



Analysis of Interleukin-10 promoter single nucleotide polymorphisms and risk of Non-Hodgkin's lymphoma in a Malaysian population

Journal:	<i>Leukemia and Lymphoma</i>
Manuscript ID:	GLAL-2014-0023.R1
Manuscript Type:	Original Article – Clinical
Date Submitted by the Author:	n/a
Complete List of Authors:	Lim, Yat Yuen; University of Malaya, Faculty of Science, Institute of Biological Sciences Chin, Yoon Ming; University of Malaya, Faculty of Science, Institute of Biological Sciences Tai, Mei Chee; University of Malaya, Faculty of Science, Institute of Biological Sciences Fani, Somayah; University of Malaya, Faculty of Science, Institute of Biological Sciences Chang, Kian; Ampang Hospital, Department of Hematology Ong, Tee Chuan; Ampang Hospital, Department of Hematology Bee, Ping Chong; University of Malaya, Faculty of Medicine, Department of Medicine Gan, Gin Gin; University of Malaya, Faculty of Medicine, Department of Medicine Ng, Ching Ching; University of Malaya, Faculty of Science, Institute of Biological Sciences
Keywords:	Cytokine production and paraneoplastic conditions < Neoplasia, Genetic and other predisposing conditions < Neoplasia, Lymphoma and Hodgkin disease < Neoplasia

SCHOLARONE™
Manuscripts

1
2
3 **Analysis of Interleukin-10 promoter single nucleotide polymorphisms and risk of Non-Hodgkin's lymphoma**
4 **in a Malaysian population**
5
6

7 **Abbreviated title:** Interleukin-10 and Non-Hodgkin's lymphoma
8
9

10 Yat-Yuen Lim¹, Yoon-Ming Chin¹, Mei-Chee Tai¹, Somayah Fani¹, Kian-Meng Chang³, Tee-Chuan Ong³, Ping-
11 Chong Bee², Gin-Gin Gan^{2,*}, Ching-Ching Ng^{1,*}
12
13

14
15 ¹Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

16 ²Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

17 ³Haematology unit, Ampang Hospital, Malaysia

18 *Shared last author
19
20
21

22 **Correspondence:**

23 Ng Ching Ching

24 Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

25 Email: ccng@um.edu.my, Tel: +60379675872, Fax: +60379675908
26
27

28 Gan Gin Gin

29 Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

30 Email: gangg@ummc.edu.my, Tel: +60379492429, Fax: +60379556936
31
32
33

34 **Keywords:** NHL; genetic susceptibility; SNP; IL10
35
36

37 **Abstract**

38 We evaluated the association of two *IL10* SNPs (rs1800896 and rs1800871) with Non-hodgkin's lymphoma risk in
39 the three major races of Malaysian population (Malay, Chinese and Indian; 317 cases and 330 controls). Our initial
40 screening demonstrated that SNP rs1800871 but not rs1800896 was significantly associated with increased NHL
41 risk in the Malays ($P_{\text{Malay-Rec}}=7 \times 10^{-3}$) and Chinese only ($P_{\text{Chinese-Rec}}=3.9 \times 10^{-2}$). Subsequent combined analysis of the
42 Malay and Chinese race revealed significant association of SNP rs1800871 with ALL-NHL subtypes ($P_{\text{Meta-ALL-NHL-Rec}}=1 \times 10^{-3}$), ALL B-cell subtypes ($P_{\text{Meta-ALL-B-cell-Rec}}=3 \times 10^{-3}$), diffused large B-cell lymphoma (DLBCL) subtype
43 ($P_{\text{Meta-DLBCL-Rec}}=2 \times 10^{-3}$) and ALL T-cell subtypes ($P_{\text{Meta-ALL-T-cell-Rec}}=3.1 \times 10^{-2}$). SNP rs1800896 only showed
44 increased risk towards follicular lymphoma (FL) ($P_{\text{Meta-FL-Dom}}=4 \times 10^{-4}$). We also detected a male-specific association
45 of SNP rs1800871 with increased NHL risk ($P_{\text{Meta-Male-ALL-NHL-Rec}}=6 \times 10^{-3}$) in the combined analysis. To our
46 knowledge, this is the first report on the association of *IL10* promoter SNPs towards NHL susceptibility in the three
47 major races of Malaysia.
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Although the survival rate of Non-Hodgkin lymphoma (NHL) has improved substantially, the incidence of the disease is still increasing steadily worldwide in the last two decades. The prevalence of NHL is highest in North America and western Europe and lower in eastern Europe and Asia [1]. In Malaysia, NHL was the sixth most common cancer with an overall cancer incidence of 4.3% per 100,000 and was more prevalent in males than females according to the National Cancer Registry 2007 report [2]. The pathogenesis of NHL is still relatively unknown. Currently, immune-suppression remains one of the most well-defined risk factor reported to be associated with the development of NHL. Patients with acquired immunodeficiency syndrome (AIDS), post-organ transplant immune deficiencies and other hereditary abnormalities such as inherited immunodeficiency syndrome have increased risks of developing NHL [3].

