Clin Genet 2007: 71: 468–470
Printed in Singapore. All rights reserved

© 2007 The Authors
Journal compilation © 2007 Blackwell Munksgaard

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2007.00776.x

Letter to the Editor

RMRP mutations in hematological disorders

To the Editor:

RMRP (ribonuclease mitochondrial RNA processing) is a highly polymorphic gene encoding an untranslated RNA. *RMRP* mutation was first linked to disease in association with cartilage-hair hypoplasia (CHH; OMIM 250250) (1), a recessive condition characterized by fine hair and skeletal abnormalities (2, 3). CHH involves significant hematological abnormalities, which coupled with *RMRP*'s involvement in fundamental cellular processes suggest the gene's involvement in additional blood dyscrasias. Here we expand the scope of *RMRP* sequence analysis to aplastic anemia (AA), a rare disease defined by failure of bone marrow hematopoiesis. Although usually autoimmune mediated, a minority of AA cases encompass an array of separate etiologies including genetic mutation in modulators of the immune response and components of the telomerase complex (4).

Blood samples were obtained from 108 consecutive patients with AA treated at the Hematology Branch of the National Heart, Lung, and Blood Institute, National Institutes of Health. Diagnosis of AA was based on the criteria of the International Agranulocytosis and Aplastic Anemia Study (5). Of the 108 patients, 9% were Asians, 30% were Blacks, 45% were Caucasians, and 16% were Hispanics. Patients or their guardians provided written informed consent approved by NHLBI institutional review board. Samples from 248 healthy persons were analyzed as controls: 10% Asian [from SNP500Cancer (http://snp500cancer.nci.nih.gov/home_1.cfm?CFID=1670627&CFTOKEN=50064305)], 42% Black [from Human Variation Panel HD100AA Coriell Cell Repositories (http://ccr.coriell.org/nigms/cells/humdiv.html) and SNP500Cancer], 40% Caucasian (from Human Variation Panel HD100CAU and SNP500Cancer), and 10% Hispanic (from SNP500 Cancer) (6). DNA was extracted from peripheral blood leukocytes using standard technique, and polymerase chain reaction amplification and subsequent sequencing of *RMRP* coding and pre-coding regions were performed as described (7).

A clear association between *RMRP* mutations and AA was not identified. Six of 108 patients with AA were found to possess novel *RMRP* single-nucleotide variants (Table 1). A known pathogenic mutation, 218 A>G, was identified in one patient (G) in heterozygosis. This mutation has been previously observed in the Japanese, and hematological abnormalities have not been reported in the six patients described to date (8, 9). Among 248 control samples, 10 harbored novel single-nucleotide variants and a known pathogenic mutation, 238 C>T, was found in one individual (Table 2). No mutations or novel variants were demonstrated in homozygosis or compound heterozygosis.

RMRP pre-coding region single-nucleotide variants have not been associated with disease (7). In this study, one novel pre-coding region variant, −21 C>G, was found in multiple individuals, including three controls, suggesting its neutrality (Table 1). The remaining novel pre-coding region variants are of nucleotides not strongly conserved evolutionarily and thus also unlikely to carry functional significance.

The seven novel coding region variants identified were examined for likelihood of pathogenicity. Recent studies have ascribed functional consequence to certain *RMRP* mutations, including involvement in 5.8S rRNA maturation (10) and transcription of cytokine and cell cycle regulatory genes (8). Additionally, predictive analyses based on secondary structure conformation and intraspecies conservation have proven effective in characterizing the myriad of *RMRP* variants (9–12). Two of this study's novel variants, 234 C>G in patient H, and 175 G>A in an Asian healthy control, are at positions characterized as evolutionarily conserved; both additionally participate in base pairing. No clinical information is available for the anonymous Asian control.

