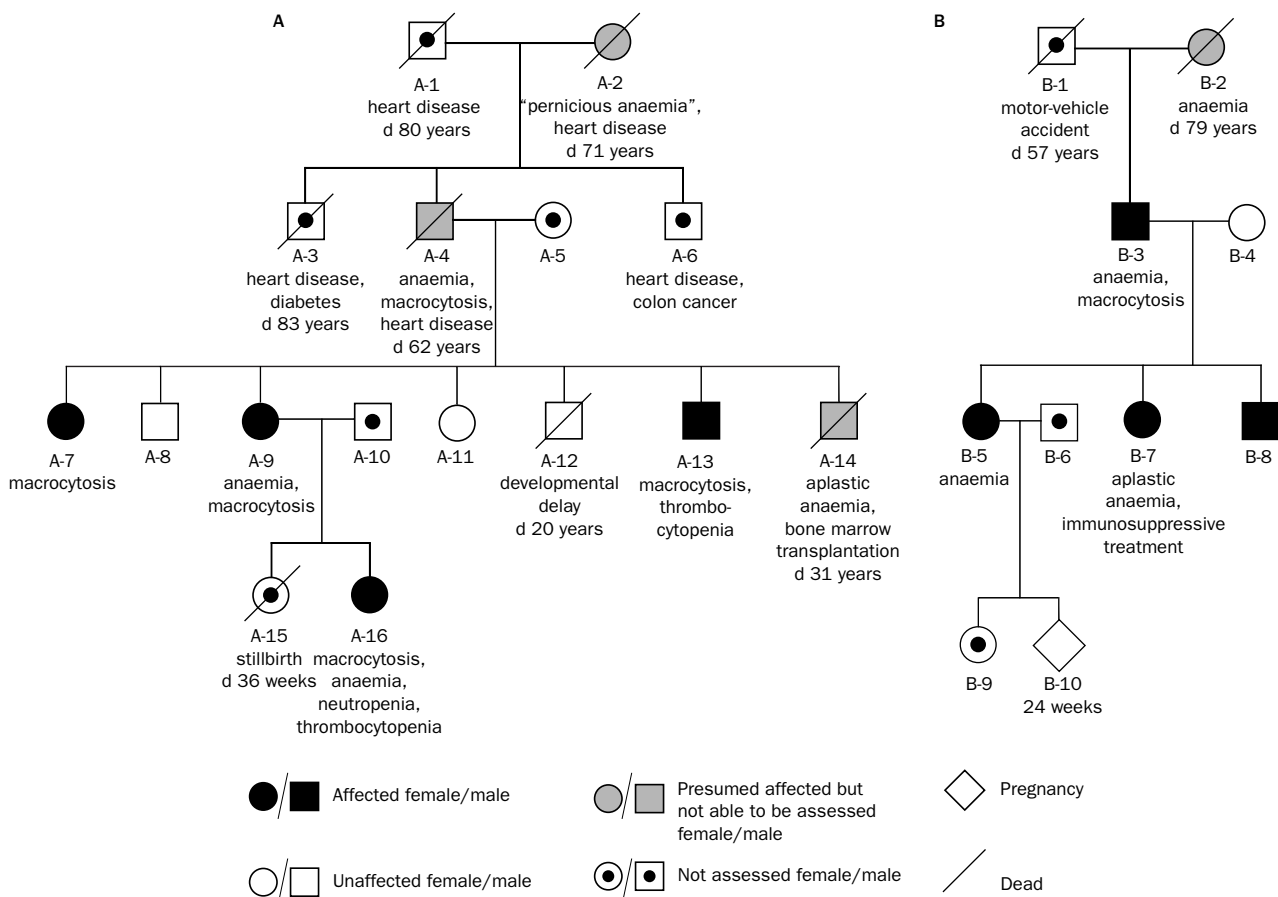
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**Aplastic anaemia in adults is usually acquired, but rarely constitutional types of bone marrow failure can occur late in life. We assessed two families with onset of pancytopenia in adults and detected two novel point mutations in the telomerase RNA gene (*TERC*) in each family. This gene is abnormal in some kindreds with dyskeratosis congenita. Individuals in our families with mutated *TERC* did not have physical signs of dyskeratosis congenita, and their blood counts were nearly normal, but all had severely shortened telomeres, reduced haemopoietic function, and raised serum erythropoietin and thrombopoietin. Bone marrow failure of variable severity due to dyskeratosis congenita, historically characterised by associated physical anomalies and early pancytopenia, may be present in otherwise phenotypically normal adults, and can masquerade as acquired aplastic anaemia.**

