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# To the editor:

## Evaluating a CLL susceptibility variant in *ITGB2* in families with multiple subtypes of hematological malignancies

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The application of rapid and affordable genome sequencing technologies to families with a high incidence of hematological malignancies (HMs) provides significant opportunities for understanding the genetic causes of these cancers.[1] In one of the few studies published to date using this approach, Goldin et al recently reported that a rare variant (rs2230531) within *ITGB2* is associated with genetic predisposition to chronic lymphocytic leukemia (CLL).[2] In this study, whole-exome sequencing was performed in a discovery set of 20 high-risk CLL

**Table 1. Characteristics of *ITGB2* rs2230531 variant carriers**

| Individual | Sex | Cohort | HM diagnosis | Age (y)* | Relationship to closest HM case |
|---|---|---|---|---|---|
| LK0016-104 | Male | TFHMS | Unaffected | 62 | Offspring of parent with DLBCL and AML, DNA of parent unavailable |
| LK0051-159 | Female | TFHMS | DLBCL | 58 | NA |
| LK0600-005 | Female | TFHMS | Unaffected | 71 | 3 affected siblings (2 with MM and 1 with HL) all negative for variant |
| LK0800-001 | Male | TFHMS | AML | 59 | Nonfamilial |
| LK0933-001 | Male | TFHMS | HCL | 66 | Nonfamilial |
| LK2042-056 | Female | TFHMS | Unaffected | 69 | Offspring of parent with NHL, DNA of parent unavailable |
| LK2042-290 | Female | TFHMS | ET | 52 | NA |
| DVA 538 | Male | TPC | Unaffected | 69 | No known family history of HMs |
| DVA 655 | Male | TPC | Unaffected | 61 | No known family history of HMs |
| PCT0-166 | Male | TPC | Unaffected | 84 | No known family history of HMs |
| DVA 1519 | Male | TPC | Unaffected | 70 | No known family history of HMs |
| DVA 1527 | Male | TPC | Unaffected | 70 | No known family history of HMs |
| DVA 1737 | Male | TPC | Unaffected | 80 | No known family history of HMs |
| PCT0-010 | Male | TCC | Unaffected | 84 | No known family history of HMs |
| PCC0-011 | Male | TCC | Unaffected | 72 | No known family history of HMs |
| DVA 88 | Male | TCC | Unaffected | 80 | No known family history of HMs |
| DVA 100 | Male | TCC | Unaffected | 82 | No known family history of HMs |
| PCT0-146 | Male | TCC | Unaffected | 77 | No known family history of HMs |
| DVA 394 | Male | TCC | Unaffected | 70 | No known family history of HMs |
| DVA 1005 | Male | TCC | Unaffected | 78 | No known family history of HMs |
| DVA 1022 | Male | TCC | Unaffected | 77 | No known family history of HMs |
| DVA 1025 | Male | TCC | Unaffected | 70 | No known family history of HMs |
| DVA 1029 | Male | TCC | Unaffected | 71 | No known family history of HMs |
| DVA 1059 | Male | TCC | Unaffected | 68 | No known family history of HMs |
| PC46-002 | Male | TCC | Unaffected | 93 | No known family history of HMs |
| PC65-001 | Male | TCC | Unaffected | 82 | No known family history of HMs |

AML, acute myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; ET, essential thrombocythemia; HCL, hairy cell leukemia; HL, Hodgkin lymphoma; MM, multiple myeloma; NA, not applicable; NHL, non-Hodgkin lymphoma (519 males); TCC, Tasmanian non-HM cancer cases; TPC, Tasmanian population controls (344 males undiagnosed with HMs according to Tasmanian Cancer Registry records up to December 2014).

*Age at diagnosis for HM cases, and current age for non-HM samples.