IL10 which codes for anti-inflammatory cytokine, is one of the critical mediators of Th1/Th2 balance involved in the immune response [4]. IL10 immunosuppressive effect appears to inhibit cell-mediated immune responses against cancer cells [5]. In lymphoma, IL10 has been reported to act as an auto or paracrine growth factor for the survival of B-cell lymphoma cells [6-10]. In fact, NHL tumors were reported to produce IL10 and other cytokines to increase its cell proliferation [11] while increased serum IL10 level was associated with poorer lymphoma patient outcome [12-16]. Furthermore, inhibition of IL10 sensitized B-cell NHL cells to apoptosis whereas IL10 knockout mice developed less age-related malignant B-cell lymphoma [17]. On the contrary, IL10 has also been reported to contain tumor-inhibiting properties by supporting an effective immune attack against malignant cells *in vivo* mainly in breast cancer and melanoma [18]. Interestingly, Cervenak et al. (2000) reported anti-angiogenic and anti-tumorigenicity of IL10 in Burkitt lymphoma *in vivo* [19]. Nonetheless, the literature at large is consistent with IL10 playing a tumor-promoting role in lymphoma. As immune-suppression is thought to be at the underlying basis of lymphomagenesis, much interest has focused upon the regulation of IL10 gene.

Single nucleotide polymorphisms (SNPs) are the most abundant DNA sequence variations. These SNPs can alter the transcription and protein expression levels or function of a gene product and therefore alter inter-individual's susceptibility to certain disease including cancer [20]. In the last decade, studies have shown that SNPs in *IL10* gene are associated with increased susceptibility to NHL [21-24]. Perhaps the most noteworthy is the InterLymph consortium report of SNP rs1800890-AA in increasing the risk of NHL and diffuse large B-cell lymphoma (DLBCL) [22]. SNP rs1800896-GG was also reported to increase risk for DLBCL in the same study [22] and in Burkitt lymphoma [25]. These SNPs were found in the promoter of *IL10* and have been demonstrated to cause variation in IL10 production. For example, rs1800890-AA genotype variant is associated with low IL10 expression [26], rs1800871-CC with high IL10 expression [27,28] while SNP rs1800896-GG is reported to be associated with both high [29] and low [30] IL10 expression using different IL10 stimulation methodologies. Another study by Zeng et al. (2009) reported increased lipopolysaccharide (LPS)-induced IL10 cytokine with SNP rs1800896 G variant and rs1800872 C variant. However, SNP rs1800871 C variant which show similar trend was not statistically significant in that study [31]. Similarly, the GCC, ACC, ATA haplotype of three *IL10* SNPs (rs1800896, rs1800871

1
2
3 and rs1800872) that are in close linkage disequilibrium are associated with high, moderate and low IL10 expression
4 respectively [32]. In general, higher IL10-producing SNPs are associated with poorer prognosis in NHL but the
5 underlying mechanism is not understood [33,34]. To date, studies have shown that increase levels of IL10 up-
6 regulates anti-apoptotic gene BCL2 to increase the survival of NHL cells [35-37] while a more recent study by
7 Gupta et al. (2012) showed that in certain DLBCL tumors, high IL10 levels lead to aberrant activation of JAK2
8 pathway which further induce c-Myc expression, an important driver of oncogenic transformation [38,39].
9
10
11

12
13 Most *IL10* SNP studies to date have yielded inconsistent association of IL10 production with NHL risk [22,24,40].
14 In addition, most studies focus on Caucasians while few have studied the effect of these SNPs in Asian populations
15 or other ethnic origins. Hoffmann et al. (2002) have shown that different ethnic groups differ in the IL10 cytokine
16 polymorphism frequency. Importantly, there is a pronounced difference in allelic and genotype frequency of *IL10*
17 between the Caucasians and the Asians. Compared to the Caucasians, majority of the Asians are low IL10 producers
18 [41-44], therefore, one might expect differences in the development of diseases associated with IL10 regulation.
19 Furthermore, genetic and environmental heterogeneity may cause the effect of genotype on disease phenotype to
20 vary between populations [45,46]. The reasons above indicate the need for further investigation of the association
21 between SNPs in the IL10 locus with NHL susceptibility in other population or ethnicity.
22
23
24
25
26
27

28 Here, we genotyped two *IL10* SNPs (rs1800896 and rs1800871) which have been shown to alter IL10 expressions *in*
29 *vitro* [26,29,32,47] in a case-control study of NHL consisting of the three major ethnic groups of Malaysia (Chinese,
30 Indian and Malay).
31
32
33

34 **MATERIALS AND METHODS**

35 **Study Population**

36 The association analysis was performed in a cohort of 317 NHL patients and 330 healthy controls from 3 major
37 ethnic groups of Malaysia (Chinese, Indian and Malay). All NHL patients and healthy controls were unrelated
38 individuals from different states of Peninsular Malaysia and recruited from University Malaya Medical Centre
39 (UMMC) and Ampang Hospital, Kuala Lumpur. The characteristics of study participants are shown in Table I.
40 Samples were not matched for both age and gender. Of the 317 cases, ~53% were male and their ages ranged from
41 14 to 84 years old (mean \pm S.D = 54 \pm 14.3). Of the 330 controls, 62.5% were male, and their ages ranged from 16
42 to 81 years old (mean \pm S.D = 41.9 \pm 14.3). The younger control subjects were randomly recruited from various
43 blood donation campaign and the older control subjects were recruited from outpatient clinics and were free from
44 any underlying malignancies. All participants gave their written informed consent. This study was approved by the
45 Medical Ethics Committee of University Malaya Medical Centre and MOH Research and Ethics Committee
46 (MREC).
47
48
49
50
51
52
53
54
55
56
57
58
59
60