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Aplastic anaemia, defined by low peripheral blood cell counts and hypocellular bone marrow, typically presents in adults as a sporadic illness and responds to immunosuppressive therapy. By contrast, constitutional bone marrow failure is usually diagnosed in children with a strong family history of blood diseases, and each syndrome includes typical physical anomalies. In Fanconi's anaemia, the paradigmatic syndrome, pancytopenia occurs early in life and is accompanied by abnormalities of growth and development, with especially marked skeletal and urogenital system anomalies. Fanconi's anaemia is diagnosed by examination of chromosomes in peripheral blood lymphocytes after exposure to agents such as diepoxybutane or mitomycin. Application of these diagnostic methods has led not only to the discovery of Fanconi's anaemia in adult patients with apparently new onset aplastic anaemia, but also to the recognition that most patients with Fanconi's anaemia have extremely subtle signs often not apparent on routine physical examination, or no characteristic findings at all.

We describe two families in which an adult was diagnosed with acquired aplastic anaemia. In each kindred, family history was initially not thought to be a contributory factor. The index patient of family A (A-14, figure) was a 30-year-old man who presented with severe pancytopenia (table) to another hospital. Bone marrow examination revealed severe hypoplasia with no evidence of dysplastic morphology, consistent with acquired aplastic anaemia.



**Pedigrees of families A and B showing clinical features and inheritance of *TERC* mutations**

d=age at death.

	A-14*	A-9	A-16	A-7	A-13	A-8	A-11	B-7	B-8	B-5†	B-3	B-4
<b>Characteristic</b>												
Age (years)	30	50	24	54	40	53	47	30	33	31	62	58
Sex	Male	Female	Female	Female	Male	Male	Male	Female	Male	Female	Male	Female
Leucocytes ( $\times 10^9/L$ )	1.2	3.6	3.2	3.6	3.8	6.9	6.1	2.1	5.2	5.4	4.0	6.2
Haemoglobin (g/L)	30	118	120	126	138	155	133	72	135	118	107	146
Mean corpuscular volume (fL)	106	113	103	105	108	92	98	136	94	88	121	93
Platelets ( $\times 10^9/L$ )	19	232	109	180	105	266	309	25	188	389	103	332
Erythroid/myeloid colonies (number)‡	—	4	4	5	17	49	58	0	27	117	10	159
Peripheral blood CD34 ( $\times 10^6/\mu L$ )	—	0.3	0.2	0.3	0.2	1.0	1.1	0.05	0.6	1.0	0.5	1.5
Thrombopoietin (ng/L)	—	393	322	427	726	160	28	1645	679	81	1266	148
Erythropoietin (U/L)	—	182	55	90	125	16	14	3212	19	110	344	8
Telomere length (kb)§												
Family member	—	3.9	5.2	4.0	3.8	—	—	3.7	4.2	4.8	—	—
Age-matched reference	—	6.8	7.7	6.7	7.0	—	—	7.4	7.3	7.4	—	—
Bone-marrow cellularity	Low	Low	Low	Low	Low	Normal	Normal	Low	Low	Low	Low	Normal

Age-matched telomere length values were derived from a cohort of normal individuals. Reference values are: peripheral blood CD34+ cells  $0.6-2.9 \times 10^6/\mu L$ ; thrombopoietin  $<120$  ng/L; erythropoietin 4–16 U/L. \*Individual A-14 died before haemopoietic function was assessed. †Individual B-5 was 24 weeks' pregnant at the time of biochemical and genetic analysis. ‡Erythroid/myeloid colonies were enumerated per 1000 peripheral blood mononuclear cells plated at low density in culture with methylcellulose and supplemental growth factors. §Telomere length values are an average of values for a diverse subset of leucocytes (granulocytes, naive T lymphocytes, memory T lymphocytes, natural killer/natural-killer-T cells, and B cells).

#### Clinical characteristics of family members

The proband's healthy 48-year-old sister (A-9), a six-antigen HLA match, was a donor for haemopoietic stem cell transplantation. Her bone marrow harvest and subsequent peripheral blood stem mobilisation, however, yielded suboptimal numbers of CD34+ cells. Transplantation was complicated by delayed engraftment with long-lasting neutropenia and, on day 188 after surgery, the patient died from sepsis. The family later approached our clinic for evaluation.

The proband of family B (B-7, figure) presented at age 21 years with moderate pancytopenia. Bone marrow examination showed hypocellularity, and a test for Fanconi's anaemia was negative. At age 26 years, because of worsening anaemia, immunosuppressive therapy was started, first with ciclosporin and later with antithymocyte immunoglobulin, without sustained benefit. Haemopoietic stem cell transplantation was planned from the patient's healthy, HLA-matched brother (B-8, figure), who had normal blood counts. However, mobilisation of haemopoietic progenitor cells proved unsuccessful, and bone marrow examination of the donor showed marked hypocellularity. The proband was later treated with androgens, resulting in clinical improvement that rendered transfusion unnecessary. Neither proband had abnormalities of the skin or fingernails.

