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VEGFA and *VEGFR2* genetic polymorphisms and survival in patients with diffuse large B cell lymphoma

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We evaluated the impact of functional polymorphisms in the vascular endothelial growth factor *A* (*VEGFA*) and vascular endothelial growth factor *2* (*VEGFR2*) genes on the survival of patients with diffuse large B cell lymphoma (DLBCL). Five potentially functional polymorphisms in the *VEGFA* (rs699947, rs2010963 and rs3025039) and *VEGFR2* (rs1870377 and rs2305948) genes were assessed in 494 DLBCL patients treated with rituximab plus CHOP chemotherapy. The associations of genotype and haplotype with overall survival (OS) and progression-free survival (PFS) were analyzed. Of the five polymorphisms, *VEGFR2* rs1870377T>A was significantly associated with both OS and PFS; in the dominant model, patients with the AA + TA genotypes had significantly better OS ($P = 0.002$) and PFS ($P = 0.004$) than those with the TT genotype. The association between significantly better OS and the AA + TA genotypes was observed separately in patients with low (0–2; $P = 0.035$) and high (3–5; $P = 0.043$) International Prognostic Index scores. Multivariate analysis showed that, relative to the AA + TA genotypes, the TT genotype was an independent prognostic factor for poor OS (HR, 1.71; 95% CI, 1.21–2.43; $P = 0.002$) and PFS (HR, 1.57; 1.13–2.17; $P = 0.004$). Other independent significant predictors of survival in patients with DLBCL were International Prognostic Index score, age > 60 years, lactate dehydrogenase concentration >normal, extranodal disease >1 and presence of B symptoms. The *VEGFR2* rs1870377 polymorphism might affect survival in patients with DLBCL, suggesting that angiogenesis might be related to poor survival in these patients. (*Cancer Sci* 2012; 103: 497–503)

Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma worldwide. The introduction of rituximab has clearly changed the prognosis of patients with DLBCL, with approximately half of patients achieving long-term disease-free survival.[1–4] Outcomes, however, are highly variable, reflecting tumor heterogeneity, with patients having different genetic abnormalities, clinical features, responses to treatment and prognoses. Therefore, effective risk-adapted strategies are needed to improve the outcome of patients with DLBCL. Although the International Prognostic Index (IPI) or revised IPI have been used as the standard clinical tool to predict outcomes,[5,6] outcomes differ significantly within IPI categories. Therefore, new biological markers that reflect the heterogeneity of DLBCL have been evaluated to better determine patient outcomes.[7–10]

Angiogenesis is a fundamental process in the growth and metastatic dissemination of both solid tumors and hematologic malignancies. The vascular endothelial growth factor (*VEGF*) pathway is one of the key regulators of this process. Activation of the *VEGF*-receptor pathway triggers a network of signaling processes that promote endothelial cell growth, migration and differentiation, as well as vascular permeability and the mobilization of endothelial progenitor cells from the bone marrow to distant sites of neovascularization. *VEGFA* (commonly referred to as *VEGF*) is produced by a variety of tumor cells as well as tumor-associated stromal cells and binds to two related receptor tyrosine kinases, *VEGFR1* and *VEGFR2*. *VEGFR2* is the predominant mediator of most of the downstream angiogenic effects of *VEGF*. *VEGF* expression is regulated by the transcriptional factor hypoxia-induced factor (*HIF*)-1 and the Von Hippel–Lindau (*VHL*) tumor suppressor gene in response to tissue hypoxia.[11] Angiogenesis and angiogenic factors are increased in most lymphomas, including peripheral T-cell lymphomas and DLBCL.[12,13] Moreover, angiogenesis has been associated with adverse outcomes and more aggressive behavior of malignant lymphomas.[14,15]

There is substantial inherited genetic variability within *VEGF* and one of its receptors, *VEGF receptor 2* (*VEGFR2*), including multiple single nucleotide polymorphisms (SNP). The level of *VEGF* expression varies depending on the presence of a genetic polymorphism.[16,17] Moreover, *VEGFR2* gene polymorphisms affect the binding of *VEGF* to *VEGFR2*.[18] Several SNP within *VEGF* and *VEGFR2* have biologic importance in predicting the prognosis of patients with breast, lung, colorectal and prostate cancer, as well as hematologic malignancies.[19–23] However, little is known about the functional role of *VEGF* polymorphisms in malignant lymphoma. Therefore, we assessed the association between *VEGFA* and *VEGFR2* polymorphisms and survival outcomes in patients with DLBCL.

