Defects in lymphocyte telomere homeostasis contribute to cellular immune phenotype in patients with cartilage-hair hypoplasia

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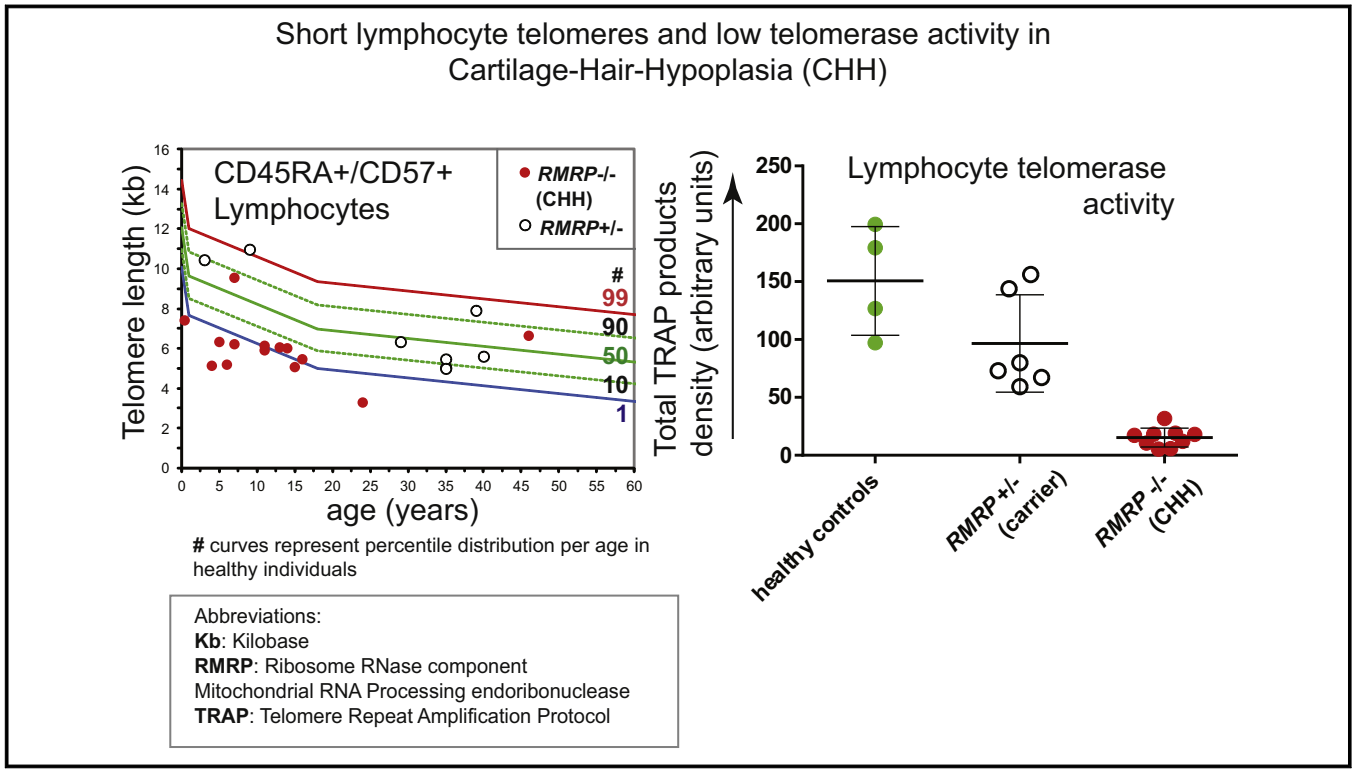
GRAPHICAL ABSTRACT



Background: Mutations in the long noncoding RNA RNase component of the mitochondrial RNA processing endoribonuclease *(RMRP)* give rise to the autosomal recessive condition cartilage-hair hypoplasia (CHH). The CHH disease phenotype has some overlap with dyskeratosis congenita, a well-known "telomere disorder." *RMRP* binds the telomerase reverse transcriptase (catalytic subunit) in some cell lines, raising the possibility that *RMRP* might play a role in telomere biology.

Objective: We sought to determine whether a telomere phenotype is present in immune cells from patients with CHH and explore mechanisms underlying these observations.

Methods: We assessed proliferative capacity and telomere length using flow–fluorescence *in situ* hybridization (*in situ* hybridization and flow cytometry) of primary lymphocytes from patients with CHH, carrier relatives, and control subjects. The role of telomerase holoenzyme components in gene expression

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and activity were assessed by using quantitative PCR and the telomere repeat amplification protocol from PBMCs and enriched lymphocyte cultures.
Results: Lymphocyte cultures from patients with CHH display growth defects *in vitro*, which is consistent with an immune deficiency cellular phenotype. Here we show that telomere length and telomerase activity are impaired in primary lymphocyte subsets from patients with CHH. Notably, telomerase activity is affected in a gene dose-dependent manner when comparing heterozygote *RMRP* carriers with patients with CHH. Telomerase deficiency in patients with CHH is not mediated by abnormal telomerase gene transcript levels relative to those of endogenous genes.
Conclusion: These findings suggest that telomere deficiency is implicated in the CHH disease phenotype through an as yet unidentified mechanism. (J Allergy Clin Immunol 2017;140:1120-9.)

