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Host genetic variants of *ABCB1* and *IL15* influence treatment outcome in paediatric acute lymphoblastic leukaemia

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Background: Host germline variations and their potential prognostic importance is an emerging area of interest in paediatric ALL.

Methods: We investigated the associations between 20 germline variations and various clinical end points in 463 children with ALL.

Results: After adjusting for known prognostic factors, variants in two genes were found to be independently associated with poorer EFS: *ABCB1* T/T at either 2677 (rs2032582) or 3435 (rs1045642) position ($P=0.003$) and *IL15* 67276493G/G (rs17015014; $P=0.022$). These variants showed a strong additive effect affecting outcome ($P<0.001$), whereby patients with both risk genotypes had the worst EFS ($P=0.001$), even after adjusting for MRD levels at the end of remission induction. The adverse effect of *ABCB1* T/T genotypes was most pronounced in patients with favourable cytogenetics ($P=0.011$) while the *IL15* 67276493G/G genotype mainly affected patients without common chromosomal abnormalities ($P=0.022$). In both cytogenetic subgroups, increasing number of such risk genotypes still predicted worsening outcome ($P<0.001$ and $=0.009$, respectively).

Conclusion: These results point to the prognostic importance of host genetic variants, although the specific mechanisms remain unclarified. Inclusion of *ABCB1* and *IL15* variants may help improve risk assignment strategies in paediatric ALL.

The successful incorporation of risk-associated factors in contemporary treatment regimens has rendered paediatric acute lymphoblastic leukaemia (ALL) to be one of the most curable forms of cancer, with cure rates exceeding 80% in developed countries (Pui *et al*, 2011). Presenting parameters, such as age, leukocyte counts and cytogenetics are widely used (Schultz *et al*, 2007). In addition, early response to therapy measured by minimal residual disease (MRD) levels after initial remission induction therapy is an independent predictor of the eventual outcome and is increasingly used for risk assignment in many contemporary protocols (Pui *et al*, 2009; Conter *et al*, 2010; Yeoh *et al*, 2012).

Clinically, inter-patient variations in treatment response are seen even in the same leukaemia subtype. For instance, up to 28% of patients with poor prognosis *t*(9;22)/*BCR-ABL1*-positive ALL can be cured (Arico *et al*, 2000), whereas up to 15% of patients with good-prognosis *t*(12;21)/*ETV6-RUNX1* fusion or hyperdiploidy (>50 chromosomes) relapse on a contemporary treatment protocol (Pui *et al*, 2009; Pui *et al*, 2011; Yeoh *et al*, 2012).

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Host-related factors may account for a significant proportion of inter-patient variation in treatment response and toxicity. The *polymorphic thiopurine methyltransferase* (*TPMT*) gene is a well-known example that affects 6-mercaptopurine (6-MP) metabolism (Relling *et al*, 1999; McLeod *et al*, 2000; Stanulla *et al*, 2005a), a drug widely used in treatment of ALL.

Paediatric ALL protocols use multi-drug combinations, which may be affected by polymorphisms participating in the transport, metabolism as well as detoxification of the individual drugs (Krajinovic *et al*, 2002; de Jonge *et al*, 2005; Rocha *et al*, 2005; Costea *et al*, 2006; Cunningham and Aplenc, 2007; Davidsen *et al*, 2008; Xu *et al*, 2012). However, the impact of such genomic variations on treatment outcome, especially in relation to conventional risk assignment strategies, remains to be fully elucidated. Using a candidate gene approach targeting 20 germline polymorphisms in 11 genes implicated in treatment response in paediatric ALL, we evaluated their influence on treatment outcome in 463 children enrolled in the Malaysia-Singapore ALL 2003 study. The selected candidates (Supplementary Table S1) have been reported either to influence the metabolism of the standard chemotherapy drugs used in treatment of ALL (e.g. *MTHFR* 677C>T (de Jonge *et al*, 2005) and *ABCB1* 3435C>T (Jamroziak *et al*, 2004)) or affect MRD level at the end of induction (e.g. *CCR5* 246A>G (Davies *et al*, 2008) and *IL15* SNPs (Yang *et al*, 2009)), or event-free survival (EFS, e.g. *GSTM1* null (Rocha *et al*, 2005) and *NQO1* 609C>T (Krajinovic *et al*, 2002).