Materials and Methods

Patients. This study included 494 patients with de novo DLBCL treated at five hospitals throughout Korea (Asan Medical Center, Yeungnam University Medical Center, Gyeongsang National Hospital, Catholic University Hospital of Daegu and Ewha Womans University Hospital) from August 2001 through August 2009. Patients were included if they were: (i) ethnic Korean; (ii) had blood samples taken at diagnosis; (iii) had been treated with R-CHOP (rituximab, cyclophosphamide,

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adriamycin, vincristine and prednisolone) with curative intent; and (iv) were available for follow up at the treating institution. Median follow up for surviving patients was 33.6 months. The study protocol was approved by the Institutional Review Board of each center, and all patients gave written informed consent before enrollment.

R-CHOP chemotherapy was repeated every 3 weeks for six to eight cycles, at the discretion of the attending physician. Response was evaluated at the end of every three treatment cycles and at the end of R-CHOP therapy. Follow-up visits were scheduled every 3 months for the first 2 years and every 6 months thereafter. Autologous stem cell transplantation (ASCT) was recommended for younger patients (<65 years) with chemo-sensitive relapse. A total of 32 patients received ASCT after second-line chemotherapy.

Candidate polymorphisms. Genes and polymorphisms known to modulate angiogenesis were selected. Criteria included: (i) SNP involved in the *VEGF* pathway; (ii) potentially functional SNP predicting alterations in protein function; (iii) SNP relevant to outcomes in other settings; and (iv) SNP with a minor allele frequency >5% in the study population. We selected five genotypes: three in the *VEGFA* gene (−2578C>A [rs699947], +405GT>C [rs2010963] and +936C>T [rs3025039]) and two in the *VEGFR2* gene (+1416T>A [rs1870377] and +1192A>G [rs2305948]).

Single nucleotide polymorphism genotyping. Genotyping was performed using the Sequenom iPLEX platform according to the manufacturer's instructions (http://www.sequenom.com; Sequenom, San Diego, CA, USA). DNA was extracted from each sample using the Qiagen kit (Qiagen, Valencia, CA, USA). SNP were detected by analyzing primer extension products generated from previously amplified genomic DNA using a Sequenom chip-based matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry platform. Multiplex SNP assays were designed using modified Assay Designer 4.0 software (Sequenom, San Diego, CA, USA). Five ng DNA were added to each well of a 384-well plate and amplified by PCR according to the manufacturer's specifications. Unincorporated nucleotides present in the PCR product were deactivated using shrimp alkaline phosphatase. Allele discrimination reactions were performed by adding the extension primers, DNA polymerase and di-deoxynucleotide triphosphates to each well. MassARRAY clean resin (Sequenom) was added to the mixture to remove extraneous salts that could interfere with MALDI-TOF analysis. The primer extension products were cleaned, and aliquots of each sample were spotted onto a 384 SpectroChip (Sequenom), which was subsequently read by the MassARRAY Compact Analyzer (Sequenom). Duplicate samples and negative controls were included to check genotyping quality. Genotypes were determined using MassARRAY Typer version 4.0 software (Sequenom). (The primer sequences are summarized in Table S1.) Overall, genotype was successfully determined in 96.4% of samples (range, 96.3–97.0%).

Statistical analysis. Hardy–Weinberg equilibrium of the five SNP was evaluated using the chi square test. Genotype frequencies were calculated using Haploview (available at http://www.broad.mit.edu/mpg/haploview). Additive, dominant and recessive models were used to assess the association between each SNP and treatment outcomes. Haplotype analysis was applied to neighboring SNP with high linkage disequilibrium. Individual haplotypes and haplotype frequencies were estimated based on a Bayesian algorithm using the phase program (available at http://www.stat.washington.edu/stephens/phase.html). Demographic and clinical information was compared across genotypes using chi square-tests for categorical variables. Overall survival (OS) was measured from the day of diagnosis to the day of last follow up or death. Progression-free survival (PFS) was calculated from the day of diagnosis to the day of progression or death from any cause. Survival estimates were calculated using the Kaplan–Meier method, with differences in OS and PFS across genotypes compared using the log-rank test. Hazard ratios (HR) and 95% confidence intervals were estimated using multivariate Cox proportional hazard models.

