Genetic variation in *TNF* and *IL10* and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium

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Summary

Background **Common genetic variants in immune and inflammatory response genes can affect the risk of developing non-Hodgkin lymphoma. We aimed to test this hypothesis using previously unpublished data from eight European, Canadian, and US case-control studies of the International Lymphoma Epidemiology Consortium (InterLymph).**

Methods **We selected 12 single-nucleotide polymorphisms for analysis, on the basis of previous functional or association data, in nine genes that have important roles in lymphoid development, Th1/Th2 balance, and proinflammatory or anti-inflammatory pathways (*IL1A, IL1RN, IL1B, IL2, IL6, IL10, TNF, LTA,* and *CARD15*). Genotype data for one or more single-nucleotide polymorphisms were available for 3586 cases of non-Hodgkin lymphoma and for 4018 controls, and were assessed in a pooled analysis by use of a random-effects logistic regression model.**

Findings **The tumour necrosis factor (*TNF*) –308G→A polymorphism was associated with increased risk of non-Hodgkin lymphoma (p for trend=0·005), particularly for diffuse large B-cell lymphoma, the main histological subtype (odds ratio 1·29 [95% CI 1·10–1·51] for GA and 1·65 [1·16–2·34] for AA, p for trend <0·0001), but not for follicular lymphoma. The interleukin 10 *(IL10)* –3575T→A polymorphism was also associated with increased risk of non-Hodgkin lymphoma (p for trend=0·02), again particularly for diffuse large B-cell lymphoma (p for trend=0·006). For individuals homozygous for the *TNF* –308A allele and carrying at least one *IL10* –3575A allele, risk of diffuse large B-cell lymphoma doubled (2·13 [1·37–3·32], p=0·00083).**

Interpretation **Common polymorphisms in *TNF* and *IL10*, key cytokines for the inflammatory response and Th1/Th2 balance, could be susceptibility loci for non-Hodgkin lymphoma. Moreover, our results underscore the importance of consortia for investigating the genetic basis of chronic diseases like cancer.**

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Introduction

The mechanisms underlying differences in immune response between individuals are complex and include inherited genetic variation and cumulative antigenic exposure to infectious and other environmental challenges that give rise to immunological memory. Common variations in genes of the immune system have evolved through selective pressure to ensure host-pathogen coexistence. However, variants selected to protect against infection could inadvertently lead to a greater risk of other diseases that are less susceptible to selection. Such variants might be expected to predispose to chronic inflammatory disease and malignant diseases of the lymphoid system.

Lymphoid development and differentiation and T-helper (Th)1/Th2 balance (ie, cellular *vs* humoral immunity) are regulated in part by key cytokines including interleukin (IL)1, IL2, IL6, IL10, tumour necrosis factor (TNF)α, and lymphotoxin (LT)α.[1–7] Furthermore, deregulated concentrations of several cytokines (eg, IL6, IL10, and TNFα) have been detected in patients with lymphoma and were associated with an adverse prognosis.[8–10] Evidence that genetic susceptibility plays a part in lymphomagenesis is provided by strong and consistent findings from registry and population-based epidemiological studies that show an increased risk of non-Hodgkin lymphoma in individuals with a family history of this or other haemopoietic malignant diseases.[11,12]

Here, we tested the hypothesis that single-nucleotide polymorphisms in nine candidate genes that have important roles in lymphoid development, proinflammatory or anti-inflammatory pathways, and Th1/Th2 balance[7] are associated with risk of non-Hodgkin lymphoma.

Methods

Study characteristics

The International Lymphoma Epidemiology Consortium (InterLymph, http://epi.grants.cancer.gov/InterLymph) is a voluntary consortium established in 2000 to facilitate collaboration between epidemiological studies of

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	Number with genotype data		Specific study characteristics
	Cases	Controls	
EPILYMPH—Italy*	144	113	Both sexes, all subtypes
EPILYMPH—Spain[15]†	354	569	Both sexes, all subtypes
University of California San Francisco[16]‡	309	685	Both sexes, all subtypes
EPILYMPH—Germany[17]§	482	481	Both sexes, all subtypes
Connecticut[18]¶	497	561	Women only, all subtypes
UK[19]‖	461	461	Both sexes, DLBCL and follicular lymphoma only
NCI-SEER[12]**	963	747	Both sexes, all subtypes
British Columbia††	376	401	Both sexes, all subtypes

DLBCL=diffuse large B-cell lymphoma. *Cagliari, Nuoro, and Oristano, Italy. †Barcelona, Tortosa, Reus, and Madrid, Spain. ‡Santa Clara, San Mateo, San Francisco, Marin, Contra Costa, and Alameda counties, San Francisco Bay Area, CA, USA. §Ludwigshafen/Upper Palatinate, Heidelberg/Rhine-Neckar County, Würzburg/Lower Frankonia, Hamburg, Bielefeld, and Munich, Germany. ¶Connecticut, USA. ‖Counties of North, East, and West Yorkshire; Lancashire, district of South Lakeland; Caradon district of Cornwall, South Devon, Dorset, and South Hampshire, UK. **Detroit, Iowa, Los Angeles, Seattle, USA. ††Greater Victoria and Vancouver, Canada.

Table 1: **Description of studies participating in InterLymph genotyping project**

lymphoma worldwide.[13,14] It was formed to coordinate selected analyses across similarly designed studies of lymphoma; to increase statistical power to detect associations, especially for histological subtypes of non-Hodgkin lymphoma that might have differing causes; and to provide protection against false-negative and false-positive findings. In this study, we analysed data from the eight studies in InterLymph who were willing and able to participate in the genotyping project. Detailed information on participant recruitment and pathology review has been published for six of the eight studies.[12,15–19] The EPILYMPH—Italy study enrolled controls from a random sample of the general population by use of population lists, and the British Columbia study enrolled controls from a random sample of the population by use of Provincial Health Insurance records. Both studies used WHO classification for non-Hodgkin lymphoma.[20] All studies were population-based, with the exception of the EPILYMPH—Spain study, which was hospital-based. We excluded patients with non-Hodgkin lymphoma who were HIV-positive, and included only white participants, almost all of whom were of European descent, to keep population homogeneity to a maximum.

