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To the editor:

## Analysis of *ITGB2* rare germ line variants in chronic lymphocytic leukemia

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Two recent publications have investigated the role of the *ITGB2* variant p.E573K in familial chronic lymphocytic leukemia (CLL) and other hematologic malignancies. The first paper, "Whole exome sequencing in families with CLL detects a variant in Integrin β 2 associated with disease susceptibility," by Goldin et al,[1] reported the identification of a rare, nonsynonymous coding variant in *ITGB2*, p.E573K, which was found in 6 out of 59 families with CLL. The penetrance of the variant within the families was not reported, although the variant was also found in 3 individuals with CLL from among 173 unrelated CLL clinic patients. All 3 patients were diagnosed at an early age and had a family history of CLL or lymphoid malignancy. A follow-up paper, in which the frequency of this variant was studied among Tasmanian patients with CLL and other hematologic malignancies, was recently published.[2] In this population, the minor allele frequency of this variant was 0.013, and the authors found that the variant was not enriched among families with hematologic malignancies compared with Tasmanian population controls or publicly available non–TCGA (The Cancer Genome Atlas), non-Finnish European controls from the Exome Aggregation Consortium database.[3] The authors of this latter paper suggest that given the conflicting findings, a gene-based analysis of *ITGB2* should be performed to determine whether multiple rare deleterious variants in this gene may contribute to disease risk.

These findings and this suggestion are of particular interest to us, as we have just recently completed such a gene-based rare variant association analysis in CLL.[4] We focused on rare germ line variants (<1% minor allele frequency) identified by whole-exome sequencing in a cohort of 646 CLL patients of European descent (composed of 80 familial individuals, 178 sporadic individuals, and 388 individuals of unknown familial status) compared with 8920 ethnically matched population controls without cancer at sampling. We included only 1 representative proband from each of the 80 families in the study cohort, and therefore, the 80 familial samples were all from unrelated individuals. *ITGB2* was not significant in an exome-wide gene burden analysis (q = 0.99).

We next queried our data set to compare the incidence of the *ITGB2* variant p.E573K in our patient cohort to that reported in the Goldin et al study. We found 7 CLL patients heterozygous for this germ line variant out of 554 CLL patients studied (only patients who had coverage at that genomic site sufficient to make a high-confidence genotype call were included). In the control cohort, we found 68 individuals with heterozygous calls at p.E573K out of a total of 8669 samples with sufficient coverage for a high-confidence call. The allele frequency of p.E573K in our cases (0.0063) appears to be about the same as the allele frequency observed by Goldin et al in their validation cohort (0.0087, or 3 out of 346 alleles in 173 patients). Furthermore, in our data set, the allele frequency of p.E573K in cases was slightly enriched over the frequency in controls (0.0039). However, a 1-sided Fisher's exact test comparing the ratio of alternate to reference alleles in cases vs controls shows that this slight enrichment in cases is not statistically significant, with a *P* value of .163 (Table 1). We note that the same 1-sided Fisher's exact test performed on Goldin et al's validation cases compared with a cohort of non-Finnish European controls taken from the Exome Aggregation Consortium database is also not statistically significant, with a 1-sided Fisher *P* value of .456 (Table 1).

Given that rare variants in *ITGB2* were not significantly associated with CLL in our exome-wide gene burden analysis and the p.E573K variant was not specifically enriched, we analyzed all of the rare variants identified in *ITGB2* in cases vs controls in our data set. Out of a total of 110 rare, nonsynonymous, biallelic coding variants found in *ITGB2* in our study cohort, only 13 were present in CLL cases, of which only 5 were present in more than one individual with CLL. The p.E573K variant was the most recurrent variant, with 7 heterozygous affected individuals, followed by the missense variant p.R529W, which was found in 5 heterozygous CLL patients. Of the 30 CLL cases carrying a

**Table 1. Allele frequency and odds ratio of *ITGB2* p.E573K in cases and controls from different cohorts**

| Case cohort | Control cohort | Allele frequency in cases (%) | Allele frequency in controls (%) | Fisher's OR (95% CI) | Fisher's *P* |
|---|---|---|---|---|---|
| Tiao et al[4] unrelated CLL cases (familial and sporadic, n = 646) | 8920 ethnically matched European control samples[4] | 7/1108* (0.63) | 68/17 338* (0.39) | 1.62 (0.73-Inf) | .163 |
| Goldin et al[1] families (n = 171; 59 families) | N/A | Not reported (6 families with ≥1 carrier/individual) | N/A | N/A | N/A |
| Goldin et al[1] unrelated CLL clinic patients (n = 173) | Exome Aggregation Consortium (non-Finnish European)[3] | 3/346 (0.87) | 461/62 646† (0.74) | 1.21 (0.32-Inf) | .456 |

CI, confidence interval; Inf, infinity; N/A, not applicable; OR, odds ratio.
*Only patients who had coverage at the genomic site of interest sufficient to make a high-confidence genotype call were included.
†Control cohort included 5 individuals homozygous for p.E573K.

rare variant in *ITGB2*, only 7 had known familial vs sporadic status, and all 7 were sporadic. None of the variants found in cases was significantly enriched compared with controls (supplemental Table 1, available on the *Blood* Web site).

Of the 646 CLL patients in our cohort, 259 were CLL patients enrolled in a prospective cohort natural history study at the Dana-Farber Cancer Institute (DFCI),[5-8] 281 were from the German CLL Study Group's randomized CLL8 clinical trial,[9,10] and 106 were from published whole-exome sequencing data from the International Cancer Genome Consortium.[11] Of the 7 CLL patients in this data set who carry the p.E573K germ line variant, 6 were sampled from the German CLL Study Group and were of unknown familial status; the remaining patient was from the DFCI cohort and was a sporadic case. The preponderance of heterozygous p.E573K samples from the German CLL8 study as compared with DFCI samples is suggestive, as it raises the possibility that the observed frequency of the p.E573K variant in the study by Goldin et al may be related to a specific European subpopulation represented by their study subjects. Without information on the ethnic background of the subjects in the Goldin et al study, or the association of the variant with CLL within families, it is difficult to fully assess this possibility. If the familial patients in the Goldin et al study were sampled from a relatively homogeneous European subpopulation with a higher frequency of p.E573K, it may be a benign allele that marks a particular subpopulation rather than a true predisposition allele. Alternatively, it may contribute to disease development within that subpopulation; assessing this would require a large control group from within the same subpopulation.

We believe these studies illustrate the ongoing challenges of disentangling germ line predisposition to CLL, when intrinsic population variability must be taken into account and predisposing alleles may be specific to small subpopulations. This is further complicated by the likelihood that the genetic basis of CLL within families may differ from that of the general population.[4] Larger case cohorts categorized by familial vs sporadic status, as well as case cohorts analyzed by ethnicity together with genetically matched control groups, are likely to be required to better define the heritability of CLL.

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