Pediatr Neonatol 2010;51(6):326–329

ORIGINAL ARTICLE

Novel Mutation in Boy With Cartilage-hair Hypoplasia

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Received: Dec 28, 2009
Revised: Feb 24, 2010
Accepted: Mar 17, 2010

KEY WORDS:
cartilage-hair hypoplasia;
immunodeficiency;
RNA component of the mitochondrial RNA processing endoribonuclease gene (*RMRP*);
short stature

Background: Cartilage-hair hypoplasia (MIM 250250) is an autosomal recessive disease with diverse clinical manifestations. The clinical phenotypes include variable degrees of bone and hair dysplasia, deficient cellular and/or humoral immunity, and a predisposition to malignancy.
Methods: We performed genetic studies of a patient with disproportionate short stature and brittle scalp hair. Genetic studies were also carried out in the patient's parents.
Results: A novel maternal mutation that consisted of a duplication of 14 nucleotides at position −13 of the RNA component of the RNA component of mitochondrial RNA processing endoribonuclease gene (*RMRP*; g. −26 to −13 dupTACTACTCTGTGAA, promoter region) and a paternal mutation base substitution of C to T at nucleotide +230 (designated as +1 in the transcription initiation site) in the coding sequence of *RMRP* were detected in this patient.
Conclusion: A novel maternal *RMRP* mutation was found in a Chinese boy with typical cartilage-hair hypoplasia.

1. Introduction

Cartilage-hair hypoplasia (CHH) is an autosomal recessive disorder of metaphyseal chondrodysplasia with multiple systemic manifestations. Disproportional short-limb dwarfism and sparse brittle hair are two of the most prominent features found in CHH patients. Other common features include defective immunity,[1] predisposition to several malignant tumors such as lymphoma,[2] ligamentous laxity,[3] hypoplastic anemia,[4] Hirschprung's disease,[5] and impaired spermatogenesis.[6]

Defective immunity has been reported to increase mortality in CHH patients.[7] A large number of CHH patients have T-cell immunodeficiency, which may present as mild to moderate lymphopenia, decreased delayed hypersensitivity, and impaired *in vitro* responsiveness of lymphocytes to mitogens.[8] Humoral immunodeficiency or combined immunodeficiency has also been reported in patients affected with CHH.[9]

CHH is caused by mutations in the RNA component of mitochondrial RNA processing endoribonuclease gene (*RMRP*) and is transmitted as an autosomal recessive trait.[10] The human *RMRP* is an untranslated, intronless 267-base pair (bp) gene, transcribed by the DNA-dependent RNA polymerase III. The exact pathogenesis of the disease phenotypes is still unclear. Only a small number of *RMRP* mutations have been reported in the Oriental population.[11,12] In our

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study, we examined *RMRP* mutations in a Chinese boy with typical CHH and identified one novel mutation.

2. Patient and Methods

A 15-year-old boy was referred to our genetics clinic because of failure to thrive, chronic diarrhea, complaint of bone pain, and recurrent pulmonary infections. The boy's birth weight was 3210 g (50^{th}–75^{th} percentile for gestational age), and his length was 42 cm ($<10^{th}$ percentile). Postnatal growth deficiency was observed after the age of 12 months, when the first respiratory tract infection developed. Since then, he had been suffering from frequent episodes of recurrent respiratory and gastrointestinal infections. At the age of 14 years, his body height was 100 cm (−4 SD) and body weight was 18.6 kg (−4 SD). A physical examination revealed sparse and brittle scalp hair, disproportionate short stature, short limbs, genu valgus, lordosis, and generalized laxity with limitation of elbow extension. A bone radiology examination showed bony scalloping, irregular sclerosis, cystic changes of the widened metaphyses, and metaphyseal dysplasia (Figure 1). A radiograph of the chest showed mixed alveolar and interstitial infiltration over both lungs. A chest computed tomography image showed bronchiectasis in both lungs. A lung function test revealed combined obstructive and restrictive respiratory dysfunction. We did not find growth hormone deficiency or other endocrinopathies.