To validate the effect of genetic variation on patient outcome, we performed a bootstrap algorithm based on 1000 replications. All statistical analyses were performed using SPSS version 17.0 (SPSS 17.0, Chicago, IL, USA) and R package, version 2.12.1 (available at http://cran.r-project.org).

Results

Clinical characteristics at diffuse large B cell lymphoma diagnosis and treatment outcome. We assessed 494 patients newly diagnosed with DLBCL at five institutions in Korea, representing the entire population of patients treated with R-CHOP with curative intent at those institutions during the study period and from whom blood samples taken at diagnosis were available. The clinical characteristics of these patients are illustrated

Table 1. Patients and disease characteristics and their associations with the *VEGFR2* rs1870377 genotype at the time of diagnosis (*N* = 494)

Characteristics	Overall		*VEGFR2* rs1870377 genotype (*N* = 476)		*P*-value
	N = 494	%	TT genotype	TA + AA genotype	
Gender					
Male	286	57.9	106	173	0.177
Female	208	42.1	63	134	
Age (median, range)	57 (17–89)				
≤60 years	289	58.5	90	189	0.078
60 years	205	41.5	79	118	
Stage					
I–II	223	45.1	72	143	0.404
III–IV	271	54.9	97	164	
LDH					
Normal	239	48.4	76	155	0.249
Elevated	267	51.4	93	152	
ECOG PS					
0–1	415	84.0	140	261	0.533
≥2	79	16.0	29	46	
Extranodal sites					
0–1	377	76.3	124	242	0.177
≥2	117	23.7	45	65	
IPI score					
0–2	325	65.8	108	207	0.437
3–5	169	34.2	61	100	
B symptoms					
Absent	365	73.9	129	222	0.340
Present	129	26.1	40	85	
Bone marrow involvement					
Absent	433	87.7	147	271	0.680
Present	61	12.3	22	36	
Bulky disease					
No	447	90.5	147	284	0.075
Yes	46	9.3	21	23	
Primary extranodal disease					
No	235	47.6	83	141	0.505
Yes	259	52.4	86	166	

ECOG PS, Eastern Cooperative Oncology Group Performance Score; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

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in Table 1. Median patient age was 57 years (range 17–89 years). At the end of R-CHOP treatment, 76% of patients showed a complete response (CR), including unconfirmed CR, with 3-year OS and PFS rates of 71.2 and 65.4%, respectively.

Genotype frequencies and effects on clinical characteristics. Genotype frequencies of the *VEGFA* and *VEGFR2* polymorphisms are listed in Table 2. All five polymorphisms were in Hardy–Weinberg equilibrium. None was significantly associated with patient-related or tumor-related factors, including age, sex or international prognostic index (IPI) variables (Table S2). However, patients with the *VEGFR2* rs1870377 TT genotype tended to be aged >60 years ($P = 0.078$), to have bulky disease ($P = 0.072$) and to not achieve CR ($P = 0.068$), compared with patients with the AA + TA genotypes (Table 1).

***VEGFR2* polymorphism and survival.** Of the five polymorphisms, only *VEGFR2* rs1870377T>A was significantly associated with both OS and PFS. Using a dominant model for the variant allele, the log-rank *P*-values for OS and PFS were 0.002 and 0.004, respectively (Table 2). Table 3 and Figure 1 show the effects of the *VEGFR2* rs1870377 polymorphism on survival outcomes. Patients with the *VEGFR2* AA + TA genotypes had significantly better OS and PFS than those with the TT genotype (Fig. 1A–D). The association between significantly better OS and the AA + TA genotypes was observed separately in patients with low (0–2; $P = 0.035$) and high (3–5; $P = 0.043$) IPI scores (Fig. 2A,B). The AA + TA genotypes had better PFS in patients with low (0–2; $P = 0.042$) and high (3–5; $P = 0.061$) IPI scores (Fig. 2C,D). Univariate analysis showed that, among pretreatment parameters, age, stage, Eastern Cooperative Oncology Group performance status, extranodal disease, IPI score, B symptoms, bone marrow involvement and *VEGFR2* genotype were significantly associated with OS and PFS (Table S3).

Haplotype analysis. The *VEGFA* rs699947 and rs2010963 polymorphisms were closely linked (correlation coefficient, $R = 0.221$; Lewontin's $D' = 0.93$), whereas linkage with the rs3025039 polymorphism was weaker (correlation coefficient, $R = 0.056$; Lewontin's $D' = 0.313$). The *VEGFR2* rs1870377 and rs2305948 polymorphisms were moderately linked (correlation coefficient, $R = 0.094$; Lewontin's $D' = 0.67$). Three haplotypes of the *VEGFA* gene for alleles rs699947 and rs2010963 were generated: CC (33.1%), CG (41.8%) and AG (25.1%), although haplotype analyses showed no significant associations between *VEGFA* haplotype and survival of patients with DLBCL.

