

Novel Mutation of the *TINF2* Gene in a Patient with Dyskeratosis Congenita

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Key Words

Dyskeratosis congenita · *TINF2* · Mutation · Direct sequencing

Abstract

Dyskeratosis congenita (DKC) is a rare inherited disease that is characterized by abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia. DKC is caused by an abnormality in a component of the telomerase and shelterin complexes. *TINF2* encodes a protein in the shelterin complex and *TERC* encodes a component of the telomerase complex. Mutations of both genes have been associated with DKC. This study examined mutations in *TINF2* and *TERC* by direct DNA sequencing in a Thai patient with DKC. We identified a novel mutation (c.845G>T) that is located in exon 6 of *TINF2* and changes an arginine to leucine (Arg282Leu). This identified mutation could be applied for molecular genetic diagnosis and genetic counseling of patients with DKC.

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Introduction

Dyskeratosis congenita (DKC) is a rare inherited syndrome caused by a defect in telomere maintenance. The incidence of DKC is approximately 1/1,000,000 individuals [1]. DKC is characterized by the classic clinical triad of abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia [2]. Almost 80% of DKC patients develop bone marrow failure, which is a major cause of early death. Moreover, DKC is associated with pulmonary fibrosis

and other malignancies [3, 4]. DKC is caused by mutations in six genes that encode protein components of the telomerase complex: dyskerin – *DKC1*, telomerase RNA component – *TERC*, telomerase reverse transcriptase – *TERT*, NHP2 ribonucleoprotein – *NHP2*, nucleolar protein 10 – *NOP10*, and shelterin complex (TERF1-interacting nuclear factor 2 – *TINF2*). These genes encode proteins for maintenance of telomeres, which are located at the ends of chromosomes [5]. There are three modes of inheritance of DKC, including X-linked recessive, autosomal dominant and autosomal recessive.

TINF2- and *TERC*-related DKC are inherited in an autosomal dominant pattern. *TINF2* is located on chromosome 14q12 and consists of 6 exons. The TIN2 protein is a component of the shelterin complex, which is involved in the protection of telomeres. Mutations in *TINF2* have been reported in 11–20% of all patients with DKC [6]. The patients present the classic clinical triad with severe aplastic anemia. *TERC* is located on chromosome 3q26 and comprises one exon with 451 base pairs. *TERC* is a component of the telomerase complex and functions in the extension of telomeres. Mutations in *TERC* have been reported in 6–10% of all DKC patients. Mutations of *TERC* are not only found in DKC, but also in other hematological disorders including aplastic anemia, myelodysplasia syndrome and non-hematological disorders such as pulmonary fibrosis and liver cirrhosis. Patients may present with aplastic anemia without typical mucocutaneous features [7].

This study examined mutations in *TINF2* and *TERC* by direct DNA sequencing in a Thai patient with DKC who had the classic clinical triad and bone marrow failure. We identified a novel mutation in the *TINF2* gene (c.845G>T).

Methods

Subject and DNA Extraction

A blood sample was collected from a Thai patient with DKC at the Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand. The patient had the classic symptoms, including abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia (fig. 1). The pedigree of the patient's family is shown in figure 2. The clinical profile and laboratory data of this patient are provided in table 1. A total of 100 unrelated Thai employees were included as normal controls who had no clinical symptoms of DKC and no family history of DKC. Genomic DNA was extracted using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Mutation Analysis

Direct sequencing of all exons was used for mutation screening in *TINF2* (NG_016650.1) and *TERC* (NG_016363.1). The primers were designed using the Primer3 software [http://bioinfo.ut.ee/primer3-0.4.0/ (June 13, 2014)] and are shown in table 2. The PCR mixture consisted of 125 ng of DNA with 5X GoTaq® Flexi buffer (5 µl), 10 mM dNTP (0.5 µl), 25 mM MgCl₂ (1.5 µl), 10 µM forward primer (0.5 µl), 10 µM reverse primer (0.5 µl) and 0.5 U GoTaq® DNA polymerase (Promega, Madison, Wisc., USA). PCR was performed with a thermal cycler (Veriti®; Applied Biosystems, Foster City, Calif., USA). PCR products were purified using ExoSAP-IT® (Affymetrix Inc., Santa Clara, Calif., USA) and sequenced using the BigDye® Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI Prism Genetic Analyzer 3130 Automated DNA sequencer® (Applied Biosystems).

Denaturing High-Performance Liquid Chromatography

The nucleotide changes in the DKC patient were compared with those in 100 healthy individuals using denaturing high-performance liquid chromatography (DHPLC) analysis. The PCR products were denatured at 95°C for 5 min and re-annealed by decreasing temperature until reaching 25°C. The products (5 µl) were injected into WAVE® cartridges of a DHPLC column (Transgenomic Inc., Omaha, Nebr., USA). The conditions of DHPLC for each PCR product were adjusted for optimal resolution.