MATERIALS AND METHODS

Patients and samples. We studied a total of 463 consecutive patients who were enrolled from July 2002 to August 2009 (median follow-up of 5.7 years upon data analyses) in the Malaysia-Singapore (Ma-Spore) ALL 2003 study from four participating centers in Singapore and Malaysia (Yeoh *et al*, 2012). This smaller cohort has similar EFS (Supplementary Figure S1) as well as demographic composition (data not shown) compared with the entire Ma-Spore ALL patient group. Genotyping assays were carried out in patients who had sufficient DNA. The patients are primarily comprised of Chinese, Malays and Indians, according to self-reported ethnicity. The treatment protocol was a modification of the ALL IC-BFM 2002 backbone (Fronkova *et al*, 2008) with additional CCG-augmented BFM regimen for high-risk patients (Nachman *et al*, 1997). Risk assignment was based on clinical- and cytogenetic-presenting features as well as early response to therapy as determined by prednisolone response and molecular MRD at the end of remission induction and consolidation (Supplementary Table S2).

Mononuclear cells were separated and collected from bone marrow aspirates or peripheral blood at diagnosis and after remission, using a Ficoll-Paque (GE Healthcare, Little Chalfont, UK) density gradient centrifugation. DNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendation.

The study was approved by the local institutional review boards at all participating institutions and informed consent was obtained from patients or their parents in accordance with the Declaration of Helsinki.

Cytogenetic subgroup, MRD, and genotype. Morphological examination and immunophenotyping were performed in all patients. Karyotyping, DNA index, and oncogene fusion screening by reverse transcription PCR were performed in National University Hospital or KK Women's and Children's Hospital in Singapore. For the present study, *t*(9;22)/*BCR-ABL1*, *t*(11q23)/*MLL* rearrangements or hypodiploid ALL (modal chromosomes <45 or DNA index <1.0) were classified as unfavourable cytogenetic subgroup, while *t*(12;21)/*ETV6-RUNX1*, *t*(1:19)/*TCF-PBX1* or hyperdiploid ALL (modal chromosomes >50 or DNA index ⩾1.16) were classified as a favourable subgroup.

For MRD monitoring, patient-specific, leukaemia-associated markers targeting the *IgH*, *TCRδ*, and *IgK-Kde* rearrangements were quantitated using BIOMED-II primers and protocols (McClure *et al*, 2006; Fronkova *et al*, 2008), and results were interpreted according to the criteria of the EuroMRD Study Group (Pongers-Willemse *et al*, 1999; van der Velden *et al*, 2006).

GSTM1/*T1* present/null and *TYMS* 28-bp enhancer repeat (rs34743033) were visualised on agarose gel directly after conventional PCR. Genotyping assays for other loci were performed using in-house allele-specific primer extension by real-time PCR. A qualified genotyping result must satisfy the following criteria: (a) at least one allele signal appears before the cycle threshold (CT) of 25; (b) for a homozygous call, CT difference between two allele signals exceeds four; and (c) for a heterozygous call, CT difference should be less than one. All bi-allelic loci studied were in Hardy–Weinberg equilibrium in each ethnic group (not applicable for *GSTM1*/*T1* loci).

Statistical analyses. Homozygosity of the major allele in the single-nucleotide polymorphism (SNP) was used as the reference genotype unless otherwise specified. For each SNP, genotype was pooled as a dichotomous variable if applicable, and tested under both dominant and recessive models (1 degree of freedom). The grouping that yielded the smaller *P*-values was retained. To control for multiple testing while maintaining sufficient statistical power, we applied Benjamini–Hochberg Step-up FDR-controlling procedure for the results of preliminary test on individual SNP. Only the candidates with adjusted *P*-values of ⩽0.05 in multivariate tests were selected for further analyses. Subsequent investigation on the associations between shortlisted SNPs and various clinical end points was exploratory, where nominal *P*-values of ⩽0.05 were considered significant.

Patients who abandoned treatment for more than six continuous weeks were excluded from all analyses provided that no event occurred before the abandonment. The EFS and OS were estimated by both Kaplan–Meier (P_{KM} by log rank test) and Cox regression (P_{COX} by proportional hazards model) using PASW Statistics 18.0 (IBM, New York, NY, USA). The time-to-event was calculated from diagnosis until the occurrence of resistance (morphological blast count ⩾5% at the end of remission induction), relapse (at any site), or death (from any cause), whichever occurred first, in EFS analysis ($N=74$; the time-to-event for resistance and induction death was registered as 1 day), or until death in OS analysis ($N=39$). For patients in continuous complete remission (CCR), survival times were censored at the date of last follow-up or until 31 December 2012. Cumulative incidence of relapse (including resistance; $N=51$) was estimated in both univariate (P_G by Gray's test) and multivariate analyses (P_{FG} by Fine and Gray hazards model) using R (version 3.0.1) packages according to published guidelines (Scrucca *et al*, 2007, 2010), where death was considered as a competing event. Clinical-presenting features, that is, the race, sex, lineage, NCI risk group (the risk for infantile cases was set to 'high' in statistical analyses), and cytogenetic subgroup, were used as co-variables in all multivariate analyses. The genotypes were examined together with clinical features in a full model (that is, non-stepwise mode). The particular genotypes that were associated with poorer EFS (termed as risk genotypes) were also tested for their additive effect on EFS, with an increasing number of such risk genotypes. When appropriate, Day 33 (end-of-induction) MRD was further added in the regression model to test the robustness of significant results discovered.