SNP Genotyping

Genomic DNA was extracted from blood samples using QIAamp® DNA Blood Mini Kit (Qiagen, Germany) or conventional phenol-chloroform methods. Polymorphic sites on the *IL10* promoter region were analyzed using PCR amplification and restriction analysis (PCR-RFLP). The *IL10* SNPs were genotyped following a previous protocol of Moraes et al. (2003) [48] with minor modifications. Briefly, genotyping for rs1800896 and rs1800871 polymorphisms were carried out in a total volume of 20µl containing 15-30 ng of genomic DNA, 0.3units of Taq polymerase with 1X PCR buffer supplied by the manufacturer (Invitrogen, USA), 1.5 mM of MgCl₂, 0.2 mM of dNTP (Promega, USA) and 0.3 µM of forward and reverse primers (Oligo, Singapore), and Millipore water. All PCR reactions were performed using ABI 2720 Thermal Cycler using the following PCR conditions: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturing at 94 °C for 30 s; annealing for 30 s (primer specific temperatures); and amplification at 72 °C for 30 s. A final extension cycle was performed at 72 °C for 7 min. The annealing temperature was 58 °C for rs1800896/rs1800871 primers. Primers including both rs1800896 and rs1800871 SNPs were sense: 5'- CCA AGA CAA CAC TAC TAA GGC TTC TTGAGG A- 3' (modified nucleotides were underlined) and antisense: 5'- AGG TAG TGC TCA CCA TGA CC -3'.

Restriction fragment length polymorphism (RFLP) digestions were performed overnight at 37°C with BseRI (New England Biolabs, USA) for rs1800896; MspI (New England Biolabs, USA) for rs1800871. The digested fragments were visualized by 3% agarose gel electrophoresis. The genotype was determined by the RFLP profiles generated. For quality control, the RFLP patterns for each genotype were verified by DNA sequencing.

Statistical Analysis

SNP call rate and deviation from Hardy-Weinberg equilibrium (HWE) were calculated in PLINK [49]. All statistical analysis was performed in R program except for Meta analysis. Test of heterogeneity (Cochrane Q's statistic test) and I^2 statistics was carried out in PLINK while power analysis was performed using Genetic Power Calculator (<http://pengu.mgh.harvard.edu/~purcell/gpc/cc2.html>). Linkage disequilibrium (LD) for SNP pair rs1800871 and rs1800872 was calculated using PLINK by using a subset of 190 samples. Logistic regression was used to calculate for the odds ratios (ORs) and 95% confidence intervals (95% CI) for the association between the NHL risk and individual SNP using three model of phenotypic expression (additive, dominant and recessive). All association analysis was adjusted against age, gender and ethnicity for possible confounding effects. SNP associations showing P-value <0.05 was deemed statistically significant. SNP with power of association <70% is considered underpowered. Meta-analysis combining the association P-values and corresponding odd ratios (ORs) were performed assuming a fixed-effects model for additive, dominant and recessive genetic models. Test of heterogeneity and I^2 statistics was calculated to determine the extent of heterogeneity across the three individual ethnic groups.

RESULTS

SNPs call rates were $\geq 99\%$ and all the SNPs were in HWE. Association analysis was performed on two IL10 promoter SNPs, rs1800896 and rs1800871, in our cohort of 317 NHL patients and 330 healthy controls, stratified according to the 3 major ethnic groups of Malay, Chinese and Indian descent. We detected significant association signals implicating causal effect towards NHL susceptibility for rs1800871 in the Malays ($P_{\text{Malay-Rec}}=7 \times 10^{-03}$; OR=4.55; CI =1.59-14.93) and Chinese ($P_{\text{Chinese-Rec}}=3.9 \times 10^{-02}$; OR=2.27; CI =1.06-5.07) (Table II). The associations are depicted in a recessive genetic model with considerable power of association for the Malays (74%) but lower for the Chinese cohort (44%). No association signal was detected for rs1800896 ($P>0.05$) in all 3 ethnic groups.

To boost the power of association, we performed a meta-analysis to combine the association signals of all 3 ethnic groups, Malay, Chinese and Indian, for rs1800896 and rs1800871, considering all genetic models. We found that the inclusion of the Indian cohort greatly increased the level of heterogeneity in SNP rs1800871 across all three genetic models ($P_{\text{Coch-Q}} = 0.1-0.2$; $I^2 = 31-55\%$) (Data not shown). Given the small sample size of the Indian cohort (40 cases and 50 controls) and to reduce the heterogeneity observed among the different ethnic groups, we omitted the Indian cohort and only combined the Chinese and Malay ethnic groups in our subsequent meta-analysis.