All studies provided details of age and sex and indicated whether a case was diagnosed with diffuse large B-cell lymphoma, follicular lymphoma, or other histology. The InterLymph genotype working group decided a priori not to investigate genotype associations for other, less common histological subtypes, because the statistical power would have been very limited, even in a pooled analysis of this size. In addition, the pathological diagnosis of diffuse large B-cell lymphoma and follicular lymphoma has been stable and comparable in Canada and the USA and in Europe, allowing data to be pooled for these subgroups across studies, even though pathology samples were not reviewed centrally. Although some studies did not divide diffuse large-cell lymphomas into B and T subtypes, we use the term diffuse large B-cell lymphoma throughout because almost all diffuse large-cell tumours derive from B cells. All studies were approved by their local ethics review committee, and written informed consent was obtained from all participants. All eight studies provided genotype data for all cases of non-Hodgkin lymphoma enrolled in their study, with the exception of the UK study, for which only cases diagnosed with diffuse large B-cell lymphoma and follicular lymphoma and their individually matched controls had been genotyped; as a consequence, the UK data were used only in histology-specific analyses.

Laboratory analysis

We chose 12 single-nucleotide polymorphisms (minor allele frequency range 0·02–0·44), each of which could be functionally important, in nine genes for co-ordinated genotyping and analysis: in location 2q14, *IL1A* –889C→T (rs1800587), *IL1B* –511C→T (rs16944), and *IL1B* –31C→T (rs1143627); in 2q14.2, *IL1RN* 9589A→T (rs454078); in 4q26–27, *IL2* –384T→G (rs2069762); in 7p21, *IL6* –174G→C (rs1800795) and *IL6* –597G→A (rs1800797); in 1q31–32, *IL10* –1082A→G (rs1800896) and *IL10* –3575T→A (rs1800890); in 6p21.3, *TNF* –308G→A (rs1800629) and *LTA* 252A→G (rs909253); and in 16q21, *CARD15* Ex11–35→C (rs2066847).

DNA samples were analysed at one of six laboratories. Five laboratories used the Taqman™ platform (Applied Biosystems, Foster City, CA, USA) exclusively or mainly for genotyping, and one laboratory, which analysed samples for the EPILYMPH—Germany study, used Pyrosequencing™ or allele-specific PCR. Sequence data and assay conditions for Taqman™ assays are available on the NCI SNP500 website http://snp500cancer.nci.nih.gov. To ensure that genotyping results were consistent across studies, every laboratory analysed the same set of DNA samples from 102 ethnically diverse individuals that had previously been sequenced and genotyped on one or more platforms as part of the SNP500Cancer project.[21] All laboratories completed genotype analysis before a comparison with the publicly available genotypes on the NCI SNP500 website. We assessed concordance across laboratories and rechecked quality control data for assays not in Hardy-Weinberg equilibrium at $p<0·05$ to confirm accuracy.

Statistical analysis

To investigate the association between the single-nucleotide polymorphisms and risk of non-Hodgkin lymphoma, risk estimates were estimated with a random-effects logistic regression model that adjusted for age (<50, 50–59, 60–69, ⩾70 years), sex, and study centre. An exact test was used to calculate risk estimates