To investigate potential sampling bias, patients enrolled were split into two halves according to the time of enrolment at each

participating center, and the additive effect of risk genotypes was re-examined. In addition, as a validation we performed re-sampling to generate 100 test sets each comprising 50% of events and 50% of continuous complete remission randomly selected from the original cohort and evaluated the additive effect of risk genotypes in each set. In both scenarios, clinical co-variables were included in analyses.

Binary logistic regression was used to identify the genotypes associated with failure to achieve complete remission (that is, resistance or induction death; $N=30$) and treatment-related mortality (defined as the death during remission induction or in complete remission without prior leukemic relapse; $N=23$) after adjusting for clinical and cytogenetic features. The genotype frequencies in different categorical groups were compared by using the Pearson Chi-square test.

Haplotype frequencies of *ABCB1* and *IL15* genes were calculated using Haploview (version 4.2) (Barrett *et al*, 2005).

RESULTS

Identification of polymorphisms of ABCB1 and IL15 as predictors of EFS. The clinical and cytogenetic features at presentation and the prevalence of alleles studied are summarised in Table 1, Supplementary Tables S3a and S3b, respectively. All bi-allelic loci were in Hardy–Weinberg equilibrium in each ethnic group (not applicable for *GSTM1/T1* loci). Of the presenting features examined, unfavourable cytogenetics was associated with a significantly poorer EFS ($P_{COX}<0.001$; Table 2), while NCI high-risk status had borderline significance ($P_{COX}=0.091$; Table 2); self-reported ethnicity, gender, and cell type were not significantly related to EFS. The complete genotype data as well as patient characteristics are available for research purposes upon request.

Among the 20 polymorphisms examined individually in preliminary test, polymorphisms in *ABCB1* (rs2032582 and rs1045642) and *IL15* (rs17015014) were found eligible for further analyses (adjusted $P=0.038$ and 0.048, respectively; Supplementary Table S4). They were subsequently analysed together in a Cox regression model adjusting for presenting clinical and cytogenetic features (Table 2). The homozygosity of either *ABCB1* 2677T/T or 3435T/T was still significantly related with a poorer EFS compared with other genotypes at these two loci ($P_{KM}=0.001$; $P_{COX}=0.003$, HR = 2.11, 95% CI = 1.29–3.45; Figure 1A). There was moderate linkage disequilibrium (LD) between 2677T and 3435T (details shown in later section). *IL15* 67276493G/G was also associated with significantly poorer EFS compared with 67276493G/C and C/C ($P_{KM}=0.050$; $P_{COX}=0.022$, HR = 1.84, 95% CI = 1.09–3.08; Figure 1B). Adjusting for MRD at the end of remission induction (Supplementary Figures S2 A–B) or excluding the cases of resistance from EFS analyses (Supplementary Table S5 and Supplementary Figures S3A–B) did not alter the significance of these polymorphisms identified.

The risk genotypes, that is, *ABCB1* T/T at 2677 or 3435 or both as well as *IL15* 67276493G/G, exhibited an additive effect ($P_{KM}<0.001$; $P_{COX}<0.001$, HR = 1.97, 95% CI = 1.38–2.82) demonstrating progressively poorer EFS in patients with an increasing number of risk genotypes (Table 2 and Figure 1C). Compared with the patients without any risk genotype, the hazard of adverse events raised 2.2 folds in patients with one risk genotype ($P_{KM}=0.005$; $P_{COX}=0.003$, HR = 2.20, 95% CI = 1.30–3.70) and 3.6 folds in those with two risk genotypes ($P_{KM}=0.002$; $P_{COX}=0.001$, HR = 3.64, 95% CI = 1.68–7.92). After adjusting for MRD at the end of remission induction (Supplementary Table S6 and Supplementary Figure S2C) or excluding the cases of resistance (Supplementary Table S7 and Supplementary Figure S3C), this additive effect remained significant. There was no

Table 1. Distribution of patients according to demographic and prognostic categories

