Published in final edited form as:

Pediatr Blood Cancer. 2013 June ; 60(6): . doi:10.1002/pbc.24389.

A Family with Hoyeraal-Hreidarsson Syndrome and Four Variants in Two Genes of the Telomerase Core Complex

Paraskevi Vogiatzi, M.D¹, Nieves Perdignes, PhD¹, Philip J. Mason, PhD¹, David B. Wilson, MD PhD², and Monica Bessler, MD PhD^{1,3}

¹Division of Hematology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA, USA

²Department of Pediatrics, Washington University, St. Louis Children's Hospital, St. Louis, MO, USA

³Division of Hematology/Oncology, Department of Internal Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

We describe an African American family with Hoyeraal Hreidarrson syndrome (HHS) in which 2 *TERT* mutations (causing P530L and A880T amino acid changes) and two in the *DKC1* variants (G486R and A487A) were segregating. Both genes are associated with dyskeratosis congenita and HHS. It was important to determine the importance of these mutations in disease pathogenesis to counsel family members. From genetic analysis of family members, telomere length and X-inactivation studies we concluded that compound heterozygosity for the *TERT* mutations was the major cause of HHS and the *DKC1* G486R variant is a rare African variant unlikely to cause disease.

Keywords

aplastic anemia; bone marrow failure; dyskeratosis congenita; telomerase; telomere

Introduction

Hoyeraal-Hreidarsson syndrome (HHS) (MIM #300240) is a severe variant of dyskeratosis congenita (DC) characterized by intrauterine growth retardation, developmental delay, microcephaly, cerebellar hypoplasia, immunodeficiency, or aplastic anemia [1-3]. All of the genes responsible for HHS discovered to date have encoded products that are important in telomere maintenance, though not all causative genes have been identified. Patients with HHS usually die before the age of 5 years of bone marrow failure and its complications. The aplastic anemia and immunodeficiency can be treated by bone marrow transplantation.

Correspondence Monica Bessler, Pediatric & Adult Comprehensive Bone Marrow Failure Center, Department of Pediatrics-Hematology/Oncology Division, Children's Hospital of Philadelphia, Abramson Research, 3615 Civic Center Boulevard, ARC302D, Philadelphia, PA 19104, USA. Phone: international+215-615-6555; Fax: international+215-615-6599. besslerm@email.chop.edu.

Authorship and Disclosures

PV and BN performed the laboratory work for this study, interpreted the results of the assays and wrote the manuscript. PJM and MB were responsible for project design, and assisted in the writing of the paper. DBW enrolled the patient and her family into the study. PV, PJM, DBW and MB approved the manuscript as submitted. The authors declare no relevant conflict of interest.

Case Report

We evaluated the family of an African American female who presented with classical clinical features of HHS. By commercial clinical sequencing, 4 sequence variants in 2 of 3 sequenced genes of the telomerase core complex were identified in the family. The patient with HHS had 3 sequence variants; two missense mutations in *TERT*, c.2638G>A and c.1589C>T, causing amino acid substitutions A880T and P530L, and one mutation in *DKC1*, c.1456G>A, causing a G486R substitution. The mother and 3 other family members, but not the HHS patient, had a synonymous *DKC1* polymorphism, c.1461C>T (A487A, see Figure 1). No mutations were found in *TERC*, the RNA component of telomerase. In order to assess disease in the HHS patients and to counsel family members and to identify possible polymorphisms it was important to determine the contribution of these variants on disease pathogenesis in this family.

Telomere lengths[4], which were very short (<1st percentile) in all family members with the exception of the grandfather, and the segregation of the sequence variants in the family are shown in Figures 1a and 1b. The mother had a history of pulmonary disease, but all other family members were clinically asymptomatic.

