Hoyeraal-Hreidarsson Syndrome Due to *PARN* Mutations: Fourteen Years of Follow-up

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ABSTRACT

Introduction: Hoyeraal-Hreidarsson syndrome is a dyskeratosis congenita-related telomere biology disorder that presents in infancy with intrauterine growth retardation immunodeficiency, and cerebellar hypoplasia in addition to the triad of nail dysplasia, skin pigmentation, and oral leukoplakia. Patients with Hoyeraal-Hreidarsson syndrome often develop bone marrow failure in early childhood. Germline mutations in *DKC1*, *TERT*, *TINF2*, *RTEL1*, *ACD*, and *PARN* cause about 60% of Hoyeraal-Hreidarsson syndrome cases.

Case Report: We report 14 years of follow-up for a patient with Hoyeraal-Hreidarsson syndrome who initially presented as an infant with intrauterine growth retardation, microcephaly, and central nervous system calcifications. He was diagnosed with Hoyeraal-Hreidarsson syndrome at age six and had a complicated medical history including severe developmental delay, cerebellar hypoplasia, esophageal and urethral stenosis, hip avascular necrosis, immunodeficiency, and bone marrow failure evolving to myelodysplastic syndrome requiring hematopoietic cell transplantation at age 14. He had progressive skin pigmentation changes, oral leukoplakia, and nail dysplasia leading to anonychia. Whole exome sequencing identified novel biallelic variants in *PARN*.

Conclusions: This case illustrates that the constellation of IUGR, central nervous system calcifications, and cerebellar hypoplasia, esophageal or urethral stenosis, and cytopenias, in the absence of congenital infection, may be due to Hoyeraal-Hreidarsson syndrome. Early diagnosis of Hoyeraal-Hreidarsson syndrome is important to optimize medical management and provide genetic counseling.

KEY WORDS: dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome, telomere, *PARN*, microcephaly, CNS calcification

INTRODUCTION

Dyskeratosis congenita is an inherited bone marrow failure syndrome caused by dysfunctional telomere maintenance^{1, 2}. It is diagnosed by the presence of the classic triad of nail dysplasia, lacy skin pigmentation, and oral leukoplakia. Additional clinical features seen in dyskeratosis congenita and related telomere biology disorders are shown in Table 1. (Table 1)^{1, 3}. Blood leukocyte telomere lengths less than the first percentile for age are consistent with the diagnosis of dyskeratosis congenita and a consequence of germline mutations in telomere biology genes⁴. X-linked recessive dyskeratosis congenita is caused by mutations in *DKC1* (MIM 300126). Autosomal dominant dyskeratosis congenita can be caused by mutations in *TERC* (MIM 602322), *TERT* (MIM 187270), *RTEL1* (MIM 608833), or *TINF2* (MIM 604319). Autosomal recessive inheritance of mutations in *TERT*, *RTEL1*, *CTCI* (MIM 613129), *NOP10* (MIM 606471), *NHP2* (MIM 606470), *WRAP53* (MIM 612661), *ACD* (MIM 609377) or *PARN* (MIM 604212) also cause dyskeratosis congenita^{1, 5-7}. Germline mutations in these genes account for approximately 70-80% of dyskeratosis congenita cases.

Hoyeraal-Hreidarsson syndrome is a form of dyskeratosis congenita with very early age at onset (Table 1). In addition to features of dyskeratosis congenita, patients with Hoyeraal-Hreidarsson syndrome have immunodeficiency^{8, 9}, intrauterine growth retardation, developmental delay, and cerebellar hypoplasia; the latter is characteristic of Hoyeraal-Hreidarsson syndrome¹⁰⁻¹².

Patients with Hoyeraal-Hreidarsson syndrome have extremely short leukocyte telomeres, even in comparison with other dyskeratosis congenita patients⁴. Mutations in a subset of dyskeratosis congenita-associated genes (*DKC1*, *TINF2*, *TERT*, *RTEL1*, *ACD*, and *PARN*) have been shown to cause Hoyeraal-Hreidarsson syndrome.

Mutations in *PARN*, which encodes poly(A)-specific ribonuclease, a deadenylase, have been linked with autosomal dominant familial pulmonary fibrosis¹³ and autosomal recessive Hoyeraal-Hreidarsson syndrome⁵. Monoallelic deletions in *PARN* have recently been described in individuals with developmental delay or mental illness¹⁴. Mutations in *PARN* have been suggested to alter mRNA stability, DNA damage response, downregulate certain telomere biology proteins, and cause short telomeres^{5, 14}.