Clinical feature	Frequency (%), $N=463$
Race	
Chinese	215 (46.4)
Malays	183 (39.5)
Indians and others[a]	65 (14.0)
Sex	
Male	259 (55.9)
Female	204 (44.1)
Age	
1 to 10 years	371 (80.1)
Younger than 1 or older than 10 years	92 (19.9)
WBC	
Below $50\times10^9\,l^{-1}$	343 (74.1)
Over $50\times10^9\,l^{-1}$	119 (25.7)
Unknown[b]	1 (0.2)
Lineage	
B cell	424 (91.6)
T cell	39 (8.4)
NCI risk group	
Standard	291 (62.9)
High[c]	171 (36.9)
Unknown[b]	1 (0.2)
Cytogenetic subtype (B-ALL only)	
Favourable	
t(12;21)/*ETV6-RUNX1*	86 (20.3)
t(1;19)/*TCF3-PBX1*	22 (5.2)
Hyperdiploidy	84 (19.8)
Unfavourable	
t(9;22)/*BCR-ABL1*	19 (4.5)
t(11q23)/*MLL* rearrangements	14 (3.3)
Hypodiploidy	5 (1.2)
Others[d]	191 (45.0)
Unknown[b]	3 (0.7)
Day 33 (end-of-induction) MRD	
Less than 10^{-4}	188 (40.6)
10^{-4} to 10^{-2}	174 (37.6)
More than 10^{-2}	41 (8.9)
Unknown[b]	60 (13.0)
Outcome (the 1st event occured)	
CCR	368 (79.5)
Abscondment[e]	21 (4.5)
Resistance	20 (4.3)
Relapse (any site) after CR	31 (6.7)
Treatment-related mortality	
During induction	10 (2.2)
After CR	13 (2.8)

Abbreviations: CCR = continuous complete remission; CR = complete remission; MRD = minimal residual disease.
[a]'Others' refer to a few of Caucasian, Vietnamese, Indonesian, and Philippines.
[b]Information is missing due to sample unavailability or unqualified assay.
[c]The risk for infantile cases was set to 'high' in statistical analyses.
[d]'Others' refer to B-ALL with normal karyotype or other uncommon abnormalities.
[e]Patients who abandoned treatment for more than six continuous weeks.

significant EFS difference between patients with only *ABCB1* risk genotype and those with only *IL15* risk genotype (data not shown).

To further investigate the impact of *ABCB1* 2677G>T/A/3435C>T and *IL15* 67276493G>C genotypes on a specific event, we examined their influence on the cumulative incidence of relapse (including resistance), failure to achieve complete remission as well as treatment-related mortality. Homozygosity of either *ABCB1* 2677T/T or 3435T/T significantly increased the risk of relapse in both univariate ($P_G = 0.001$; Supplementary Figure S4A) and multivariate models after adjusting for clinical and cytogenetic features ($P_{FG} = 0.011$, HR = 2.17, 95% CI = 1.19–3.94; Supplementary Table S8). The risk of relapse in patients with at least one T/T was 20.4% compared with 9.0% in those without any T/T. Taking the end-of-induction MRD level into account did not alter the significance ($P_{FG} = 0.027$, HR = 2.01, 95% CI = 1.08–3.73). The risk of relapse also became higher with increasing number of risk genotypes ($P_G < 0.001$; Supplementary Figure S4B; $P_{FG} = 0.011$, HR = 1.84, 95% CI = 1.15–2.93; Supplementary Table S8). The trend remained significant after adjusting for the end-of-induction MRD level ($P_{FG} = 0.026$, HR = 1.69, 95% CI = 1.07–2.68).

IL15 67276493G/G was associated with higher risk of failure to achieve complete remission when compared with G/C and C/C genotypes ($P = 0.009$, OR = 3.14, 95% CI = 1.34–7.36; Supplementary Table S9). G/G homozygosity also correlated with poorer overall survival ($P_{KM} = 0.050$; $P_{COX} = 0.005$, HR = 2.74, 95% CI = 1.35–5.58; Supplementary Table S10 and Supplementary Figure S5), primarily due to more than 3-fold higher risk of treatment-related mortality in patients with *IL15* 67276493G/G ($P = 0.015$, OR = 3.40, 95% CI = 1.27–9.11; Supplementary Table S11).

We also examined the associations of identified risk genotypes with known risk factors. Neither T/T homozygosity at *ABCB1* 2677 or 3435 nor *IL15* 67276493G/G was found to correlate with cytogenetics and MRD level after remission induction (Supplementary Table S12).