	Controls	Cases	Odds ratio (95% CI)	p	p for heterogeneity
***TNF* –308G→A**					
GG	2312 (74%)	1927 (71%)	1 (ref)	NA	NA
GA	719 (23%)	705 (26%)	1·18 (1·04–1·33)	0·009	NA
AA	87 (3%)	86 (3%)	1·25 (0·91–1·70)	0·16	0·47†
GA or AA	806 (26%)	791 (29%)	1·19 (1·05–1·33)	0·005	0·36‡
Trend	3118 (100%)	2718 (100%)	1·16 (1·04–1·28)	0·005	0·48§
***LTA* 252A→G**					
AA	1699 (48%)	1465 (47%)	1 (ref)	NA	NA
AG	1484 (42%)	1281 (42%)	1·00 (0·86–1·16)	1·00	NA
GG	326 (9%)	339 (11%)	1·18 (0·96–1·44)	0·11	0·014†
AG or GG	1810 (52%)	1620 (53%)	1·01 (0·88–1·16)	0·89	0·011‡
Trend	3509 (100%)	3085 (100%)	1·05 (0·95–1·15)	0·26	0·030§
***IL10* –3575T→A**					
TT	1419 (41%)	1172 (39%)	1 (ref)	NA	NA
TA	1604 (46%)	1423 (47%)	1·10 (0·98–1·22)	0·098	NA
AA	439 (13%)	435 (14%)	1·19 (1·00–1·41)	0·044	0·65†
TA or AA	2043 (59%)	1858 (61%)	1·11 (1·01–1·23)	0·037	0·82‡
Trend	3462 (100%)	3030 (100%)	1·09 (1·01–1·17)	0·02	0·43§
***IL10* –1082A→G**					
AA	972 (31%)	804 (30%)	1 (ref)	NA	NA
AG	1513 (49%)	1326 (49%)	1·08 (0·95–1·22)	0·23	NA
GG	623 (20%)	580 (21%)	1·13 (0·96–1·32)	0·13	0·75†
AG or GG	2136 (69%)	1906 (70%)	1·09 (0·97–1·22)	0·13	0·86‡
Trend	3108 (100%)	2710 (100%)	1·06 (0·99–1·14)	0·11	0·55§
***IL1A* –889C→T**					
CC	1740 (50%)	1494 (49%)	1 (ref)	NA	NA
CT	1436 (42%)	1306 (43%)	1·06 (0·96–1·18)	0·26	NA
TT	281 (8%)	253 (8%)	1·07 (0·89–1·29)	0·47	0·67†
CT or TT	1717 (50%)	1559 (51%)	1·06 (0·96–1·17)	0·23	0·61‡
Trend	3457 (100%)	3053 (100%)	1·05 (0·97–1·13)	0·25	0·42§
***IL1B* –511 C→T**					
CC	1559 (45%)	1371 (45%)	1 (ref)	NA	NA
CT	1566 (45%)	1338 (44%)	0·99 (0·89–1·10)	0·86	NA
TT	365 (10%)	358 (12%)	1·11 (0·92–1·35)	0·28	0·14†
CT or TT	1931 (55%)	1696 (55%)	1·01 (0·92–1·12)	0·79	0·073‡
Trend	3490 (100%)	3067 (100%)	1·03 (0·96–1·11)	0·39	0·035§
***IL1B* –31C→T**					
TT	1520 (44%)	1362 (45%)	1 (ref)	NA	NA
CT	1569 (45%)	1304 (43%)	0·95 (0·85–1·05)	0·32	NA
CC	379 (11%)	364 (12%)	1·07 (0·90–1·28)	0·43	0·096†
CT or CC	1948 (56%)	1668 (55%)	0·97 (0·88–1·07)	0·58	0·044‡
Trend	3468 (100%)	3030 (100%)	1·01 (0·93–1·08)	0·86	0·032§
***IL1RN* 9589A→T**					
AA	1870 (54%)	1558 (52%)	1 (ref)	NA	NA
AT	1345 (39%)	1230 (41%)	1·10 (0·99–1·22)	0·086	NA
TT	254 (7%)	232 (8%)	1·13 (0·93–1·37)	0·21	0·4†
AT or TT	1599 (46%)	1462 (48%)	1·10 (1·00–1·22)	0·053	0·22‡
Trend	3469 (100%)	3020 (100%)	1·08 (1·00–1·17)	0·062	0·55§
***IL2* –384T→G**					
TT	1755 (51%)	1491 (49%)	1 (ref)	NA	NA
TG	1389 (40%)	1281 (42%)	1·09 (0·97–1·23)	0·14	NA
GG	310 (9%)	267 (9%)	0·99 (0·79–1·25)	0·96	0·073†
TG or GG	1699 (49%)	1548 (51%)	1·07 (0·97–1·18)	0·16	0·34‡
Trend	3454 (100%)	3039 (100%)	1·04 (0·96–1·12)	0·31	0·40§
***IL6* –174G→C**					
GG	1277 (36%)	1097 (36%)	1 (ref)	NA	NA
GC	1658 (47%)	1470 (48%)	1·02 (0·92–1·14)	0·66	NA
CC	564 (16%)	499 (16%)	1·01 (0·87–1·18)	0·86	0·54†
GC or CC	2222 (64%)	1969 (64%)	1·02 (0·92–1·13)	0·68	0·47‡
Trend	3499 (100%)	3066 (100%)	1·01 (0·94–1·08)	0·78	0·33§
***IL6* –597G→A**					
GG	1151 (38%)	998 (38%)	1 (ref)	NA	NA
GA	1423 (46%)	1243 (47%)	1·01 (0·90–1·13)	0·92	NA
AA	494 (16%)	417 (16%)	0·95 (0·81–1·12)	0·56	0·41†
GA or AA	1917 (62%)	1660 (62%)	0·99 (0·89–1·11)	0·89	0·23‡
Trend	3068 (100%)	2658 (100%)	0·98 (0·91–1·06)	0·65	0·17§

(continues)

(continued)

	Controls	Cases	Odds ratio (95% CI)	p	p for heterogeneity
CARD15 Ex11–35→C					
– –	3347 (96%)	2926 (95%)	1 (ref)	NA	NA
– +	149 (4%)	141 (5%)	1·08 (0·85–1·37)	0·54	NA
+ +	1 (<1%)	2 (<1%)	2·29 (0·12–135)	0·60	0·31†
– + or + +	150 (4%)	143 (5%)	1·09 (0·86–1·39)	0·47	0·27‡
Trend	3497 (100%)	3069 (100%)	1·10 (0·87–1·40)	0·41	0·25§

Data are number of individuals (%) unless otherwise stated. NA=not applicable. *Includes up to seven studies that enrolled all histological types of non-Hodgkin lymphoma with genotype data for specific single-nucleotide polymorphisms. †Test for heterogeneity for codominant model. ‡Test for heterogeneity for dominant model. §Test for heterogeneity for additive model (ie, trend).

Table 2: **Pooled genotype frequencies and risks for all histologies by single-nucleotide polymorphism***

and p values for homozygous carriers of the *CARD15* Ex11–35→C variant, and for homozygous carriers of *TNF* –308G→A for diffuse large B-cell lymphoma in the National Cancer Institute-Surveillance Epidemiology and End Results (NCI-SEER) Detroit centre, because there was only one homozygous case or control in these analyses.

Heterogeneity across studies was assessed by comparison of the logistic-regression model with and without the cross-product terms of the genotypes and study centre by use of a likelihood-ratio test. Heterogeneity between subtypes of non-Hodgkin lymphoma was assessed by comparing them directly in a logistic-regression model and testing for differences in the genotype association. The test for trend was assessed with an additive model—that is, with a single variable for genotype coded as the number of variant alleles, in the logistic-regression model. All genotype analyses were done with STATA version 8.2.