Key words: *Primary immune deficiency, telomere length, cartilage-hair hypoplasia, mitochondrial RNA processing endoribonuclease, lymphocyte*

Cartilage-hair hypoplasia (CHH; [MIM: 250250]) is an autosomal recessive condition characterized by metaphyseal dysplasia, bone marrow failure, increased risk of hematologic malignancy, and variable immunodeficiency. In particular, patients with CHH can have severe combined immunodeficiency and/or susceptibility to pathogens typically cleared by T-cell and natural killer (NK) cell–mediated mechanisms, such as the Herpesviridae varicella zoster virus, EBV, or human herpes virus 6. Mutations in the long noncoding (lnc) RNA component of the mitochondrial RNA processing endoribonuclease gene (*RMRP*; encoded on chromosome 9p)[1,2] or its regulatory elements cause CHH. At present, the dominant molecular mechanisms underlying the CHH phenotype are not clearly defined; however, some disease manifestations and risk factors described in patients with CHH overlap with those of patients with known telomere deficiency diseases, such as dyskeratosis congenita.[3-5]

RMRP has been shown to have several important molecular functions, including processing of mitochondrial RNA, cleavage of ribosomal RNA, and cleavage of specific mRNAs to their active form, including cyclin B2. All these known functions can both individually and collectively impair cellular metabolism, cellular function, and proliferation when *RMRP* is mutated (reviewed by Thiel and Rauch[6]). Furthermore, several independent reports have implicated *RMRP* binding to the telomerase reverse transcriptase (catalytic subunit) [TERT]),[7-9] potentially associating *RMRP* function with telomere homeostasis.

Telomeres consist of G-rich DNA repeats and bound proteins at the end of linear chromosomes that effectively provide a protective "capping" structure preventing activation of DNA damage and repair responses. Telomere length in human cells and cell types is heterogeneous, and loss of telomere repeats was shown to occur with cell division and age. Replicative senescence or apoptosis caused by short telomeres has been clearly demonstrated, and critical loss of a few telomeres or telomere molecular uncapping (which can be seen in the absence of global telomere length compromise) has been implicated in genomic instability and neoplastic transformation, as well as in several age-related diseases (reviewed by Aubert and Lansdorp[10]).

Abbreviations used
CHH: Cartilage-hair hypoplasia
FISH: Fluorescence *in situ* hybridization
GAPDH: Glyceraldehye-3-phosphate dehydrogenase
lnc: Long noncoding
NK: Natural killer
RMRP: Mitochondrial RNA processing endoribonuclease
RPLPO: Ribosomal protein lateral stalk subunit P0
RT-qPCR: Real-time quantitative PCR
TERC: Telomerase RNA component
TERT: Telomerase reverse transcriptase (catalytic subunit)
TRAP: Telomere repeat amplification protocol
UPN: Unique patient number

Unlike the majority of somatic cells, lymphocytes upregulate telomerase expression at specific developmental stages, as well as in response to antigen receptor or cytokine stimulation.[11,12] This selective and controlled activation of expression peaks 3 days after *in vitro* antigen receptor stimulation and has been shown to support the telomere length maintenance and proliferative potential of these cells, both of which are essential to sustaining appropriate cell lineage development, cell division, and clonal expansion inherent in immune function.[12,13]

We investigated the telomere length, telomerase activity, and disease status of 15 patients with CHH and 8 haploinsufficient carriers from 6 families with CHH, all carrying the common founder mutation *RMRP* n.70A>G. *In vitro* proliferative capacity of primary lymphocytes and telomerase molecular parameters were examined to further characterize the cellular immune phenotype and telomere biology phenotype correlated with specific genotypes in heterozygote carriers and patients with CHH.

METHODS

Ethical statement and patients' characteristics

All samples from patients with CHH and their relatives were obtained with informed consent and approval of local ethical review boards in accordance with the Declaration of Helsinki. The patients were cared for at the Clinic for Special Children in Strasburg, Pennsylvania. Clinical and molecular studies were performed with oversight from the Lancaster General Hospital Institutional Review Board. All reported subjects underwent laboratory and molecular testing, as previously described.[14] The patients had clinical examination features consistent with the metaphyseal dysplasia classic for CHH and were found to have homozygous lncRNA *RMRP* n.70 A>G variants.

Telomere length measurements using flow–fluorescence *in situ* hybridization and statistics

Telomere length measurements with flow–fluorescence *in situ* hybridization (flow-FISH) was performed, as previously described.[15] Briefly, white blood cells were isolated by means of osmotic lysis of erythrocytes in whole blood with NH_4Cl. The white blood cells were then mixed with bovine thymocytes of known telomere length (which serve as an internal control), denatured in formamide at 87°C, and hybridized with a fluorescein-conjugated $(CCCTAA)_3$ peptide nucleic acid probe specific for telomere repeats and counterstained with LDS751 DNA dye. The fluorescence intensity in granulocytes, total lymphocytes, and lymphocyte subsets defined by labeled antibodies specific for CD20, CD45RA, and CD57 relative to internal control cells and unstained controls was measured on a FACSCalibur instrument or Fortessa instrument (Becton Dickinson, Franklin Lakes, NJ) to calculate the median telomere length from duplicate measurements. The 99th, 90th, 10th, and 1st percentile curves represent regression curves representative of data