Table 2. The influence of *ABCB1* 2677G>T/A/3435C>T and *IL15* 67276493G>C genotypes on EFS for patients enrolled in the Malaysia-Singapore ALL 2003 Study

Variable	HR (95% CI)	*P*-value
NCI risk group		
Standard	Ref.	
High	1.56 (0.93–2.60)	0.091
Cytogenetic subgroup		<0.001
Favourable	Ref.	
Others	2.10 (1.13–3.89)	0.018
Unfavourable	6.48 (3.09–13.57)	<0.001
ABCB1, 2677G>T/A (rs2032582) and 3435C>T (rs1045642)		
Other genotypes	Ref.	
2677T/T or 3435T/T or both	2.11 (1.29–3.45)	0.003
IL15, 67276493G>C (rs17015014)		
G/C and C/C	Ref.	
G/G	1.84 (1.09–3.08)	0.022
NCI risk group		
Standard	Ref.	
High	1.56 (0.93–2.60)	0.090
Cytogenetic subgroup		<0.001
Favourable	Ref.	
Others	2.12 (1.14–3.92)	0.017
Unfavourable	6.61 (3.18–13.74)	<0.001
The number of risk genotypes[a]		0.001
0	Ref.	
1	2.20 (1.30–3.70)	0.003
2	3.64 (1.68–7.92)	0.001
Additive effect	1.97 (1.38–2.82)	<0.001

Abbreviations: CI = confidence interval; HR = hazards ratio. Regression was adjusted for patients' race, sex, lineage, NCI risk group, and cytogenetic subgroup. Only variables with nominal *P* of <0.1 are shown in the table. A total of 413 cases with complete information were eligible for this analysis.
[a]Risk genotypes refer to *ABCB1* T/T at either 2677 or 3435 position and *IL15* 67276493G/G.

Prognostic significance of ABCB1 and IL15 in patient subgroups. In patients with favourable cytogenetics, T/T homozygosity at either *ABCB1* 2677 or 3435 position was associated with a significantly poorer EFS ($P_{KM} = 0.011$; $P_{COX} = 0.011$, HR = 4.02, 95% CI = 1.38–11.75; Figure 2A). The EFS in patients carrying *ABCB1* 2677T/T or 3435T/T was 78.2% ± 7.6%, compared with 94.4% ± 1.9% in those carrying other genotypes. Such an association became even stronger after adjusting for MRD level ($P_{COX} < 0.001$, HR = 19.26, 95% CI = 4.43–83.72; 80.6% ± 8.6% *vs* 98.9% ± 0.8%). In contrast, the impact of *IL15* 67276493 genotype was more pronounced in patients who do not carry chromosomal abnormalities of known prognosis, showing a significantly worse EFS in G/G carriers than the others ($P_{KM} = 0.038$; $P_{COX} = 0.022$, HR = 2.20, 95% CI = 1.12–4.30; Figure 2C), with EFS of 73.9% ± 5.6% for G/G carriers and 86.7% ± 2.8% for G/C and C/C carriers, respectively. Adjustment for MRD level did not

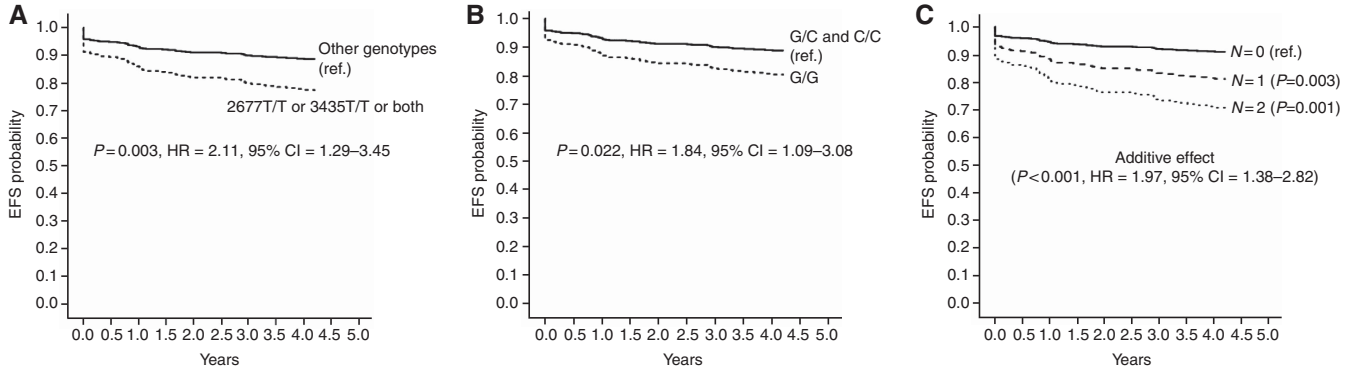

Figure 1. Plots of EFS probability for 413 patients enrolled in the Malaysia-Singapore ALL 2003 Study. Regression was adjusted for patients' race, sex, lineage, NCI risk group, and cytogenetic subgroup. The patients were stratified by respective genotypes of (**A**) *ABCB1* 2677G>T/A/3435C>T, (**B**) *IL15* 67276493G>C, and by (**C**) the number of risk genotypes.

