
Red Cell Disorders

Low frequency of VHL gene mutations in young individuals with polycythemia and high serum erythropoietin

In one out of six young individuals with polycythemia and high erythropoietin levels we found a heterozygous VHL gene mutation (430G→A; Gly144Arg). The man's unaffected mother and sister carry the same mutation. No other VHL genomic or expression alterations were found. In one other patient different genetic conditions were found.

Letters to the Editor

The molecular basis of congenital and familial polycythemas is largely unknown. Chuvash polycythemia (CP) is an autosomal recessive polycythemia with increased serum erythropoietin (Epo) caused by bi-allelic mutations of the von Hippel-Lindau (VHL) gene. VHL mutations are also responsible for von Hippel-Lindau disease, an autosomal dominant syndrome predisposing to the development of multiple tumors.

Since 50% of sporadic cases of congenital CP with high serum Epo are attributed to bi-allelic VHL gene mutations, we searched for VHL gene mutations in 4 previously described children with congenital polycythemia and unexplained high levels of serum Epo and in 2 other individuals with a similar phenotype. While in 5 of the cases polycythemia appeared to be congenital, in 1 case it was not possible to date the age of onset of the disease precisely. All patients were Italian from the Venetian region; none of them was of Chuvashian origin. None had splenomegaly or detectable causes of secondary erythrocytosis. In one patient neurofibromatosis type 2 was present without other structural or transcriptional alterations, other as yet unknown factors might contribute to determining the polycythemia phenotype. However, the molecular mechanism of erythrocytosis in these cases remains to be elucidated.

It is worth noting that we found the association of mononuclear cells using standard methods.1

Genomic DNA was extracted from peripheral blood leukocytes by conventional methods after signed informed consent, according to the Declaration of Helsinki. Mutation analysis was conducted on the entire coding sequence and intron-exon boundaries of the VHL gene by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis, followed by direct sequencing of the amplicons, as previously reported.2 Total cytoplasmin RNA was extracted from peripheral blood with RNAzol (RNA-Be, Tel-Test, Inc.) and retro-transcribed by random-priming.

The VHL-specific cDNA was amplified with primers 1CF (5′-GTGCTCGGCTGGTGAATCTC-3′) and 3R (5′-CAAGACTCATCACGTACCATACAAAGCTG-3′) which obtained amplification of two different isoforms, due to the normal alternative splicing of exon 2. The VHL isoform, including exon 2 was then sequenced with primers 2Fi (5′-agg tca cct ttg gct ctt cag a 3′) and 3R. Since CP is considered an autosomal recessive condition, we hypothesize that when the heterozygous VHL mutation is present without other structural or transcriptional alterations, other as yet unknown factors might contribute to determining the polycythemia phenotype. However, the molecular mechanism of erythrocytosis in these cases remains to be elucidated.
polycythemia with neurofibromatosis type 2. In this case Epo might be stimulated through other still unknown mechanisms.

Maria Luigia Randi,* Alessandra Murgia,*
Maria Caterina Putti,*
Maddalena Martella,*
Alberto Casarin,* Giuseppe Opocher,*
Fabrizio Fabris

Internal Medicine and *Endocrinology Dept of Medical and Surgical Sciences, *Dept of Pediatrics, *Clinic Pediatric Oncology-Hematology, Dept. of Pediatrics, University of Padua Medical School, Padua, Italy

MLR, AM and MCP contributed equally to this study

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Correspondence: Maria Luigia Randi, Dept. of Medical and Surgical Science, via Ospedale 105, 35128 Padua, Italy. Phone: international +39.049.8212668. Fax: international +39.049.82111391. E-mail: marialuigia.randi@unipd.it

Table 1. Main clinical and laboratory characteristics of our patients.

