Association between CYP2B6 c.516G>T variant and acute leukaemia

A protocol for systematic review and meta-analysis

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Abstract
Background: Acute leukemia (AL) is a kind of malignant tumor of hematopoietic system. A number of studies have suggested that Single Nucleotide Polymorphisms are significantly associated with risk of AL. Present study performs meta-analysis to evaluate the association between CYP2B6 c.516G>T variant and AL risk.

Methods: Databases including PubMed, EMBASE, Chinese National Knowledge Infrastructure (CNKI), and Wanfang were searched for literatures to September 30, 2019, both in English and Chinese. Relative risk and its 95% confidence intervals were used to assess the associations. Statistical analyses of this meta-analysis were conducted by using STATA 13.0. software.

Results: A total of 7 studies, including 1038 cases and 1648 controls, were analyzed. Our results indicated that CYP2B6 c.516G>T variant was significantly related to an increased the risk of AL under dominant model, recessive model, homozygote model, and allelic model. In addition, subgroup analyses were also performed by disease classification, country, and study design. No significant associations were obtained between CYP2B6 c.516G>T variant and the risk of AL under the recessive model in the design of hospital-based (relative risk = 0.98; 95% confidence interval: 0.95–1.01; P = 0.118).

Conclusion: Our meta-analysis indicated that the CYP2B6 variant is significantly associated with AL risk, in which CYP2B6 c.516G>T is related to an increased risk of AL.

Abbreviations: AL = acute leukemia, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, CIs = confidence intervals, CNKI = China National Knowledge Infrastructure, HWE = Hardy-Weinberg equilibrium, RR = relative risk.

Keywords: cytochrome P-450 CYP2B6, leukemia, meta-analysis

1. Introduction
Acute leukemia (AL) is a kind of malignant tumor of hematopoietic system characterized by the enhanced self-renewal and proliferation as well as inhibited differentiation and apoptosis of leukemia cells.[1] The malignant of hematopoietic cells leads to aggregation of leukemia cells and subsequential extensive infiltration into bone marrow, liver, spleen, lymph nodes and other organs, which eventually leads to bleeding, anemia infection and other phenomena.[2-4] According to the involved cell types, AL can be divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).[5] ALL originated from the malignant clonal disease of hematopoietic stem/progenitor cells, and its molecular mechanism has not been fully defined.[6] In the United States, about
5000 new cases of ALL are diagnosed each year, more than half of them in children.[7] ALL is the most common malignant tumor in pediatrics, accounting for more than 25% of cancer in children.[8] The peak incidence of all was 2 to 5 years’ old, slightly higher in men than in women, and the risk for white people was twice that of African Americans.[9,10] Genetic factors such as trisomy 21 (Down syndrome) have a 15-fold increased relative risk (RR).[11] Other inducing conditions include immune deficiency and chromosome breakage syndrome, and most of them cannot be found in these potential diseases. Epstein Barr virus infection is associated with a small number of mature B cells ALL.[12,13] Increasing evidences suggest that the environmental exposure, such as benzene exposure, formaldehyde exposure, ionizing radiation, and particulate matters increases the risk of ALL.[14-16]

AML is a group of heterogeneous diseases characterized by uncontrolled proliferation of myeloid precursor cells which gradually replace normal hematopoiesis of bone marrow.[17] Genetic changes in tumor clones lead to molecular cascade reactions, which in turn lead to abnormal proliferation and differentiation of malignant cells and inhibit normal hematopoiesis, but its molecular origin is still unclear.[18] The variant of cytochrome P450 enzyme has an important effect on biotransformation of chemicals, especially pre carcinogens.[19] CYP2B6 enzyme is widely distributed in macrophages, peripheral blood, lymphocytes, brain, liver, kidney, lung, small intestine, endometrium, and alveoli of bronchioles, and participates in the synthesis and metabolism of various endogenous and exogenous substances.[20,21] CYP2B6 gene is located in 19q12–13.2, with a total length of 27.1 kb, including 11 exons which encode 491 amino acids.[22] A number of variants have been found in CYP2B6 such as NM_000767.5:c.516G>T (CYP2B6 c.516G>T) variant which affects the activity of CYP2B6, and reduces the rate of human transformation of carcinogenic substances into inactive metabolites, which leads to the accumulation of carcinogenic substances, and a series of diseases.[23-26]

To date, many studies have been performed to detect the correlation between CYP2B6 c.516G>T variant and AL. However, the results of published studies are inconsistent and inconclusive, which may be attributed to differences in sample size and ethnic diversity of the population. Therefore, we carried out this meta-analysis to investigate the association between CYP2B6 variant and AL.