Patients G and H, who, respectively, harbored, both in heterozygosis, a known and a predicted pathogenic mutation, had clinical profiles consistent with acquired AA. Patient G, a 9-year-old Caucasian woman, was revealed by genetic analysis to have inherited the mutation from her father, previously diagnosed with chronic lymphocytic leukemia (CLL). Her deceased

Table 1. Novel *RMRP* single-nucleotide variants and pathogenic mutations in patients with aplastic anemia

Patient	*RMRP* variant	Race or ethnicity	Allele frequency	Base pairing	Species conservation (12)
A, B	−21 C>G	C, B	2/216	—	8/8
C	−7 G>A	A	1/216	—	6/8
D	53 C>T	B	1/216	No	3/11
D, E	139 A>G	B	2/216	No	4/11
F	149 A>G	H	1/216	No	9/11
G	218 A>G[a]	C	1/216	No	11/11
H	234 C>G	B	1/216	Yes	10/11

A, Asian; B, Black; C, Caucasian, H, Hispanic.
[a]Demonstrated pathogenic mutations.

Table 2. Novel *RMRP* single-nucleotide variants and pathogenic mutations in controls

RMRP variant	Race or ethnicity	Allele frequency	Base pairing	Species conservation (12)
−55 T>C	C	1/496	—	4/8
−25 A>G	B	1/496	—	4/8
−22 A>C	B	1/496	—	4/8
−21 C>G	B	3/496	—	8/8
−13 A>C	B	1/496	—	6/8
−11 C>T	B	1/496	—	4/8
−4 C>T	B	1/496	—	4/8
119 C>T	C	1/496	Yes	7/11
175 G>A	A	1/496	Yes	11/11
227 C>T	C	1/496	No	4/11
238 C>T[a]	C	1/496	No	11/11

A, Asian; B, Black; C, Caucasian.
[a]Demonstrated pathogenic mutations.

paternal grandfather also suffered from CLL; however, sample acquisition was not possible. Patient H, a 55-year-old Black woman, had no family history of blood dyscrasia. No extrahematological conditions, such as short stature or skeletal or hair abnormalities, were observed in either patient or their families.

CLL carries the highest familial association among hematological cancers, but the responsible alleles have not yet been identified. It is posited that many low-risk alleles contribute cumulatively to CLL risk (13). No evidence exists for monoallelic *RMRP* mutations causing disease, and although CHH patients are at increased risk for developing lymphoproliferative disorders, specific association with CLL or other leukemias has not been reported (14). Whether *RMRP* mutation contributes to risk of CLL will require elucidation in future studies.

Two haplotypes have been observed to contribute six relatively common single-nucleotide polymorphisms (SNPs) in *RMRP*: one consists of the −56 A>G, −48 C>A, −6G>A, 156 G>C, and 177 C>T SNPs and the second of −58 T>C and −48 C>A (7, 12). When stratified by racial/ethnic background, the frequency of these common SNPs varied markedly (Table 3). Caucasian and Hispanic populations shared similar SNP frequencies, agreeing with separate studies of comparable populations (7, 12). The

Table 3. Allele frequency of common *RMRP* single-nucleotide polymorphisms stratified by race/ethnicity

	n	−58 T>C (%)	−56 A>G (%)	−48 C>A (%)	−24 C>G (%)	−6 G>A (%)	127 G>C (%)	156 G>C (%)	177 C>T (%)
Asian-C	48	46	0	42	0	2	0	2	2
Asian-P	20	40	0	40	0	0	0	0	0
Asian-T	68	45	0	42	0	2	0	2	2
Black-C	206	12	26	47	1	26	1	27	26
Black-P	64	8	38	52	2	36	0	38	38
Black-T	270	11	29	48	1	28	1	30	29
Caucasian-C	196	31	10	40	0	10	1	10	10
Caucasian-P	98	37	10	47	0	10	0	10	10
Caucasian-T	294	32	10	41	0	10	1	10	10
Hispanic-C	46	20	11	28	0	11	0	11	15
Hispanic-P	34	35	15	50	0	15	0	15	15
Hispanic-T	80	26	13	37	0	13	0	13	15

C, controls; *n*, allele number; P, patients; T, total.