Multivariate analysis. A multivariate survival analysis using Cox's proportional hazard model showed that the rs1870377 TT genotype was an independent prognostic factor for poor OS (hazard ratio [HR], 1.73; 95% CI, 1.23–2.44; $P = 0.002$) and PFS (HR, 1.64; 1.19–2.25; $P = 0.002$) compared with the AA + TA genotypes when IPI score was included (Table 4). These significance levels were also maintained with multivariate analysis including IPI variables ($P = 0.005$ and $P = 0.006$, respectively). In the multivariate analysis that included IPI score, other significant independent predictors of OS and PFS in patients with DLBCL were IPI score ($P = 0.0001$ each) and B symptoms ($P = 0.002$ and $P = 0.004$, respectively). Predictors of OS and PFS in multivariate analyses that included IPI variables included age >60 years ($P = 0.0001$ each), lactate dehydrogenase concentration >normal ($P = 0.003$ and $P = 0.001$, respectively), extranodal disease >1 ($P = 0.018$ and $P = 0.033$, respectively) and presence of B symptoms ($P = 0.0001$ each).

We validated these results using the bootstrap method. Table 4 shows the probabilities of each variable to be accepted in the final multivariate model. When IPI scores and IPI variables were incorporated in multivariate analysis, the rs1870377 genotype (TT *vs* AA + TA) showed high probabilities (0.926 each).

Discussion

We have investigated the prognostic impact of five potentially functional polymorphisms in the *VEGF* and *VEGFR2* genes in a large population of patients with DLBCL. We found that the AA + TA alleles of the *VEGFR2* +1416T>A (rs1870377) polymorphism were associated with significantly better survival outcomes among patients treated with R-CHOP chemotherapy within the same IPI category. VEGFR2 mediates most

Table 2. Genotype frequencies and log-rank *P* values for *VEGFA* and *VEGFR2* polymorphisms in patients with diffuse large B cell lymphoma

Gene	ID no.	Base change	Genotype		Log-rank *P* for OS			Log-rank *P* for PFS			MAF in healthy population			
			MAF	HWE *P*	Additive	Dominant	Recessive	Additive	Dominant	Recessive	Korean	Asian	European†	African†
VEGFA	rs699947	−2578C>A	0.226	0.997	0.429	0.629	0.194	0.530	0.485	0.464	0.340‡	0.318	0.408	0.117
	rs2010963	+405G>C	0.337	0.687	0.882	0.991	0.650	0.812	0.940	0.562	0.519§	0.352	0.431	0.340
	rs3025039	+936C>T	0.154	0.055	0.497	0.543	0.437	0.289	0.348	0.318	0.206§	0.182	0.186	0.068
VEGFR2	rs1870377	+1416T>A	0.322	0.253	0.004	0.002	0.021	0.010	0.004	0.049	–	0.344	0.275	0.117
	rs2305948	+1192G/A	0.121	0.463	0.966	0.823	0.929	0.997	0.968	0.953	–	0.078	0.067	0.292

In the reference sequence, the transcription start site was designated nt +1. †Information about polymorphism ID and MAF in other ethnic populations (Asian, European and African-American) were obtained from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). ‡From reference 43. §From reference 44. HWE, Hardy–Weinberg equilibrium; ID, identification; MAF, minor allele frequency; OS, overall survival; PFS, progression-free survival; *VEGFA*, vascular endothelial growth factor A; *VEGFR2*, vascular endothelial growth factor receptor 2.

Table 3. Overall survival and progression-free survival according to the *VEGFR2* rs1870377T>A genotype

Polymorphism/genotype	No. of patients	%	Overall survival						Progression-free survival					
			No. of deaths	%	3-year OS	HR	95% CI	*P**	No. of events	%	3-year PFS	HR	95% CI	*P**
VEGFR2 rs1870377														
TT	169	35.5	61	36.1	63.7	1		0.004	69	40.8	56.7	1		0.010
TA	219	46.0	58	26.5	73.4	0.656	0.458–0.940	0.022	67	30.6	68.0	0.676	0.483–0.947	0.023
AA	88	18.5	15	17.0	81.6	0.427	0.243–0.751	0.003	21	23.9	76.7	0.519	0.318–0.846	0.009
TA + AA	307	64.5	73	23.8	75.7	0.591	0.420–0.830	0.002	88	28.7	70.5	0.631	0.460–0.865	0.004

*P-values < 0.025 were statistically significant for Bonferroni correction for multiple testing. OS, overall survival; PFS, progression-free survival.

