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Vascular endothelial growth factor (VEGF) gene (*VEGFA*) polymorphism can predict the prognosis in acute myeloid leukaemia patients

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Summary

Increased angiogenesis, mediated by vascular endothelial growth factor (VEGF), was associated with poor prognosis in acute myeloid leukaemia (AML) patients. The current study investigated the impact of VEGF gene (*VEGFA*) single nucleotide polymorphisms (SNPs) on treatment outcomes for AML. Four *VEGFA* SNPs were analysed for −2578C>A (rs699947), −460T>C (rs833061), +405G>C (rs2010963) and +936 C>T (rs3025039) loci in 138 AML patients. The +936 CC/CT genotype showed strong correlation with favourable leukaemia-free survival (LFS) at 2 years (51·3%) *versus* with +936 CC genotype (33·6%, $P = 0{\cdot}03$). Strong linkage disequilibrium was noted among loci −2578, −460 and +405, but not with +936. Accordingly, four haplotypes were generated based on the genotypes of −2578, −460 and +405 as follows: CTC (40·2%), CTG (35·0%), ACG (22·0%) and ATC (1·2%). The LFS and event-free survival (EFS) inversely correlated with CTG haplotype ($P = 0{\cdot}03$ for LFS; $P = 0{\cdot}05$ for EFS). We scored the *VEGFA* polymorphism marker based on +936 C>T genotype and CTG haplotype for −2578, −460 and +405, which demonstrated a good correlation with the treatment outcomes: LFS ($P = 0{\cdot}01$), EFS ($P = 0{\cdot}03$) and overall survival ($P = 0{\cdot}01$). The *VEGFA* +936 C>T genotype and CTG haplotype seemed to have an additive effect to predict the prognosis in AML patients.

Keywords: vascular endothelial growth factor, single nucleotide polymorphism, acute myeloid leukaemia.

Angiogenesis, the process of new blood vessel formation from endothelial precursors, is involved in the pathogenesis and progression of acute myeloid leukaemia (AML) (Hussong *et al*, 2000; Lee *et al*, 2001). Vascular endothelial growth factor (VEGF), a soluble, 34–46 kDa, heparin-binding glycoprotein dimer, is a potent angiogenic peptide with diverse biological activities including angiogenesis (Thomas, 1996).

Previous investigations demonstrated that a higher level of VEGF was detected in patients with AML compared with healthy controls, and has been correlated with worse prognosis (Aguayo *et al*, 1999, 2002; de Bont *et al*, 2002). Dysregulation of VEGF production was suggested to have a major impact on leukaemic growth and constitutes an important step in the progression of AML (Aguayo *et al*, 2000; Hussong *et al*, 2000; Lee *et al*, 2001; Thomas *et al*, 2001). Via paracrine pathways, AML cells increase the production of VEGF, which activates endothelial cells in the marrow to secrete several growth factors or cytokines (such as macrophage colony-stimulating factor, granulocyte colony-stimulating factor and interleukin-6) that stimulate AML cell growth (Fiedler *et al*, 1997; Bellamy *et al*, 1999). Via autocrine pathways, AML cells increase the production of VEGF, which binds to the VEGF receptor-2 on leukaemic cells, increasing AML cell survival (Dias *et al*, 2000).

VEGF gene (*VEGFA*) expression is regulated by a variety of growth factors, cytokines, hormones and hypoxia (Klagsbrun & D'Amore, 1996). Furthermore, recent investigations have demonstrated that *VEGFA* polymorphisms can contribute to the inter-individual variants in VEGF expression. *VEGFA* is located on chromosome 6p21·3 and consists of eight exons and

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seven introns (Tischer *et al*, 1991; Vincenti *et al*, 1996). The polymorphisms in the promoter region (loci −2578C>A and −460T>C), 5′-untranslated region (+405C>G) or 3′-untranslated region (+936C>T) have been associated with different levels of VEGF expression (Brogan *et al*, 1999; Renner *et al*, 2000; Watson *et al*, 2000; Stevens *et al*, 2003; Koukourakis *et al*, 2004; Prior *et al*, 2006).

As the prognosis of AML patients may correlate with the degree of angiogenesis and VEGF, the current study evaluated the ability of *VEGFA* polymorphisms to predict prognosis in AML patients. Moreover, using data from the +936C>T genotype and CTG haplotype of loci −2578, −460 and +405, we generated a *VEGFA* polymorphism marker score and assessed its predictive capability in determining the prognosis of AML patients.