Table 4. Chi-square values (and p-values) comparing frequencies of common single-nucleotide polymorphisms representative of two major haplotypes across populations (patients and controls combined)

	Asian	Black	Caucasian	Hispanic
Asian	—	25.5 (<0.001)	7.3 (<0.01)	9.1 (<0.01)
Black	43.7 (<0.001)	—	33.1 (<0.001)	8.8 (<0.01)
Caucasian	4.3 (<0.050)	36.7 (<0.001)	—	0.5 (>0.1)
Hispanic	6.0 (<0.025)	11.4 (<0.001)	1.1 (>0.1)	—

Lower left half of table: −58 T>C; upper right half of table: −56 A>G.

Asian and Black populations, however, diverged from this pattern and were themselves dissimilar. These differences were statistically significant, as demonstrated in Table 4 for representative SNPs −58 T>C and −56 A>G. These differences in *RMRP* haplotypes between populations, while not apparently associated with AA, may benefit future *RMRP* mutation screening efforts.

SA Graf[a,b,*]
RT Calado[a,*]
S Kajigaya[a]
NS Young[a]

[a]Hematology Branch, National Heart, Lung, and Blood Institute and [b]Howard Hughes Medical Institute, National Institutes of Health Research Scholars Program, Bethesda, MD, USA

*These authors contributed equally to this work.

References

1. Ridanpaa M, van EH, Pelin K et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia. Cell 2001: 104: 195–203.
2. McKusick VA, Eldridge R, Hostetler JA, Ruangwit U, Egeland JA. Dwarfism in the amish. II Cartilage-hair hypoplasia. Bull Johns Hopkins Hosp 1965: 116: 285–326.
3. Makitie O. Cartilage-hair hypoplasia in Finland: epidemiological and genetic aspects of 107 patients. J Med Genet 1992: 29: 652–655.
4. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 2006: 108: 2511–2521.
5. Kaufman DW, Kelly JP, Levy M, Shapiro S. The drug etiology of agranulocytosis and aplastic anemia. New York: Oxford, 1991.
6. Packer BR, Yeager M, Staats B et al. SNP500Cancer: a public resource for sequence validation and assay development for genetic variation in candidate genes. Nucleic Acids Res 2004: 32: D528–D532.
7. Bonafe L, Schmitt K, Eich G, Giedion A, Superti-Furga A. RMRP gene sequence analysis confirms a cartilage-hair hypoplasia variant with only skeletal manifestations and reveals a high density of single-nucleotide polymorphisms. Clin Genet 2002: 61: 146–151.
8. Hermanns P, Bertuch AA, Bertin TK et al. Consequences of mutations in the non-coding RMRP RNA in cartilage-hair hypoplasia. Hum Mol Genet 2005: 14: 3723–3740.
9. Hirose Y, Nakashima E, Ohashi H et al. Identification of novel RMRP mutations and specific founder haplotypes in Japanese patients with cartilage-hair hypoplasia. J Hum Genet 2006: 51: 706–710.
10. Thiel CT, Horn D, Zabel B et al. Severely incapacitating mutations in patients with extreme short stature identify RNA-processing endoribonuclease RMRP as an essential cell growth regulator. Am J Hum Genet 2005: 77: 795–806.
11. Hermanns P, Tran A, Munivez E et al. RMRP mutations in cartilage-hair hypoplasia. Am J Med Genet A 2006: 140: 2121–2130.
12. Bonafe L, Dermitzakis ET, Unger S et al. Evolutionary comparison provides evidence for pathogenicity of RMRP mutations. PloS Genet 2005: 1: e47.
13. Sellick GS, Catovsky D, Houlston RS. Familial chronic lymphocytic leukemia. Semin Oncol 2006: 33: 195–201.
14. Makitie O, Pukkala E, Teppo L, Kaitila I. Increased incidence of cancer in patients with cartilage-hair hypoplasia. J Pediatr 1999: 134: 315–318.

Correspondence:
Neal S Young, MD
Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health
10 Center Drive, Building, 10, CRC, Room 3E-5140
Bethesda, MD 20892-1202,
USA
Tel.: +1 301 496 5093
Fax: +1 301 496 8396
e-mail: youngns@mail.nih.gov