Haemopoietic function was assessed in family members of the index patients by standard laboratory techniques on blood and marrow samples (table). All samples were obtained with written informed consent under protocols approved by the NHLBI Institutional Review Board.

Most family members showed no or only slight perturbations in haemoglobin, white cell, and platelet counts (table). The most consistent finding in members of family A was above-average mean corpuscular volume. Bone marrow biopsy samples from multiple individuals in both families were markedly hypocellular. Affected individuals in both families had low peripheral blood CD34+ cell concentrations (median  $0.5 \times 10^6/L$  [range  $0.05-1.0 \times 10^6/L$ ]), compared with unaffected individuals ( $1.1 \times 10^6/L$ ;  $1.0-1.5 \times 10^6/L$ ), and a marked decrease in progenitor cells in colony culture assays in affected individuals (7.5 colonies [0–119]) versus unaffected individuals (58 colonies [45–163]). Median concentrations of serum haemopoietic growth factors of affected individuals in both families were markedly higher (erythropoietin 118 U/L [19–3212], thrombopoietin 553 ng/L [81–1645]) than those of unaffected relatives

(14 U/L [8–16] and 148 ng/L [28–160], respectively) suggesting a compensatory increase in these stimulating cytokines.

Telomere length was measured in subsets of leucocytes.<sup>1</sup> Affected individuals in both families had extremely short telomeres in all cell types analysed (table) compared with age-matched telomere lengths derived from a reference group of 400 controls (GMB and PML, unpublished data). We amplified and sequenced *TERC*.<sup>1</sup> A novel heterozygous C→T mutation was identified in the pseudoknot domain (CR2/CR3) of *TERC* at position 116 in all affected members of family A. A heterozygous 204 C→G mutation in *TERC* was present in all affected individuals in family B. Neither of these mutations was observed in a series of 194 phenotypically normal individuals.<sup>1</sup>

Dyskeratosis congenita was first described in the early 20th century in case reports that emphasised the ectodermal dysplasia that was ultimately regarded as a prominent part of the syndrome. The classic diagnostic triad includes hyperpigmentation, dystrophic nails, and oral leukoplakia. *DKC1* is mutated in the X-linked form of the disease,<sup>2</sup> and mutations in *TERC* cause autosomal dominant dyskeratosis congenita.<sup>3</sup> The product of *TERC*, telomerase RNA, interacts with telomerase reverse transcriptase to form functional telomerase complexes that can add telomere repeats to the ends of chromosomes.

In our families, poor mobilisation of the donor was the clue to a hereditary basis for the bone marrow failure in the index patients. The mutation in family A affects the functionally important pseudoknot domain, a region that has been implicated in other kindreds with constitutional aplastic anaemia.<sup>4</sup> Although the mutation in family B is located outside of currently appreciated functional domains, it also seemed to result in severe shortening of telomeres.

Telomeres also are short in some patients with acquired aplastic anaemia, suggesting that such patients have cryptic dyskeratosis congenita as a result of mutations in genes encoding members of the telomerase complex. Telomere length is not correlated with severity of pancytopenia, and the shortest telomeres often occur in patients with modest, but chronic, low blood counts.<sup>5</sup> However, mutations in *TERC* are rare in individuals presenting with bone marrow failure.<sup>1</sup>

Low stem cell numbers in a putative donor for a patient with aplastic anaemia, or a family history of even mild blood count abnormalities, should suggest the possibility of an underlying hereditary syndrome. Confirmatory genetic testing for dyskeratosis congenita mutations serves to direct

these patients away from immunosuppressive treatments, suggests the benefit of a trial of androgens, and leads to modification of the conditioning programme before stem cell transplantation. As the clinical syndrome of dyskeratosis congenita becomes better defined by molecular studies, the traditional emphasis on signs of dyskeratosis in diagnosis will probably be replaced by identification of the characteristic haemopoietic stem cell defect resulting from disturbed telomere maintenance.

#### Contributors

P Fogarty, A Wiestner, and N S Young conceived the idea for the study, accrued patients, and contributed to data analysis and interpretation. H Yamaguchi sequenced *TERC*. G Baerlocher measured telomere length and contributed to data analysis and interpretation. E Sloand contributed to data analysis and accrued patients. W S Zeng did haemopoiesis assays. E J Read did flow cytometry of peripheral blood progenitor cells. P M Lansdorp contributed to data analysis and interpretation. All investigators contributed to the writing and review of the paper. P Fogarty and H Yamaguchi contributed equally to this work.

#### Conflict of interest statement

None declared.

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