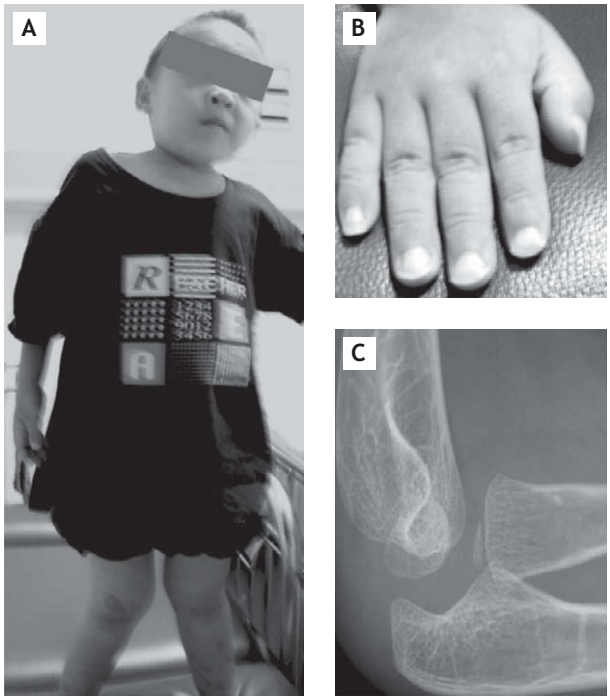

Figure 1 Clinical and X-ray characteristics of our patient with Cartilage-hair hypoplasia. (A) Photographs show a short stature and genu valgus, and (B) brachydactyly of the hands. (C) A radiograph shows cystic changes of widened metaphyses and metaphysial dysplasia of the patient's elbow.

2.1. Immunological studies

Serum immunoglobulin levels and cellular immune assays were measured by nephelometry. Lymphocyte proliferation studies were performed by stimulation with phytohemagglutinin antigen (5 μg/mL). A nitroblue tetrazolium test and chemotaxis assay were performed to assay polymorphonuclear neutrophil function.

2.2. Detection of RMRP mutations

Peripheral blood or hair was obtained with informed consent from the patient and his parents. Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. A genomic sequence of *RMRP* (M29916: the transcription initiation site was designated as +1) was obtained from a public database. Its entire transcribed region and the approximately 500-bp promoter region were amplified by polymerase chain reaction (PCR) from genomic DNA, and it was screened for mutations through direct sequencing. PCR primers used were RM3F (5′-GGCCAGACCATATTTGCATAAG-3′) and RM3R (5′-CAGTGAGCCGTGGTATCG-3′) (−226 to +295), and RM2F (5′-GGAGGATACAGGCGAGTCAG-3′) and RM2R (5′-GCAGAATAGCTAATAGACACGAAATG-3′) (−548 to −148). PCR conditions were 95ºC for 3 minutes, and then 35 cycles of 95ºC for 30 seconds, 60ºC for 30 seconds, 72ºC for 30 seconds, and finally 72ºC for 3 minutes. PCR products were sequenced for both strands using an ABI Prism 3700 automated sequencer (Applied Biosystems, Foster City, CA, USA). To confirm mutations on the individual alleles, the PCR fragments were subcloned using the TGem Easy cloning kit (Invitrogen, Carlsbad, CA, USA), and several clones were sequenced using M13 universal primers.