We assessed the robustness of the findings by calculating the false-discovery rate,[22] defined as the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses, which yields a p value corrected for multiple comparisons, and by application of the false-positive report probability method.[23] Before analysis, investigators were asked to provide a range of prior probabilities of association with non-Hodgkin lymphoma for every single-nucleotide polymorphism, based on their interpretation of all sources of information; prior probability values for *TNF* –308G→A and *IL10* –3575T→A varied from 0·001 (ie, that a given single-nucleotide polymorphism has a one in one thousand chance of being truly associated with non-Hodgkin lymphoma) to 0·1. We divided these values by two and applied them to the two histology-specific results presented here, using the observed risk estimates from the additive model. A false-positive report probability value rejection criterion of 0·2 was used to designate findings as noteworthy.[23]

	Controls	Cases						p for difference between histological types
		Diffuse large B-cell lymphoma			Follicular lymphoma			
		n (%)	Odds ratio (95% CI)	p	n (%)	Odds ratio (95% CI)	p	
***TNF* –308G→A**								
GG	2597 (73%)	716 (66%)	1 (ref)	NA	576 (71%)	1(ref)	NA	..
GA	854 (24%)	312 (29%)	1·29 (1·10–1·51)	0·002	209 (26%)	1·03 (0·86–1·23)	0·78	..
AA	113 (3%)	53 (5%)	1·65 (1·16–2·34)	0·006	25 (3%)	0·92 (0·55–1·54)	0·74	..
GA or AA	967 (27%)	365 (34%)	1·33 (1·14–1·55)	0·00021	234 (29%)	1·01 (0·85–1·21)	0·88	..
Trend	3564 (100%)	1081 (100%)	1·29 (1·14–1·46)	<0·0001	810 (100%)	1·00 (0·86–1·16)	0·98	0·0037
***LTA* 252A→G**								
AA	1876 (47%)	519 (44%)	1 (ref)	NA	424 (48%)	1 (ref)	NA	..
AG	1701 (43%)	491 (42%)	1·06 (0·88–1·29)	0·52	371 (42%)	0·91 (0·78–1·07)	0·25	..
GG	380 (10%)	159 (14%)	1·47 (1·18–1·84)	0·001	97 (11%)	1·04 (0·80–1·33)	0·78	..
AG or GG	2081 (53%)	650 (56%)	1·13 (0·96–1·33)	0·14	468 (52%)	0·93 (0·80–1·08)	0·37	..
Trend	3957 (100%)	1169 (100%)	1·16 (1·04–1·29)	0·007	892 (100%)	0·98 (0·87–1·10)	0·71	0·015
***IL10* –3575T→A**								
TT	1593 (41%)	422 (36%)	1 (ref)	NA	323 (37%)	1 (ref)	NA	..
TA	1816 (46%)	567 (48%)	1·20 (1·04 –1·39)	0·015	418 (47%)	1·10 (0·93–1·29)	0·28	..
AA	512 (13%)	180 (15%)	1·28 (1·04–1·57)	0·02	142 (16%)	1·24 (0·99–1·55)	0·066	..
TA or AA	2328 (59%)	747 (64%)	1·22 (1·06–1·40)	0·006	560 (63%)	1·13 (0·97–1·32)	0·13	..
Trend	3921 (100%)	1169 (100%)	1·15 (1·04–1·26)	0·006	883 (100%)	1·11 (1·00–1·24)	0·059	0·62
***IL10* –1082A→G**								
AA	1089 (31%)	294 (27%)	1 (ref)	NA	227 (28%)	1 (ref)	NA	..
AG	1734 (49%)	537 (50%)	1·14 (0·97–1·36)	0·12	388 (48%)	1·02 (0·85–1·23)	0·83	..
GG	742 (21%)	253 (23%)	1·23 (1·00–1·52)	0·053	194 (24%)	1·12 (0·90–1·40)	0·30	..
AG or GG	2476 (69%)	790 (73%)	1·17 (1·00–1·37)	0·048	582 (72%)	1·05 (0·88–1·25)	0·57	..
Trend	3565 (100%)	1084 (100%)	1·11 (1·00–1·22)	0·043	809 (100%)	1·06 (0·95–1·18)	0·31	0·49
***TNF* –308G→A and *IL10* –3575T→A**								
GG/TT	1077 (30%)	258 (24%)	1 (ref)	NA	223 (28%)	1 (ref)	NA	..
GG/TA	1180 (33%)	342 (32%)	1·26 (1·04–1·52)	0·016	258 (32%)	1·03 (0·85–1·27)	0·74	..
GA/TT	339 (10%)	115 (11%)	1·44 (1·11–1·86)	0·006	64 (8%)	0·87 (0·64–1·19)	0·39	..
GA/TA	390 (11%)	143 (13%)	1·51 (1·18–1·92)	0·00094	115 (14%)	1·27 (0·98–1·65)	0·07	..
GG/AA	322 (9%)	109 (10%)	1·39 (1·07–1·81)	0·015	91 (11%)	1·24 (0·94–1·65)	0·13	..
AA/TT	46 (1%)	16 (2%)	1·57 (0·86–2·86)	0·14	6 (1%)	0·60 (0·25–1·45)	0·25	..
GA/AA	120 (3%)	51 (5%)	1·69 (1·17–2·43)	0·0047	29 (4%)	1·01 (0·65–1·56)	0·98	..
AA/TA or AA	66 (2%)	36 (3%)	2·13 (1·37–3·32)	0·00083	19 (2%)	1·18 (0·69–2·04)	0·55	..
***IL1A* –889C→T**								
CC	1962 (50%)	601 (51%)	1 (ref)	NA	435 (49%)	1 (ref)	NA	..
CT	1631 (42%)	485 (41%)	0·97 (0·84–1·11)	0·62	372 (42%)	0·99 (0·84–1·16)	0·88	..
TT	319 (8%)	84 (7%)	0·85 (0·65–1·11)	0·23	80 (9%)	1·00 (0·68–1·48)	1·00	..
CT or TT	1950 (50%)	569 (49%)	0·95 (0·83–1·08)	0·42	452 (51%)	1·00 (0·86–1·16)	1·00	..
Trend	3912 (100%)	1170 (100%)	0·94 (0·85–1·05)	0·27	887 (100%)	1·02 (0·90–1·14)	0·79	0·18
***IL1B* –511C→T**								
CC	1744 (44%)	517 (45%)	1 (ref)	NA	396 (44%)	1 (ref)	NA	..
CT	1773 (45%)	513 (44%)	0·99 (0·86–1·15)	0·94	400 (45%)	1·01 (0·86–1·18)	0·89	..
TT	426 (11%)	131 (11%)	1·01 (0·81–1·26)	0·93	98 (11%)	1·02 (0·80–1·32)	0·86	..
CT or TT	2199 (56%)	644 (55%)	1·00 (0·87–1·14)	0·98	498 (56%)	1·01 (0·87–1·18)	0·86	..
Trend	3943 (100%)	1161 (100%)	1·00 (0·91–1·11)	0·97	894 (100%)	1·01 (0·90–1·13)	0·84	1·00
***IL1B* –31C→T**								
TT	1707 (44%)	517 (45%)	1 (ref)	NA	396 (45%)	1 (ref)	NA	..
CT	1778 (45%)	508 (44%)	0·96 (0·84–1·11)	0·62	385 (44%)	0·95 (0·81–1·12)	0·55	..
CC	437 (11%)	135 (12%)	1·01 (0·81–1·26)	0·92	99 (11%)	0·99 (0·77–1·27)	0·93	..
CT or CC	2215 (56%)	643 (55%)	0·97 (0·85–1·11)	0·70	484 (55%)	0·96 (0·82–1·12)	0·59	..
Trend	3922 (100%)	1160 (100%)	0·99 (0·90–1·10)	0·88	880 (100%)	0·98 (0·88–1·10)	0·73	0·83
***IL1RN* 9589A→T**								
AA	1870 (54%)	474 (53%)	1 (ref)	NA	350 (51%)	1 (ref)	NA	..
AT	1345 (39%)	354 (40%)	1·07 (0·91–1·26)	0·43	285 (41%)	1·14 (0·96–1·36)	0·14	..
TT	254 (7%)	63 (7%)	1·00 (0·72–1·39)	1·00	52 (8%)	1·19 (0·85–1·67)	0·32	..
AT or TT	1599 (46%)	417 (47%)	1·06 (0·91–1·23)	0·48	337 (49%)	1·15 (0·97–1·36)	0·10	..
Trend	3469 (100%)	891 (100%)	1·03 (0·92–1·16)	0·59	687 (100%)	1·11 (0·98–1·27)	0·11	0·43
***IL2* –384T→G**								
TT	1977 (51%)	605 (52%)	1 (ref)	NA	431 (48%)	1 (ref)	NA	..
TG	1585 (40%)	459 (39%)	0·93 (0·79–1·09)	0·35	383 (43%)	1·06 (0·91–1·24)	0·44	..
GG	349 (9%)	102 (9%)	0·92 (0·68–1·23)	0·55	75 (8%)	1·00 (0·76–1·31)	0·98	..
TG or GG	1934 (49%)	561 (48%)	0·92 (0·81–1·06)	0·24	458 (52%)	1·05 (0·91–1·22)	0·51	..
Trend	3911 (100%)	1166 (100%)	0·95 (0·86–1·05)	0·34	889 (100%)	1·02 (0·91–1·15)	0·68	0·37