Meta-analysis of rs1800896 and rs1800871 was performed in the Malay-Chinese combined cohort considering all NHL subtypes (ALL NHL) as well as stratifying into All B-cell, All T-cell and for All B-cell lymphoma, it was further divided into DLBCL and follicular lymphoma (FL). In ALL NHL, only rs1800871 showed increased risk to NHL susceptibility ($P_{\text{Meta-ALL-NHL-Rec}}=1 \times 10^{-03}$; OR=2.87; CI = 1.55-5.48) (Table III). The association signal from the meta-analysis of rs1800871 improved upon the strength and power of association observed in single ethnic cohorts ($P_{\text{Malay-Rec}}=7 \times 10^{-03}$; $P_{\text{Chinese-Rec}}=3.9 \times 10^{-02}$). Direction of the effect size for rs1800871 is consistent across both Malay and Chinese cohorts with low heterogeneity observed ($P_{\text{Coch-Q}}>0.05$; $I^2<1.65$) (Data not shown). Meta-analysis failed to detect association for rs1800896 in ALL NHL in the Malay-Chinese combined cohort.

When stratified into the different NHL subtypes, the combined Malay-Chinese cohort detected association signals in a recessive model for rs1800871 in ALL B-cell ($P_{\text{Meta-ALL-B-cell-Rec}}=3 \times 10^{-03}$; OR=2.72; CI=1.43-5.3) and ALL T-cell lymphoma ($P_{\text{Meta-ALL-T-cell-Rec}}=3.1 \times 10^{-02}$; OR=3.1; CI=1.03-8.34) (Table III). A more detailed analysis of rs18700871 in B-cell subtypes detected association in DLBCL ($P_{\text{Meta-DLBCL-Rec}}=2 \times 10^{-03}$; OR = 2.95; CI = 1.48-5.96) but not in FL (Table III). The data suggests rs18700871 to be an informative molecular marker for DLBCL susceptibility in the Malay-Chinese cohort in Malaysia. SNP rs1800896 was only associated with FL subtype susceptibility ($P_{\text{Meta-FL-Dom}}=4 \times 10^{-04}$; OR = 4.5; CI = 1.94-10.44) in the combined Malay-Chinese cohort (Table III). Unlike other IL10 signals in our study, rs1800896 displays a causal dominant effect towards increased risk to FL.

In stratified analysis involving gender, association signal for rs18700871 is only observed in a recessive model in the combined Malay-Chinese male cohort for all NHL subtypes ($P_{\text{Meta-Male-all-NHL-Rec}}=6 \times 10^{-03}$; OR=3.23; CI=1.43-7.7) (Table IV). SNP rs18600896 is not associated with NHL susceptibility for any gender in the Malay-Chinese cohort. Further stratified analysis of gender for each NHL subtype was not performed due to limitations of the study sample size.

DISCUSSION

We report an association signal implicating *IL10* gene in NHL susceptibility in the Malaysian Malay and Chinese ethnic groups. SNP rs1800871-CC was found to be significantly associated with increased risk of NHL in both the Malay and Chinese cohorts (Table II). To boost the power of association for rs1800871, meta-analysis combining the Malay and Chinese cohorts for rs1800871 was performed. Association signals were detected for ALL NHL as well as ALL B-cell, ALL T-cell and DLBCL subtypes in the combined Malay-Chinese cohort in a recessive genetic model (Table III). SNP rs1800871-CC increased NHL risk, but only in male NHL patients in the combined Malay-Chinese cohort (Table IV). It is highly possible that SNPs that increased IL10 production including rs1800871-CC reported here contribute to a higher NHL incidence observed in males [2,50]. Interestingly, all rs1800871 associations are detected in a recessive genetic model. We noted that prospective associations linking rs1800871 towards NHL susceptibility in the Chinese ethnic cohort as well as the all T-cell subtype (ALL T-cell) are underpowered and should be interpreted with caution (Table II; power of association <50% and Table III; power of association <60% respectively).

SNP rs1800896 generally was not associated with NHL susceptibility with the exception of the FL subtype in the combined Malay-Chinese cohort in a dominant genetic model (Table III). The strong power of association implies a potential risk marker for the FL subtype in both Malay and Chinese ethnic groups. Replication in a separate cohort would be necessary to ascertain this association signal.

Association signals of rs1800896 and rs1800871 revealed inconsistent results with respect to NHL or NHL sub-type susceptibility within similar Asian populations as well as the more distinct Caucasian populations. Our findings associating rs1800871 with increased risk for NHL as well as ALL B-cell and DLBCL subtypes, contradicts the findings of Rothman groups which reported *IL10* associations at rs1800890 and rs1800896 but not rs1800871 [21,22,24]. This is perhaps due to the difference in the allelic distribution of these SNPs in our Malaysian population (rs1800896, Malay MAF=0.08; Chinese MAF=0.05; rs1800871, Malay=0.25; Chinese MAF=0.27) compared to the Caucasian population as reported by the Rothman group (rs1800896, MAF=0.42-0.45; rs1800871 MAF=0.75). Our data also contradicts the recent findings of Hosgood et al. (2013), whereby SNP rs1800896 but not rs1800871 (as reported in our current study) is associated with NHL and other subtypes in an Asian population consisting of participants from Hong Kong, South Korea and Jinan, China [51]. This observation is despite consistent allelic frequencies for rs1800896 and rs1800871 between our study and Hosgood et al. (2013). However, our data is consistent with the reports of Wong et al. (2010) and Breen et al. (2003) in AIDS-related NHL utilizing the Multicenter AIDS Cohort samples as well as a recent meta-analysis study by Cao et al. (2013). Wong et al. (2010) demonstrated that individuals with rs1800871-CT or -TT show decreased risk of developing AIDS-related NHL [52] which is in agreement with our data whereby rs1800871-CC increases the risk of developing NHL. Breen et al. (2003) reported that rs1800872-CC, which is tightly linked to rs1800871 in our study ($r^2=0.95$), is associated with increased risk of AIDS-related NHL [23]. Similarly, data from Cao et al. (2013) also suggest that rs1800872-CC and rs1800871-CC increase DLBCL susceptibility although these associations were not significant after correction for multiple testing [53]. Thus, these studies provided further evidence that rs1800871-CC may be a NHL risk variant in the Malaysian Malay and Chinese ethnic groups.