Materials and methods

Patient characteristics and treatment

A retrospective analysis of 138 patients with newly diagnosed AML between June 1996 and October 2004 at Kyungpook National University Hospital was evaluated. The patient characteristics and treatment are summarized in Table I. Median age was 42·5 years (range 15–86 years) and the male to female ratio was 57%:43%. Twenty-seven per cent had favourable risk cytogenetics ($n = 32$), 58% intermediate-risk ($n = 69$) and 15% unfavourable-risk groups ($n = 18$) (Grimwade *et al*, 1998). At presentation, the mean white blood cell (WBC) count (± standard error) was $34{\cdot}3 \pm 4{\cdot}4 \times 10^9$/l, peripheral blasts were 40 ± 3% and serum lactate dehydrogenase (LDH) 1245 ± 150 IU/l (Table I).

Of the 119 patients receiving remission induction chemotherapy, the majority (84%) were treated with idarubicin 12 mg/m^2/d IV for three consecutive days and cytarabine 100 mg/m^2/d continuous infusion IV for seven consecutive days; the remaining 19 patients (16%) received daunorubicin 45 mg/m^2/d for three consecutive days plus cytarabine 100 mg/m^2/d continuous infusion IV for seven consecutive days. For patients over 55 years ($n = 48$), the anthracycline dose was reduced by one-third. Thirteen patients did not have a bone marrow examination because of early treatment-related mortality (TRM; sepsis, $n = 7$; bleeding $n = 2$; disseminated intravascular coagulopathy, $n = 2$) or unknown ($n = 2$). Of 106 evaluable patients, 74 patients (70%) achieved a complete remission (CR). Ten patients subsequently achieved a CR after receiving second or third course of induction chemotherapy, and further 10 patients achieved a CR after salvage therapy with allogeneic stem cell transplant. Patients' treatment flow during the study period is summarized in Fig S1.

All patients who achieved a CR received consolidation therapy with two cycles of high-dose cytarabine (3 g/m^2/d continuous infusion IV twice a day on days 1, 3, 5). Stem cell transplantations (SCT) (allogeneic donor, $n = 52$; autologous, $n = 7$) were performed as a consolidative treatment ($n = 49$) or salvage therapy ($n = 10$) (Kim *et al*, 2005). The patients who did not receive a SCT ($n = 35$) were given one additional cycle of high-dose cytarabine.

Table I. The characteristics and treatment outcomes in overall AML patients.

Clinical variables	No patients (%)
Sex (male/female)	79/59 (57/43)
Age (years, range)	42·5 (15–86)
Age, ≥50 years	48 (35)
FAB classification	
M0/M1/M2	7/7/72 (5/5/52)
M4/M5/M6	21/13/6 (15/9/4)
M7/unclassified	4/8 (3/6)
Cytogenetic risk	
Available	119 (85)
Favourable	32 (27)
Intermediate	69 (58)
Unfavourable	18 (15)
Laboratory (mean ± SE)	
Peripheral white cell counts (x109/l)	34·3 ± 4·4
Peripheral blasts (%)	40 ± 3
Serum LDH (IU/l)	1240 ± 150
First line induction chemotherapy	
Treated cases	119 (86)
Daunorubicin + AraC*	19 (16)
Idarubicin + AraC2†	100 (84)
Stem cell transplantation	59 (43)
Allogeneic	52 (38)
Autologous	7 (5)

Values in parenthesis are expressed as % or range.
AML, acute myeloid leukaemia; FAB classification, French-American-British classification of AML; SE, standard error; LDH, lactate dehydrogenase; AraC, cytarabine.
*Daunorubicin 45 mg/m^2/d for three consecutive days plus cytarabine 100 mg/m^2/d continuous infusion IV for seven consecutive days.
†Idarubicin 12 mg/m^2/d IV for three consecutive days plus cytarabine 100 mg/m^2/d continuous infusion IV for seven consecutive days.

Genotyping of the VEGFA polymorphism

For *VEGFA* genotyping, the genomic DNA was extracted from patients' peripheral blood using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA). The *VEGFA* −2578C>A (rs699947), −460T>C (rs833061), +405C> G (rs2010963) and 936C>T (rs3025039) genotypes were determined using a polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method as previously described (Han *et al*, 2004; Lee *et al*, 2005; Nam *et al*, 2005). To confirm the genotyping results, selected PCR-amplified DNA samples ($n = 2$, for each genotype) were examined by DNA sequencing (Lee *et al*, 2005). The current study was approved by the Kyungpook National University Hospital Institutional Research Board; each patient gave written informed consent.

Cytogenetic risk group

Cytogenetic studies on the marrow samples at presentation were performed using standard G-banding with trypsin-Wright's staining after unstimulated short-term (24 h) cultures. The criteria used to describe a cytogenetic clone and the karyotype followed the recommendations of the International System for Human Cytogenetic Nomenclature (Mitelman, 1995). At least 20 bone marrow metaphase cells were analysed in the patients designated as having a normal karyotype. The cytogenetic risk groups were classified according to the Medical Research Council AML10 trial criteria (Grimwade *et al*, 1998).