<table>
<thead>
<tr>
<th>Case (sex)</th>
<th>Age* (years, months)</th>
<th>Follow up duration (years)</th>
<th>RBC volume (M/L/kg)</th>
<th>Hb (g/L)</th>
<th>HCT (%)</th>
<th>WBC (&gt;10^9/L)</th>
<th>Platelets (&gt;10^9/L)</th>
<th>EEC cultures</th>
<th>EPO (UI/mL)</th>
<th>Associated clinical features</th>
<th>VHL Genetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD (M)</td>
<td>13 y 17</td>
<td>(NW 25-30)</td>
<td>55.8</td>
<td>180</td>
<td>56</td>
<td>10.9</td>
<td>535</td>
<td>normal</td>
<td>207.3</td>
<td>none</td>
<td>Exon 2 VHL</td>
</tr>
<tr>
<td>RM (F)</td>
<td>3 y 6 m</td>
<td>(NW ~25)</td>
<td>49.3</td>
<td>194</td>
<td>65</td>
<td>5.98</td>
<td>290</td>
<td>not performed</td>
<td>31.7</td>
<td>Multiple cerebral ischemic lesions</td>
<td>normal</td>
</tr>
<tr>
<td>DGL (F)*</td>
<td>3 y 8 m</td>
<td>(NW ~25)</td>
<td>67.1</td>
<td>167</td>
<td>56.8</td>
<td>6.2</td>
<td>307</td>
<td>normal</td>
<td>128</td>
<td>headache, normal</td>
<td></td>
</tr>
<tr>
<td>DGG (M)*</td>
<td>4 m</td>
<td>#</td>
<td>156</td>
<td>(NW 9.5-12.5)</td>
<td>57.8</td>
<td>8.0</td>
<td>363</td>
<td>normal</td>
<td>&gt;200</td>
<td>none</td>
<td>normal</td>
</tr>
<tr>
<td>MA (M)</td>
<td>23 y 16</td>
<td>(NW &lt;36)</td>
<td>53</td>
<td>173</td>
<td>50.1</td>
<td>6.2</td>
<td>840</td>
<td>normal</td>
<td>51</td>
<td>Budd-Chiari syndrome</td>
<td></td>
</tr>
<tr>
<td>LN (F)</td>
<td>26 y 7</td>
<td>(NW &lt;32)</td>
<td>32</td>
<td>158</td>
<td>48.2</td>
<td>6.7</td>
<td>619</td>
<td>not performed</td>
<td>35</td>
<td>none</td>
<td>normal</td>
</tr>
</tbody>
</table>

*Age at first observation; NV: expected normal values for age (from Price DC, Ries C, in Nuclear Medicine in Clinical Pediatrics 1975 and Dallman PR, in Pediatrics, 1977); RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; EPO: erythropoietin; NF2: neurofibromatosis type 2; *patients DGL and DGG are siblings; “not performed because of the young age and the reluctance to use radioactive substances.

Table 2. Heterozygous VHL mutations in individuals with erythrocytosis reported in the literature.

<table>
<thead>
<tr>
<th>Individuals (sex)</th>
<th>VHL genotype</th>
<th>wt mRNA expression</th>
<th>Associated clinical features</th>
<th>Carrier family members</th>
<th>Ethnicity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (M, F) siblings</td>
<td>376G→T / wt</td>
<td>Normal</td>
<td>F: renal subcapsular hemangioma</td>
<td>Father (asymptomatic)</td>
<td>Ukrainian</td>
<td>2</td>
</tr>
<tr>
<td>1 (M)</td>
<td>598C→T / wt</td>
<td>Unknown</td>
<td>None</td>
<td>Mother and son (asymptomatic)</td>
<td>English</td>
<td>7</td>
</tr>
<tr>
<td>1 (F)</td>
<td>598C→T / wt</td>
<td>Normal</td>
<td>None</td>
<td>?</td>
<td>German</td>
<td>9</td>
</tr>
<tr>
<td>1 (F)</td>
<td>311G→T / wt</td>
<td>Normal</td>
<td>None</td>
<td>?</td>
<td>German (?)</td>
<td>9</td>
</tr>
<tr>
<td>1 (F)</td>
<td>523A→G / wt</td>
<td>Normal</td>
<td>Ataxia-teleangectasia</td>
<td>Father (asymptomatic)</td>
<td>Portuguese</td>
<td>10</td>
</tr>
<tr>
<td>1 (M)</td>
<td>430C→A / wt</td>
<td>Normal</td>
<td>None</td>
<td>Mother and sister (asymptomatic)</td>
<td>Italian</td>
<td>Present paper</td>
</tr>
</tbody>
</table>

wt: wild type.