1
2
3 The inconsistent associations of IL10 SNPs rs1800896 and rs1800871 point to possible influence of
4 environmental factors working in tandem with genetic factors to modulate IL10 production. This is supported by the
5 moderate heritability linked to IL10 production (0.5–0.75) as well as strong interaction between genetic and
6 environmental factors [47,54,55].
7
8

9 Importantly, Salhi et al (2008) reported that IL10 rs1800871-CC in the promoter region is associated with
10 elevated IL10 production in stimulated peripheral blood mononucleated cells (PBMCs) [27] while Lech-Maranda et
11 al (2004) showed that rs1800871-CC is correlated with elevated serum of IL10 in DLBCL patients [56]. Although
12 definitive ‘cause-and-effect’ study to link rs1800871-CC to elevated IL10 production has yet to be determined,
13 report from Salhi et al. (2008) demonstrated that the C allele of rs1800871 could modify nuclear factor binding [27].
14 Therefore we are encouraged that rs1800871-CC could play a causative role in NHL development in the Malaysian
15 Malays and Chinese. However, rs1800872-CC does not influence nuclear factor binding in the *IL10* promoter region
16 despite being tightly linked to rs1800871 [27].
17
18
19
20

21 To our knowledge, this is the first report on the association of *IL10* promoter SNPs towards NHL
22 susceptibility in the three major races of Malaysia. Studies have suggested that IL10 plays a tumor-promoting role in
23 lymphoma. Therefore, genetic variation in the *IL10* promoter region may play an important role in the etiology of
24 NHL in the Malaysian Malays and Chinese ethnic groups especially in the male patients. We note that our findings
25 only partially resolve susceptibility factors causing NHL and require validation involving genetic data from larger
26 cohort, environmental factors, and interaction between both factors. Functional and clinical evidence to confirm the
27 regulatory effect of these *IL10* promoter SNPs would provide strong support to the role of rs1800896 and rs1800871
28 as NHL risk biomarkers.
29
30
31
32
33

34 **ACKNOWLEDGMENT**

35 The authors would like to thank the Director of Health Malaysia for permission to publish this paper. The authors
36 thank all the participants of the study, Khor Xue Fen and Ng Wai Yan for their assistance in genotyping, staffs of the
37 blood bank, UMMC and Ampang hospital. The work was supported by a grant from the MOSTI eScience (12-02-
38 03-2023) and High Impact Research Grant UM-MOHE UM.C/625/1/HIR/MOHE/ CHAN-02 (H-50001-A000023)
39 from the Ministry of Higher Education Malaysia.
40
41
42
43

44 **CONFLICT OF INTEREST**

45 The authors declare that they have no conflict of interest. Please see disclosure forms provided.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

