

## Evaluating a CLL susceptibility variant in *ITGB2* in families with multiple sub-types 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.<sup>1</sup> 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).<sup>2</sup> In this study whole exome sequencing (WES) was performed in a discovery set of 20 high-risk CLL 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 (NHL) cases. In 20 families, 926 variants were prioritized, of which two were identified in multiple families, rs2230531 in *ITGB2* and rs201725177 in *SWI5*.

The integrin gene *ITGB2* was selected for re-sequencing in 39 additional small CLL families and 173 unrelated CLL patients from a clinical cohort. Carriers of the variant were identified in four families and three 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.<sup>2</sup>

Integrins more broadly are known for their role in cancer,<sup>3</sup> 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 suffer from recurring bacterial and fungal infections.<sup>4</sup> Across 53 tissues in the GTEx RNA-seq expression database, *ITGB2* is primarily expressed in blood,<sup>5</sup> supporting a role for this gene in diseases of this tissue type. Additionally, Rotunno *et al* have previously reported this variant in a WES study of familial Hodgkin lymphoma.<sup>6</sup>

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<sup>7-9</sup>, is a collection of familial and non-familial 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 non-familial HM cases from the TFHMS cohort

(n=379, 48.3% male), together with two Tasmanian population control datasets (n=862), detailed further in Table 1.

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

Individual	Sex	Cohort	HM Diagnosis	Age*	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	Non-familial
LK0933-001	Male	TFHMS	HCL	66	Non-familial
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

Abbreviations: HM hematological malignancy, TFHMS Tasmanian Familial Hematological Malignancies Study, TPC Tasmanian population controls comprised of 344 male Tasmanian population controls undiagnosed for HMs according to Tasmanian Cancer Registry records up to December 2014, TCC 519 male Tasmanian non-HM cancer cases, DLBCL diffuse large B-cell lymphoma, MM multiple myeloma, HL, Hodgkin lymphoma, AML acute myeloid leukemia, HCL hairy cell leukemia, NHL non-Hodgkin lymphoma, ET essential thrombocytopenia, NA not applicable. \*Age = age in years at diagnosis for HM cases and current age for non-HM samples.

We identified four HM cases as carriers of the rs2230531 variant in the TFHMS cohort. Two HM cases carrying rs2230531 had a family history of HMs, and two were non-familial 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 two 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. Supplementary Table S1 details HM subtypes included in these analyses.

To appropriately test the enrichment of rs2230531 in HM families compared to population controls, there is a need to account for the non-independence 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 SOLAR.<sup>10</sup> 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-Finnish European, non-TCGA (The Cancer Genome Atlas) component of the Exome Aggregation Consortium (ExAC), where the MAF is 0.007.<sup>11</sup> 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 to both Tasmanian population controls ( $P=0.671$ ) and publicly available population data from ExAC ( $P=0.235$ ). Additionally, rs2230531 was not enriched in Tasmanian controls, nor all Tasmanian samples together (TFHMS/TPC/TCC) in comparison to the non-TCGA, non-Finnish European component of ExAC ( $P=0.141$  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 an approximate 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.

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

Frequency Comparison	chi (1 d.f.)	P
TFHMS vs TPC/TCC	0.163	0.686
TFHMS vs non-TCGA NFE ExAC	1.408	0.235
TPC/TCC vs non-TCGA NFE ExAC	2.162	0.141
Tasmania vs non-TCGA NFE ExAC	3.3	0.069

Non-TCGA NFE ExAC refers to non-Finnish European, non-TCGA (The Cancer Genome Atlas) component of the Exome Aggregation Consortium (ExAC) with a MAF for rs2230531 = 0.0073853. TFHM refers to Tasmanian Familial Hematological Malignancy Study with a MAF = 0.013436. TPC refers to Tasmanian population control and TCC refers to the male Tasmanian non-HM cancer case samples with a combined MAF = 0.0111437. Across all Tasmanian samples (TFHMS/TPC/TCC) MAF=0.01157.

These data provide evidence that the *ITGB2* variant rs2230531 is not enriched in Tasmanian families with HMs in comparison to 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.<sup>12</sup> 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.

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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. This study is supported by the David Collins Leukaemia Foundation, Cancer Council Tasmania, the Leukaemia Foundation and the National Health and Medical Research Council, Australia. Genealogical work was supported by the Max Bruce Trust, Tasmania. J.L.D. is supported by an Australian Research Council Future Fellowship.

### **Contributions**

N.B.B., J.R.M. and J.L.D. conceived the study design. N.B.B., J.C.C and J.B. designed, performed and interpreted the genetic analysis. J.R.M. performed the TaqMan genotyping. K.A.M. and R.M.L. provided clinical input. A.B. performed genealogical analyses. N.B.B., J.R.M. and J.L.D. drafted the manuscript; and all authors contributed to the final manuscript. No conflicts to declare.

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