(continues)

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(continued)

	Controls	Cases						p for difference between histological types
		Diffuse large B-cell lymphoma			Follicular lymphoma			
		n (%)	Odds ratio (95% CI)	p	n (%)	Odds ratio (95% CI)	p	
***IL6* –174G→C**								
GG	1427 (36%)	419 (36%)	1 (ref)	NA	313 (35%)	1 (ref)	NA	..
GC	1858 (47%)	527 (45%)	0·97 (0·83–1·12)	0·66	417 (47%)	0·97 (0·82–1·15)	0·73	..
CC	664 (17%)	217 (19%)	1·08 (0·89–1·31)	0·45	163 (18%)	1·01 (0·81–1·25)	0·95	..
GC or CC	2522 (64%)	744 (64%)	1·00 (0·87–1·15)	0·96	580 (65%)	0·98 (0·84–1·15)	0·81	..
Trend	3949 (100%)	1163 (100%)	1·03 (0·93–1·13)	0·59	893 (100%)	1·00 (0·90–1·11)	0·97	0·87
***IL6* –597G→A**								
GG	1151 (38%)	300 (37%)	1 (ref)	NA	232 (39%)	1 (ref)	NA	..
GA	1423 (46%)	386 (47%)	1·04 (0·88–1·24)	0·64	270 (45%)	0·89 (0·73–1·09)	0·25	..
AA	494 (16%)	127 (16%)	0·99 (0·78–1·25)	0·92	96 (16%)	0·88 (0·68–1·16)	0·37	..
GA or AA	1917 (62%)	513 (63%)	1·03 (0·87–1·21)	0·74	366 (61%)	0·89 (0·74–1·07)	0·22	..
Trend	3068 (100%)	813 (100%)	1·00 (0·90–1·12)	0·95	598 (100%)	0·93 (0·82–1·06)	0·27	0·36
***CARD15* Ex11–35→C**								
––	3770 (96%)	1096 (95%)	1 (ref)	NA	834 (95%)	1 (ref)	NA	..
–+	161 (4%)	49 (4%)	1·01 (0·73–1·42)	0·93	43 (5%)	1·19 (0·70–2·02)	0·53	..
++	1 (<1%)	3 (<1%)	10·32 (0·83–542)	0·038	2 (<1%)	9·04 (0·47–533)	0·087	..
–+or ++	162 (4%)	52 (5%)	1·08 (0·78–1·49)	0·66	45 (5%)	1·26 (0·83–1·94)	0·28	..
Trend	3932 (100%)	1148 (100%)	1·13 (0·83–1·55)	0·43	879 (100%)	1·29 (0·82–2·02)	0·27	0·78

NA=not applicable. *Includes data for up to eight studies with data for specific single-nucleotide polymorphisms.

