Short Communication

The $P2X7$ Receptor Gene A1513C Polymorphism Does Not Contribute to Risk of Familial or Sporadic Chronic Lymphocytic Leukemia

Gabrielle S. Sellick, Matthew Rudd, Paul Eve, Ruth Allinson, Estella Matutes, Daniel Catovsky and Richard S. Houlston

Abstract

The $P2X7$ receptor, a plasma membrane ATP-gated ion channel that plays a role in lymphocyte apoptosis, has been suggested to be involved in the development of chronic lymphocytic leukemia (CLL). $P2X7$ is polymorphic with 1513A and 1513C alleles encoding fully active and nonfunctional proteins, respectively. We evaluated the significance of the $P2X7$-A1513C polymorphism on CLL risk by genotyping 424 patients and 428 healthy controls. To empower detection of an association, we included in our analysis 106 familial cases. Allele frequencies were identical in cases and controls irrespective of whether cases were familial or sporadic (frequency of the C allele was 0.17 and 0.17, respectively). The odds ratio of CLL associated with the C allele was 1.03 (95% confidence interval: 0.80-1.31). A meta-analysis of this study and five other smaller published studies provides no evidence of relationship between this $P2X7$ polymorphism and risk of CLL (odds ratio = 0.99, 95% confidence interval: 0.74-1.32).

Introduction

B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia accounting for around 30% of all cases (1). The risk of CLL is increased ~7-fold in first-degree relatives of CLL cases (2). To date, no gene which when mutated has been shown to unambiguously confer susceptibility to the disease (3). While part of the residual familial risk could be due to high penetrance mutations in as yet unidentified genes, a polygenic mechanism may provide a more plausible alternative explanation. Alleles conferring relative risks of ~2.0 will rarely cause multiple-case families and are difficult or impossible to identify through linkage (4). The search for low penetrance alleles has, therefore, centered on association studies based on comparing the frequency of polymorphic genotypes in cases and controls.

The $P2X7$ receptor, a plasma membrane ATP-gated ion channel that plays a role in lymphocyte apoptosis, has been suggested as a contributory factor to the pathogenesis of CLL (5). The $P2X7$ gene that resides on chromosome 12q24 is polymorphic, the 1513A and 1513C alleles of the gene encoding fully active and nonfunctional proteins, respectively. Wiley et al. (5) have recently reported that the A1513C polymorphism is associated with risk of CLL. To examine this proposition further, we have undertaken a large case-control study including a high proportion of familial CLL cases, because this strategy can enrich for individuals with an inherited genetic risk empowering the detection of an association (6).

Patients and Methods

Patients. Four hundred and twenty-four CLL patients (275 males, 149 females; mean age at diagnosis 59 years, SD = 12) referred to the Royal Marsden Hospital NHS Trust were studied. One hundred and six of the cases had one or more first-degree relatives affected with CLL. Age-matched controls consisted of 428 healthy spouses of patients enrolled in another United Kingdom cancer study (283 males, 145 females; mean age 58 years, SD = 12). None of these individuals had a personal or family history of malignancy. Both cases and controls were British Caucasians with no obvious geographical distinction between the two groups. All samples were obtained with informed consent and ethical review board approval.

Detection of $P2X7$-A1513C Genotypes. Genotypes were generated using TaqMan technology (Applied Biosystems, Foster City, CA) using an Applied Biosystems 7900HT sequence detection system. PCR reactions contained 6.25 μL ABI TaqMan PCR Master Mix, 0.3125 μL ABI SNP assay-by-design master mix based on minus strand DNA sequence containing 900 nmol/L forward.
hardy-weinberg equilibrium. the power to detecting genotypes in controls was tested for a departure from using the module metan (10) implemented in stata asymmetrical. statistical manipulations were undertaken conversely, if there is bias, the funnel plot will be plot resembles a symmetrical inverted funnel (9). logOR estimate. in the absence of publication bias, the heterogeneity between studies was present; ref. 8). effects and a random-effects model (if significant, (7). Studies were analyzed jointly using both a fixed-design. Pooled estimates of the OR were obtained by this analyses were primary references and of case-control abstracts of hematology meetings. Articles included for articles. Additional studies were ascertainment through references cited in these publications and through abstracts of hematology meetings. Articles included for these analyses were primary references and of case-control design. Pooled estimates of the OR were obtained by calculating a weighted average of the logarithm of ORs (7). Studies were analyzed jointly using both a fixed-effects and a random-effects model (if significant, heterogeneity between studies was present; ref. 8).