The proband inherited the *TERT* P530L mutation from her father and the *TERT* A880T mutation from her mother (Figure 1A). The proband and her parents all had very short telomeres, with the mother's being shorter than the father's telomeres (Figure 1B). A half sister had the A880T mutation, while a paternal aunt and grandmother had the P530L mutation; all these individuals had short telomeres, but not as short, compared with age-matched controls, as the proband and her mother. These results suggest both mutations affect telomere length but A880T has a stronger effect. In an *in vitro* telomerase assay the P530L mutation had no effect on telomerase activity while the A880T mutation led to a severe reduction (Figure 1C). Amino acid A880 is perfectly conserved amongst species whereas P530 is not (Figure 2A). Together these findings suggest A880T is a pathogenic mutation. The presence of short telomeres in individuals with P530L and the association of this mutation with liver cirrhosis in another study [5] suggest that this mutation may contribute to the disease in this case. That the P530L mutation does not reduce telomerase activity *in vitro* could be explained if it disrupts telomerase assembly or stability without affecting catalysis. HHS has been previously described to result from a homozygous *TERT* mutation and, in one case, from a heterozygous *TERT* mutation[6].

The proband was heterozygous for the *DKC1* G486R polymorphism (rs150319104; minor allele frequency in 1000 genomes = 0.001). Normally females heterozygous for pathogenic *DKC1* mutations have no phenotype or are only very mildly affected, but all female carriers of disease causing *DKC1* mutations show extreme skewing of X-inactivation owing to a competitive advantage of cells expressing the wild-type allele following random X-inactivation[7]. We reasoned that a sensitive test for the status of the *DKC1* G486R mutation would be to test the mother, who is heterozygous for the mutation, for skewed X-inactivation. Because she was homozygous at the HUMARA locus the commonly used HUMARA assay for X-inactivation would be uninformative. We therefore extracted RNA from peripheral blood mononuclear cells (PBMCs) and examined the expression of *DKC1* by reverse transcription and PCR (Figure 2C). Both alleles were equally expressed, which represented strong evidence that the G486R mutation is not disease causing. This is corroborated by recent population analysis showing this mutation has a frequency of 0.3-0.46% in African Americans[8], not compatible with an allele causing a very rare disease.

Finally the synonymous *DKC1* polymorphism, c.1461C>T, (rs1127051; minor allele frequency in 1000 genomes = 0.065), also segregating in this family but unlinked to the G486R mutation (Figure 1A), is a known polymorphism with an allele frequency of 5-13% in African populations. We tested 47 African American males and 29 females[9] for c.1461C>T and G486R and obtained frequencies of 11.5% and 0% respectively.

Discussion

Thus our investigation using a combination of telomere length, in vitro and in vivo assessment of telomerase activity revealed that in this family the disease causing mutations are both *TERT* gene mutations, the A880T more severely than the P530P mutation, whereas both *DKC1* variants are polymorphisms, one relatively frequent the other very rare and both unlikely to contributing to the disease pathology. We cannot exclude that the two *DKC1* variants are hypomorphic variants that on their own will not cause disease, but, in combination with an impaired telomerase activity, might aggravate the disease. The study of a much larger DC patient population would be needed to address this question.

Why is this important? All 3 variants, the A880T, the P530P, and the G486R variant are in the database of commercial clinical sequencing as mutations identified in a patient with HHS, and are thus categorized as disease associated mutations. Consequently individuals, in the case of the G486R variant mainly of African origin, carrying the G486R variant are diagnosed with a devastating disease they do not have or excluded from serving as bone marrow donors for a family member in need of a bone marrow transplant. With the increasing number of variants that are detected with the availability of whole exome/genome sequencing the careful investigation of whether a variant associated with disease is indeed causing disease will become increasingly essential. The increased prevalence of a specific variant in patients with disease and absence in ethnically matched control individuals, the segregation with clinical or laboratory markers of the disease in affected family members, and an impaired function of the gene product in an in vitro assay are helpful supportive criteria in determining the pathogenicity of a sequence variant.

Acknowledgments

We thank the family for participating in the study and acknowledge Maria Nieves Perdignes and Bai-Wei Gu for fruitful discussions.

