Single-nucleotide variants in TGFB1, TGFBR2, IL17A, and IL17F immune response genes contribute to follicular lymphoma susceptibility and aggressiveness

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Follicular lymphoma (FL) is the most frequent subtype of indolent non-Hodgkin lymphoma (NHL) worldwide, encompassing 35% of cases1. Immune dysregulation is one of the hallmarks of lymphomagenesis2, and cytokines act on shifting immune responses. In this setting, disruption of the balance among cytokines may be a key event in the susceptibility to NHL3. The major immune pathways encompass Th1 (cellular) response, which is regulated by cytokines such as interleukin 12A (IL12A) and IL2, and T helper type 2 (Th2) (humoral) response, which relies on molecules such as IL10 and transforming growth factor-β (TGFβ)4,5. It is also known that TGFβ binds to two receptors (TGFBR1 and TGFBR2) that act as important regulators of downstream effectors6. More recently, a novel immune-response pathway dependent on the production of IL-17A and IL-17F, the Th17, was also described6.

From the genetic point of view, one mechanism responsible for differential cytokine production is the presence of single-nucleotide variants (SNVs) in coding genes3. Some previous reports already addressed the role of SNVs within cytokine genes in the risk and/or prognosis of lymphomas, including mixed NHL cohorts, diffuse large B cell lymphoma, and, to a lesser extent, FL3,7. However, there are still potentially functional SNVs in key cytokine genes that remain largely unexplored in FL pathogenesis.

We aimed, in the present study, to test whether 16 SNVs in eight immune-response genes (IL12A, IL2, IL10, TGFB1, TGFBR1, TGFBR2, IL17A, and IL17F) affected FL susceptibility (Supplementary Table 1). We selected SNVs with previous evidence of functionality, either due to the modulation of immune responses or due to previous associations with cancer development or prognosis.

The FL cohort comprised newly diagnosed cases, 71 men and 88 women, followed at the Hematology and Hemotherapy Center of the University of Campinas (Campinas, Brazil) and A.C. Camargo Cancer Center (São Paulo, Brazil), and the control group (blood donors) was composed of 109 men and 110 women from the Hematology and Hemotherapy Center of the University of Campinas. All subjects were unrelated individuals and controls denied any previous neoplasm. The gender distribution between groups was similar, but patients were older than controls (median ages of 56.0 and 50.0 years) (Supplementary Table 2). The study was conducted according to the Declaration of Helsinki and was approved by the institutional ethics committees (# 32177014.3.0000.5404 and # 32177014.3.3001.5432). All subjects signed the informed consent.

We extracted genomic DNA from peripheral blood samples of FL patients, and for genotypic comparisons we also extracted genomic DNA of controls’ peripheral blood samples. DNA was extracted using the lithium chloride technique, and final concentrations were set to 50 ng/µL. Genotyping experiments were performed in pre-designed,
personalized genotyping plates from the Taqman Openarray® QuantStudio™ 12K Real-Time PCR System (Life Technologies Inc., Carlsbad, CA, USA).

For all SNVs, the Hardy–Weinberg equilibrium (HWE) was calculated using the χ² test; HWE was admitted if p > 0.05. The pairwise linkage disequilibrium (LD) analysis in the Haploview 4.2 software was used to ensure that markers were appropriate for inclusion in haplotype estimates. In this setting, both the data (genotypic distributions) and the locus information (name and location of SNVs) files were uploaded to Haploview in the linkage format and processed by the program (https://www.broadinstitute.org/haploview/downloads). The output of these data was an LD plot that expressed the LD coefficient (D’) among groups of SNVs. A D’ value higher than 0.80 was considered significant for haplotype formation.

To assess the role of an SNV or haplotype on FL susceptibility, we performed age-adjusted logistic regression analyses, and risks were expressed using odds ratios (ORs) and 95% confidence intervals (95% CIs).

Control samples were in HWE in all tested loci, except in five of them (IL2 rs2069762, TGFB1 rs334348, TGFB2 rs3087465, IL17A rs3748067, and IL17F rs7637870), and patients lacked HWE in loci of four SNVs: IL12A rs568408, IL2 rs2069762, IL10 rs3024491, and TGFB1 rs3334348 (Supplementary Table 1). The analyses revealed a strong LD among IL12A rs583911, rs568408, and rs485497 (D’ = 0.93), and among IL10 rs3024491, rs1800872, and rs1800890 (D’ = 0.92) (Supplementary Fig. 1).

Individuals with TGFB1 rs1800469 CC or CT genotypes had a higher risk of FL development when compared to those with the TT genotype (OR = 2.13, 95% CI: 1.06–4.28) (Table 1). TGFB1 rs1800469 CC or CT genotypes were also more common in patients with Ann Arbor stages III or IV than in controls (93.3% vs. 84.5%, p = 0.02); carriers of these genotypes were under a 2.63 (95% CI: 1.16–5.97)-fold increased risk of developing aggressive FL than others.