Results

All exons in *TINF2* and *TERC* were screened for mutations in a Thai female patient with DKC. A novel missense mutation (c.845G>T) was identified in *TINF2* (fig. 3). The mutation generates a codon change from arginine to leucine (Arg282Leu) in exon 6 of *TINF2*. This is a hot spot for mutation in DKC. The TIN2 protein consists of two domains, including a TPP1/TRF2-binding domain and a TRF1-binding domain. The hot spot for *TINF2* mutations is found in a small region (amino acids 269–298) near the TRF1-binding domain. This missense mutation (c.845G>T) was detected in the DKC patient but not in 200 alleles of healthy individuals. The DHPLC chromatogram was different between the patient and control group (fig. 4). The patient showed a heterozygous mutation in *TINF2* consistent with the autosomal dominant inheritance mode of this gene. The pedigree of the patient is shown in figure 2. The patient's father, sister and brother also suffered from DKC. Their symptoms are similar to those of the patient (fig. 1, table 1). The mutation in *TINF2* in this patient resulted in a phenotype consistent with the classic clinical triad [8, 9]. The clinical characteristics of the patient are also similar to the phenotype of *TINF2* mutations from a previous study [8].

Discussion

In this study, we identified a novel mutation (c.845G>T) in *TINF2*. Approximately 11–20% of DKC patients have mutations in the *TINF2* gene [6]. Missense mutations are the most common type of mutation in DKC. Most of the mutations are found in the *DKC1* gene that is inherited in an X-linked recessive manner. The family history revealed that there was a male-to-male transmission (father-to-son), which firmly excluded X-linked recessive inheritance. Therefore, the mutation in this female Thai patient may be found in either autosomal dominant or autosomal recessive modes. Due to the pattern of inheritance in the patient's family members consistent with autosomal dominant fashion (fig. 2), the mutation in *TINF2* is the most likely cause of disease. The identified mutation in this patient (c.845G>T) is located in exon 6, which is a frequently mutated region of *TINF2*.

We compared the sequence to other species such as rat, cattle, rhesus macaque and chimpanzee using MultAlin (<http://multalin.toulouse.inra.fr>) (August 4, 2014) and the alignment shows that the *TINF2* gene is highly conserved in evolution (fig. 5). *TINF2* encodes the TIN2 protein, which is a component of the shelterin complex. This protein is involved in protection of telomeres from DNA damage. This mutation (c.845G>T) occurs in amino acid residue 282, which is located in a region adjacent to the TRF1-binding domain of TIN2. This mutation changes an arginine to leucine (Arg282Leu), converting a positively charged group to a non-polar group, which may cause dysfunction of TIN2. A previous study reported that a mutation in *TINF2* reduces the capability of TIN2 to associate with telomerase, leading to

telomere shortening [10]. Thus, the novel mutation reported here may disrupt the telomere-protective function of TIN2, thereby resulting in shortened telomeres.

Other reports found that mutation at codon 845 (c.845G>A) results in an arginine change to histidine [8, 9]. The patients in those studies were diagnosed with DKC, DKC/Hoyeraal-Hreidarsson syndrome or DKC/Revesz syndrome. Clinical characteristics varied in terms of skin pigmentation, nail dystrophy, leukoplakia, aplastic anemia, epiphora, retinopathy and developmental delay. In addition, pulmonary fibrosis was found in another patient with *TINF2* mutation in exon 6 [4]. However, the mechanism of pulmonary fibrosis in DKC is still unknown. Therefore, genotype-phenotype correlations between mutation in *TINF2* and patient characteristics may be postulated. DKC is a rare disease and a case report was published on a Thai male patient with mutation in *DKC1* (c.1058C>T) [11]. This is the first report of a female patient in Thailand with DKC. This study will help with molecular genetic diagnosis of DKC patients and family members. Furthermore, the data will be useful for genetic counseling and treatment of DKC patients.

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Statement of Ethics

The patient gave written informed consent for this study. The study was approved by the Institutional Review Board (COA No. Si391/2014).

Disclosure Statement

The authors have no conflicts of interest to disclose.

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Table 1. Clinical profile and laboratory data

Clinical data and laboratory test	Result
Diagnosis	DKC
Classic clinical triad	
Nail dystrophy	+
Skin abnormality	+
Mucosal leukoplakia	–
Aplastic anemia	+
Pulmonary fibrosis	+
Hb, g/dl	4.4
Hct, %	14.6
Platelets, $\times 10^3/\mu\text{l}$	8
WBC, $\times 10^9/\text{l}$	5.74

