

## Identification of novel *RMRP* mutations and specific founder haplotypes in Japanese patients with cartilage-hair hypoplasia

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Received: 18 April 2006 / Accepted: 10 May 2006 / Published online: 11 July 2006  
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**Abstract** Cartilage-hair hypoplasia (CHH), or metaphyseal dysplasia, McKusick type, is an autosomal recessive disease with diverse clinical manifestations.

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CHH is caused by mutations in *RMRP* (ribonuclease mitochondrial RNA processing), the gene encoding the RNA component of the ribonucleoprotein complex RNase MRP. A common founder mutation, 70A>G has been reported in the Finnish and Amish populations. We screened 11 Japanese patients with CHH for *RMRP* mutations and identified mutations in five probands, including three novel mutations (16-bp dup at +1, 168G>A, and 217C>T). All patients were compound heterozygotes for an insertion or duplication in the promoter or 5'-transcribed regions and a point mutation in the transcribed region. Two recurrent mutations were unique to the Japanese population: a 17-bp duplication at +3 and 218A>G. Haplotype analysis revealed that the two mutations common in Japanese individuals were contained within distinct haplotypes. Through this analysis, we have identified a unique mutation spectrum and founder mutations in the Japanese population.

**Keywords** *RMRP* · Mutation · Founder haplotype · Japanese · Cartilage-hair hypoplasia

### Introduction

Cartilage-hair hypoplasia (CHH; MIM 250250), or metaphyseal dysplasia, McKusick type, is an autosomal recessive disease that has diverse clinical manifestations. The most prominent features are short stature and hypoplastic hair. Other common features include metaphyseal dysplasia of the long and short tubular bones, defective immunity, predisposition to malignant tumors including lymphoma, ligamentous laxity, hypoplastic anemia, and Hirschsprung disease.

Variations in clinical severity are remarkable both between and within families (Makitie and Kaitila 1993). In addition, CHH variants with only skeletal manifestations (metaphyseal dysplasia without hypotrichosis; MIM 250460) have been reported (Verloes et al. 1990; Castriota-Scanderbeg et al. 2001; Bonafe et al. 2002; Nakashima et al. 2003).

CHH is caused by mutations in the *RMRP* gene (Ridanpaa et al. 2001), which encodes a 267-nucleotide RNA component of RNase MRP (*RMRP*: ribonuclease mitochondrial RNA processing) (Ridanpaa et al. 2001; Bonafe et al. 2002). To date, 73 *RMRP* mutations have been reported (Ridanpaa et al. 2001, 2002; Bonafe et al. 2002, 2005; Nakashima et al. 2003; Kuijpers et al. 2003; Harada et al. 2005; Hermanns et al. 2005; Thiel et al. 2005). The 70A>G mutation is the most prevalent; it comprises 92% of mutations seen in the Finnish population and is commonly seen in other populations (Ridanpaa et al. 2002). *RMRP* mutations also cause a variant form of CHH (Bonafe et al. 2002), anauxetic dysplasia (MIM 607095) (Thiel et al. 2005), and Omenn syndrome (Roifman et al. 2006). Mutations in *RMRP* can be classified into three categories according to the position of the mutation, described as follows: (1) promoter mutations involve duplication or insertion of several nucleotides between the TATA box and the transcription initiation site; (2) transcript mutations are one- or two-nucleotide changes within the transcribed region; (3) 5' end mutations are insertions or duplications in the 5' end of the transcript (Ridanpaa et al. 2002). The molecular pathogenesis of these mutations and genotype–phenotype associations are yet to be clarified.

To explore further the range of *RMRP* mutations in CHH, we examined the *RMRP* gene in Japanese CHH patients. Our study reveals several novel and common mutations, and characterizes a unique mutation spectrum and founder mutations in the Japanese population.

## Subjects and methods

### Patients and mutation analysis

A total of 11 CHH patients were included in this study. The inclusion criteria were generalized metaphyseal dysplasias of long and short tubular bones with or without extra-skeletal disorders known to be associated with CHH, including hair hypoplasia and immunological disorders. *RMRP* mutations had previously

been identified in two patients (CHH1, 2) (Nakashima et al. 2003).

Peripheral blood, hair, or fingernails were obtained with informed consent from the patients and their parents. Genomic DNA was extracted using standard procedures. Polymerase chain reaction (PCR) direct sequencing analysis for *RMRP* was performed as described (Nakashima et al. 2003).