Definition and end points

Complete remission was defined as the presence of no more than 5% blast cells in the bone marrow aspirates and a trephine bone marrow biopsy and an absolute neutrophil count of $\geq 1 \times 10^9$/l and platelets of $>100 \times 10^9$/l without evidence of extramedullary leukaemia. Relapse was defined as marrow infiltration by more than 5% blast cells in previously normal bone marrow or evidence of extramedullary leukaemia.

Event-free survival (EFS) was defined as the interval from the date of treatment to the date of a confirmed relapse, persistence of disease or death from any cause. Leukaemia-free survival (LFS) was defined as the interval from the date of confirmed CR to the date of a confirmed relapse or death from any cause. Overall survival (OS) was defined as the length of time from the date of diagnosis to the date of death from any cause.

A *VEGFA* polymorphism marker score was generated based on the genotype at locus +936 and the CTG haplotype on loci −2578/−405/+460. A score of 1 was assigned to favourable alleles (i.e. +936 CT or TT genotype, or 0 copy of CTG haplotype) and a score of 0 was assigned to unfavourable alleles (i.e. +936 CC genotype, or 1 or 2 copies of CTG haplotype). After summing up the scores, three risk groups were obtained: Low (composite score 2), Intermediate (composite score 1) and High risk (composite score 0).

Statistical analysis

The results were analysed according to information available as of July 2005. Patients were stratified based on the following four clinical parameters: age: (< or ≥55 years), WBC count (< or $\geq 50 \times 10^9$/l), marrow blast percentage (< or ≥50%) and LDH (< or ≥1000 IU/l). Univariate associations between CR and the individual clinical parameters were analysed using a chi-square test, Fisher's exact test, or Mann–Whitney's *U*-test.

The haplotype analysis and control for deviation from the Hardy–Weinberg equilibrium for the *VEGFA* polymorphisms was conducted using Haploview software (available at http://www.broad.mit.edu/mpg/haploview). Subsequently, haplotype frequencies were estimated with linkage disequilibrium (LD) coefficient, D. Individual haplotypes were determined based on a Bayesian algorithm using the Phase program (Stephens *et al*, 2001) (available at http://www.stat.washington.edu/stephens/phase.html).

The frequencies of the genotypes or haplotypes were compared using a chi-square test or Fisher's test according to the patients' characteristics or achievement of CR after first cycle of remission induction chemotherapy. A logistic regression analysis was performed to identify independent predictive factor for induction failure (such as failure to achieve CR or early TRM), including the WBC count, age, serum LDH level, cytogenetic risk, the +936 C>T genotype (CC vs. CT/TT) and *VEGFA* haplotype based on −2578/−405/+460 (0 copy vs. 1 or 2 copies of CTG haplotype). The odds ratio (OR) and 95% confidence interval (CI) for the relative risks were also estimated.

The EFS, LFS and OS estimates were calculated using the method of Kaplan and Meier, and the differences between the EFS, LFS or OS rates compared using a log-rank test. Multivariate survival analyses using Cox's proportional hazard model were performed to define the prognostic factors for EFS, LFS and OS using a backward conditional procedure until the *P*-value for the likelihood ratio test was >0·05. The following variables were included for the analyses: the risk group defined by the *VEGFA* polymorphism marker score [high-risk group (i.e. score 0) vs. intermediate or low risk group (i.e. score 1 or 2)], age, WBC count, serum LDH level, cytogenetics and the use of SCT. The hazard ratio (HR) and 95% CI were also estimated. A cut-off *P*-value of 0·05 was adopted for all statistical analyses. The statistical data were obtained using the Statistical Package for the Social Sciences (SPSS) software (SPSS 13·0 Inc., Chicago, IL, USA).

Results

Of 119 patients receiving induction chemotherapy, 106 patients were evaluable for response. Seventy per cent (95% CI: 60–79%) of patients achieved a CR. With a median follow-up of 14·5 months (range, 0·5–101 months), the 2-year OS was 42·3 ± 5·3% and median OS, 16 months. The 2-year EFS and LFS were 37·1 ± 5·3% and 39·9 ± 5·5% respectively. Median EFS and LFS were 10 and 13 months respectively. No difference in the probability of achieving a CR or OS was noted between patients receiving idarubicin and those receiving daunorubicin.

The genotype frequencies of VEGFA polymorphisms

The genotype frequencies for the *VEGFA* polymorphisms are summarized in Table II. The frequency of each genotype of *VEGFA* polymorphisms was comparable to previous results in Korean (Han *et al*, 2004; Lee *et al*, 2005) or Han-Chinese populations, (Kataoka *et al*, 2006) but different from Caucasian population (Table SI) (Brogan *et al*, 1999; Freathy *et al*,

Table II. The genotype frequency of VEGFA polymorphism at −2578/−460/+405/+936 loci.