Funding

This work was supported by the Buck Family Endowed Chair in Hematology and National Institutes of Health/ National Cancer Institute Grant (NHI/ NCI 2R01CA105312) to MB, and grant (NIH/NCI R01 CA1/06995) to PJM.

References

1. Hoyeraal HM, Lamvik J, Moe PJ. Congenital hypoplastic thrombocytopenia and cerebral malformations in two brothers. *Acta Paediatr Scand.* 1970; 59(2):185–191. [PubMed: 5442429]
2. Hreidarsson S, Kristjansson K, Johannesson G, et al. A syndrome of progressive pancytopenia with microcephaly, cerebellar hypoplasia and growth failure. *Acta Paediatr Scand.* 1988; 77(5):773–775. [PubMed: 3201986]
3. Knight SW, Heiss NS, Vulliamy TJ, et al. Unexplained aplastic anaemia, immunodeficiency, and cerebellar hypoplasia (Hoyeraal-Hreidarsson syndrome) due to mutations in the dyskeratosis congenita gene, *DKC1*. *Br J Haematol.* 1999; 107(2):335–339. [PubMed: 10583221]
4. Du HY, Pumbo E, Ivanovich J, et al. *TERC* and *TERT* gene mutations in patients with bone marrow failure and the significance of telomere length measurements. *Blood.* 2009; 113(2):309–316. [PubMed: 18931339]

5. Calado RT, Brudno J, Mehta P, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. *Hepatology*. 2011; 53(5):1600–1607. [PubMed: 21520173]
6. Marrone A, Walne A, Tamary H, et al. Telomerase reverse-transcriptase homozygous mutations in autosomal recessive dyskeratosis congenita and Hoyeraal-Hreidarsson syndrome. *Blood*. 2007; 110(13):4198–4205. [PubMed: 17785587]
7. Vulliamy TJ, Knight SW, Dokal I, et al. Skewed X-inactivation in carriers of X-linked dyskeratosis congenita. *Blood*. 1997; 90(6):2213–2216. [PubMed: 9310472]
8. Campbell CD, Sampas N, Tsalenko A, et al. Population-genetic properties of differentiated human copy-number polymorphisms. *Am J Hum Genet*. 2011; 88(3):317–332. [PubMed: 21397061]
9. Huang Q, Robledo S, Wilson DB, et al. A four base pair insertion in exon 1 of the RPS19 gene is a common polymorphism in African-Americans. *Br J Haematol*. 2006; 135(5):745–746. [PubMed: 17107358]

Figure 1.

A. Family pedigree and genotypes. The proband is shaded. **B.** Flow Fish Telomere lengths of the family members in PBMCs isolated by Ficoll gradient centrifugation using fluorescein isothiocyanate (FITC)-conjugated (C3TA2)3 peptide nucleic acid (PNA) probe flow cytometry[4]. Relative telomere length (RTL) results by comparing PBMCs with a control cell line, GM03671C, a tetraploid cell line with telomere length of 100%. The numbers refer to the individuals in the pedigree in panel A. **C.** TRAP (telomerase repeat amplification protocol) assay. Mutations identified in *TERT* genes were introduced into plasmids containing the wild-type (WT) gene using a QuickChange site-directed mutagenesis kit (Stratagene). Mutant or wild-type *TERT* and wild-type *TERC* plasmids (4 μ g) were then transfected using Lipofectamine 2000 into WI-38 VA13 cells at 90% confluence. After 48 hours, diluted protein cell lysates (1:5) were assayed for telomerase activity using a PCR-based TRAP assay (Chemicon International). WT: wild-type *TERT* and wild-type *TERC*, HI: Heat Inactivated, LB: Lysis Buffer, IC: Internal Control (36-bp) for PCR amplification, ON: overnight.

Figure 2.
A. Conservation of the *TERT* and *DKC1* variants in different species. **B.** Sanger sequencing of *DKC1* cDNA prepared from RNA from PBMCs of the proband’s mother. The pedigree in Figure 1A establishes that the 1456G>A and 1461 C>T mutations are on different alleles.