2. Materials and methods

This meta-analysis was performed on the basis of the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement.

2.1. Search strategy

PubMed, EMBASE, CNKI, and Wanfang databases were systematically searched for relevant studies until September 30, 2019. The search was limited to the studies published in English or Chinese with the combination of the keyword: “polymorphism” OR “polymorphisms” OR “variant” OR “mutation” OR “genotype” OR “allele” OR “SNP”) AND (“Cytochrome P-450 CYP2B6” OR “Cytochrome P450 CYP2B6” OR “P-450 CYP2B6, Cytochrome” OR “CYP1B6” OR “1,4-Cineole 2-exo-Monoxygenase” OR “1,4-Cineole 2 exo Monoxygenase” OR “2-exo-Monoxygenase, 1,4-Cineole” OR “Cytochrome P450 2B6” OR “P450 2B6, Cytochrome” OR “CYP2B6”) AND (“leukemia” OR “Leukemias” OR “Leucocythemia” OR “Leucocythaemias” OR “Leucocythemia” OR “Leucocystemia”).

2.2. Inclusion and exclusion criteria

Studies enrolled in this meta-analysis were screened according to the following criteria: CYP2B6 were investigated, case–control, genotype distribution were available in both cases and controls, publication in English or Chinese. The main exclusion criteria of this meta-analysis were as follow: obviously irrelevant studies, reviews or meta-analysis, duplicate publication in bi languages, not English or Chinese.

2.3. Data extraction and document quality evaluation

Two different individuals reviewed all eligible studies. The following data of articles were recorded: first author, year of publication, country and ethnicity of study, study design, genotyping method, the number of cases and controls, genotype/allele distribution in cases and controls, and P value of Hardy-Weinberg equilibrium (HWE). In addition, the methodological quality of studies was assessed using the Newcastle-Ottawa scale. The Newcastle-Ottawa scale scale scores vary from 0 to 9 points: studies with scores of 0 to 4 were considered low quality and 5 to 9 were considered high quality.

2.4. Statistical analysis

This meta-analysis was performed using STATA13 software (STATA Corp., College Station, TX). The association between CYP2B6 variant and AL were estimated using RR and its 95% confidence intervals (CIs). Heterogeneity was expressed using the $I^2$-based Cochrane Q test and $I^2$ index. A random-effects model was used when heterogeneity was observed in studies ($I^2 > 50\%$); otherwise, ORs were pooled by the fixed-effects model ($I^2 < 50\%$). HWE in the controls was calculated using the $\chi^2$ test and $P > .05$ was considered as consistent with HWE. Sensitivity analysis was used to estimate the stability of the results by omitting individual study sequentially. An estimate of publication bias was calculated using Begg rank correlation test and Egger linear regression test. A probability level of $P < .05$ was considered statistically significant.[27]

3. Results

3.1. 1. Search results and study characteristics

The selection process in this meta-analysis was shown in Figure 1. A total of 451 articles were identified in the initial search. After exclusion of irrelevant records by screening titles and abstracts, 9 articles were assessed for further evaluation. Finally, a total of 4 articles including 7 studies and 1038 cases and 1648 controls were enrolled in this meta-analysis, the main characteristics of enrolled studies were summarized in Table 1.[28-31] In all eligible studies, 3 studies were involved ALL and 4 were involved AML. Among the studies, 4 were conducted in Asians, and others were white. All the studies were considered to be high quality. Apart from that, the design of these studies was based on hospital or population. After HWE test, except Yu’s study, other studies were in accordance with HWE equilibrium ($P > .05$).
3.2. Meta-analysis results

This meta-analysis’ results of association between CYP2B6 c.516G>T variant and AL were summarized in Table 2. Our meta-analysis results showed that CYP2B6 c.516G>T variant was significantly related to an increased the risk of AL under four model (dominant model: RR = 0.77; 95% CI: 0.72–0.83; \( P = .000 \); recessive model: RR = 0.97; 95% CI: 0.95–0.99; \( P = .000 \); homozygote model: RR = 0.84; 95% CI: 0.80–0.89; \( P = .000 \); allelic model: RR = 0.88; 95% CI: 0.84–0.92; \( P = .000 \) (Fig. 2).