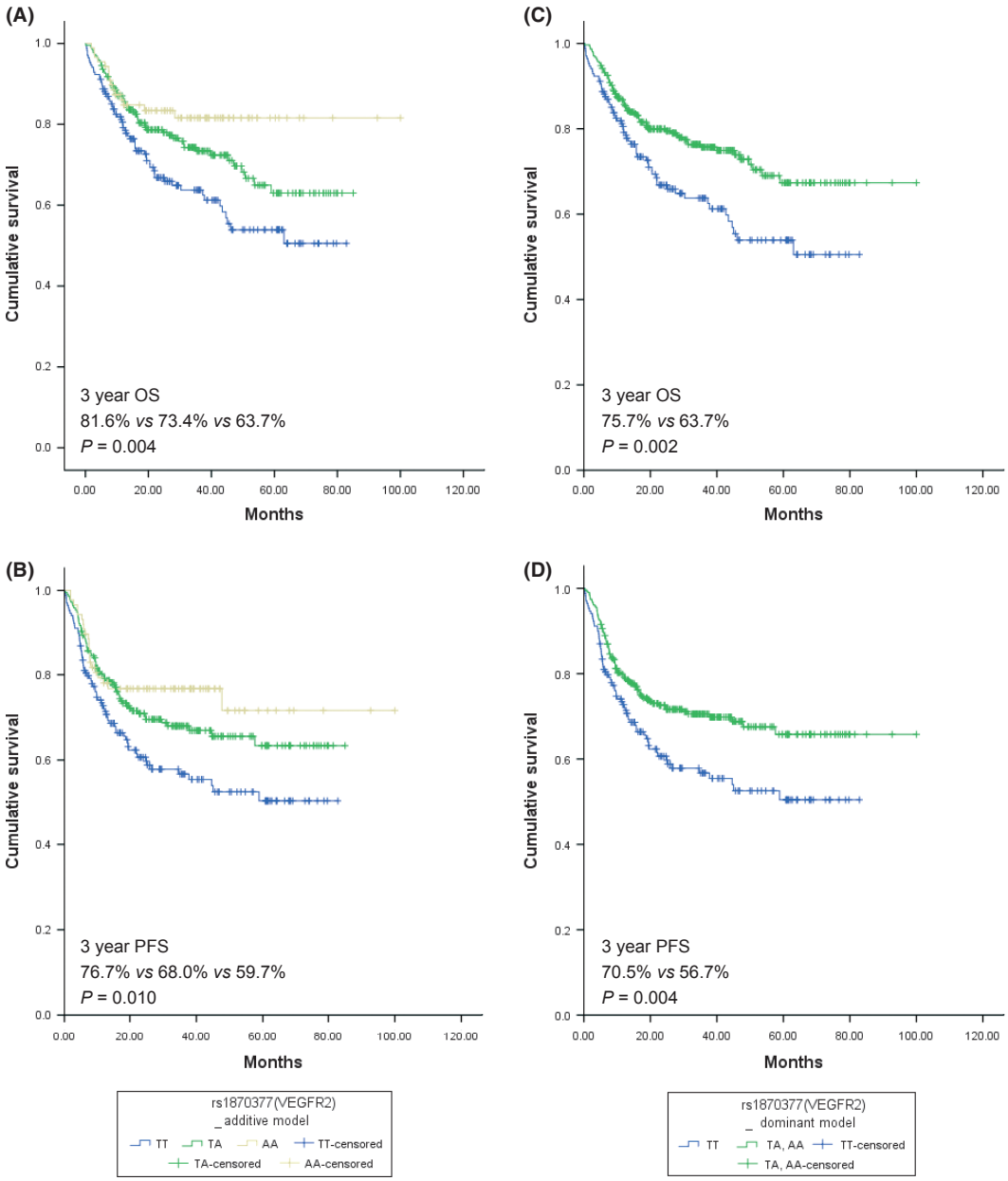

Fig. 1. Overall survival (OS) and progression-free survival (PFS) curves according to *VEGFR2* rs1870337 genotype. (A,B) OS and PFS for *VEGFR2* rs1870377 genotype by additive model ($P = 0.004$ and $P = 0.010$, respectively, by log-rank test); (C,D) OS and PFS for *VEGFR2* rs1870377 genotype by dominant model ($P = 0.002$ and $P = 0.004$, respectively, by log-rank test).

of the downstream effects of VEGF in angiogenesis,[24] and the mutant allele of rs1870377 might reduce the affinity of VEGF for its receptor,[18,25] suggesting that increased angiogenic activity might be related to tumor progression in patients with DLBCL.