### Haplotype analysis

Seven patients (CHH1, 2, 4–8) who had *RMRP* mutations common in Japanese CHH were subjected to haplotype analysis. We constructed linkage disequilibrium (LD) blocks containing the *RMRP* gene using genotype data from 44 Japanese individuals in the HapMap phase I database (International HapMap Consortium 2005). The haplotype structure with its tag SNPs was determined using Haploview (Barrett et al. 2005). We genotyped five tag SNPs using the Taqman assay with a PRISM 7900 sequence detector (Applied Biosystems). Haplotypes for chromosomes harboring the two common Japanese mutations were determined by genotyping tag SNPs for seven patients and their parents.

## Results

### Identification of *RMRP* mutations

We screened nine patients for *RMRP* mutations and identified mutations in six (Table 1). Of these patients, four had hair hypoplasia or immunological disorders, and two showed skeletal changes only (CHH variant). Three mutations were novel, and three were recurrent (Nakashima et al. 2003; Bonafe et al. 2005). Other than 182G>T, these mutations have not been seen in populations other than the Japanese. We suppose that the three novel mutations are not polymorphisms because they were not detected in 65 Japanese controls. Four mutations were point mutations in the transcribed region, and two were duplications in the promoter region. All patients were compound heterozygotes.

### Genotype–phenotype association

Eight Japanese patients with *RMRP* mutations were evaluated. None of these patients possessed the full spectrum of skeletal, hair, and immunological disorders characteristic of classical CHH. Three had skeletal changes only (CHH variant), and hair hypoplasia was

**Table 1** *RMRP* mutations and clinical phenotypes

Patient	Age	Sex	Mutation		Clinical phenotype					
			Paternal allele	Maternal allele	BH	BW	PH	SC	HH	ID
CHH1 <sup>a</sup>	17	F	182G>A <sup>b</sup>	17-bp dup at +3	46.5	2,900	-10.0	+	-	+
CHH2 <sup>a</sup>	7	F	17-bp ins at -20	218A>G	48.0	3,200	-3.2	+	-	-
CHH3	14	M	16-bp dup at +1	168G>A	42.5	2,160	-8.0	+	-	+
CHH4	12	M	217C>T	218A>G	49.0	3,000	-2.0	+	-	-
CHH5	8	F	17-bp dup at +3	218A>G	48.2	3,138	-2.9	+	-	-
CHH6	4	F	218A>G	17-bp dup at +3	48.5	2,950	-3.1	+	+	-
CHH7	3	M	218A>G	17-bp dup at +3	49.2	3,086	-2.2	+	+	-
CHH8	3	M	17-bp dup at +3	182G>T	48.2	2,750	-4.0	+	-	+
NC1	14	F	-	-	47.2	2,650	-4.8	+	-	-
NC2	3	M	-	-	40.0	2,840	-7.0	+	-	-
NC3	8	F	-	-	ND	ND	-4.0	+	-	+

*BH* birth height (cm); *BW* birth weight (g); *PH* present height (SD); *SC* skeletal change; *HH* hair hypoplasia; *ID* immunological disorder; *ND* no data available

<sup>a</sup>Previously described (Nakashima et al. 2003)

<sup>b</sup>de novo mutation

seen in just two patients, who were siblings. In most cases, birth length and height were not severely affected, although two patients who were short from birth developed very severe short stature (<-8 SD). Patient CHH4, who had transcript mutations on both alleles, showed a mild phenotype.

#### Haplotype analysis of mutation alleles

Including the two previously screened cases (CHH1 and 2), the two recurrent mutations (17-bp dup at +3 and 218G>A) were found in five patients, respectively. Because these mutations are so common among Japanese CHH cases, we suspected the presence of founders. To investigate this possibility, we determined the haplotype structure of *RMRP* in seven patients (CHH1, 2, 4–8) who had the common mutations.

Haplotypes were assessed by PCR direct sequencing, using the four SNPs within the *RMRP* region that showed minor allelic frequencies greater than 10% in the Japanese population (Nakashima et al. 2003; International HapMap Consortium 2005). Our analysis revealed that the two recurrent mutations were contained within distinct haplotypes (Fig. 1).