In subgroup analysis based on ALL, AML, white, Asian, and design of PB studies, there were significant associations under all models, but under the recessive model (TT vs GT + GG) in the design of HB studies, as Figure 3, no obvious associations between CYP2B6 c.516G>T variant and AL were found (RR = 0.98, 95% CI: 0.95–1.01; \( P = .118 \)).

3.3. Heterogeneity, publication bias and sensitivity analysis

As shown in Table 2, no significant heterogeneity was detected under each model (\( I^2 < 50\% \)). The Begg funnel plot did not find any obvious publication bias \( (P = .230) \) under Allelic model Fig. 4, but the Egger’s test shows the there are some publication bias in those studies \( (P = .028) \). With trim and fill method, there is no more study needed \( (k = 0) \), it means no publication bias.

Sensitivity analysis was performed by removing one single study from the studies for all models. The RRs and 95% CIs were not materially altered, suggesting this meta-analysis was robust and credible. From the fail-safe number method, >66 studies should change the conclusion. Sensitivity analysis showed that the omission of any individual study did not substantially influence the risk estimates, which supported the credibility and reliability of this meta-analysis.

Table 1

| Study ID      | Year | Racial descent | Source of control | Case | Control | Genotype distribution |
|---------------|------|----------------|-------------------|------|---------|-----------------------|
|               |      |                |                   |      |         | Case                  |
|               |      |                |                   |      |         | Control               |
| ALL           |      |                |                   |      |         |                       |
| Berköz and Yalin[28] | 2009 | White          | PB                | 44   | 100     | GG 18 26 0 67 33 0 0.048 |
| Yuan et al[29] | 2011 | Asian          | HB                | 96   | 348     | GT 55 36 5 258 83 7 0.914 |
| Yu[30]        | 2010 | Asian          | PB                | 45   | 161     | TT 25 13 7 124 25 12 0.000 |
| AML           |      |                |                   |      |         |                       |
| Berköz and Yalin[28] | 2009 | Caucasian      | PB                | 36   | 100     | GG 18 18 0 67 33 0 0.048 |
| Yuan et al[29] | 2011 | Asian          | HB                | 164  | 348     | GT 97 61 6 258 83 7 0.914 |
| Daraki et al[31] | 2014 | Caucasian      | PB                | 572  | 430     | TT 297 222 53 279 128 23 0.107 |
| Yu[30]        | 2010 | Asian          | PB                | 81   | 161     | HWE 47 22 12 124 25 12 0.000 |

ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, HB = hospital-based, HWE = Hardy-Weinberg equilibrium, PB = population-based.
4. Discussions

The molecular mechanism of AL (including ALL and AML) is not clear at present. Lan et al.\(^{[32]}\) found that the levels of single chromosome, trisomy, tetrasomy and SCA in 29 workers exposed to relatively high levels of formaldehyde were higher than those in 23 unexposed workers. The increase of these markers is common in acute myeloid leukemia.

Carlos-wallace et al.\(^{[33]}\) performed meta-analysis and uncovered a correlation between household benzene exposure and childhood leukemia. Related studies have shown that these adverse environmental factors are closely related to the occurrence of AL.

CYP2B6 enzyme can metabolize and eliminate the activity of some substances, and the decrease of its enzyme activity may result in the inactivation of harmful substances in the environment and excessive accumulation of toxic substances in the body.

Tsuchiya et al.\(^{[34]}\) found that patients with homozygous (TT) for a specific allele of the CYP2B6 gene have significantly higher concentrations of Efavirenz. Haas et al.\(^{[35]}\) found that the negative neurological response of Efavirenz drug in patients was related to the TT genotype, whereas Gounden et al.\(^{[36]}\) found the blood concentration of Efavirenz in patients with TT type was higher.