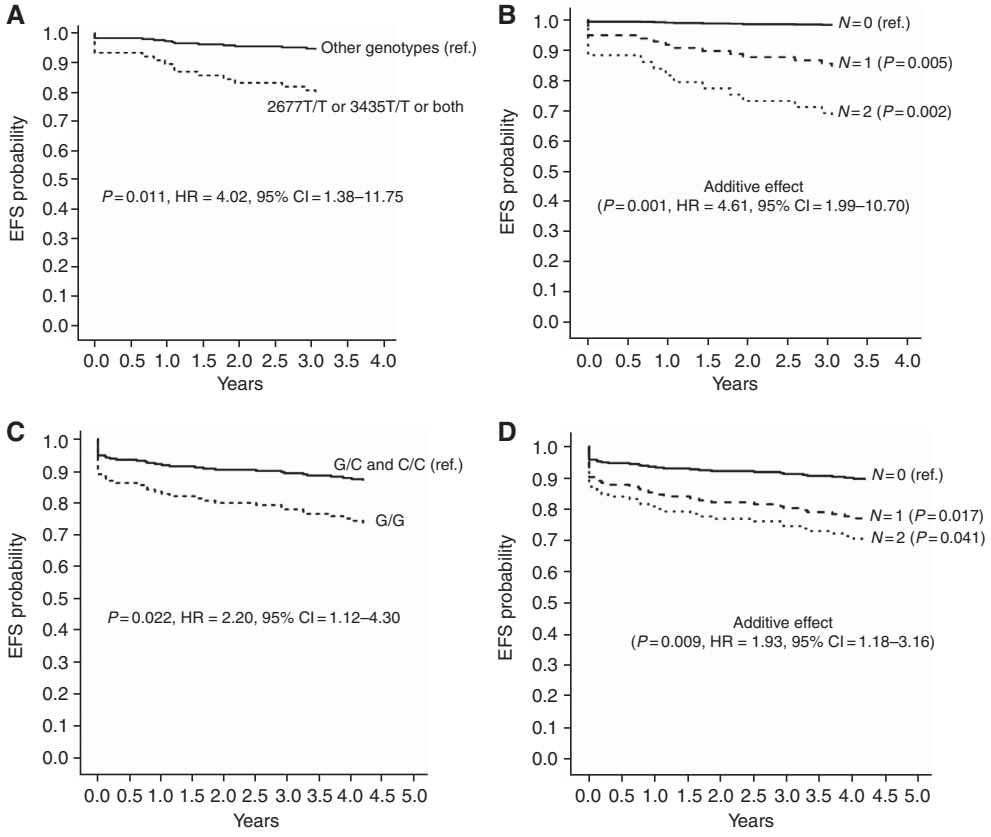

Figure 2. Plots of EFS probability for patients without high-risk cytogenetic lesions. Regression was adjusted for patients' race, sex, lineage, and NCI risk group. The patients were stratified by (**A**) *ABCB1* 2677G > T/A/3435C > T ($N = 184$) and (**B**) the number of risk genotypes ($N = 174$) in favourable cytogenetic subgroup, and (**C**) *IL15* 67276493G > C ($N = 209$) and (**D**) the number of risk genotypes in the subgroup without common chromosomal abnormalities ($N = 208$).

moderate the significance ($P_{COX} = 0.022$, HR = 2.30, 95% CI = 1.13–4.70; 78.6% ± 5.4% *vs* 90.2% ± 2.7%).

In both cytogenetic subgroups, the additive effect of risk genotypes was found ($P_{KM} < 0.001$ and $P_{COX} < 0.001$, Figure 2B; and $P_{KM} = 0.013$ and $P_{COX} = 0.009$, Figure 2D) and remained significant after adjusting for MRD level ($P_{COX} < 0.001$ and $= 0.008$ respectively). In addition, we also examined the influence of risk genotypes in the two major ethnic groups, the Chinese and Malays, respectively. In both races, the additive effect of risk genotypes was also found ($P_{KM} < 0.001$ and $P_{COX} = 0.001$ in the Chinese, Supplementary Figure S6A; $P_{KM} = 0.075$ and $P_{COX} = 0.036$ in the Malays, Supplementary Figure S6B) and remained significant after adjusting for MRD level ($P_{COX} = 0.003$ and $= 0.006$, respectively).