Next, we determined the haplotype block structure for the region surrounding *RMRP* using genotype data from 44 Japanese individuals in the HapMap database. *RMRP* was contained in a block spanning ~20 kb (Fig. 2 top). The haplotype block was represented by seven haplotypes with >1% frequency (Fig. 2 bottom). The haplotype of the chromosome containing the 17-bp dup at +3 mutation was determined by parental–children transmission in three (CHH1, 5, 8) of five disease chromosomes. All three had haplotype III

(AAAGG), which is seen in 13.6% of the Japanese population. The other two patients might have this haplotype, but the phase was not determined. Likewise, we determined the haplotype in two of five chromosomes containing the 218A>G mutation (CHH2 and CHH5). Both had a rare haplotype (ATTGA) that was not seen among the 44 Japanese individuals used in the haplotype block analysis. The three other patients might also possess this haplotype, but the phase was not determined (Fig. 3).

#### Discussion

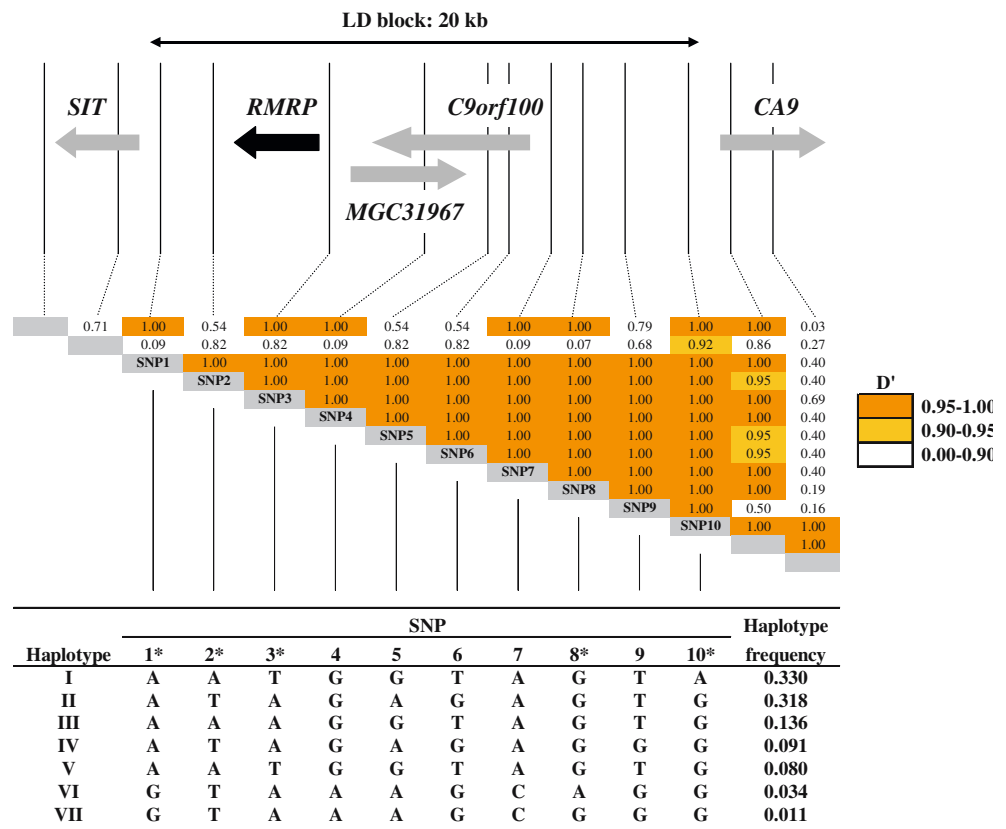
The mutation spectrum for *RMRP* in the Japanese population is unique. The 70A>G founder mutation that is prevalent in Western populations (Ridanpaa et al. 2002) has not been reported in Japanese individuals. On the other hand, the 218A>G and 17-bp dup at +3 mutations are common in Japanese patients, but have not been reported in other populations. We have shown that the two common mutations are contained within rare distinct haplotypes, indicating the presence of unique founders among Japanese CHH patients. Because these haplotypes have been defined by tag SNPs, our results will be useful in detecting CHH mutations and their carriers in Japanese CHH patients.