The presence of publication bias was examined by plotting ORs in order according to the variance of the logOR estimate. In the absence of publication bias, the plot resembles a symmetrical inverted funnel (9). Conversely, if there is bias, the funnel plot will be asymmetrical. Statistical manipulations were undertaken using the module metan (10) implemented in stata version 7.0 (stata corporation, college station, TX).

to test for population stratification, the distribution of genotypes in controls was tested for a departure from hardy-weinberg equilibrium. the power to detecting an association between the P2X7-A1513C polymorphism and CLL in studies was computed using the method published by fleiss et al. (11), implemented in the statistical program power (Epicenter software, Version 1.30).

results and discussion

The frequency of the AA, AC, and CC genotypes were similar in cases and controls—69% (291/424), 28% (118/424), and 3% (15/424) and 70% (299/428), 26% (112/428), and 4% (17/428), respectively. The frequency of the A and C alleles in controls was similar to that reported in other European populations, 83% and 17%, respectively (5, 6), and genotypes showed no evidence of a departure from hardy-weinberg equilibrium indicative of population stratification. The frequency of AA, AC, and CC genotypes were similar if cases were sporadic or familial—70% (74/106), 24% (26/106), and 6% (6/106) and 68% (217/318), 29% (92/318), and 3% (9/318), respectively. The ages at diagnosis of CLL in cases were not significantly different in individuals with the three genotypes. Using all the data generated, the OR of CLL associated with possession of the C allele was 1.03 (95% CI: 0.80-1.31).

Association studies are capricious if based on small sample numbers and it is not uncommon that subsequent studies fail to replicate initial findings. The initial study by wiley et al. (5) was based on only 36 cases and 46 controls. To our knowledge, four other studies have examined the relationship between the A1513C polymorphism and risk of CLL (fig. 1; refs. 12-15). Combining the findings of the five published studies and our study provides data on 989 cases and 1,271 controls. A meta-analysis shows no evidence for a relationship between the P2X7-A1513C polymorphism and risk of CLL (OR = 0.96; 95% CI: 0.82-1.12). There is, however, clear evidence of heterogeneity (Phet = 0.02) with inclusion of the wiley et al. (5) study. Under a random effects model, the pooled OR is 0.99 (95% CI: 0.74-1.32). Excluding the study reported by wiley et al. (5), the combined OR remains non-significant (OR = 0.91, 95% CI: 0.74-1.11, Phet = 0.21).

The analyses of the P2X7-A1513C polymorphism published to date serve to illustrate some of the pitfalls in the design of association studies. Most common variants are unlikely to confer more than a 2-fold difference in risk of CLL. None of the published P2X7-A1513C studies have 80% power to demonstrate a genotypic risk of less than 2.0, stipulating a significance threshold of 0.01. Population stratification is also an issue in association studies, because this can lead to spurious evidence for or against an association between the marker and disease. It is noteworthy that in the study reported by thunberg et al. (14), there was an overrepresentation of homozygosity for the wild-type allele among controls (P = 0.035).

variation in P2X7 defined by the A1513C polymorphism has been shown in some, but not all studies to affect severity and progression of CLL (12-14). Because our study was based on an analysis of both incident and

<table>
<thead>
<tr>
<th>Study</th>
<th>OR (95% CI)</th>
<th>% Weight</th>
</tr>
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<tbody>
<tr>
<td>This study (2004)</td>
<td>1.03 (0.80, 1.31)</td>
<td>41.6</td>
</tr>
<tr>
<td>Zhang et al. (2003)</td>
<td>0.84 (0.66, 1.34)</td>
<td>19.7</td>
</tr>
<tr>
<td>Thunberg et al. (2003)</td>
<td>0.62 (0.40, 0.96)</td>
<td>13.5</td>
</tr>
<tr>
<td>Dasgupta et al. (2003)</td>
<td>1.19 (0.74, 1.90)</td>
<td>11.5</td>
</tr>
<tr>
<td>Stanczyński et al. (2003)</td>
<td>0.74 (0.46, 1.20)</td>
<td>10.8</td>
</tr>
<tr>
<td>Wiley et al. (2002)</td>
<td>3.75 (1.46, 9.64)</td>
<td>2.8</td>
</tr>
<tr>
<td>Overall</td>
<td>0.96 (0.82, 1.12)</td>
<td></td>
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Figure 1. Plot of the OR of CLL associated with the P2X7-A1513-C allele in the current study and five previously published reports. Studies were weighted according to the inverse of the variance of the log of the OR. Horizontal lines, 95% confidence intervals (95% CI). Box, OR point estimate; its area is proportional to the weight of the study. Diamond (and broken line), overall summary estimate, with confidence interval given by its width. Unbroken vertical line, at the null value (OR = 1.0).
prevalent cases, genotype-specific survivorship could theoretically affect the frequency of carrier state in affected individuals. Accepting this caveat, our study and a meta-analysis of all available data leads us to question whether the P2X7-A1513C polymorphism affects the risk of CLL.

The published studies of the P2X7-A1513C polymorphism serve to illustrate the issues of study design pertinent to association analyses aimed at the identification of susceptibility alleles. Future studies of polymorphic variants as risk factors for CLL and other haematological malignancies should be based on adequate sample sizes commensurate with the detection of small genotypic risks and avoidance of design issues that can lead to spurious associations.

Acknowledgments
We are grateful to the patients and their clinicians for participation in this study.

References
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