- [1] Evans LS, Hancock BW. Non-Hodgkin lymphoma. *Lancet* 2003;362:139-146.
- [2] Ariffin OZ, Saleha ITN. NCR Report 2007. Ministry of Health, Malaysia 2011.
- [3] Shankland KR, Armitage JO, Hancock BW. Non-Hodgkin lymphoma. *Lancet* 2012;380:848-857.
- [4] Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nature reviews. Immunology* 2010;10:170-181.
- [5] Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nature reviews. Cancer* 2005;5:263-274.
- [6] Khatri VPac, M.A. A review of the association between interleukin-10 and human B-cell malignancies. *Cancer Immunol. Immunother* 1998;46:239-244.
- [7] Hofmann SR ER, Zhou YJ, et al. . Cytokines and their role in lymphoid development, differentiation and homeostasis. *Curr Opin Allergy Clin Immunol* 2002;2:495-506.
- [8] MacNeil IA, Suda T, Moore KW, Mosmann TR, Zlotnik A. IL-10, a novel growth cofactor for mature and immature T cells. *Journal of immunology* 1990;145:4167-4173.
- [9] Rousset F, Garcia E, Defrance T, et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 1992;89:1890-1893.
- [10] Go NF, Castle BE, Barrett R, et al. Interleukin 10, a novel B cell stimulatory factor: unresponsiveness of X chromosome-linked immunodeficiency B cells. *The Journal of experimental medicine* 1990;172:1625-1631.
- [11] Voorzanger N, Touitou R, Garcia E, et al. Interleukin (IL)-10 and IL-6 are produced in vivo by non-Hodgkin's lymphoma cells and act as cooperative growth factors. *Cancer research* 1996;56:5499-5505.
- [12] Inagaki A, Ishida T, Ishii T, et al. Clinical significance of serum Th1-, Th2- and regulatory T cells-associated cytokines in adult T-cell leukemia/lymphoma: high interleukin-5 and -10 levels are significant unfavorable prognostic factors. *International journal of cancer. Journal international du cancer* 2006;118:3054-3061.
- [13] Blay JY, Burdin N, Rousset F, et al. Serum interleukin-10 in non-Hodgkin's lymphoma: a prognostic factor. *Blood* 1993;82:2169-2174.
- [14] Lech-Maranda E, Bienvenu J, Michallet AS, et al. Elevated IL-10 plasma levels correlate with poor prognosis in diffuse large B-cell lymphoma. *European cytokine network* 2006;17:60-66.
- [15] el-Far M, Fouda M, Yahya R, el-Baz H. Serum IL-10 and IL-6 levels at diagnosis as independent predictors of outcome in non-Hodgkin's lymphoma. *Journal of physiology and biochemistry* 2004;60:253-258.
- [16] Stasi R, Zinzani PL, Galieni P, et al. Prognostic value of serum IL-10 and soluble IL-2 receptor levels in aggressive non-Hodgkin's lymphoma. *British journal of haematology* 1994;88:770-777.
- [17] Czarneski J, Lin YC, Chong S, et al. Studies in NZB IL-10 knockout mice of the requirement of IL-10 for progression of B-cell lymphoma. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K* 2004;18:597-606.
- [18] Mocellin S, Marincola FM, Young HA. Interleukin-10 and the immune response against cancer: a counterpoint. *Journal of leukocyte biology* 2005;78:1043-1051.
- [19] Cervenak L, Morbidelli L, Donati D, et al. Abolished angiogenicity and tumorigenicity of Burkitt lymphoma by interleukin-10. *Blood* 2000;96:2568-2573.
- [20] Costeas PA, Koumas L, Koumouli A, Kyriakou-Giantsiou A, Papaloizou A. Cytokine polymorphism frequencies in the Greek Cypriot population. *Eur J Immunogenet* 2003;30:341-343.
- [21] Lan Q, Zheng T, Rothman N, et al. Cytokine polymorphisms in the Th1/Th2 pathway and susceptibility to non-Hodgkin lymphoma. *Blood* 2006;107:4101-4108.