Locus	% tested	Allele frequency		HWE
		Major	Minor	*P*-value
−2578	92·0	C: 0·754	A: 0·246	0·6536
−460	96·3	T: 0·777	C: 0·223	0·5580
+405	97·0	G: 0·583	C: 0·417	0·8945
+936	100	C: 0·830	T: 0·170	0·1301

HWE, Hardy–Weinberg equilibrium.

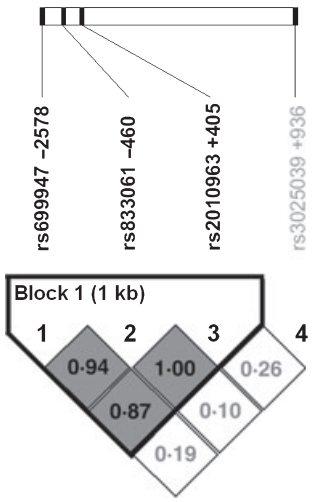

Fig 1. Pairwise linkage disequilibrium in the study population. SNPs selected for haplotype reconstruction are shown in bold.

2006; Girnita *et al*, 2006). Hardy–Weinberg equilibrium was observed for all polymorphisms (Table II).

The LD of *VEGFA* polymorphisms at loci −2578/−460/+405/+936 are shown in Fig 1. There were strong LDs between loci −460 and +405 (D′ = 1·00), −2578 and −460 (D′ = 0·94) and −2578 and +405 (D′ = 0·87). However, linkages of +936 with −2578 (D′ = 0·19), −460 (D′ = 0·10) or +405 (D′ = 0·26) were weak (D′ < 0·5). Accordingly, we generated the haplotype of *VEGFA* polymorphisms based on three genotypes at loci −2578, −460 and +405. The frequencies of haplotypes at loci −2578/−460/+405 were CTC (40·2%), CTG (35·0%), ACG (22·0%) and ATC (1·2%).

VEGFA polymorphism and achievement of complete remission after first induction chemotherapy

The disease characteristics, such as cytogenetic group, peripheral WBC counts, sex or age at diagnosis did not show any significant association with the four genotypes of *VEGFA* polymorphisms (data not shown). In addition, no difference of the use of SCT was noted according to the four genotypes of *VEGFA* polymorphisms (data not shown). In univariate analyses, the CR rates was not statistically different according to the +936C>T genotype or the CTG haplotype for loci −2578/+460/−405 (Table III).

VEGFA polymorphism and survival

Patients with +936CT/TT genotype or with 1 or 2 copies of CTG haplotype showed better survival. As shown in Table III, 2-year LFS were significantly in favour of the group with +936 CT/TT genotype compared to those with +936 CC genotype (51·3 ± 10·4% vs. 33·6 ± 6·3%, $P = 0·03$; Fig 2A). In addition, 2-year OS was also in favour of the group with the +936 CT/TT genotype compared to those with +936 CC genotype (57·6 ± 9·1% vs. 33·6 ± 6·1%, $P = 0·02$ for OS). Other genotypes were not found to be associated with improved 2-year LFS, EFS or OS except for +405G>C genotype for LFS: patients with the +405 CC genotype showed better LFS than those with the +405 CG/GG genotype ($P = 0·05$).

With respect to the haplotype analyses, patients with 0 copy of the CTG haplotype for loci −2578/−460/+405 demonstrated a better 2-year LFS ($P = 0·04$; Table III and Fig 2B) and EFS ($P = 0·04$) compared to those with 1 or 2 copies of CTG haplotype.

Table III. Response to induction chemotherapy and survival by genoptype at position, +936 C>T and by CTG haplotype for loci −2578/−460/+405.

	Overall patients	+936 C>T genotype			CTG haplotype at −2578/−460/+405		
		C/C	C/T or T/T	*P*-value	0 copy	1 or 2 copies	*P*-value
Follow-up duration (months, median)	14·3 (0·5–101)	13 (0·5–78)	18·5 (0·5–101)		12 (0·5–74)	18·5 (0·5–101)	
Response to induction chemotherapy							
Evaluated patients/overall patients, *n* (%)	106/115	70/77	36/38		46/48	60/67	
Early treatment-related mortality, *n* (%)	9 (8)	7 (9)	2 (5)	NS	2 (4)	7 (10)	NS
Complete remission, *n* (%)	74 (63)	53 (69)	23 (61)	NS	36 (75)	41 (61)	NS
Survival at 2 years (%)							
Leukaemia-free survival	39·9 ± 5·5	36·6 ± 6·3	51·3 ± 10·4	0·02	51·3 ± 8·1	30·6 ± 7·3	0·04
Event-free survival	37·1 ± 5·3	25·9 ± 6·0	57·5 ± 9·0	NS	48·3 ± 7·9	26·7 ± 6·9	0·03
Overall survival	42·3 ± 5·3	33·6 ± 6·1	57·6 ± 9·1	0·02	53·4 ± 7·7	34·2 ± 6·9	NS

NS, not significant.