Table 2. Primers used for PCR amplification

Fragment	Exon	Primer	Product size, bp
<i>TINF2</i>			
1	1	F: 5'-GTGGCCTTCTGACGCACCGT-3' R: 5'-GCTCGCATCCCGCCCTTTC-3'	642
2	2+3	F: 5'-GCCCACAGCTTGGGGATGAG-3' R: 5'-CTGGGCGACAGAGCAAGATT-3'	484
3	4+5	F: 5'-GCCTGGCCACTAACCAC-3' R: 5'-TCTTATGCCCGGAGCCCA-3'	422
4	6.1	F: 5'-CGGGCATAAGAAACCAGTTA-3' R: 5'-TTCTCCTCAGAGCCCTTCC-3'	527
5	6.2	F: 5'-GCCTGAGAGCAAGGAAGAAC-3' R: 5'-TTCTGCTCCTTCCCACTCAC-3'	553
6	6.3	F: 5'-TGGTTTCCCTCCTACAGTGTG-3' R: 5'-AGTTTGGGAAGGGCCAGTTCT-3'	652
<i>TERC</i>			
1	1	F: 5'-CCAGCCGCCCCGAGAGAGTG-3' R: 5'-TGCAGCACACTGGCCAGTC-3'	672



Fig. 1. A 37-year-old female who had skin pigmentation (a) and nail dystrophy (b).

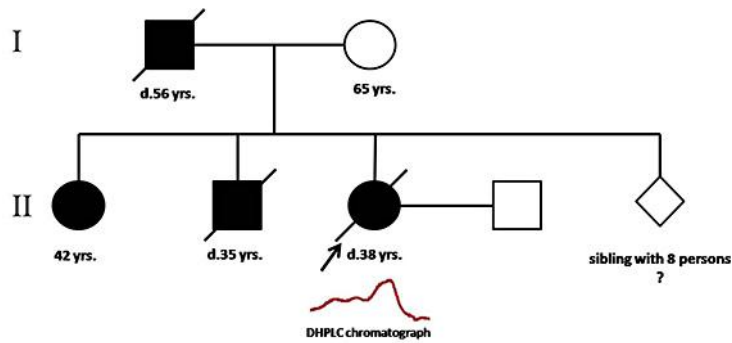


Fig. 2. The pedigree of a Thai patient with DKC. Her father, sister and brother also had clinical characteristics similar to that of the patient.

A T A A G G A G C **K** C C C C A C A G T

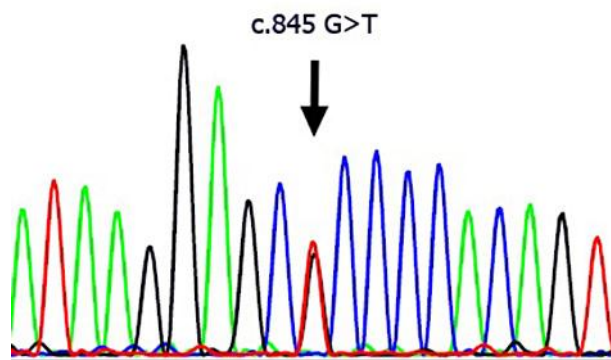


Fig. 3. Direct sequencing of *TINF2* shows heterozygous c.845G>T in exon 6.

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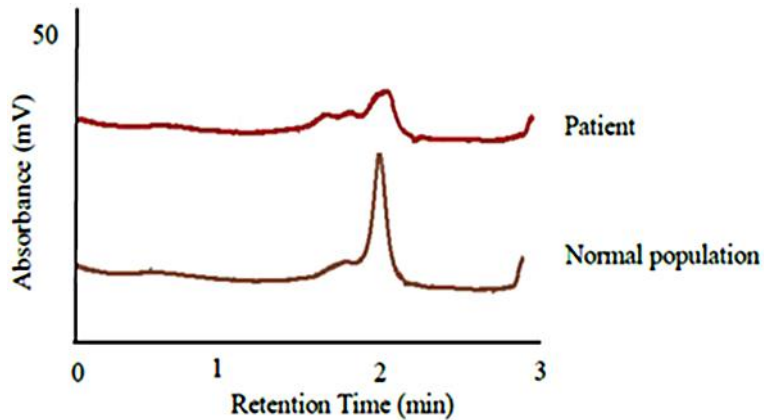


Fig. 4. The heteroduplex from DHPLC analysis of the DKC patient compared with the normal control population.

	260	270	280	290	300
Rat	NLAPLGKRRKCP	SQHTSAKACH	KERPTVML	FPRNTGLPSQ	DIPNP
Cattle	NLAPLGRRRIQP	QWAPT	SRGHKERPTV	MLL	PFRDVGSPAQVTSKP
Rhesus	NLAPLGRRRVQS	QWASTRGGH	KERPTVML	FPRNLGSPTQ	VISKP
Chimpanzee	NLAPLGRRRVQS	QWASTRGGH	KERPTVML	FPRNLGSPTQ	VISKP
Human	NLAPLGRRRVQS	QWASTRGGH	KERPTVML	FPRNLGSPTQ	VISKP
Consensus	NLAPLGrRr	.qsQWast	.gHKERPTVML	fPFR#.GsP	.Qviskp

Fig. 5. Alignment of TIN2. The conserved residues are shown in red and low consensus residues are shown in blue.