Angiogenesis is highly important in several lymphoma subtypes. Increased serum and plasma VEGF concentrations have been linked to increased tumor burden, advanced stage and poor prognosis in patients with non-Hodgkin's lymphoma.[14,26] VEGF, VEGFR1 and VEGFR2 are coordinately expressed in primary human DLBCL specimens,[27] and DLBCL cell lines in culture proliferate in response to VEGF stimulation.[28] Angiogenesis-related biomarkers might predict clinical outcomes in patients with DLBCL. For example, VEGFR2 expression has been found to correlate with expression of HIF-1α.[29] Moreover, expression of VEGFR2 might predict poor survival in DLBCL patients treated with R-CHOP,[30] and HIF-1α expression has been associated with clinical outcomes in DLBCL patients treated with R-CHOP.[31] At least two distinct angiogenic mechanisms augment lymphoma progression: autocrine stimulation through expression of VEGF and VEGF receptors on lymphoma cells, and paracrine influences of the proangiogenic tumor microenvironment on both local neovascularization and recruitment of inflammatory cells.[26] Recently, the importance of the tumor microenvironment has been emphasized in the neoplastic progression and growth of non-Hodgkin's lymphoma.[10] Gene expression profiling studies demonstrate that genetic signatures expressed by stromal and infiltrating immune cells define distinct prognostic groups. Moreover, lymphoma-associated macrophages or circulating endothelial progenitor cells might predict poor survival of patients with non-Hodgkin's lymphoma.[32–34]

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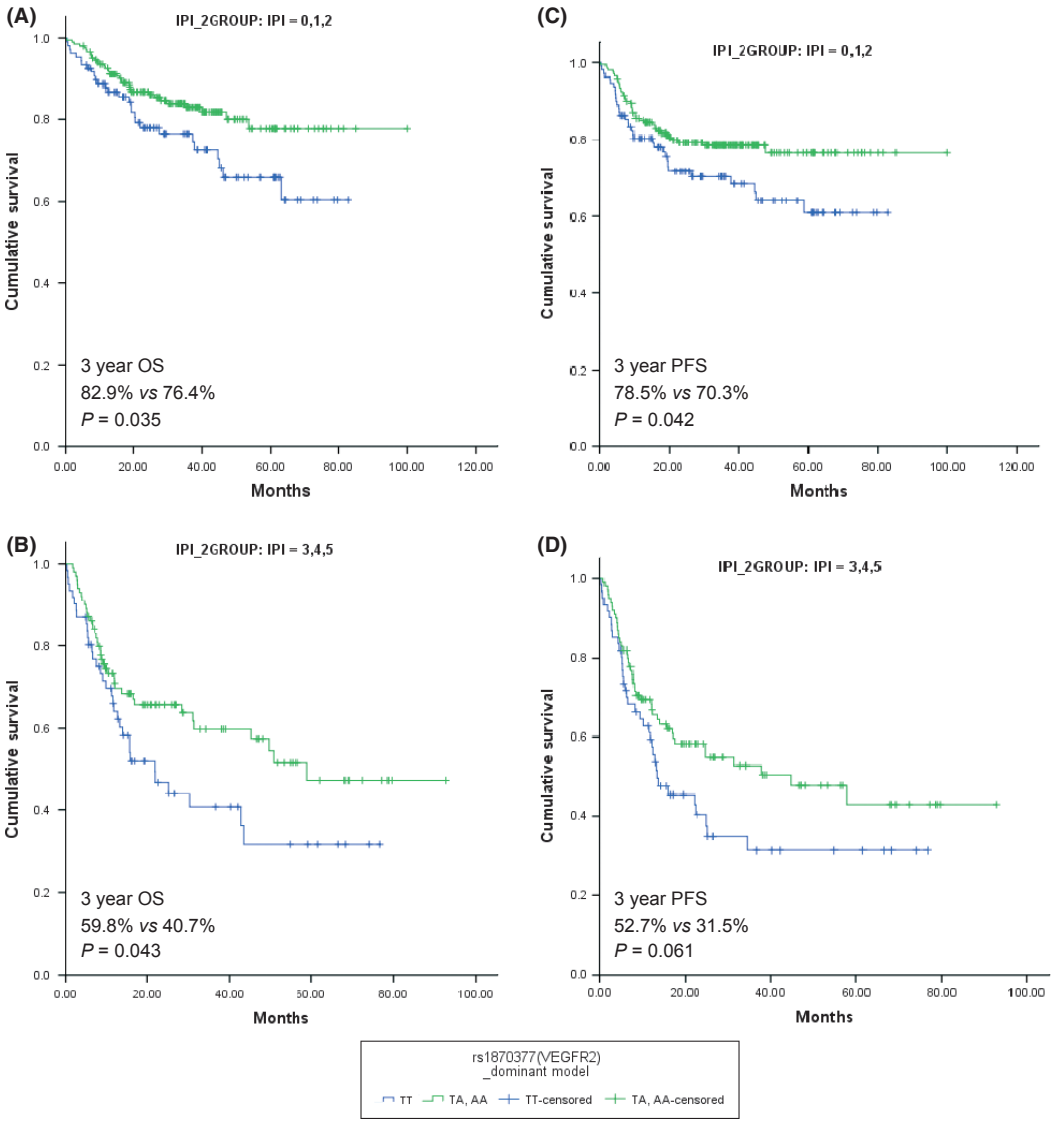