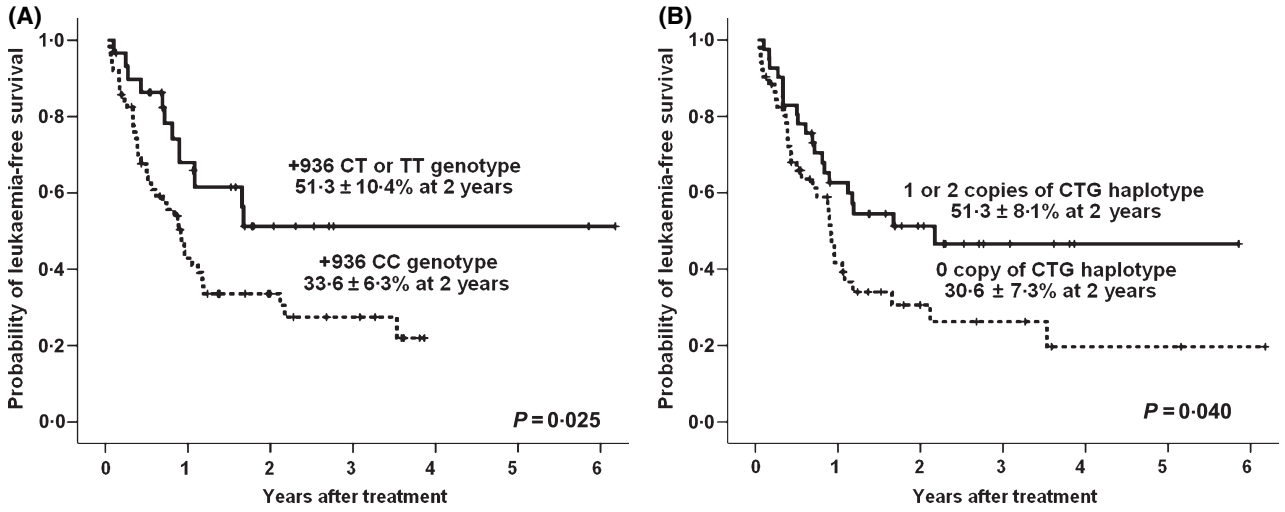

Fig 2. Leukaemia-free survival in AML patients according to *VEGFA* polymorphism by +936 C>T genoptype (A) and by CTG haplotype for −2578/−460/+405 (B).

Scoring of the VEGFA polymorphism marker based on the +936C>T genotype and the CTG haplotype for −2578/−460/+405 and its association with prognosis

As the above results demonstrated that the prognostic significance of the +936 C>T genotype and the CTG haplotype in patients with AML, a *VEGFA* polymorphism marker score was generated using data from the +936 C>T genotype and the CTG haplotype for loci −2578/−460/+405. Using the *VEGFA* polymorphism marker score, three risk groups were identified and classified as low (score 2), intermediate (score 1) and high risk (score 0). The *VEGFA* polymorphism markers also did not show any significant associations with disease characteristics, such as cytogenetic group, peripheral WBC counts, sex or age at diagnosis or the use of SCT (data not shown).

As shown in Fig 3, risk groups identified by *VEGFA* polymorphism marker could have prognostic significances in a dose-dependent manner. In univariate analyses, LFS at 2 years for the low-, intermediate-, or high-risk group was 56·7 ± 12·5%, 45·2 ± 9·3 or 25·6 ± 7·6%, respectively ($P = 0·01$; $P = 0·003$ in a dominant model for *VEGFA* polymorphism marker); 2-year EFS 62·5 ± 11·3%, 37·6 ± 8·9% or 20·5 ± 7·2%, respectively ($P = 0·03$; $P = 0·01$ in a dominant model); and 2-year OS 62·2 ± 11·4%, 48·9 ± 8·6% or 26·7 ± 7·4% respectively ($P = 0·01$; $P = 0·005$ in a dominant model).

Multivariate analysis

Logistic regression analysis was used to identify predictive factors for the treatment failure with first induction chemotherapy. Two independent predictive factors were identified including cytogenetics and WBC counts, but neither +936C>T genotype nor the CTG haplotype was found to be associated with treatment failure after first induction chemotherapy (Table IV).