Table 3: **Pooled genotype frequencies and risks for diffuse large B-cell lymphoma and follicular lymphoma***

Haplotypes were estimated from single-nucleotide polymorphisms within the same chromosomal region using the expectation-maximisation algorithm.[24] Measures of **linkage disequilibrium**, D′ and r^2, were assessed with Haploview.[25] Overall differences in the haplotype distribution between cases and controls were assessed with a global score test,[26] which was adjusted for age, sex, and study centre. The effects of individual haplotypes were estimated from the additive model by fitting of a logistic-regression model and use of the estimated probabilities of the haplotypes as weights to update the regression coefficients in an iterative manner.[26] All haplotype analyses were done with the statistical package **Haplo Stats** version 1.1.0. Fixed-effects pooled analyses for haplotype associations are reported, although similar results were obtained when haplotypes were estimated for each individual study and then combined in a meta-analysis with random-effects models (not shown).

Linkage disequilibrium
The non-random association of two or more genetic markers on the same chromosome, usually in close proximity, that tend to be inherited together either more or less frequently in any given population than would be expected from the distance between them.

Haplo Stats
http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm

Role of the funding source

The study sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 shows brief details of the eight case-control studies of the InterLymph consortium that participated in this project. The median response rate for participants who were both interviewed and provided a source of genomic DNA (blood, or in some studies, buccal cells) was 71·2% (range 45·4–84·6) for cases and 49·6% (27·6–63·9) for controls. Sensitivity analyses showed that these results were unchanged after exclusion of the study with the lowest response rate for either cases or controls (not shown).

The study populations included only adults; the mean age of cases was 58·7 years (SD 12·9) and of controls was 58·1 years (14·0). All studies provided data for all single-nucleotide polymorphisms at the time analysis began, except the UK study, which did not have data for *IL1RN* 9589A→T or IL6 –597G→A, and the British Columbia study, which did not have data for *IL6* –597G→A, *IL10* –1082A→G, or TNF –308G→A.

IL2 –384T→G and *TNF* –308G→A in the EPILYMPH—Spain study and *IL1B* –511C→T and *TNF* –308G→A in the University of California San Francisco study were not consistent with Hardy-Weinberg equilibrium at 0·01>p>0·001. *IL1B* –31C→T in the University of California San Francisco study; *IL1RN* 9589A→T, *IL10* –1082A→G, and *LTA* 252A→G in the EPILYMPH—Germany study; *IL1RN* 9589A→T in the Connecticut study; and *IL6* –174G→C in the UK study were not consistent with Hardy-Weinberg equilibrium at 0·05>p>0·01. Exclusion of studies with a single-nucleotide polymorphism out of Hardy-Weinberg equilibrium had a minimum effect on risk estimates for the polymorphisms or for haplotypes containing the polymorphism (not shown).

Table 2 shows results from the analyses of single-nucleotide polymorphisms for all cases of non-Hodgkin lymphoma, and table 3 for diffuse large B-cell lymphoma and follicular lymphoma separately. *TNF* –308G→A was associated with increased risk of