2.3. Expression analysis

Total RNAs from peripheral lymphocytes were extracted using TRIzol reagent (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's protocol. First-strand cDNAs were synthesized by reverse transcription of 5–10 μg of total RNA with 200 U of Superscript II RNase H-Reverse transcriptase and a gene-specific primer (5′-AGCCGCGCTGAGAATGAG-3′) (248 ← 265). *RMRP* cDNA (5–257) was amplified using the primers RM-RTF (5′-GTGCTGAAGGCCTGTATCCT-3′) and RM-RTR (5′-TGAGAATGAGCCCCGTGT-3′). PCR

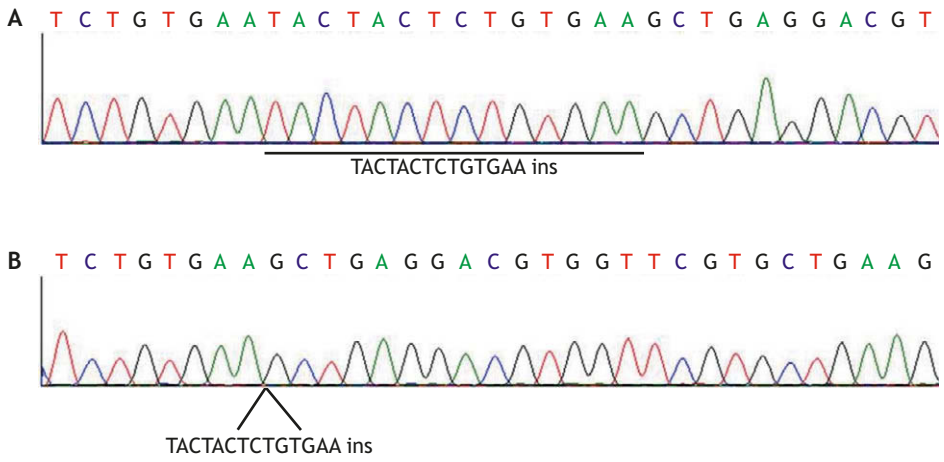

Figure 2 Mutations found in the patient. (A) A novel 14bp insertional mutation (g. −26 to −13 dupTACTACTCTGTGAA) in the patient's maternal allele is shown. (B) A direct sequencing tract of a normal control is shown.

conditions were as described above. PCR products were analyzed by gel electrophoresis and direct sequencing.

3. Results

3.1. Hematological and immunological studies

Hematological evaluation showed microcytic anemia (Hb, 11.5 g/dL; mean corpuscular volume, 74.3 fL), leukocytosis (white blood cell count, 13.1×10^9/L) and thrombocytopenia (7.5×10^9/L), iron deficiency (21 mg/100 mL; normal, 50–150 mg/100 mL), low ferritin (32 ng/mL; normal, 20–200 ng/mL), zinc deficiency (50.2 μg/dL; normal, 100–125 μg/dl), and a low calcium level (7.6 mg/dL).

An immunological evaluation showed a deficit of both cellular immunity and dysgammaglobulinemia. Lymphocyte subsets showed lymphopenia (CD3+ T cells: 517 cells/L, 40.62%; CD19+ B cells: 175 cells/L, 13.72%) but there was a relative increase in natural killer cells (CD56+ cells: 382 cells/L, 29.98%). A high CD4/CD8 ratio (2.2) contributed to relatively low CD8+ cell counts (7.39%). A lower IgG2 (73.80 mg/dL) was also observed compared with age-adjusted reference values of healthy children (IgA: 423 mg/dL; IgE: <18.40 IU/ml; IgM: 49.30 mg/dL; IgG1: 1493.80 mg/dL; IgG2: 273.80 mg/dL; IgG3: 37.95 mg/dL; IgG4: 13.74 mg/dL). Delayed-type hypersensitivity to *Candida* antigens (1:10) and the results of a tuberculin test (5 TU/0.1 mL) were weakly positive. The stimulation index of the patient's lymphocyte proliferation was approximately 50% of normal controls. Neutrophil function tests including a chemotaxis assay, nitroblue tetrazolium test, and adhesion molecule expression were all normal. Levels of C3 and C4 were normal. Currently, this patient receives monthly intravenous gamma globulin administration to prevent recurrent infections.