In a multivariate survival analysis using Cox's proportional hazard model (Table IV), the patients in the high-risk group by *VEGFA* polymorphism marker exhibited decreased LFS compared to those in the intermediate- or low-risk groups ($P = 0·04$, HR 1·84). Unfavourable cytogenetics was also an independent prognostic factor for shortened LFS ($P = 0·04$, HR 2·02). High-risk group by *VEGFA* polymorphism marker ($P = 0·05$, HR 1·82), unfavourable karyotypes ($P = 0·03$, HR 1·98) and high WBC counts at presentation ($P = 0·01$, HR 2·26) were associated with poor OS.

Discussion

The current study demonstrates the importance of *VEGFA* polymorphism for predicting the prognosis in patients with AML. The *VEGFA* polymorphisms, especially the +936C>T genotype and the CTG haplotype for loci −2578/−460/+405, were significantly associated with the treatment outcomes in AML patients. Furthermore, the *VEGFA* polymorphism marker score (generated using data from the +936C>T genotype and the CTG haplotype for −2578/−460/+405) appears to have prognostic significance in AML patients.

Previous investigations indicated that higher *VEGFA* expression levels are associated with poor outcomes in patients with acute lymphoblastic leukaemia, (Koomagi *et al*, 2001) non-Hodgkin lymphoma (Salven *et al*, 1997; Niitsu *et al*, 2002; Hazar *et al*, 2003) and AML (Aguayo *et al*, 1999, 2000, 2002, 2003; Aguayo, 2004). The VEGF produced by the leukaemic cell itself may promote the growth and proliferation of AML cells via a paracrine (Fiedler *et al*, 1997; Bellamy *et al*, 1999) or autocrine pathway (Dias *et al*, 2000). Moreover, VEGF may inhibit apoptotic cell death by upregulating MCL1 expression (Katoh *et al*, 1998; Kuramoto *et al*, 2002) or via Hsp90-mediated induction of Bcl-2 expression (Dias *et al*, 2002). Levels of VEGF expression may correlate with *VEGFA*