In addition, subjects harboring TGFB2 GG (OR = 2.10, 95% CI: 1.34–3.29), IL17A CC (OR = 4.10, 95% CI: 2.48–6.75), or IL17F TT (OR = 3.74, 95% CI: 2.32–6.04) genotypes were under increased risks of developing FL when compared to those with other genotypes (Table 1). The remaining SNVs (Supplementary Table 3) or haplotypes (Supplementary Table 4) did not alter FL susceptibility.

Herein, we showed novel associations between four SNVs and FL risk. First, we found that the “C” allele of TGFB1 rs1800469 (CC or CT genotypes) was associated with an increased risk of developing FL, like what has already been described for a solid malignancy. It is noteworthy that this association was retained in patients with more aggressive disease (Ann Arbor stages III and IV). It was previously demonstrated that individuals with the TT genotype of rs1800469 produce the highest levels of TGFβ compared to the remaining genotypes. In this setting, subjects harboring the CC or CT genotypes may have a less prominent TGFβ-mediated immunosuppression compared to TT individuals. This, ultimately, may allow the proliferation of

| Single-nucleotide variant | Patients, n (%) | Controls, n (%) | OR (95% CI) | p value |
|---------------------------|----------------|----------------|-------------|---------|
| **TGFB1 rs1800469**       |                |                |             |         |
| CC                        | 68 (42.7)      | 81 (37.0)      | 2.32 (1.10–4.92) | 0.02    |
| CT                        | 78 (49.1)      | 104 (47.5)     |             |         |
| TT                        | 13 (8.2)       | 34 (15.5)      | 1.00 (reference) |         |
| **TGFB2 rs3087465**       |                |                |             |         |
| GG                        | 86 (55.5)      | 89 (41.0)      | 2.33 (0.88–6.12) | 0.08    |
| GA                        | 62 (40.0)      | 113 (52.1)     |             |         |
| AA                        | 7 (4.5)        | 15 (6.9)       | 1.00 (reference) |         |
| **IL17A rs3748067**       |                |                |             |         |
| CC                        | 113 (76.4)     | 104 (48.8)     | 0 (0–infinite) | 0.99    |
| CT                        | 35 (23.6)      | 106 (49.8)     |             |         |
| TT                        | 0 (0.0)        | 3 (1.4)        | 1.00 (reference) |         |
| **IL17F rs7637870**       |                |                |             |         |
| TT                        | 104 (68.4)     | 90 (42.5)      | 0 (0–infinite) | 0.99    |
| TC                        | 46 (30.3)      | 122 (57.5)     |             |         |
| CC                        | 2 (1.3)        | 0 (0.0)        | 1.00 (reference) |         |
| TT                        | 104 (68.4)     | 90 (42.5)      | 3.74 (2.32–6.04) | <0.001  |
| **rs1800469**             |                |                |             |         |
| TT                        | 148 (100.0)    | 210 (98.6)     | 0 (0–infinite) | 0.99    |
| CC                        | 2 (1.3)        | 0 (0.0)        | 1.00 (reference) |         |

Rs reference number. OR odds ratio (adjusted only by age using logistic regression estimates), 95% CI 95% confidence interval. Significant values of p are presented in bold letters. The numbers of patients and controls differed from the total numbers enrolled in study because it was not possible to obtain genotypes in some cases.
lymphocytes, neoplastic transformation, and disease dissemination in CC or CT individuals.

We also demonstrated strong associations between TGFBR2 GG, IL17A CC, and IL17F TT genotypes and increased risks of developing FL. Intriguingly, there was a lack of HWE in controls for these three SNVs. Since they were previously implicated on immunological modulation, we hypothesize that strong selective pressures could be responsible for this phenomenon. Non-compliance to HWE in controls, for the same SNVs, was also observed in other populations.

Furthermore, in one study conducted to investigate the role of SNVs within immune-response genes in NHL, Chen et al. found interactions between IL12A rs568408, body mass index, and susceptibility for FL in females. We could not replicate this result in our population, probably due to differences in study designs, and the presence of different genetic backgrounds from a diverse ancestry in Brazil.

In summary, we present, for the first time, preliminary evidence demonstrating that inherited variants in immune-response genes (TGFBR1 rs1800469, TGFBR2 rs3087465, IL17A rs3748067, and IL17F rs763780) alter susceptibility to FL and disease aggressiveness. These findings, following proper validation, may represent easily assessable and potentially targetable biomarkers reflecting immune alterations that lead to FL. We believe that further epidemiological and functional studies regarding these genes and SNVs should be conducted with the purpose of data validation and to better understand the biological role of these variants in FL.

Acknowledgements
G.R.A.-M. and G.J.L. performed the experiments and statistical analyses and wrote the paper. M.T.D., V.C.C.d.L., G.W.B.C., and C.A.d.S. assisted the patients, collected the data, and wrote the paper. F.A.S., C.S.P.L., and J.V. conceived the study and wrote the paper.

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Conflict of interest
The authors declare that they have no conflict of interest.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41408-020-00365-4).

Received: 5 May 2020 Revised: 11 August 2020 Accepted: 11 September 2020

Published online: 06 October 2020

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Blood Cancer Journal