	TNF −308G→A				*IL10* −3575T→A				
	Controls	Cases	Odds ratio (95% CI)	p	Genotype	Controls	Cases	Odds ratio (95% CI)	p
EPILYMPH—Italy									
GG	100 (88%)	54 (90%)	1·0 (ref)	NA	TT	73 (65%)	34 (58%)	1·0 (ref)	NA
GA	13 (12%)	5 (8%)	0·75 (0·25–2·27)	0·61	TA	37 (33%)	22 (37%)	1·26 (0·64–2·48)	0·51
AA	0	1 (2%)	NA	NA	AA	2 (2%)	3 (5%)	3·03 (0·47–19·55)	0·24
GA or AA	13 (12%)	6 (10%)	0·92 (0·32–2·61)	0·88	TA or AA	39 (35%)	25 (42%)	1·35 (0·70–2·61)	0·37
Trend	113 (100%)	60 (100%)	1·10 (0·43–2·85)	0·84	Trend	112 (100%)	59 (100%)	1·40 (0·79–2·50)	0·25
EPILYMPH—Spain*									
GG	434 (79%)	55 (72%)	1·0 (ref)	NA	TT	271 (49%)	34 (44%)	1·0 (ref)	NA
GA	103 (19%)	16 (21%)	1·15 (0·63–2·11)	0·64	TA	233 (42%)	39 (51%)	1·40 (0·85–2·31)	0·18
AA	15 (3%)	5 (7%)	2·65 (0·92–7·62)	0·07	AA	50 (9%)	4 (5%)	0·68 (0·23–2·00)	0·48
GA or AA	118 (21%)	21 (28%)	1·34 (0·77–2·31)	0·30	TA or AA	283 (51%)	43 (56%)	1·28 (0·79–2·07)	0·32
Trend	552 (100%)	76 (100%)	1·39 (0·90–2·14)	0·14	Trend	554 (100%)	77 (100%)	1·06 (0·73–1·54)	0·76
University of California San Francisco†									
GG	487 (72%)	61 (62%)	1·0 (ref)	NA	TT	238 (35%)	35 (38%)	1·0 (ref)	NA
GA	160 (24%)	32 (33%)	1·55 (0·97–2·48)	0·069	TA	343 (51%)	41 (44%)	0·77 (0·47–1·25)	0·28
AA	26 (4%)	5 (5%)	1·57 (0·57–4·30)	0·38	AA	96 (14%)	17 (18%)	1·16 (0·62–2·20)	0·64
GA or AA	186 (28%)	37 (38%)	1·55 (0·99–2·43)	0·055	TA or AA	439 (65%)	58 (62%)	0·85 (0·54–1·34)	0·49
Trend	673 (100%)	98 (100%)	1·40 (0·97–2·00)	0·07	Trend	677 (100%)	93 (100%)	1·01 (0·73–1·40)	0·95
EPILYMPH—Germany									
GG	338 (71%)	87 (67%)	1·0 (ref)	NA	TT	192 (40%)	47 (36%)	1·0 (ref)	NA
GA	130 (27%)	37 (29%)	1·10 (0·71–1·71)	0·66	TA	215 (45%)	64 (50%)	1·25 (0·81–1·92)	0·32
AA	11 (2%)	5 (4%)	1·77 (0·59–5·28)	0·31	AA	71 (15%)	18 (14%)	1·12 (0·60–2·07)	0·73
GA or AA	141 (29%)	42 (33%)	1·16 (0·76–1·76)	0·50	TA or AA	286 (60%)	82 (64%)	1·21 (0·81–1·83)	0·35
Trend	479 (100%)	129 (100%)	1·18 (0·82–1·70)	0·37	Trend	478 (100%)	129 (100%)	1·10 (0·83–1·46)	0·52
Connecticut									
GG	402 (72%)	103 (66%)	1·0 (ref)	NA	TT	240 (43%)	55 (35%)	1·0 (ref)	NA
GA	139 (25%)	49 (32%)	1·36 (0·91–2·02)	0·13	TA	268 (48%)	74 (48%)	1·20 (0·81–1·78)	0·35
AA	18 (3%)	3 (2%)	0·65 (0·19–2·27)	0·50	AA	53 (9%)	26 (17%)	2·12 (1·22–3·70)	0·0081
GA or AA	157 (28%)	52 (34%)	1·28 (0·87–1·87)	0·22	TA or AA	321 (57%)	100 (65%)	1·36 (0·94–1·97)	0·11
Trend	559 (100%)	155 (100%)	1·15 (0·82–1·60)	0·42	Trend	561 (100%)	155 (100%)	1·39 (1·06–1·82)	0·016
UK									
GG	285 (64%)	154 (61%)	1·0 (ref)	NA	TT	174 (38%)	82 (31%)	1·0 (ref)	NA
GA	135 (30%)	82 (32%)	1·12 (0·80–1·57)	0·50	TA	212 (46%)	128 (49%)	1·29 (0·92–1·82)	0·14
AA	26 (6%)	17 (7%)	1·22 (0·64–2·33)	0·54	AA	73 (16%)	52 (20%)	1·51 (0·97–2·35)	0·069
GA or AA	161 (36%)	99 (39%)	1·14 (0·83–1·57)	0·42	TA or AA	285 (62%)	180 (69%)	1·35 (0·97–1·86)	0·071
Trend	446 (100%)	253 (100%)	1·11 (0·87–1·43)	0·40	Trend	459 (100%)	262 (100%)	1·24 (1·00–1·54)	0·054
NCI—SEER									
GG	551 (74%)	202 (65%)	1·0 (ref)	NA	TT	286 (39%)	107 (35%)	1·0 (ref)	NA
GA	174 (23%)	91 (29%)	1·48 (1·09–2·01)	0·012	TA	329 (45%)	151 (49%)	1·28 (0·95–1·73)	0·10
AA	17 (2%)	17 (5%)	2·83 (1·41–5·70)	0·0035	AA	116 (16%)	51 (16%)	1·20 (0·81–1·80)	0·36
GA or AA	191 (26%)	108 (35%)	1·60 (1·20–2·14)	0·0015	TA or AA	445 (61%)	202 (65%)	1·26 (0·95–1·67)	0·10
Trend	742 (100%)	310 (100%)	1·56 (1·22–1·99)	0·0003	Trend	731 (100%)	309 (100%)	1·13 (0·93–1·37)	0·21
Detroit‡									
GG	83 (73%)	45 (75%)	1·0 (ref)	NA	TT	46 (41%)	21 (36%)	1·0 (ref)	NA
GA	29 (25%)	14 (23%)	0·86 (0·38–1·92)	0·71	TA	49 (44%)	28 (48%)	1·20 (0·57–2·54)	0·63
AA	2 (2%)	1 (2%)	0·92 (0·02–18·18)	1·00	AA	16 (14%)	9 (16%)	0·97 (0·34–2·78)	0·95
GA or AA	31 (27%)	15 (25%)	0·84 (0·38–1·82)	0·65	TA or AA	65 (59%)	37 (64%)	1·14 (0·56–2·31)	0·72
Trend	114 (100%)	60 (100%)	0·84 (0·42–1·68)	0·62	Trend	111 (100%)	58 (100%)	1·03 (0·63–1·69)	0·91
Iowa‡									
GG	189 (73%)	68 (64%)	1·0 (ref)	NA	TT	82 (32%)	36 (34%)	1·0 (ref)	NA
GA	65 (25%)	32 (30%)	1·40 (0·84–2·34)	0·19	TA	128 (50%)	50 (47%)	0·99 (0·58–1·67)	0·96
AA	5 (2%)	6 (6%)	3·42 (1·00–11·72)	0·05	AA	46 (18%)	20 (19%)	1·06 (0·55–2·07)	0·86
GA or AA	70 (27%)	38 (36%)	1·55 (0·95–2·52)	0·079	TA or AA	174 (68%)	70 (66%)	1·01 (0·62–1·65)	0·97
Trend	259 (100%)	106 (100%)	1·56 (1·03–2·37)	0·03	Trend	256 (100%)	106 (100%)	1·02 (0·74–1·42)	0·88
Los Angeles‡									
GG	97 (80%)	39 (67%)	1·0 (ref)	NA	TT	56 (46%)	22 (38%)	1·0 (ref)	NA
GA	25 (20%)	18 (31%)	1·89 (0·92–3·91)	0·085	TA	48 (40%)	29 (50%)	1·75 (0·87–3·51)	0·11
AA	0	1 (2%)	NA	NA	AA	17 (14%)	7 (12%)	1·10 (0·39–3·07)	0·86
GA or AA	25 (20%)	19 (33%)	1·99 (0·97–4·09)	0·06	TA or AA	65 (54%)	36 (62%)	1·57 (0·81–3·02)	0·18
Trend	122 (100%)	58 (100%)	2·06 (1·03–4·13)	0·04	Trend	121 (100%)	58 (100%)	1·20 (0·76–1·89)	0·44
Seattle‡									
GG	182 (74%)	50 (58%)	1·0 (ref)	NA	TT	102 (42%)	28 (32%)	1·0 (ref)	NA
GA	55 (22%)	27 (31%)	1·91 (1·08–3·37)	0·026	TA	104 (43%)	44 (51%)	1·52 (0·88–2·64)	0·14
AA	10 (4%)	9 (10%)	3·57 (1·35–9·43)	0·01	AA	37 (15%)	15 (17%)	1·44 (0·69–3·00)	0·33
GA or AA	65 (26%)	36 (42%)	2·16 (1·28–3·66)	0·0042	TA or AA	141 (58%)	59 (68%)	1·50 (0·89–2·52)	0·13
Trend	247 (100%)	86 (100%)	1·90 (1·27–2·84)	0·0019	Trend	243 (100%)	87 (100%)	1·25 (0·88–1·77)	0·21