- 1
2
3 [22] Rothman N, Skibola CF, Wang SS, et al. Genetic variation in TNF and IL10 and risk of non-
4 Hodgkin lymphoma: a report from the InterLymph Consortium. *Lancet Oncol* 2006;7:27-38.
5 [23] Breen EC, Boscardin WJ, Detels R, et al. Non-Hodgkin's B cell lymphoma in persons with acquired
6 immunodeficiency syndrome is associated with increased serum levels of IL10, or the IL10
7 promoter -592 C/C genotype. *Clinical immunology* 2003;109:119-129.
8 [24] Purdue MP, Lan Q, Kricker A, et al. Polymorphisms in immune function genes and risk of non-
9 Hodgkin lymphoma: findings from the New South Wales non-Hodgkin Lymphoma Study.
10 *Carcinogenesis* 2007;28:704-712.
11 [25] Minnicelli C, Barros MH, Klumb CE, Romano SO, Zalcborg IR, Hassan R. Relationship of Epstein-
12 Barr virus and interleukin 10 promoter polymorphisms with the risk and clinical outcome of
13 childhood Burkitt lymphoma. *PLoS One* 2012;7:e46005.
14 [26] Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide
15 polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of
16 systemic lupus erythematosus. *Journal of immunology* 2001;166:3915-3922.
17 [27] Salhi A, Rodrigues V, Jr., Santoro F, et al. Immunological and genetic evidence for a crucial role of
18 IL-10 in cutaneous lesions in humans infected with *Leishmania braziliensis*. *Journal of*
19 *immunology* 2008;180:6139-6148.
20 [28] Sun F, Sun Y, Zhang D, Zhang J, Song B, Zheng H. Association of interleukin-10 gene
21 polymorphism with cachexia in Chinese patients with gastric cancer. *Annals of clinical and*
22 *laboratory science* 2010;40:149-155.
23 [29] Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of
24 polymorphism in the interleukin-10 gene promoter. *European journal of immunogenetics :*
25 *official journal of the British Society for Histocompatibility and Immunogenetics* 1997;24:1-8.
26 [30] Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide
27 polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-
28 10 (IL-10) locus. *Genes and immunity* 1999;1:151-155.
29 [31] Zeng L, Gu W, Chen K, et al. Clinical relevance of the interleukin 10 promoter polymorphisms in
30 Chinese Han patients with major trauma: genetic association studies. *Critical care* 2009;13:R188.
31 [32] Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the
32 interleukin-10 5' flanking region determine variable interleukin-10 transcription and are
33 associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis and rheumatism*
34 1999;42:1101-1108.
35 [33] Bogunia-Kubik K, Mazur G, Wrobel T, Kuliczowski K, Lange A. Interleukin-10 gene
36 polymorphisms influence the clinical course of non-Hodgkin's lymphoma. *Tissue antigens*
37 2008;71:146-150.
38 [34] Lee JJ, Kim DH, Lee NY, et al. Interleukin-10 gene polymorphism influences the prognosis of T-
39 cell non-Hodgkin lymphomas. *British journal of haematology* 2007;137:329-336.
40 [35] Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by
41 induction of the bcl-2 protein. *The Journal of clinical investigation* 1994;93:424-428.
42 [36] Alas S, Emmanouilides C, Bonavida B. Inhibition of interleukin 10 by rituximab results in down-
43 regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. *Clinical*
44 *cancer research : an official journal of the American Association for Cancer Research*
45 2001;7:709-723.
46 [37] Cohen SB, Crawley JB, Kahan MC, Feldmann M, Foxwell BM. Interleukin-10 rescues T cells from
47 apoptotic cell death: association with an upregulation of Bcl-2. *Immunology* 1997;92:1-5.
48 [38] Gupta M, Han JJ, Stenson M, et al. Elevated serum IL-10 levels in diffuse large B-cell lymphoma:
49 a mechanism of aberrant JAK2 activation. *Blood* 2012;119:2844-2853.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 [39] Slack GW, Gascoyne RD. MYC and aggressive B-cell lymphomas. *Advances in anatomic pathology* 2011;18:219-228.
- 4
5 [40] Howell WM, Rose-Zerilli MJ. Interleukin-10 polymorphisms, cancer susceptibility and prognosis. *Familial cancer* 2006;5:143-149.
- 6
7 [41] Meenagh A, Williams F, Ross OA, et al. Frequency of cytokine polymorphisms in populations from western Europe, Africa, Asia, the Middle East and South America. *Human immunology* 2002;63:1055-1061.
- 8
9 [42] Mok CC, Lanchbury JS, Chan DW, Lau CS. Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. *Arthritis and rheumatism* 1998;41:1090-1095.
- 10
11 [43] Hoffmann SC, Stanley EM, Cox ED, et al. Ethnicity greatly influences cytokine gene polymorphism distribution. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2002;2:560-567.
- 12
13 [44] Padyukov L, Hahn-Zoric M, Lau YL, Hanson LA. Different allelic frequencies of several cytokine genes in Hong Kong Chinese and Swedish Caucasians. *Genes and immunity* 2001;2:280-283.
- 14
15 [45] Weiss KM, Terwilliger JD. How many diseases does it take to map a gene with SNPs? *Nature genetics* 2000;26:151-157.
- 16
17 [46] Syvanen AC. Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nature reviews. Genetics* 2001;2:930-942.
- 18
19 [47] Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors--a twin study. *Genes and immunity* 2002;3:407-413.
- 20
21 [48] Moraes MO, Santos AR, Schonkeren JJ, et al. Interleukin-10 promoter haplotypes are differently distributed in the Brazilian versus the Dutch population. *Immunogenetics* 2003;54:896-899.
- 22
23 [49] Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics* 2007;81:559-575.
- 24
25 [50] Muller AM, Ihorst G, Mertelsmann R, Engelhardt M. Epidemiology of non-Hodgkin's lymphoma (NHL): trends, geographic distribution, and etiology. *Annals of hematology* 2005;84:1-12.
- 26
27 [51] Hosgood HD, 3rd, Au WY, Kim HN, et al. IL10 and TNF variants and risk of non-Hodgkin lymphoma among three Asian populations. *International journal of hematology* 2013;97:793-799.
- 28
29 [52] Wong HL, Breen EC, Pfeiffer RM, et al. Cytokine signaling pathway polymorphisms and AIDS-related non-Hodgkin lymphoma risk in the multicenter AIDS cohort study. *AIDS* 2010;24:1025-1033.
- 30
31 [53] Cao HY, Zou P, Zhou H. Genetic association of interleukin-10 promoter polymorphisms and susceptibility to diffuse large B-cell lymphoma: a meta-analysis. *Gene* 2013;519:288-294.
- 32
33 [54] Westendorp RG, Langermans JA, Huizinga TW, et al. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997;349:170-173.
- 34
35 [55] Grondal G, Kristjansdottir H, Gunnlaugsdottir B, et al. Increased number of interleukin-10-producing cells in systemic lupus erythematosus patients and their first-degree relatives and spouses in Icelandic multicase families. *Arthritis and rheumatism* 1999;42:1649-1654.
- 36
37 [56] Lech-Maranda E, Baseggio L, Biennu J, et al. Interleukin-10 gene promoter polymorphisms influence the clinical outcome of diffuse large B-cell lymphoma. *Blood* 2004;103:3529-3534.
- 38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table I Characteristics of study participants.

Characteristics	Patients n = 317 N (%)	Controls n = 330 N (%)
Age		
<50	98 (31)	238 (72)
50-60	105 (33)	52 (16)
>60	114 (36)	40 (12)
Gender		
Male	168 (53)	206 (62)
Female	149 (47)	124 (38)
Race		
Malay	130 (41)	118 (36)
Chinese	147 (46)	162 (49)
Indian	40 (13)	50 (15)
NHL subtypes		
B-cell Lymphoma	276 (87)	-
• DLBCL	164 (52)	-
• FL	61 (19)	-
• Other B-cell	51 (16)	-
T-cell Lymphoma	41 (13)	-

B-cell Lymphoma = B-cell NHL; T-cell Lymphoma = T-cell NHL;

DLBCL = Diffuse large B-cell lymphoma; FL = Follicular Lymphoma

Table II Association results of IL10 promoter SNPs towards NHL susceptibility stratified according to 3 major ethnic groups of the Malaysian population.