**Table 2. Wald test MLE group comparisons for rs2230531**

| Frequency comparison | $\chi^2$ (1 df) | *P* |
|---|---|---|
| TFHMS vs TPC/TCC | 0.163 | .686 |
| TFHMS vs non-TCGA NFE ExAC | 1.408 | .235 |
| TPC/TCC vs non-TCGA NFE ExAC | 2.162 | .141 |
| Tasmania vs non-TCGA NFE ExAC | 3.3 | .069 |

For the non-TCGA NFE population of the ExAC, the MAF for rs2230531 is 0.0073853. For the TFHMS, the MAF is 0.013436. For the TPC and TCC samples, the combined MAF is 0.0111437. Across all Tasmanian samples (TFHMS/TPC/TCC), the MAF is 0.01157.

df, degrees of freedom; non-TCGA NFE ExAC, non–The Cancer Genome Atlas, non–Finnish European component of the ExAC.

families. Analysis proceeded on a "per family basis," to identify rare (minor allele frequency [MAF] <1% in population databases), nonsynonymous variants shared in the CLL cases, as well as obligate carriers and non-Hodgkin lymphoma cases. In 20 families, 926 variants were prioritized, of which 2 were identified in multiple families, rs2230531 in *ITGB2* and rs201725177 in *SWI5*.

The integrin gene *ITGB2* was selected for resequencing in 39 additional small CLL families and 173 unrelated CLL patients from a clinical cohort. Carriers of the variant were identified in 4 families and 3 of 173 unrelated CLL patients. In summary, this variant was detected in 6 of 59 CLL families and 3 CLL cases from a clinical cohort, all with a family history of lymphoid malignancy.[2]

Integrins are broadly known for their role in cancer[3]; however, mutations in *ITGB2* have not previously been implicated in CLL. Variation in *ITGB2* presents as an interesting biological candidate for the genetic susceptibility of CLL. Mutations in *ITGB2* have been shown to cause leukocyte adhesion deficiency type 1 (OMIM: 116920), an autosomal-recessive disorder where neutrophil function is affected and patients experience recurring bacterial and fungal infections.[4] Across 53 tissues in the Genotype-Tissue Expression (GTEx) RNA-sequencing expression database, *ITGB2* is primarily expressed in blood,[5] supporting a role for this gene in diseases of this tissue type. Additionally, Rotunno et al have previously reported this variant in a whole-exome sequencing study of familial Hodgkin lymphoma.[6]

Here, we examine the *ITGB2* variant rs2230531 in an independent cohort of HMs from the Tasmanian Familial Hematological Malignancies Study (TFHMS). Our aim was to follow up the findings of Goldin et al in relation to the role of this variant in the genetic predisposition to HMs. The TFHMS cohort, previously described in detail,[7-9] is a collection of familial and nonfamilial HM cases and unaffected relatives from Tasmania, the island state of Australia. Using TaqMan genotyping (ThermoFisher Scientific, assay ID: C__15852956_10), we genotyped rs2230531 in 71 affected and 228 unaffected first-degree relatives from 47 families, in addition to 80 nonfamilial HM cases from the TFHMS cohort (n = 379, 48.3% male), together with 2 Tasmanian population control datasets (n = 862), detailed further in Table 1.

We identified 4 HM cases as carriers of the rs2230531 variant in the TFHMS cohort. Two HM cases carrying rs2230531 had a family history of HMs, and 2 were nonfamilial cases. Three unaffected relatives of HM cases were also identified as variant carriers. An unaffected relative and a familial HM case were identified in the same family; however, the 2 are only distantly related and separated by 12 meioses (based on genealogical records). We also detected the variant in our Tasmanian population non-HM cohorts (TPC and TCC; Table 1) with an overall MAF of 0.011. None of the 25 CLL cases screened, or their first-degree relatives, were found to carry the variant. Table 1 describes the characteristics of the individuals carrying this variant. Supplemental Table 1 (available on the *Blood* Web site) details HM subtypes included in these analyses.

To appropriately test the enrichment of rs2230531 in HM families compared with population controls, there is a need to account for the nonindependence of related samples in TFHMS. To do this, we used a maximum likelihood estimate (MLE) of the MAF in the TFHMS families, taking into account the pedigree structure, using Sequential Oligogenic Linkage Analysis Routines software.[10] MLEs were compared across groups using a Wald test (with one degree of freedom). As an additional population control group for comparison, allele frequency data were also leveraged for rs2230531 from 26 133 samples in the non–The Cancer Genome Atlas, non–Finnish European component of the Exome Aggregation Consortium (ExAC), where the MAF is 0.007.[11] We additionally compared the Tasmanian population control cohort alone, and in combination with the TFHMS samples as a single Tasmanian group, against the ExAC sample set.