We report 14 years of follow-up for a patient who initially presented as an infant with microcephaly, developmental delay, and central nervous system calcifications. The patient was diagnosed with Hoyeraal-Hreidarsson syndrome at six years of age and recently found to have AR mutations in the poly(A)-specific ribonuclease gene, *PARN*.

METHODS

Family NCI-165 participated in an IRB-approved longitudinal cohort study at the National Cancer Institute (NCI) entitled “Etiologic Investigation of Cancer Susceptibility in Inherited Bone Marrow Failure Syndromes” (ClinicalTrials.gov Identifier: NCT00027274). This study includes comprehensive family history and individual history questionnaires, detailed medical record review, and biospecimen collection¹⁵. Telomere length was evaluated by flow cytometry with fluorescent *in situ* hybridization (flow FISH) in leukocytes¹⁶. Comprehensive clinical evaluations were undertaken by the proband’s clinical care team.

Whole exome sequencing (WES) was performed using DNA from the proband’s brother (NCI-165-2), mother (NCI-165-3), and father (NCI-165-4) as previously described^{6, 17}; there was insufficient DNA available from the proband for WES. As most cases of Hoyeraal-Hreidarsson syndrome are caused by biallelic mutations, we hypothesized that we could infer potential compound heterozygous or homozygous variants in the proband based on the genotypes of his

first-degree relatives. We examined rare variants (MAF <0.7% in 1000 Genomes¹⁸ and the NHLBI ESP Exome Variant Server and prioritized them using *in silico* data using the Combined Annotation Dependent Depletion (CADD) scores¹⁹, PROVEAN²⁰, SIFT²¹, PolyPhen-2²², MutationTaster²³, MutationAssessor²⁴, FATHMM²⁵, and the likelihood ratio test. All variants of interest were confirmed by Sanger sequencing in the proband's DNA.

RESULTS

Clinical Case Report

The proband, NCI-165-1, was the first child of healthy, non-consanguineous parents, born at 39 weeks gestation, who weighed 2300 grams. He was noted to have microcephaly, hypotonia, and poor feeding as a neonate. A brain CT scan at three months of age showed thalamic and deep cerebral calcifications. Oral ulcers were noted before 12 months of age and his inability to tolerate oral feedings and failure to thrive prompted placement of a gastrostomy tube at 18 months. He was monitored by multiple pediatric subspecialties for severe developmental delay, esophageal stricture, undescended testes, microcephaly, seizures, and cerebellar ataxia. An MRI demonstrated cerebellar hypoplasia at age two years (Figure 1).

His family history was notable for a paternal aunt with lymphoma, paternal grandmother with breast cancer, paternal great uncle with leukemia, paternal great grandmother with breast cancer, maternal great aunt with ovarian cancer, and a maternal grandfather with emphysema or pulmonary fibrosis.

At five years of age, he was evaluated for persistent urethral meatus erosion. Physical examination revealed lingual and buccal leukoplakia, reticulated hyperpigmentation of the upper chest, and severe nail dystrophy and anonychia of several digits (Figure 1). Skin biopsy showed

mild epidermal atrophy with pigmentary incontinence and melanin-laden macrophages. A complete blood count showed thrombocytopenia and mild macrocytosis but normal white blood cell count and hemoglobin. A bone marrow biopsy at age six showed 40% cellularity with left-shifted myeloid maturation. Laboratory testing of telomere length by flow FISH in leukocytes determined the patient's telomeres to be far below the 1st-percentile compared with age-matched controls (Figure 2). This constellation of findings is diagnostic of the Hoyeraal-Hreidarsson syndrome variant of dyskeratosis congenita.

The proband developed worsening thrombocytopenia at eight years of age. A repeat bone marrow examination identified continued hypocellularity of 30% with left-shifted myeloid maturation and worsening reduction of erythrocytic and megakaryocytic progenitors.

Cytogenetic analysis was normal (46XY[20]) and FISH for monosomy 7 and for trisomy 8 was negative. He was managed with periodic blood and platelet transfusions in addition to the androgen, oral oxymetholone, resulting in gradual improvement of his blood counts and transfusion independence.