We saw no patient who possessed all of the skeletal, hair, and immunological features characteristic of classical CHH. Extra-skeletal features are not prevalent in the Japanese population, although immunological disorders may yet develop in this young set of patients. As described previously (Nakashima et al. 2003), all patients were compound heterozygotes for

**Fig. 1** The haplotype structure of Japanese CHHs in the *RMRP* region. The two common mutations are contained within distinct haplotypes (colored), respectively. SNP positions from the transcription start site of *RMRP* (denoted as +1) are indicated at the top. The telomere is to the left. Under the *Chromosome* heading, *F* and *M* indicate the paternal and maternal chromosomes, respectively

Position (nt)	-149	-58	-48	<i>RMRP</i>		274
	SNP	T/A	T/C	C/A	Mutation	T/C
<b>Chromosome</b>						
CHH1/F	T	T	C	17-bp dup at +3		T
CHH5/F	T	T	C	17-bp dup at +3		T
CHH6/M	T	T	C	17-bp dup at +3		T
CHH7/M	T	T	C	17-bp dup at +3		T
CHH8/F	T	T	C	17-bp dup at +3		T
CHH2/M	A	C	A	218A>G		T
CHH4/M	A	C	A	218A>G		T
CHH5/M	A	C	A	218A>G		T
CHH6/F	A	C	A	218A>G		T
CHH7/F	A	C	A	218A>G		T
CHH1M	T	T	C	182G>A		T
CHH2/F	A	C	A	17-bp ins at -20		T
CHH4/F	T	T	A	217C>T		T
CHH8/M	A	C	A	182G>T		T

**Fig. 2** The linkage disequilibrium (*LD*) block and haplotype structure around *RMRP* in the Japanese population. *Top* Genomic structure and the *LD* block containing *RMRP*. *LD* was evaluated using *D'* statistical analysis. *Bottom* The haplotype structure and frequency of the *LD* block containing *RMRP*. The block was represented by seven haplotypes. \*Tag SNPs: SNP1, rs10972549; SNP2, rs1538537; SNP3, 3750430; SNP8, rs4878625; SNP10, rs2071675



insertions or duplications in the promoter or 5' transcribed regions and point mutations in the transcribed region. The former mutations cause marked decreases in gene transcription and, hence, the substantial effects on phenotype; in contrast, the latter mutations have only mild effects on transcription (Nakashima et al. 2003). This is consistent with the mild phenotype ob-

served in patient CHH4, who had two point mutations. Although it is clear that *RMRP* mutations produce a spectrum of phenotypes, from CHH variants with only skeletal manifestations (Bonafe et al. 2002) at the mild end to anauxetic dysplasia (Thiel et al. 2005) at the severe end, further studies are necessary to better delineate these phenotype–genotype correlations.

**Fig. 3** The haplotype structure of the region around *RMRP*. The two common mutations are contained within distinct haplotypes (colored). The telomere is to the right. SNP positions are indicated from the transcription start site of *RMRP* (denoted as +1)

Position (nt)	3536	845	<i>RMRP</i>			-149	-11890	-15877
	1	2	←			3	8	10
Tag SNP	A/G	A/T	Mutation			A/T	G/A	G/A
Chromosome								
CHH1/F	A	A	17-bp dup at +3			A	G	G
CHH5/F	A	A	17-bp dup at +3			A	G	G
CHH6/M	A	A	17-bp dup at +3			A	G	G/A
CHH7/M	A	A	17-bp dup at +3			A	G	G/A
CHH8/F	A	A	17-bp dup at +3			A	G	G
CHH2/M	A	T	218A>G			T	G	A
CHH4/M	A	A/T	218A>G			T	G	G/A
CHH5/M	A	T	218A>G			T	G	A
CHH6/F	A	T	218A>G			T	G	G/A
CHH7/F	A	T	218A>G			T	G	G/A
CHH1M	A	A	182G>A			A	G	G
CHH2/F	A	T	17-bp ins at -20			T	G	G
CHH4/F	A	A/T	217C>T			A	G	G/A
CHH8/M	A	T	182G>T			T	G	A

**Acknowledgments** Eiji Nakashima, Hirofumi Ohashi, Gen Nishimura, and Shiro Ikegawa are members of the Japanese Skeletal Dysplasia Consortium, Tokyo, Japan. We thank the patients and their relatives who co-operated in this study. This work was supported by a grant-in-aid from Research on Child Health and Development (contract grant no. 17C-1).

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