Validation tests. Patients were split into two halves based on the time of enrolment at each participating centre. In both cohorts, the additive effect of T/T homozygosity at *ABCB1* 2677 or 3435 and *IL15* 67276493G/G still remained significant (Supplementary Figure S7A, C), even after adjusting for MRD levels at the end of induction (Supplementary Figure S7B, D). In addition, we generated 100 random test sets by re-sampling. Of these 100 test scenarios, the significance was achieved for 86 test sets (Supplementary Figure S8A), and all results consistently showed that increasing number of risk genotypes corresponded to a poorer EFS (i.e., HR > 1; Supplementary Figure S8B).

Linkage disequilibrium. As multiple loci in *ABCB1* and *IL15* were genotyped, we calculated the linkage disequilibrium (LD) among different alleles. Two loci, *ABCB1* 2677 (rs2032582) and *ABCB1* 3435 (rs1045642), were in a moderate LD block. The LD was slightly stronger in the Chinese ($r^2 = 0.58$ for entire patient cohort and $r^2 = 0.74$ for the Chinese; Supplementary Figure S9), with two major haplotypes, 2677G-3435C (52.3%) and 2677T-3435T (36.2%). *IL15* 67276493G > C (rs17015014) was not in LD with any other studied loci of the *IL15* gene (Supplementary Figure S10).

DISCUSSION

In childhood ALL, there is significant inter-individual variation in response to chemotherapy, which is not fully predicted by presenting features such as age and leukaemic cytogenetics. Newly described markers such as *IKZF1* and *CRLF2* have demonstrated prognostic impact in many studies (Mullighan *et al*, 2009; Buitenkamp *et al*, 2012; Chen *et al*, 2012; Feng and Tang, 2013), but their values still remain inconclusive in the Malaysia-Singapore ALL 2003 study according to our pilot investigations (data not shown). Here we showed that host germline variants of *ABCB1* and *IL15* genes are significant predictors of treatment outcome, suggesting that the characterisation of these genes' variants at diagnosis could contribute to risk-stratification strategies. In our study, the *ABCB1* 2677T/T or 3435T/T genotypes were associated with an increased risk of relapse/resistance. *ABCB1* 2677G > T/A introduces an Ala-to-Ser/Thr change in exon 21 while 3435C > T is a synonymous polymorphism in exon 26, which affects transport by altering substrate specificity (Kimchi-Sarfaty *et al*, 2007). Various studies have shown that not all synonymous SNPs are considered silent; they can cause changes in protein expression, conformation or function and are increasingly

implicated in human disease risk and other complex traits (Sauna and Kimchi-Sarfaty, 2011).

Large studies in patients with acute myocardial infarction on oral anti-coagulant clopidogrel, a substrate of P-glycoprotein (P-gp), reported lower drug levels and poorer cardiovascular outcomes in patients with *ABCB1* 3435T/T homozygotes (Simon *et al*, 2009; Mega *et al*, 2010). Oral dexamethasone, used extensively in paediatric ALL treatment including our Ma-Spore ALL 2003 protocol, is also a known substrate for P-gp. Patients with *ABCB1* 2677T/T or 3435T/T may have lower systemic dexamethasone exposure due to higher *ABCB1* expression on the apical villi of enterocytes (Nakamura *et al*, 2002), causing enhanced extrusion of drugs into the intestinal lumen. Supporting this hypothesis, Yang *et al* (2012) recently reported two *ABCB1* variants (rs10264856 and rs4728709) that affected oral dexamethasone clearance and were associated with increased risk of relapse in the St Jude and COG cohorts, although we did not observe any LD between rs10264856/rs4728709 and rs2032582/rs1045642 in our study as well as the data from 1000 Genomes Project (the maximum r^2 is only 0.14). Taken together with our findings on *ABCB1* 2677 and 3435, these strongly suggest the importance of *ABCB1* variants in treatment of childhood ALL.

However, Jamroziak *et al* (2004) and Stanulla *et al* (2005b) previously reported lower EFS ($N=111$) and higher CNS relapse ($N=34$), respectively, in *ABCB1* 3435C/C carriers treated on the BFM-ALL 86/90/95 protocols. Differences in the type of steroid used during induction therapy (prednisolone in the BFM-ALL 86/90/95 compared with dexamethasone in Ma-Spore ALL 2003) may account for this observed difference. In addition, the lower frequency of *ABCB1* 2677T and 3435T alleles among African Americans (10.0% and 20.2%, respectively) (Kroetz *et al*, 2003) compared with our studied population (43.6% and 40.5%, respectively) may mask the effect of *ABCB1* variants on treatment outcome in the previously reported St Jude Total XIIIB protocol (Rocha *et al*, 2005).