Over the next several years, he experienced several significant medical events, including hip dislocation that required surgical correction and was complicated by delayed wound closure. He was treated for esophageal stenosis with serial dilations; however he continued to require all feedings via gastrostomy tube. His mucocutaneous findings progressed to include lacrimal duct stenosis with chronic epiphora, persistent urethral meatal stenosis, progressive hair loss with brittle thin hair, increasing skin hyperpigmentation, and advancement of nail dystrophy to complete anonychia (Figure 1).

By age 13, he had developed pancytopenia; bone marrow biopsies showed myelodysplastic changes with cellularity of 10%. Karyotyping revealed a clonal abnormality in all analyzed

metaphases: 47, XY, +der(1)i(1)(q10)inv(q10;q25) in which there were two normal chromosomes 1 and an isochromosome of the long arm of chromosome 1 with an inversion. Progressive immunodeficiency was noted and he had prolonged pneumonitis due to *Pneumocystis jiroveci*. Severe, recurrent hematochezia developed requiring platelet and red blood cell transfusions. MRI enterography showed normal bowel wall thickness, but colonoscopy revealed multiple vascular telangiectatic lesions in the cecum and ascending colon (Figure 1). Oxymetholone was discontinued due to loss of hematologic response. Interestingly, after that, the hematochezia improved and repeat colonoscopy demonstrated resolution of telangiectasias.

The patient's cerebellar function was poor and he had only nonverbal communication and was in special education programs. Ophthalmologic examinations at age 13 years were normal with no cataracts, vitritis, retinopathy, or chorioretinitis. Thoracic CT found no evidence of pulmonary fibrosis at age 13, but a CT the following year showed interstitial thickening with associated bronchiectasis.

He underwent a nonmyeloablative hematopoietic cell transplant at age 14 because of life-threatening pancytopenia and high risk of developing acute myeloid leukemia associated with the myelodysplastic bone marrow changes with clonal abnormality. His donor was his 9/10 HLA-matched sibling. The preparative regimen consisted of Campath (3 mg test dose then 10 mg on day -21, 15 mg on day -20, and 20 mg on day -19), fludarabine 30 mg/m²/dose once daily on days -6 to -2, and total body irradiation of 200 cGy on day -1. Graft versus host disease prophylaxis consisted of cyclophosphamide 50 mg/kg on day +3, mycophenolate mofetil, and tacrolimus. Neutrophil engraftment occurred on day +26 and platelets were sustained at over 50,000 without transfusion on day +31. His post-transplant course was complicated by two

episodes of *E. coli* bacteremia. Subsequently, he developed coagulase negative *Staphylococcus* bacteremia, cytomegalovirus reactivation, posterior reversible leukoencephalopathy syndrome complicated by a seizure, upper and lower gastrointestinal bleeding with duodenal hematoma, and acute graft versus host disease of the gastrointestinal tract and liver that were refractory to steroids. He developed respiratory failure requiring intubation due to pulmonary hemorrhage and died on day +119 post transplant.

Whole Exome Sequencing

WES identified 14 genes for which both parents carried one heterozygous rare variant and the brother inherited at most one of the two variants (Supplementary Table 1). Each parent had a different variant in *PARN*, a gene recently found to be mutated in patients with Hoyeraal-Hreidarsson syndrome⁵. Sanger sequencing revealed that the proband inherited a maternal nonsense mutation (chr16:14698077G>A [hg19], c.C709T [NM_002582.3], p.R237X [NP_002573.1]) and a paternal variant in the 5' untranslated region (UTR, chr16:14724045G>A, c.-63C>T [NM_002582.3]); the healthy brother inherited neither variant.

The transcript bearing the premature stop codon, p.R237X, is predicted to be degraded by the nonsense mediated decay pathway. This mutation is extremely rare, with a MAF of 0.002% in the Exome Aggregation Consortium (ExAC) database (Exome Aggregation Consortium (ExAC), Cambridge, MA; <http://exac.broadinstitute.org> [accessed July 2015]); it is not present in either the ESP6500 dataset from the Exome Variant Server or the 1000 Genomes database. It is predicted to be deleterious by *in silico* algorithms, and its CADD score places it in the top 10% most deleterious mutations. If translated, this mutation would encode a protein lacking 402 amino acids (over 60% of the protein NP_002573.1), including part of an R3H domain, a

ribonuclease domain, an RNA recognition motif, a nuclear localization sequence, and a C-terminal domain (Figure 2B).

