

Recurrent A 353 V mutation in a Thai family with X-linked dyskeratosis congenita

We report on two Thai brothers who presented with classical features of dyskeratosis congenita (DKC). Both developed pancytopenia at 3 and 6 years of age. Molecular analysis of the dyskerin gene revealed a mutation in exon 11 (A353V) inherited from the mother. This mutation is an important cause of DKC worldwide.

Dyskeratosis congenita (DKC) is a rare inherited multisystem syndrome with a triad of characteristic phenotypes: abnormal skin pigmentation, mucosal leukoplakia and nail dystrophy.¹ Bone marrow failure is a common finding ($\approx 80\%$ of patients) and is the most common cause of early death in patients. There are three possible modes of inheritance, autosomal dominant, autosomal recessive and X-linked recessive. Only in the X-linked form, which accounts for the majority of cases, have the affected gene (DKC1, dyskerin) and its mutations been characterized.^{2,3} Most mutations are missense and no null mutation has been observed suggesting that such mutations are probably lethal.

Recently, we studied two patients affected by this syndrome from a family in Bangkok, Thailand. The older brother presented with chronic anemia and fatigue at 6 years of age. We found the classical phenotypes described above, as shown in Figure 1A-E. The hematologic study revealed pancytopenia (Hb 9 g/dL, Hct 30%, white blood count $3.4 \times 10^9/L$, ANC $1.475 \times 10^9/L$, platelet count $26 \times 10^9/L$) and bone marrow examination showed hypocellularity and aplastic changes.

Subsequent physical examination and hematologic analysis of the proband's younger brother revealed similar findings and the X-linked mode of inheritance in this family was suspected.

The molecular analysis of DKC1 was carried out using genomic DNA extracted from peripheral leukocytes from both patients and their parents. Using a modified screening method from Knight *et al.*,⁷ we found a missense mutation (C \rightarrow T) in exon 11 at position 1058, which results in the loss of a *MspA11* restriction site (data not shown).

Both patients inherited this mutation from their mother who was ascertained to be heterozygous for this change. This mutation was confirmed by direct genomic sequencing of exon 11 using the primer set, *forward* 5' TAAAGTGGCATAACAAGTAG 3' and *reverse* 5' ACCTGGCAGGGCAGCAAC 3' as the amplification and sequencing primers (annealing temperature at 65°C) (Figure 2).

The dyskerin gene is highly conserved and the protein ubiquitously expressed. It has been proposed that dyskerin has many functions in various tissues.¹ By sequence homology, dyskerin contains the TruB pseudouridine(ψ)synthase motif, PUA domain (pseudouridine synthases and archaeosine-specific transglycosylases) and multiple phosphorylation sites which may play a role in rRNA biosynthesis, ribosomal subunit assembly, pseudouridination and also centromere/microtubule binding important for chromosomal segregation and cell division. It has been postulated that viable mutations in dyskerin may exert effects largely in specific tissues that have a high turnover rate such as bone marrow and skin.^{1,4} Recently, dyskerin has been shown to be associated with the RNA component (hTR) of telomerase and its deficiency may cause telomerase destabilization and decreased telomerase activity.⁵ This could lead to shortened telomeres that have been detected in DKC patients and indeed might explain some clinical phenotypes of this syndrome.^{5,6} However, the precise function of dyskerin and its interacting proteins and RNAs in the cell remains unclear and further study is required.

The exon 11, 1058 C \rightarrow T, substitution resulting in missense mutation A353V has previously been reported in patients of Caucasian origin. So far, this mutation has been found as DKC1



Figure 1. A 6-year old boy presented with reticulated hyperpigmentation predominantly in the head and neck region, anterior chest wall and ears (a, b), dysmorphic finger- and toe-nails (c,d) and mucosal leukoplakia (e).

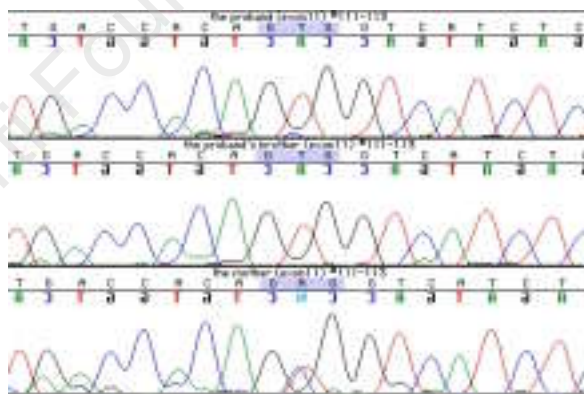


Figure 2. Direct genomic sequencing of DKC1 gene showed hemizygosity of 1058 C \rightarrow T mutation in exon 11 in both brothers and heterozygosity in the mother.

mutation in 17 different families making it the most common missense mutation responsible for the syndrome.^{1,3,7} There are few reported cases of this syndrome in the Far East and none from Thailand.^{8,9} It is unlikely that this recurrent mutation has a common founder because it has been detected independently in patients from different ethnic origins. Hydrolytic deamination of 5' methylated-cytosine is the most likely cause, changing meCpG \rightarrow TpG with poor repair process for T:G mispairing.¹⁰ There is a high frequency of CG dinucleotide to TG or CA mutations related to pathologic phenotypes in the human genome and second to mutations arising from deletion or chromosomal rearrangement.¹⁰ Screening for such of mutation hot spots can lead to the optimization of mutation search strategies. The occurrence of the A353V mutation in a Thai family in the pre-

sent study, suggests this is likely to be a common cause of Dyskeratosis congenita and highlights the importance of screening for this mutation in other patients in the future.

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