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 SNP500Cancer) (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
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 extrahepatic 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 1. Novel RMRP single-nucleotide variants and pathogenic mutations in patients with aplastic anemia |
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| **RMRP** variant | Race or ethnicity | Allele frequency | Base pairing | Species conservation |
| --- | --- | --- | --- | --- |
| 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 | C | 1/216 No 11/11 |
| H 234 C>G | B | 1/216 Yes 10/11 |

A, Asian; B, Black; C, Caucasian, H, Hispanic.

aDemonstrated pathogenic mutations.

| Table 2. Novel RMRP single-nucleotide variants and pathogenic mutations in controls |
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| **RMRP** variant | Race or ethnicity | Allele frequency | Base pairing | Species conservation |
| --- | --- | --- | --- | --- |
| −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 |
| 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 | C | 1/496 No 11/11 |

A, Asian; B, Black; C, Caucasian.

aDemonstrated pathogenic mutations.

| Table 3. Allele frequency of common RMRP single-nucleotide polymorphisms stratified by race/ethnicity |
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| n | −56 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 |
| Asian-P | 20 | 40 | 0 | 40 | 0 | 0 | 0 | 0 |
| Asian-T | 68 | 45 | 0 | 42 | 0 | 2 | 0 | 2 |
| Black-C | 206 | 12 | 26 | 47 | 1 | 26 | 1 | 27 |
| Black-P | 64 | 8 | 38 | 52 | 2 | 36 | 0 | 38 |
| Black-T | 270 | 11 | 29 | 48 | 1 | 28 | 1 | 30 |
| Caucasian-C | 196 | 31 | 10 | 40 | 0 | 10 | 1 | 10 |
| Caucasian-P | 98 | 37 | 10 | 47 | 0 | 10 | 0 | 10 |
| Caucasian-T | 294 | 32 | 10 | 41 | 0 | 10 | 1 | 10 |
| Hispanic-C | 46 | 20 | 11 | 28 | 0 | 11 | 0 | 11 |
| Hispanic-P | 34 | 35 | 15 | 50 | 0 | 15 | 0 | 15 |
| Hispanic-T | 80 | 26 | 13 | 37 | 0 | 13 | 0 | 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) |
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| **Asian** | **Black** | **Caucasian** | **Hispanic** |
| −58 T>C | 25.5 (0.001) | 7.3 (0.01) | 9.1 (0.01) |
| −56 A>G | 43.7 (<0.001) | — | 33.1 (<0.001) |
| −48 C>A | 4.3 (<0.05) | 36.7 (<0.001) | — |
| −24 C>G | 6.1 (<0.05) | 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 $-58 \text{T>C}$ and $-56 \text{A>G}$. 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.

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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