The 5' UTR variant, c.-63C>T, is in a highly conserved block upstream of the *PARN* start codon, and therefore may alter transcription of *PARN* (Figure 2C). The affected nucleotide is in a region of H3K27 acetylation as reported by the ENCODE consortium, adding further support to its role in transcriptional regulation of *PARN*. Additionally, in one of the three *PARN* transcript variants reported in the RefSeq database (NM_001134477), this variant is within two base pairs of the intron-exon border, and is therefore predicted to affect splicing of this transcript. This variant is not covered by the exome sequencing data in the ExAC database, and is not present in the 1000 Genomes data; the CADD score ranks this variant among the 10% most deleterious.

DISCUSSION

This case illustrates the diagnostic and clinical management challenges often seen in Hoyeraal-Hreidarsson syndrome. In early infancy, the proband was thought to have congenital cytomegalovirus infection, although the antibody titers were not entirely consistent with that diagnosis. He was thoroughly evaluated for IUGR, congenital infections, severe developmental delay, failure to thrive, seizures, and esophageal and urethral stenosis by numerous experts. The diagnosis of Hoyeraal-Hreidarsson syndrome was not made until age six when his medical problems had progressed and the constellation of findings, including thrombocytopenia, prompted further evaluation.

In addition to advancing skin pigmentation abnormalities, nail dysplasia, and oral leukoplakia, our patient had progressive urethral and esophageal stenosis that required intervention. He had

significant developmental delay associated with cerebellar hypoplasia and CNS calcifications. His cytopenias worsened with age and corresponded with a reduction in bone marrow cellularity. Notably, his platelet and red blood cell counts improved with oxymetholone but declined again with the onset of myelodysplasia.

Gastrointestinal ectasias are a common feature of the related telomere biology disorder, Coats plus, but this finding has not been widely reported in Hoyeraal-Hreidarsson syndrome. Our patient had widespread ectasias of the large bowel with resultant severe hematochezia. He also had CNS periventricular calcifications, another feature of the Coats-plus syndrome²⁶, but he did not have the characteristic exudative retinopathy, retinitis or retinal vascular malformation when examined at age 13.

Germline variants in *PARN* were first linked to human disease with the discovery of autosomal dominant mutations in pulmonary fibrosis patients with short telomeres¹³. Subsequently, autosomal recessive *PARN* mutations were connected with dyskeratosis congenita/Hoyeraal-Hreidarsson syndrome and shown to likely affect telomere biology through widespread effects on mRNA stability, DNA damage response defects, and an association with down regulation of telomere biology proteins⁵. *PARN* deficiency also appears to be associated with loss of specific H/ACA box RNAs, snoRNAs and TERC¹⁴.

Tummala et al described two cases with novel biallelic *PARN* variants; in both cases, one copy of *PARN* was severely truncated⁵. Two patients with developmental delay and one with mental illness were reported to have monoallelic deletions of part of *PARN* by Dhanraj et al¹⁴; all had normal brain MRIs and no known hematopoietic phenotype. Notably, patient 1 in the same report had a deletion and a missense variant of *PARN* and features consistent with Hoyeraal-

Hreidarsson syndrome, including very short lymphocyte telomeres, global developmental delay, and small cerebellum.

Similarly, the patient described here bears a variant (R237X) resulting in a significant *PARN* truncation. Unlike the variants described previously, the second *PARN* allele in our patient is affected by a potential regulatory variant in the 5' UTR. This mutation affects a highly conserved sequence in the *PARN* promoter region (Figure 2C), and may also alter splicing between the first and second exons of *PARN* transcript NM_001134477.

The cases reported by Tummala et al have marked similarity to our patient. All patients had significant developmental delay, with microcephaly and cerebellar hypoplasia, and developed bone marrow failure⁵. Additionally, DCR380 case 1, DCR380 case 2, DCR270 case 3, and our patient, NCI-165-1, had the dyskeratosis congenita mucocutaneous triad. The facial features shown in DCR 380 case 2 are remarkably similar to those in our patient, suggesting that recognition of similar features could be helpful in making future diagnoses.

In summary, this case illustrates that the constellation of IUGR, CNS calcifications, and cerebellar hypoplasia, esophageal or urethral stenosis, and cytopenias, in the absence of congenital infection, may be due to Hoyeraal-Hreidarsson syndrome. Early diagnosis of this complex disorder is important to optimize medical management and provide genetic counseling and testing.

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DISCLAIMER

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army, U.S. Navy or the Department of Defense.