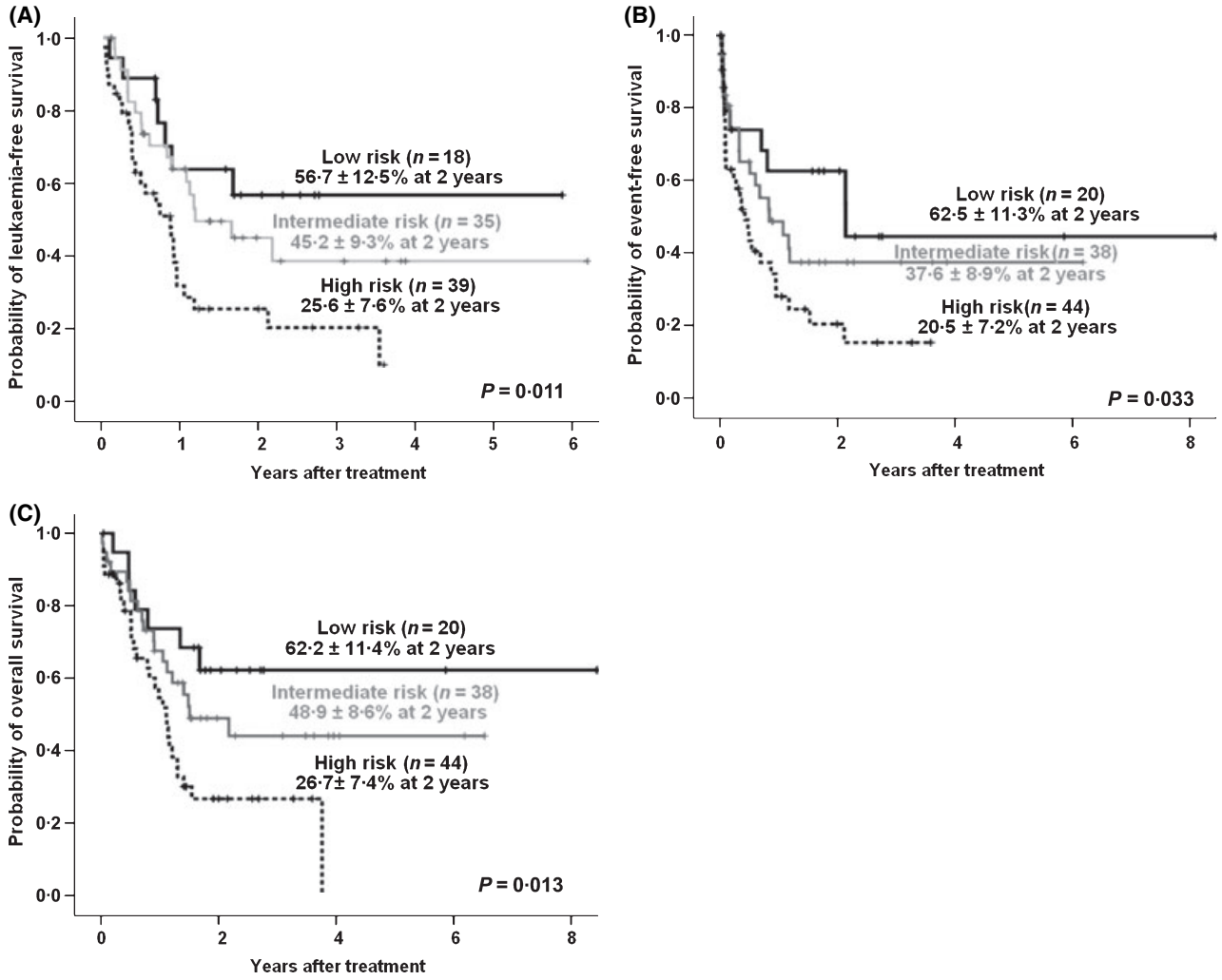

Fig 3. Leukaemia-free (A), event-free (B) and overall survival (C) by the *VEGFA* polymorphism marker based on +936C>T genoptype and CTG haplotype for −2578/−460/+405.

polymorphisms. The −460/+405 haplotype appears to correlate with VEGF expression levels (Stevens *et al*, 2003). Watson *et al* (2000) reported that the +405G allele is associated with higher VEGF production than the +405C allele. In contrast, Awata *et al* (2005) reported that +405C allele correlated with higher VEGF production than the +405G allele. Furthermore, the −2578C allele may be associated with increased VEGF expression compared with the +2578A allele (Shahbazi *et al*, 2002).

Genetic polymorphisms involving genes such as *VEGFA*, may serve as prognostic markers in patients with AML (Illmer *et al*, 2002; Kim *et al*, 2006; Monzo *et al*, 2006). Monzo *et al* (2006) reported the results of a genomic polymorphism study in intermediate-risk patients with AML; multiple genes including *VEGFA* +405G>C (−634 from translation start site; rs833061) and Ex7-913A>G genotype (Monzo *et al*, 2006). The *VEGFA* +405 genotype may possibly be associated with risk of relapse in intermediate-risk patients with AML ($P = 0{\cdot}06$). In the current study, we examined three other *VEGFA* loci as potential prognostic markers in AML [i.e. −2578C>A (rs699947), −460T>C (rs833061) and +936 C>T (rs3025039)], in addition to the +405G>C genotype. The +936C>T genotype could discriminate AML patients with inferior LFS, EFS and OS.

As LD appears to be highly structured into conserved blocks of sequence that are separated by recombination hot spots, (Daly *et al*, 2001) determination of recombination hot spots is required to generate appropriate and relevant haplotypes in polymorphism studies. The Haploview program provided us with a graphical representation of LDs among VEGF gene loci (Fig 2) (Barrett *et al*, 2005). We selected three gene loci (i.e. −2578/−460/+405) based on D′s to perform the subsequent haplotype analyses. Previous studies have usually generated a *VEGFA* haplotype consisting of a gene locus in the promoter or 5′-untranslated region together with the +936C>T gene locus in 3′-untranslated region. However, the distance between these two loci is fairly large (*c.* 14–16 K base pairs). Moreover, our study indicates that the D′ between the +936C>T genotype

Table IV. Multivariate survival analysis.

