Risk-Reducing Genetic Variant of \textit{Wilms Tumor 1} Gene rs16754 in Korean Patients With \textit{BCR-ABL1}-Negative Myeloproliferative Neoplasm

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The genetic variant rs16754 of \textit{Wilms tumor} gene 1 (\textit{WT1}) has recently been described as an independent prognostic factor in AML patients. It is of great interest to test whether \textit{WT1} single nucleotide polymorphism can be used as a molecular marker in other types of cancer, to improve risk and treatment stratification. We performed sequencing analysis of exons 7 and 9 of \textit{WT1}, which are known mutational hotspots, in a total of 73 patients with \textit{BCR-ABL1}-negative myeloproliferative neoplasm (MPN) and 93 healthy controls. No previously reported \textit{WT1} mutations were identified in the present study. In Korean patients with \textit{BCR-ABL1}-negative MPN, \textit{WT1} genetic variant rs16754 had no significant impact on clinical outcomes. We observed a significant difference in the allelic frequencies of \textit{WT1} rs16754 in Koreans between \textit{BCR-ABL1}-negative MPN cases and healthy controls. Individuals carrying variant G alleles of \textit{WT1} rs16754 showed a relatively low prevalence of \textit{BCR-ABL1}-negative MPN, compared with those carrying wild A alleles of \textit{WT1} rs16754 (Hazard ratio 0.10-0.65, \textit{P}<0.05). Therefore, possession of the variant G allele of \textit{WT1} rs16754 may reduce the risk of developing \textit{BCR-ABL1}-negative MPN.

\textbf{Key Words:} Myeloproliferative neoplasm, \textit{WT1}, rs16754

Recently, molecular markers have been described that likely contribute to disease pathogenesis and determine the clinical outcome in \textit{BCR-ABL1}-negative myeloproliferative neoplasms (MPN). Most common are genetic mutations in exon 12 of \textit{JAK2}, myeloproliferative leukemia virus (\textit{MPL}), casitas B-lineage lymphoma proto-oncogene (\textit{CBL}), Src homology 2 B3 (\textit{SH2B3}), \textit{Wilms tumor 1} (\textit{WT1}), serine/arginine-rich splicing factor 2 (\textit{SRSF2}), and epigenetic mutations in TET oncogene family member (\textit{TET2}), isocitrate dehydrogenase (\textit{IDH})1/2, additional sex combs-like 1 (\textit{ASXL1}), enhancer of zeste homolog 2 (\textit{EZH2}), and DNA methyltransferase (\textit{DNMT3A}).

The \textit{WT1} gene, on chromosome 11p13, is a potent transcriptional regulator of genes involved in cell survival, differentiation, and proliferation [1]. The precise role of \textit{WT1} in hematopoiesis and its contribution to leukemogenesis are not fully understood. Recently, interest in \textit{WT1} has grown, with the discovery of mutations (most in a “hotspot” in exon 7) in patients with AML [2, 3]. However, despite the large number of patients analyzed, controversies remain about the prognostic impact of these mutations in AML. \textit{WT1} mutations are also seen in MDS; increased \textit{WT1} expression is associated with higher blast counts and portends an early progression to acute leukemia [4]. Recently, several publications have emphasized the possible impact of the genetic variant rs16754, located in exon 7 of \textit{WT1}, on the outcome for both pediatric and adult AML patients [5]. Becker et al. [6] demonstrated that AML patients carrying homozygotes for a genetic...
variant of rs16754 (GG) had a more favorable outcome in a study of a subset of patients with FLT3-ITD. However, in a Korean cohort, different genotypes of rs16754 did not have a significant impact on clinical outcome in AML [7]. Therefore, it is of interest to test whether the WT1 rs16754 genetic variant shows potential as a molecular marker in BCR-ABL1-negative MPN, to improve risk and treatment stratification.

We performed sequencing analyses of WT1 mutational hotspots in exons 7 and 9 in 75 patients with BCR-ABL1-negative MPN and 93 healthy controls from a Korean population; we also included genetic variant rs16754, located in exon 7 of WT1.

A total of 75 patients (32 with essential thrombocytosis [ET], 25 with polycythemia vera [PV], 10 with primary myelofibrosis [PMF], and eight with unclassifiable MPN) were enrolled. The diagnoses of PV, ET, and PMF were made according to the WHO criteria [8, 9]. All patients were diagnosed between June 2007 and March 2012 at Pusan National University Hospital, Busan, Korea. The JAK2 V617F mutation was identified in 20 (80%) of 25 patients with PV, 18 (56.2%) of 32 patients with ET, five (50%) of 10 patients with PMF, and five (62.5%) of eight patients with unclassifiable MPN. The patients comprised 40 males and 35 females (median age = 57.3 yr, range = 19-83 yr). All patients provided informed consent. This research was reviewed and approved by full committee review of the Institutional Review Board at Pusan National University Yangsan Hospital (No. 05-2014-058).