FIGURE LEGENDS

Figure 1. Clinical Features of the Proband. The proband at age 6 years (left column) and 14 years (middle column). **A)** Facial features (top panels) include protuberant ears, broad nasal tip, broad prominent nasal root, full lips. Also noted are thin hair, microcephaly, and reticulated hyperpigmentation that have been progressive with age. **B, C)** Dry thickened skin of the hands and feet with progressive nail dystrophy leading to anonychia. **D)** Reticulated hyperpigmentation with areas of hypopigmentation, covering the entire skin surface became more pronounced with age. **E)** MRI illustrating cerebellar hypoplasia (yellow arrow) at age 2 years. **F)** Skin biopsy at 6 years of age shows mild epidermal atrophy with pigmentary incontinence and melanin-laden macrophages. **G)** Leukoplakia and ulcers of the tongue and small teeth at age 13 years. **H)** Colonoscopy at age 14 with multiple diffuse telangiectasias.

Figure 2. Molecular Features of the Proband. **A)** Lymphocyte telomere length, as measured by flow-FISH, for family NCI-165: proband, filled blue squares at age 5 and 13; brother, open blue diamond, father, open blue square, mother open blue circle. **B)** PARN contains three RNA-binding domains (the R3H domain [red], the RRM domain [RNA recognition motif, blue], two catalytic nuclease domains [green]), and a C-terminal domain (CTD, orange). A predicted bipartite NLS motif is located between the RRM domain and CTD (grey)²⁷. Black arrows/brackets indicate previously reported *PARN* mutations associated with Hoyeraal-Hreidarsson syndrome^{5, 14}; The blue bracket indicates the deletion reported here. **C)** The histogram represents conservation amongst vertebrates across the *PARN* promoter region. Bases -89 to -19 (the region encompassing the 5' UTR mutation detected in NCI-165-1) are shown in the detailed multiple sequence alignment below.

Table 1. Clinical Manifestations of Dyskeratosis Congenita, Hoyeraal-Hreidarsson Syndrome, and Related Disorders. The + symbol indicates the feature has been reported in at least 50% of affected individuals; +/- denotes present in some affected individuals but absent in others and could develop with age; features with – have not yet reported in the disorder. Table derived from literature review^{1, 10, 26, 28-30} and unpublished data from the NCI cohort study of inherited bone marrow failure syndromes.

System	Feature	Dyskeratosis Congenita	Hoyeraal-Hreidarsson Syndrome	Revesz Syndrome	Coats plus
Neurologic	Intracranial calcifications	+/-	+/-	+	+
	Cerebellar hypoplasia	+/- ^b	+ ^a	+/-	+/-
	Microcephaly	+/-	+/-	+/-	+/-
	Developmental delay	+/-	+	+/-	+/-
	Progressive neurologic complications	+/-	+	+/-	+/-
	Leukodystrophy	-	-	-	+
	Parenchymal brain cysts	-	-	-	+/-
Growth	Intrauterine growth retardation	+/-	+/-	+/-	+
	Extrauterine growth retardation	+/-	+/-	+/-	+
Dermatologic	Nail dysplasia	+	+/-	+	+/-
	Lacy, reticular skin pigmentation	+	+/-	+/-	+
	Oral leukoplakia	+	+	+/-	-
	Early gray or sparse hair	+/-	+/-	+/-	+
	Nail dysplasia	+	+/-	+	+/-
Ophthalmologic	Exudative retinopathy	-	-	+ ^a	+
	Retinal telangiectasias, hemorrhages	+/-	+/-	-	+
	Lacrimal duct stenosis	+/-	+/-	-	-
Gastrointestinal	Gastrointestinal vascular ectasias	-	-	-	+
	Esophageal stenosis	+/-	+/-	-	-
	Diarrhea, colitis	-	+/-	-	-
	Non-alcoholic liver disease	+/-	-	-	-
Pulmonary	Pulmonary fibrosis	+/-	-	-	-
Hematopoietic	Immunodeficiency	+/-	+	-	-
	Bone marrow failure	+/-	+	+/-	+

Orthopedic	Osteopenia, fractures, poor bone healing	+/-	-	-	+
	Avascular necrosis of hips or shoulders	+/-	-	-	-
Urogenital	Urethral stenosis (males)	+/-	+/-	-	-
Other	Increased risk of cancer	+/-	-	-	-

^aRequired for diagnosis

^bIndividuals reported as dyskeratosis congenita with cerebellar hypoplasia may have been misclassified and actually have Hoyeraal-Hreidarsson syndrome.

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Figure 1

Figure 2