Table 2 shows the results of Wald test MLE comparisons. Overall, rs2230531 was not enriched in HM families in comparison with both Tasmanian population controls ($P = .671$) and publicly available population data from ExAC ($P = .235$). Additionally, rs2230531 was not enriched in Tasmanian controls or all Tasmanian samples together (TFHMS/TPC/TCC) in comparison with the non-TCGA, non–Finnish European component of ExAC ($P = .141$ and 0.069, respectively). A post-hoc power analysis was performed to consider the power within the TFHMS dataset to detect a difference in allele frequencies from the observed allele frequency of 0.011 in the 862 Tasmanian controls. The TFHMS dataset (of sample size 379) has 80% power to detect a difference of ~0.023 in allele frequency, 50% for a 0.014 difference, and 25% for a 0.008 difference. The measured difference was only 0.0022923, suggesting that no biologically significant difference exists between sample groups.

These data provide evidence that the *ITGB2* variant rs2230531 is not enriched in Tasmanian families with HMs in comparison with both Tasmanian and larger international population cohorts. In our cohort, we observe the variant in HM cases other than CLL, including both lymphoid and myeloid HMs, the latter being a novel observation. This suggests that if this variant does indeed contribute to HM risk (in other populations), it may contribute more broadly across HM subtypes and thus may warrant further analysis in other familial HM cohorts and HM subtypes.

An alternative interpretation of these data is that rs2230531 is a rare polymorphism not associated with disease and present in the population at large. This may be a feature of the historically stable and isolated Tasmanian population. The established founder effect in Tasmania can lead to the observation that some rare variants, reported in large publicly available databases, are enriched in the Tasmanian population (occur at a slightly higher frequency in the Tasmanian population; unpublished observation). In our non-HM Tasmanian samples, rs2230531 has a MAF of 0.011, whereas in 26 133 samples from ExAC the MAF is 0.007. This difference, however, is not statistically significant.

Our findings do not necessarily rule out a role for *ITGB2* in CLL or HM genetic predisposition. HM is a multifactorial disease, and mutations in *ITGB2* may play but one part in the larger context of HM risk. Our study does serve to provide a necessary sequel to the study of Goldin et al, as the evaluation of a disease-associated variant in multiple cohorts is required to establish a new genetic cause of disease, as outlined in guidelines produced by MacArthur et al.[12] We propose that the current conflicting findings of the rs2230531 in familial HMs, together with the biological role of *ITGB2* in blood cells, creates an a priori hypothesis that genetic variation in *ITGB2* contributes to HM

risk. Further, this finding should encourage other researchers in the field to step beyond the single variant and perform gene-based screening of *ITGB2* to determine whether multiple, rare, deleterious variants contribute to HM risk.

The TFHM study was approved by the Health and Medical Human Research Ethics Committee (Tasmanian network; reference number H8551), and written informed consent was obtained from all participating individuals. The Tasmanian population samples used in this study were approved by the Health and Medical Human Research Ethics Committee (Tasmanian network) reference number H9999 and H7740.

The online version of this article contains a data supplement.

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# To the editor:

## Silica particles contribute to the procoagulant activity of DNA and polyphosphate isolated using commercial kits

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Recent advances in understanding the contributions of the contact pathway to thrombosis, inflammation, and innate immunity[1] have renewed interest in identifying (patho)physiologic activators of this pathway. Candidates include collagen,[2] misfolded proteins,[3] DNA,[4] RNA,[4] and polyphosphate (polyP).[5] Contributions of extracellular nucleic acids to coagulation and inflammation are under intense investigation, with several studies reporting the procoagulant effects of DNA,[6-8] particularly in the context of neutrophil extracellular traps (NETs).[9-11] In this study, we attempted to compare the clotting activity of polyP versus cellular DNA. To do this, we isolated DNA from human cells using Qiagen kits, as previously done by others (Table 1). We now report that DNA purified by using these kits was contaminated with highly procoagulant silica particles. Some studies have employed Qiagen kits to isolate polyP from human cells (Table 1), and we also now report that polyP isolated by using such kits risks contamination with procoagulant silica.

Detailed methods used to purify and analyze DNA and polyP are described in the supplemental Methods. Briefly, DNA was purified from HEK 293 cells or human NETs by using DNeasy Blood and Tissue kits (Qiagen) or phenol/chloroform extraction. Some preparations of λ phage DNA or short-chain polyP were repurified on DNeasy kits.