A total of 75 DNA samples were extracted from the bone marrow of MPN patients between 2007 and 2012. All samples were obtained at initial diagnosis of MPN. Genomic DNA was extracted from cryopreserved mononuclear cells using an AccuPrep Genomic DNA extraction kit (Bioneer, Daejeon, Korea). The BCR-ABL1 gene rearrangement was assessed by reverse transcription-polymerase chain reaction using an in-house method. Mutational analysis of coding regions previously described as mutational hotspots for WT1 (exons 7 and 9) was performed by using PCR amplification and bidirectional direct sequencing. Primers were designed by using the Primer 3 software (http://frodo.wi.mit.edu/primer3/). The 20-μL reaction mixture for amplification contained 1 μL of DNA template, 1 μL of each primer, 17 μL of water-solubilized AccuPower HotStart PCR Premix (Bioneer), and directly sequenced in both directions on an ABI 3100 analyzer using BigDye chemistry (Applied Biosystems, Foster City, CA, USA). The sequence data files were analyzed by using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Electropherograms were also read manually to identify mutations below the detection threshold of the software.

No mutations were detected in WT1 exons 7 or 9, but c.1107A > G was detected in 88.0% of patients; this known genetic variant (rs16754) is listed in the National Center for Biotechnology Information Single Nucleotide Polymorphism (SNP) Database (dbSNP; http://ncbi.nlm.nih.gov/projects/SNP/). WT1 rs16754 is located in the mutational hotspot of WT1 exon 7. We aimed to genotype the WT1 rs16754 locus and analyze the clinical impact of WT1 rs16754 genotypes on clinical outcomes in Korean adult patients with MPN. A total of 93 DNA samples were acquired from healthy controls. The MPN patients and healthy controls were divided into three groups based on genotype at rs16754 in WT1 exon 7: wild type (AA), heterozygotes (AG), and homozygotes (GG).

The frequencies of the three genotypes for WT1 rs16754 were 12.0% (nine patients) AA, 49.3% (37 patients) AG, and 38.7% (29 patients) GG in the 75 patients with MPN (Table 1). In the 93 healthy controls, the AA, AG, and GG genotypes were observed in 1.1% (one individual), 40.9% (38 individuals), and 58.1% (54 individuals), respectively. The genotype frequencies showed no significant difference. The A allele was present at a frequency of 36.7% in MPN patients, compared with 21.5% in controls (P = 0.002). A significant difference in the allelic frequencies of WT1 rs16754 was noted in Koreans between BCR-ABL1-negative MPN cases and healthy controls.

Genotype-specific risks were estimated as odds ratios (OR) for the heterozygote and homozygote types, with the wild type as the baseline category, using the χ² test and logistic regression for the three different genetic models (co-dominant, dominant, and recessive). The results showed a risk-reducing association with MPN for WT1 rs16754 with an OR of 0.11 (95% confidence interval 0.04-0.36).

**Table 1.** Allele frequencies for WT1 rs16754 (n = 168)

| Genotype | MPN (n = 75) | Control (n = 93) |
|----------|--------------|-----------------|
| GG       | 29 (38.7%)   | 54 (65.1%)      |
| GA       | 37 (49.3%)   | 38 (40.9%)      |
| AA       | 9 (12.0%)    | 1 (1.1%)        |

**Allele:**

| Allele | MPN (n = 75) | Control (n = 93) |
|--------|--------------|-----------------|
| G      | 95 (63.3%)   | 146 (78.5%)     |
| A      | 55 (36.7%)   | 40 (21.5%)      |

Abbreviation: MPN, myeloproliferative neoplasm.
SNP/snp_ref.cgi?rs=16754) [10]. The allele frequencies observed in this study are similar to those for the other Asian populations and differ significantly from those for the CEU population. G was a major allele in the Korean population, but a minor allele in the CEU population. The differences were observed in both MPN patients and healthy controls.

We found evidence of a risk-reducing association with BCR-ABL1-negative MPN for WT1 rs16754, consistent with the G allele functioning as an MPN-protective allele. No significant difference in OS was detected between genotypes. The genetic function of WT1 rs16754 has not been fully elucidated. The prognostic impacts of WT1 rs16754 on clinical outcomes for AML have been investigated in several studies [5, 11, 12], but the results were conflicting. These contradictory results indicate that the influence of WT1 rs16754 should be investigated further.

In conclusion, we observed a significant difference in the allelic and genotypic frequencies of WT1 rs16754 in an Asian population, relative to the frequencies reported for a western population. The individuals carrying variant G alleles of WT1 rs16754 showed a relatively low prevalence of MPN, compared with those carrying A alleles of WT1 rs16754 (HR: 0.10-0.65, P<0.05); the G allele for WT1 rs16754 might reduce the risk of developing MPN.

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