### Table 2

| Study group | Study | Dominant model TT + GT vs GG | Recessive model TT vs GT + GG | Homozygote model TT vs GG | Allelic model T vs G |
|-------------|-------|-----------------------------|-------------------------------|---------------------------|---------------------|
|             | RR (95% CI) | P (%) | RR (95% CI) | P (%) | RR (95% CI) | P (%) | RR (95% CI) | P (%) |
| Overall     | 0.77 (0.72–0.83) | 0.000 | 0.000 | 0.97 (0.95–0.99) | 0.002 | 0.000 | 0.84 (0.80–0.89) | 0.000 | 0.88 (0.84–0.92) | 0.000 |
| ALL         | 0.73 (0.64–0.85) | 0.000 | 0.000 | 0.96 (0.92–1.01) | 0.088 | 0.000 | 0.80 (0.70–0.92) | 0.001 | 16.9% | 0.85 (0.78–0.94) | 0.001 |
| AML         | 0.79 (0.73–0.85) | 0.000 | 0.000 | 0.97 (0.94–0.99) | 0.020 | 21.9% | 0.85 (0.80–0.91) | 0.000 | 0.89 (0.84–0.93) | 0.000 |
| White       | 0.78 (0.71–0.85) | 0.000 | 0.000 | 0.96 (0.93–0.99) | 0.016 | / | 0.78 (0.65–0.96) | 0.000 | 47.8% | 0.89 (0.83–0.95) | 0.000 |
| Asian       | 0.77 (0.70–0.85) | 0.000 | 0.000 | 0.97 (0.94–1.00) | 0.045 | 15.1% | 0.84 (0.77–0.90) | 0.000 | 0.87 (0.81–0.92) | 0.000 |
| PB          | 0.77 (0.71–0.84) | 0.000 | 0.000 | 0.95 (0.92–0.98) | 0.002 | 0.000 | 0.85 (0.79–0.91) | 0.000 | 0.88 (0.83–0.93) | 0.000 |
| HB          | 0.79 (0.70–0.88) | 0.000 | 0.000 | 0.98 (0.95–1.01) | 0.118 | 0.000 | 0.84 (0.80–0.89) | 0.000 | 0.88 (0.82–0.95) | 0.001 |

ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, CI = confidence interval, HB = hospital-based, PB = population-based, RR = relative risk.

#### Figure 2.

Forest plot showing for the relationship between CYP2B6 c.516G>T variant and AL under dominant model.
than that of GG type in South Africans. The decrease of the clearance rate of TT genotype revealed the decrease of its metabolic ability to the substrate.

In this study, 1038 cases and 1648 controls, was analyzed by meta-analysis. The RR and P values of our 4 models were: dominant model: $RR = 0.77$, 95% CI: 0.72–0.83, $P = .000$; recessive model: $RR = 0.97$, 95% CI: 0.95–0.99, $P = .000$; homozygote model: $RR = 0.84$, 95% CI: 0.80–0.89, $P = .000$; allelic model: $RR = 0.88$, 95% CI: 0.84–0.92, $P = .000$; respectively. The results suggested that the CYP2B6 c.516 G>T variant is associated with AL. The RR value under dominant model ($RR = 0.77$) is less than that of Recessive model ($RR = 0.97$), suggesting that the incidence probability of GG wild type is less than that of GT. In addition, when separating the patients with non-blood-related diseases as a subgroup we didn’t find any significant relationship between incidence and gene variant under the Recessive model. Given the fact that there were only 2 studies in the subgroup and the gene distribution in the control group was not in line with HWE balance. High-quality and case control studies are needed to further assess the role of CYP2B6 gene variant in AL.

Although present meta-analysis elicits some interesting findings, limitations still exist. First, our search was limited to the studies published in English or Chinese, which may lead to a certain level of selection bias due to the limitation of language. Second, this study did not consider some detailed information of enrolled population, such as sex, age, and diseases. Thirdly, some studies in the control groups were not consistent with HWE, such as Yu’s study, which might influence the results. Finally, there are some degrees of publication bias in this meta-analysis, the results of Egger and Begg are different. The reason is not related to publication bias, but to poor methodological quality of smaller studies.

NOTE: Weights are from random effects analysis.
5. Conclusions

In conclusion, our meta-analysis indicated that the CYP2B6 c.516G>T variant is associated with the occurrence of AL under 4 models. CYP2B6 c.516 TT, TG genotype was significantly related to an increased risk of AL. However, there were no obvious associations between the risk of AL and CYP2B6 variants in the design of HB studies based on hospital because of the limitations of the baseline, and few studies were included. In the population with related genotypes, close attention should be paid to environmental contact factors to reduce their susceptibility factors.

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