3.2. Detection of *RMRP* mutations

Two *RMRP* mutations were found in our patient. The paternal mutation is a base substitution of C to T at nucleotide +230 (designated as +1 in the transcription initiation site) in the coding sequence of the *RMRP*, similar to that described by Ridanpaa et al.[11] The maternal mutation is a novel mutation that consists of a duplication of 14 nucleotides in the promoter region of *RMRP* (g. −26 to −13 dupTACTACTCTGTGAA, promoter region; see Figure 2A).

3.3. Expression analysis

To confirm that the novel maternal promoter mutation caused the silencing of the corresponding *RMRP* allele,[10] we examined the differential allelic *RMRP* expression of our patient with CHH. Direct sequencing of the patient's cDNA showed only the paternal C230T allele, suggesting silencing of the maternal allele with a promoter mutation. Gel electrophoresis revealed decreased cDNA levels compared with those of both of his parents and a normal control (Figure 3A).

4. Discussion

CHH is one of a few Mendelian disorders caused by mutations in a nuclear encoded, noncoding RNA gene. The mutations in our patient are compound heterozygote, which has different physiological consequences. The disease-causing functional impairment

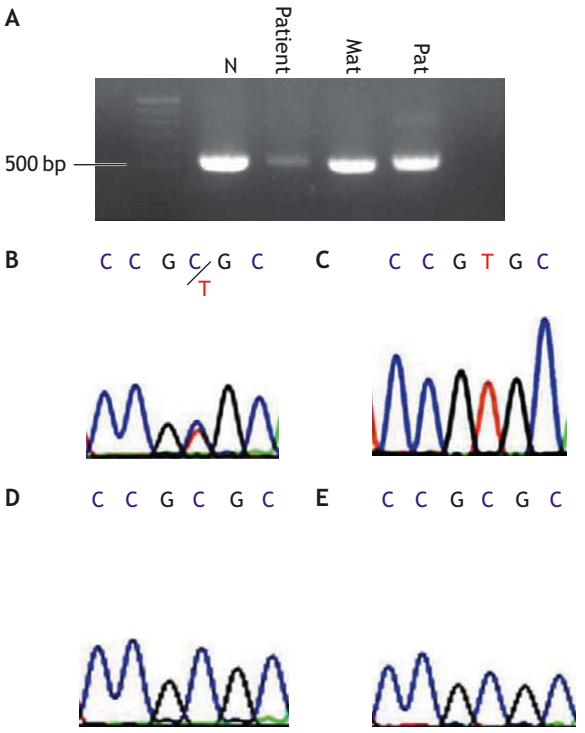

Figure 3 Differential expression of parental *RMRP* alleles in the patient. (A) Decreased expression was found in our patient by reverse transcriptase polymerase chain reaction compared with that in normal control and both of his parents. (B) Direct sequencing of *RMRP* from genomic DNA with the 230C>T mutation and (C) cDNA from the patient. Only the paternal 230T allele was transcribed. (D) Direct sequencing of the same allele from the genomic DNA and (E) cDNA from a normal control. N=normal control; Pat=paternal; Mat=maternal.

of the *RMRP* product in CHH mutations in humans is unknown.[11] No obvious genotype-phenotype correlation has been reported to date. *RMRP* mutations can be classified into two major categories: (1) duplications or insertions of some nucleotides in the promoter region, and (2) one or two changed nucleotides in the transcribed region.[11] The mutations found in our patient corresponded to the first (maternal mutation) and second (paternal mutation) categories. Mutations in the first category have been reported to cause silencing of the corresponding *RMRP* alleles, and mutations in the second category might cause the alteration of the secondary structure of the transcripts or alterations in ribosomal processing.[13]

In this study, we have investigated a patient with CHH using gene mutation studies and have found a new mutation in *RMRP* (g. −26 to −13 dupTACTACTCTGTGAA, promoter region). Therefore, this enabled the development of a prenatal diagnosis for this family.

Acknowledgments

This study was supported by Grant NSC 96-2314-B-182A-040-MY3 from the National Science Council, Taiwan, and Grant CMRPG880451 from Chang Gung Memorial Hospital, Taiwan.

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