SNP ID	Factor	MAF		Additive (1 vs 2)		Dominant (11 vs 12 + 22)		Recessive (11 + 12 vs 22)		Model	Power of Association P<0.05
		cases	ctrl	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value		
rs1800896	Chinese	0.07	0.05	1.67 (0.78-3.6)	0.187	1.67 (0.78-3.6)	0.187	NA	NA	-	-
IL10-1082 (A>G)*	Indian	0.24	0.14	1.74 (0.71-4.51)	0.235	2.09 (0.77-5.85)	0.152	0.64 (0.02-17.9)	0.763	-	-
	Malay	0.10	0.08	1.36 (0.69-2.77)	0.383	1.47 (0.7-3.13)	0.315	0.71 (0.03-19.23)	0.817	-	-
rs1800871	Chinese	0.35	0.27	1.43 (1-2.05)	0.051	1.39 (0.86-2.27)	0.181	2.27 (1.06-5.07)	0.039*	Recessive	44%
IL10-819 (T>C)*	Indian	0.32	0.37	0.65 (0.31-1.3)	0.236	0.42 (0.15-1.14)	0.096	0.99 (0.24-3.88)	0.991	-	-
	Malay	0.36	0.25	1.53 (0.99-2.4)	0.058	1.28 (0.72-2.29)	0.396	4.55 (1.59-14.93)	0.007*	Recessive	74%

Abbreviations: CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; NA, not available

* Major allele > minor allele

All OR and P-value are adjusted for gender and age.

Table III Association results of IL10 promoter SNPs towards all and common subtypes of NHL susceptibility in the combined Malay-Chinese cohort.

SNP ID	Factor	MAF		Additive (1 vs 2)		Dominant (11 vs 12 + 22)		Recessive (11 + 12 vs 22)		Model	Power of Association P<0.05
		cases	ctrl	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value		
rs1800896 IL10-1082 (A>G)*	All NHL	0.08	0.06	1.48 (0.9-2.47)	0.129	1.54 (0.91-2.62)	0.106	0.78 (0.03-20.76)	0.862	-	-
	All B-cell	0.08	0.06	1.5 (0.88-2.58)	0.129	1.59 (0.92-2.76)	0.1	NA	0.980	-	-
	DLBCL	0.05	0.06	0.89 (0.44-1.73)	0.737	0.92 (0.44-1.83)	0.815	NA	0.981	-	-
	FL	0.13	0.06	3.97 (1.78-8.89)	0.001*	4.5 (1.94-10.44)	0.0004*	NA	0.989	Dominant	81%
rs1800871 IL10-819 (T>C)*	All T-cell	0.10	0.06	1.63 (0.64-3.73)	0.265	1.54 (0.53-3.91)	0.387	5.69 (0.21-153.29)	0.234	-	-
	All NHL	0.35	0.26	1.49 (1.13-1.97)	0.005*	1.38 (0.96-2)	0.085	2.87 (1.55-5.48)	0.001*	Recessive	87%
	All B-cell	0.35	0.26	1.51 (1.13-2.03)	0.006*	1.44 (0.98-2.12)	0.068	2.72 (1.43-5.3)	0.003*	Recessive	84%
	DLBCL	0.34	0.26	1.41 (1.02-1.96)	0.036*	1.22 (0.79-1.9)	0.366	2.95 (1.48-5.96)	0.002*	Recessive	85%
	FL	0.36	0.26	1.59 (0.94-2.7)	0.086	1.84 (0.93-3.7)	0.082	1.6 (0.44-5.03)	0.441	-	-
All T-cell	0.34	0.26	1.43 (0.82-2.43)	0.197	1.18 (0.57-2.42)	0.659	3.1 (1.03-8.34)	0.031*	Recessive	58%	

Abbreviations: CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; NA, not available

*Major allele > minor allele

All OR and P-value are adjusted for gender, age and race.

Table IV Association results of IL10 promoter SNPs towards NHL susceptibility stratified according to gender in the combined Malay-Chinese cohort.

SNP ID	Factor	MAF		Additive (1 vs 2)		Dominant (11 vs 12 + 22)		Recessive (11 + 12 vs 22)		Model	Power of Association
		cases	ctrl	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value		P<0.05
rs1800896	Male	0.08	0.06	1.3 (0.66-2.6)	0.456	1.27 (0.63-2.57)	0.511	NA	0.987	-	-
IL10-1082 (A>G)*	Female	0.08	0.06	1.73 (0.81-3.86)	0.172	1.99 (0.89-4.64)	0.098	NA	0.987	-	-
rs1800871	Male	0.36	0.25	1.56 (1.08-2.27)	0.019*	1.44 (0.87-2.37)	0.154	3.23 (1.43-7.7)	0.006*	Recessive	71%
IL10-819 (T>C)*	Female	0.34	0.28	1.36 (0.9-2.08)	0.153	1.23 (0.71-2.16)	0.463	2.58 (1-7.33)	0.059	-	-

Abbreviations: CI, confidence interval; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; NA, not available

*Major allele > minor allele

All OR and P-value are adjusted for gender, age and race.