Fig. 2. Overall survival (OS) and progression-free survival (PFS) for *VEGFR2* rs1870377 genotype according to International Prognostic Index (IPI) and revised IPI scores. (A,B) OS for *VEGFR2* rs1870377 genotype according to IPI (low IPI versus high IPI) ($P = 0.035$ and $P = 0.043$, respectively, by log-rank test); (C,D) PFS for *VEGFR2* rs1870377 genotype according to IPI (low IPI versus high IPI) ($P = 0.042$ and $P = 0.061$, respectively, by log-rank test).

Single nucleotide polymorphisms, the most common type of variation among human genes, can alter gene expression and determine phenotypic variability.[35] Methods of detecting SNP are reproducible and can be easily automated. The *VEGF* and *VEGFR2* genes are both highly polymorphic. The *VEGFA* gene is located on chromosome 6 at location 6p21.1, and its 5′- and 3′-untranslated regions (UTR) have been shown to contain key regulatory elements that are sensitive to hypoxia and that contribute to the high variability in *VEGF* production among tissues. For example, the rs2010963G>C SNP in the 5′-UTR of *VEGFA* affects protein translation efficiency, and the rs3025039C>T SNP in the 3′-UTR influences the circulating plasma concentrations and tumor tissue expression of *VEGF*.[15,16,36,37] *VEGF* polymorphisms have been associated with clinical variables in several malignancies.[19–23]

The mutant alleles of two nonsynonymous SNP (rs2305948 and rs1870377) in the *VEGFR2* gene have been found to reduce the affinity of VEGF for its receptor.[18,25] The *VEGFR2* rs1870377T>A SNP causes an amino acid change, from glutamine to histidine, in the immunoglobulin (Ig)-like extracellular domain 5. *VEGFR2* Ig-like domains 4–7 have been reported to contain structural features that inhibit receptor signaling.[38] This amino acid change in domain 5 might reduce the affinity of VEGFR2 for VEGF. Moreover, the *VEGFR2* rs1870377T>A polymorphism might be associated with a change in downstream signaling following VEGF binding, a change in self-inhibition when VEGF does not bind to its receptor, a change in the autocrine function of VEGF or a change in VEGFR2 expression. In addition, the *VEGFR2* rs1870377T>A polymorphism might be a surrogate predictor of response to imatinib therapy, in that it might indicate complete cytogenetic response or treatment failure.[39] If VEGF production is maximal in response to HIF-1α under hypoxic conditions, the function of VEGFR2 might be rate-limiting and a receptor subtype with increased affinity for VEGF might increase VEGFR2 signaling. Further studies are warranted to determine the functional impact of this nonsynonymous SNP on *VEGF*-mediated signaling pathways.