Prognostic factors	Univariate		Multivariate	
Induction failure, 1st chemotherapy	*P*-value	OR [95% CI]	*P*-value	OR [95% CI]
+936 CC genotype	0·19	0·58 [0·26–1·31]	NS	–
CTG haplotype	0·15	0·55 [0·24–1·24]	NS	–
Unfavourable cytogenetics	0·04	2·76 [1·04–7·33]	0·02	3·89 [1·30–11·63]
High WBC count (≥50 × 109/l)	0·01	3·33 [1·33–8·16]	0·003	4·90 [1·74–13·77]
Older patient (≥50 years)	0·08	2·18 [0·91–5·24]	NS	–
High LDH (≥1000 IU/l)	0·3	1·58 [0·69–3·61]	NS	–
Leukaemia free survival	*P*-value	HR [95% CI]	*P*-value	HR [95% CI]
High risk VEGFA polymorphism marker*	0·003	2·21 [1·28–3·80]	0·04	1·84 [1·03–3·27]
Unfavourable cytogenetics	0·003	2·46 [1·32–4·59]	0·03	2·02 [1·05–3·86]
Older patient (≥50 years)	0·1	1·62 [0·87–3·03]	NS	–
Without SCT	0·3	1·37 [0·77–2·44]	NS	–
High WBC count (≥50 × 109/l)	0·4	0·71 [0·32–1·58]	NS	–
Event free survival	*P*-value	HR [95% CI]	*P*-value	HR [95% CI]
High risk VEGFA polymorphism marker*	0·01	1·90 [1·13–3·18]	NS	–
Unfavourable cytogenetics	0·001	2·58 [1·43–4·68]	0·01	2·20 [1·19–4·10]
Older patient (≥50 years)	0·1	1·52 [0·86–2·67]	NS	–
Without SCT	0·9	1·01 [0·61–1·68]	NS	–
High WBC count (≥50 × 109/l)	0·3	1·38 [0·73–2·61]	NS	–
Overall survival	*P*-value	HR [95% CI]	*P*-value	HR [95% CI]
High risk VEGFA polymorphism marker*	0·005	2·11 [1·23–3·60]	0·05	1·82 [1·01–3·28]
Unfavourable cytogenetics	0·002	2·58 [1·41–4·73]	0·03	1·98 [1·07–3·69]
Older patient (≥50 years)	0·03	1·89 [1·07–3·31]	NS	-
Without SCT	0·09	1·57 [0·93–264]	NS	-
High WBC count (≥50 × 109/l)	0·08	1·71 [0·93–3·13]	0·01	2·26 [1·19–4·29]

OR, Odds ratio; 95% CI, 95% confidence interval; WBC, white blood cells; LDH, lactate dehydrogenase; VEGF, vascular endothelial growth factor; HR, hazard ratio; SCT, stem cell transplantation; NS, not significant.

*By dominant model for VEGFA polymorphism marker (intermediate/low-risk group as reference *versus* high-risk group) based on the 936C>T genotype and CTG haplotype for −2578, −460 and +405.

and other loci was very weak (<0·5) (Fig 3). Therefore, the *VEGFA* haplotype used in our study excluded the +936C>T genotype. In our study, the CTG haplotype at loci −2578/−460/+405 was found to be associated with poor outcome. Risk classification using the *VEGFA* polymorphism marker (based on +936C>T genotype and the CTG haplotype), was able to discriminate different prognostic group in patients with AML (Fig 3 and Table IV). Moreover, an additive interaction between the +936C<T genotype and the CTG haplotype was observed. Therefore, these polymorphisms (i.e. +936C<T genotype and the CTG haplotype at loci −2578/−460/+405) may be useful prognostic markers in patients with AML.

In the present study, the *VEGFA* SNPs could not predict the induction failure including early TRM or failure to achieve CR, but could reflect survival of AML patients. The high-risk patients by *VEGFA* scoring showed significantly lower LFS and OS after adjusting other clinical factors in Cox's proportional hazard model, but not for EFS. Further study is strongly warranted to reach a clear conclusion on this issue using larger, more homogeneous group of AML patients.

Reports have been inconsistent as to the functional role of each *VEGFA* genotype (Renner *et al*, 2000; Krippl *et al*, 2003; Awata *et al*, 2005). The +935T allele in the +936C>T genotype had been associated with lower VEGF production in a Caucasians (Renner *et al*, 2000; Krippl *et al*, 2003) whereas, the +936C>T genotype did not correlate with VEGF levels in the Japanese population (Awata *et al*, 2005). These differences may be due to different functional role of the *VEGFA* polymorphism in different ethnic groups. Different levels of the VEGF expression by the *VEGFA* polymorphism may be due to: (i) loss of a potential binding site for the transcription factor AP-4 with the C-to-T transition; (ii) linkage disequilibrium of this polymorphism with another, yet to be identified, polymorphism elsewhere; and (iii) modification of the mRNA structure with the C-to-T (Renner *et al*, 2000).

Although we did not perform a promoter assay to evaluate the functional role of *VEGFA* haplotype, Lee *et al*, (2005) reported that Korean patients with the TG haplotype at loci −460/+405 appear to have a higher risk of lung cancer, suggesting that polymorphism of the *VEGFA* may contribute to an inherited predisposition to lung cancer. In their study, Lee *et al* (2005) proposed possible association of the TG haplotype at loci −460/+405 with higher production of VEGF.

In conclusion, the current study indicates that (i) the *VEGFA* +936 C>T genotype and the CTG haplotype correlated significantly with prognosis in patients with AML, and (ii) a strategy evaluating multiple polymorphisms in certain genes, such as the *VEGFA* polymorphism marker, could predict the prognosis in patients with AML.

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Supplementary material

The following supplementary material is available for this article online:

Fig S1. Summary of patients' flow during study period

Table SI. Comparison of genotype frequency in VEGF polymorphism according to the ethnicity

The material is available as part of the online article from:http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2141.2007.12345.x (This link will take you to the article abstract).

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