References

7. Percy MJ, McMullin MF, Potter M, Treacy M, Watson WH, Lappin TRJ. Chuvash-type congenital polycythemia in 4 fami-


**Myelodysplastic Syndromes**

**Lack of mutations of the human telomerase RNA gene (hTERC) in myelodysplastic syndrome**

Myelodysplastic syndrome (MDS), considered a pre-leukemic state, has recently been categorized as a subset of bone marrow failure syndromes. Unlike other subtypes of bone marrow failure syndromes, such as aplastic anemia or dyskeratosis congenita, little is known about genetic alterations of human telomerase in MDS, despite the fact that immune cells from patients with MDS frequently exhibit telomere attrition.

Human telomerase RNA (hTERC) is an essential component of the telomerase ribonucleoprotein complex, and mutations in hTERC can result in haploid insufficiency, reducing telomerase activity, leading to premature telomere shortening. Identification of mutations of hTERC in bone marrow failure syndromes, including myelodysplastic syndrome (MDS), may provide insights into the underlying molecular causes of these syndromes.

In the present study, we investigated mutations of the hTERC gene (NT 005612.14) using polymerase chain reaction-direct sequencing in 42 marrow samples from 35 consecutive MDS patients (34 to 80 years old); 19 had refractory anemia (RA), 14 had RA with excess blasts (RAEB), and two patients had RAEB in transformation. Seven RAEB patients were also studied at the time their disease transformed into acute myeloid leukemia. Blood samples were also obtained from 134 healthy volunteers (4 to 90 years old). All samples were collected from Japanese patients and healthy volunteers after obtaining informed consent. Telomere length and telomerase activity were measured as previously described in mononuclear cells.

We selected seven hTERC loci: C98T, the template region G38A, pseudoknot domain C72T and D110-113, CR4-CD5 domain G305A and G322A, and Box H/ACA domain G450A, to identify possible mutations of the hTERC gene. We also examined polymorphisms at 514. Direct sequencing showed no heterozygous hTERC mutations of these loci in 42 MDS samples and 134 healthy volunteers, although MDS patients had variable telomere lengths (short in 27%, normal in 69%, and long in 5%) compared to normal volunteers with low telomerase activity. We did not find allelic variations at the 514 locus in healthy populations: AA genotype (MDS 11.1% versus control 11.9%), AG genotype (MDS 55.6% versus control 51.4%), and GG genotype (MDS 33.3% versus control 36.7%) and no deviation was notable in MDS patients.

hTERC mutations at certain loci affect telomerase activity, and most MDS patients show normal to low levels of telomerase activity; nevertheless, cells from some MDS patients have telomere attrition. In one study it was reported that, out of 55 MDS patients, two black patients had a G58A change and one other patient had a G322A substitution. More recently a black MDS patient with G58A was also reported. Since the G58A substitution seems to be common in the black population (5/24 normal subjects) and no G58A mutations in the hTERC gene were detected among the normal Japanese population or in the MDS patients in our study, mutations in the hTERC gene are unlikely to be related to telomere changes observed in most MDS patients. Since some MDS patients have shortened telomeres and also low telomerase activity, it remains possible that the dysfunction of telomere regulation in MDS patients may be caused by alterations in other proteins that interact with telomerase or in the catalytic component (hTERT) itself.

Kazuma Ohyashiki,* Jerry W. Shay,* Junko H. Ohyashiki*

*First Department of Internal Medicine, Tokyo Medical University, Tokyo, Japan; °Department of Cell Biology, University of Texas Southwestern Medical Center at Dallas, TX, USA

Intractable Immune System Disease Research Center, Tokyo Medical University, Tokyo, Japan

Key words: telomerase, hTERC mutations, myelodysplastic syndrome

Correspondence: Kazuma Ohyashiki, M.D., First Department of Internal Medicine (Hematology/Oncology division), Tokyo Medical University 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan. Phone: international +81.3.33422520. Fax: international +81.3.53816651. E-mail: ohyashik@r.7mu.or.jp

**References**


**Myelodysplastic Syndromes**

**Secondary myelodysplastic syndromes following treatment with azathioprine are associated with aberrations of chromosome 7**

We report 14 cases of secondary myelodysplastic syndromes (sMDS) following treatment with azathioprine for non-malignant disorders. Long-term treatment with azathioprine seems to be associated with an increased risk of MDS and subsequent leukemic transformation.

**Letters to the Editor**

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