The manner that *ABCB1* variants affect the P-gp expression on the apical villi of enterocytes remains unresolved (Lin and Yamazaki, 2003; Leschziner *et al*, 2007; Hodges *et al*, 2011; Wolf *et al*, 2011). Nakamura *et al* (2002) reported that healthy Japanese subjects with 3435T/T had significantly higher duodenal *ABCB1* mRNA expression and lower digoxin plasma level compared with C/C or C/T genotype carriers, while Hoffmeyer *et al* (2000) and Efferth *et al* (2003) disagreed with such a correlation. The effect of *ABCB1* polymorphisms on P-gp activity is likely dependent on the substrates as well as the tissues (or cell types) studied, thus becoming a confounding factor when studying P-gp genotype–phenotype relationship. Further studies in correlating dexamethasone clearance and *ABCB1* polymorphisms are required.

Considering the drug efflux capacity of P-gp, it is rational to speculate that the impact of *ABCB1* genotype on the risk of relapse/resistance originates from its effect on MRD level. Although not statistically significant in our patients, Day 33 MRD high risk (i.e., $\geqslant 10^{-2}$) tended to be enriched in the *ABCB1* T/T group compared with the others (14.9% *vs* 8.8%, $P=0.088$, Supplementary Table S12). Published data on the relationship between *ABCB1* gene and MRD are controversial. For instance, an earlier report showed that the median *ABCB1* expression at diagnosis of childhood ALL were roughly comparable between the MRD-positive group and MRD-negative group at the end of induction therapy (Fedasenka *et al*, 2008). This finding was challenged by a very recent study reporting that mRNA expression of *ABCB1* higher than a certain cutoff would raise the risk of MRD positivity by 12-fold after 1-year treatment in children with ALL (Rahgozar *et al*, 2014). Such discrepancies are likely due to (but not limited to) the diverse grouping criteria, the quantitation methods applied, the time points investigated, and sampled populations in different studies. As the determinants on early treatment response are known to be multifactorial, a clear correlation between *ABCB1* genotype and MRD level may not be expected.

The *IL15* 67276493G/G genotype was associated with a poorer EFS and OS in our study, and carried a higher probability of treatment-related mortality. IL15 regulates T- and natural killer cell activation and proliferation, having central roles in cell-mediated immunity against microbes (Yoshikai and Nishimura, 2000). Administration of exogenous IL15 in animal models causes severe but reversible neutropenia due to redistribution of neutrophils out of the circulation (Berger *et al*, 2009; Waldmann *et al*, 2011). This SNP resides in the 3′UTR region of the gene, which exerts a negative regulatory effect on the expression of *IL15*; polymorphism at the 3′UTR may reduce this negative regulation, resulting in an enhanced expression. It is noteworthy that genetic variants in *IL15*, albeit different *IL15* SNPs, have been previously inferred to significantly correlate with the MRD level at the end of induction (Yang *et al*, 2009), a strong surrogate marker to predict treatment outcome.

ABCB1 3435T/T or 2677T/T and *IL15* 67276493G/G showed a surprising additive effect in predicting a poorer EFS probability, even after adjusting for MRD levels at the end of remission induction. P-glycoprotein mediates in transmembrane transport of cytokines. It has been reported that *ABCB1* 2677T/T–3435T/T are associated with lower secretion of cytokines in PHA-stimulated lymphocytes (Pawlik *et al*, 2005). Conversely, in HIV-infected individuals, P-glycoprotein expression and function are decreased and restored only after IL15 stimulation (Chang *et al*, 2000). Therefore, we speculate that *ABCB1* 2677T/T or 3435T/T may alter secretion of IL15, which affects cellular immune responses and treatment outcome consequently.

In summary, host genetic variations in *ABCB1* and *IL15* genes influence treatment outcomes in paediatric ALL in this predominantly Asian population treated on the Ma-Spore ALL 2003. Inclusion of host variants in *ABCB1* and *IL15* genes may help future risk assignment strategies and deserves further study in other treatment cohorts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

YL performed the data analyses; SKYK initiated the study and coordinated genotyping assays; HA coordinated the Ma-Spore ALL 2003 study at University of Malaya Cancer Research Institute, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; AMIO performed genotyping assays; HPL coordinated the Ma-Spore ALL 2003 study at Sime Darby Medical Centre, Subang Jaya, Malaysia; AMT coordinated the Ma-Spore ALL 2003 study at KK Women's and Children's Hospital, Singapore; TCQ

coordinated the Ma-Spore ALL 2003 study at National University Hospital, Singapore; AEJY, initiated the study, performed analyses, and wrote the manuscript with YL and SKYK and the input of all other authors.

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