Many antiangiogenic agents are currently undergoing clinical trials in various tumor types. Encouraging preliminary clinical evidence supports the safety, feasibility and clinical efficacy of

Table 4. Multivariate analysis of factors prognostic for overall survival and progression-free survival in patients with diffuse large B cell lymphoma

Variable	Overall survival			Progression-free survival			Bootstrap probability
	HR	95% CI	*P*	HR	95% CI	*P*	
Multivariate analysis including IPI score							
B Sx	1.780	1.234–2.567	0.002	1.857	1.321–2.609	0.0001	0.964
VEGFR2 rs1870377	1.729	1.227–2.438	0.002	1.635	1.189–2.247	0.002	0.926
IPI							
Low risk	1		0.0001	1		0.0001	0.215
Low–intermediate	1.870	1.131–3.091		1.500	0.949–2.373		
High–intermediate	2.562	1.561–4.205		2.363	1.526–3.660		
High	4.410	2.671–7.280		3.103	1.944–4.951		
Multivariate analysis including IPI variables							
Age >60 years	2.148	1.524–3.028	0.0001	1.925	1.403–2.640	0.0001	0.971
LDH >normal	1.843	1.235–2.748	0.003	1.851	1.286–2.666	0.001	0.974
Extranodal >1	1.564	1.081–2.264	0.018	1.457	1.030–2.060	0.033	0.859
B Sx	2.020	1.407–2.900	0.0001	2.035	1.459–2.838	0.0001	0.964
VEGFR2 rs1870377	1.645	1.165–2.324	0.005	1.565	1.137–2.153	0.006	0.926

95% CI, 95% confidence interval; HR, hazard ratio; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

antiangiogenic therapy in various human lymphoma subtypes, including DLBCL. In pilot studies, monotherapy with the anti-VEGF monoclonal antibody bevacizumab has shown modest clinical activity in patients with relapsed aggressive non-Hodgkin's lymphoma.[40,41] However, a phase III trial of R-CHOP-bevacizumab was closed early owing to safety issues. The effectiveness of bevacizumab in patients with malignant lymphoma requires further investigation in well-designed clinical trials.

Sunitinib and sorafenib are orally bioavailable inhibitors that affect receptor tyrosine kinases, including VEGFR2.[42] If VEGFR2 is more responsive than VEGF to angiogenic activity in patients with DLBCL, receptor tyrosine kinase inhibitors might be more effective than bevacizumab. To our knowledge, our study is the first to show that a *VEGFR2* polymorphism is significantly associated with the prognosis of patients with DLBCL. Therefore, antiangiogenic therapy might improve the clinical outcomes of patients with DLBCL. Moreover, personalized therapy with antiangiogenic agents might improve the clinical outcomes of patients with activated angiogenesis and poor prognosis.

The strengths of this study include its large sample size, its homogeneous treatment regimen, and the detailed data regarding each patient's characteristics and survival. This study, however, has several limitations. We selected only a few *VEGFA* and *VEGFR2* polymorphisms using a candidate gene approach. Although this approach aids in hypothesis generation based on biological plausibility, it has notable limitations. For example, the observed association might not be due to the candidate polymorphism chosen, but rather to a linked polymorphism. In addition, the true functions of these polymorphisms have not been determined. Moreover, many other genes are involved in angiogenesis, a complex process that includes many activating and inhibitory factors. Because peripheral blood at diagnosis was used to analyze germline genotype, the presence of lymphoma cells in peripheral blood might have affected these results.

The internal validation results using the bootstrap method and the consistent prognostic significances in subgroup analysis (i.e. patients with similar IPI scores) indicate that, although we could not show independently validated results in this report, our analysis might have clinical significance, despite the limitations inherent to retrospective analyses. However, the results we observed should be confirmed in large independent studies, as well as in studies that cover all angiogenesis pathways and are correlated with the true function of detected polymorphisms.

In summary, we found that genetic variations in the *VEGFR2* gene might be associated with survival in patients with DLBCL. More relevant prognostic models are needed following the introduction of rituximab. A better understanding of the biology of DLBCL might result in the discovery of new targets and novel therapeutics that will advance the treatment of this disease.

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Disclosure Statement

The authors have declared no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primer sequences for five genotypes of *VEGFA* and *VEGFR2* genes.

Table S2. Patients and disease characteristics and their associations with the *VEGFA* rs699947, rs2010963, rs3025039 and rs2305948 genotypes at the time of diagnosis (N = 494).

Table S3. Univariate analysis for overall survival (OS) and progression-free survival (PFS).

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