(continues)

(continued)

	TNF –308G→A				*IL10* –3575T→A				
	Controls	Cases	Odds ratio (95% CI)	p	Genotype	Controls	Cases	Odds ratio (95% CI)	p
British Columbia									
GG	..	..	..	..	TT	119 (34%)	28 (33%)	1·0 (ref)	NA
GA	..	..	..	..	TA	179 (51%)	48 (56%)	1·19 (0·70–2·01)	0·52
AA	..	..	..	..	AA	51 (15%)	9 (11%)	0·74 (0·33–1·70)	0·48
GA or AA	..	..	..	..	TA or AA	230 (66%)	57 (67%)	1·085 (0·65–1·80)	0·75
Trend	..	..	..	..	Trend	349	85	0·94 (0·66–1·35)	0·75

Data are number of individuals (%) unless otherwise indicated. NA=not applicable. *p=0·005 for test of Hardy-Weinberg equilibrium. Expected distribution of genotypes in controls is GG: 427·01 (77·36%), GA: 116·98 (21·19%), and AA: 8·01 (1·45%). Risk estimate for carrying AA genotype would be higher if control population was in Hardy-Weinberg equilibrium. †p=0·0069 for test of Hardy-Weinberg equilibrium. Expected distribution of genotypes in controls is GG: 477·70 (70·98%), GA: 178·61 (26·54%), and AA: 16·70 (2·48%). Risk estimate for carrying AA genotype would be higher if control population was in Hardy-Weinberg equilibrium. ‡Study sites for NCI–SEER.

Table 4: **Study-specific genotype frequencies and risks for *TNF* –308G→A and *IL10* –3575T→A and diffuse large B-cell lymphoma**

non-Hodgkin lymphoma for both the GA and AA genotypes (table 2). When restricted to diffuse large B-cell lymphoma, the main histological subtype, which comprised about 27% of cases of non-Hodgkin lymphoma, the risk estimates were stronger (table 3). Risk estimates for diffuse large B-cell lymphoma remained significant when any study was removed from the analysis (not shown). Although the tests for departure from Hardy-Weinberg equilibrium for *TNF* –308G→A in controls were significant for the EPILYMPH—Spain and University of California San Francisco studies, the observed frequencies differed little from that expected under Hardy-Weinberg equilibrium (eg, about 1% for homozygotes in both studies; table 4). No genotyping errors were seen when quality control samples were rechecked in either study. Furthermore, genotyping done for non-InterLymph related projects in these studies found that 95% or more of the single-nucleotide polymorphisms assessed were in Hardy-Weinberg equilibrium, as expected, which lessens the possibility that these two control populations were not in Hardy-Weinberg equilibrium. Finally, a sensitivity analysis showed that risk estimates were much the same when we excluded these two studies from the analysis (p for trend=0·0013): the odds ratio was 1·29 (95% CI 1·10–1·51) before exclusion and 1·26 (1·06–1·50) after exclusion for the *TNF* –308GA genotype, and 1·65 (1·16–2·34) before exclusion and 1·58 (1·01–2·47) after exclusion for the AA genotype.

By contrast, *TNF* –308G→A was not associated with follicular lymphoma (table 3), the second most common subtype, comprising about 19% of cases, and the association was significantly different from that seen for diffuse large B-cell lymphoma (table 3). Furthermore, this polymorphism was not associated with risk of the other histological subtypes of non-Hodgkin lymphoma combined (ie, for all non-Hodgkin lymphoma cases minus diffuse large B-cell lymphoma and follicular lymphoma): risk estimates were 1·15 (0·99–1·35) for heterozygotes and 1·00 (0·65–1·54) for homozygotes (p for trend=0·16), and the effect differed significantly from the association with diffuse large B-cell lymphoma (p=0·01). However, we cannot exclude the possibility of an effect in a small histological subgroup that we did not assess in this study.

Figure 1 shows study-specific associations between *TNF* –308G→A and diffuse large B-cell lymphoma and follicular lymphoma, under an additive model. The *TNF* –308G→A association was consistent for diffuse large B-cell lymphoma, with all studies showing risk estimates of higher than 1·0 (figure 1, table 4). The pooled estimate from the test for trend (ie, additive model) remained highly significant after adjustment by the false-discovery rate method (ie, original p value of 0·000055 adjusted for 12 comparisons with each of diffuse large B-cell lymphoma and follicular lymphoma became p=0·0013), and the finding was deemed noteworthy as determined by the false-positive report probability approach for even the lowest prior probability estimate of 0·0005.

TNF –308G→A was in linkage disequilibrium with *LTA* 252A→G (pooled D′=0·97, r^2=0·38), which is consistent with previous reports,[27] and *LTA* 252A→G was also associated with increased risk of diffuse large B-cell lymphoma (table 3). Because studies done on *LTA* 252A→G in stimulated mononuclear cells have shown raised concentrations of LTα, a potent pro-inflammatory cytokine,[28] we attempted to distinguish its effect from that of the TNF variant by estimating haplotypes. Assuming an additive model, we found that the haplotype with both *TNF* –308G→A and *LTA* 252A→G (ie, AG haplotype) was associated with increased risk of diffuse large B-cell lymphoma, with risk estimates of more than 1·0 for all studies (figure 2). By contrast, the GG haplotype was not associated with risk of diffuse large B-cell lymphoma (figure 2). The two haplotypes AG and GG differed significantly in risk of diffuse large B-cell lymphoma (p=0·003).

The other key finding of our study was that *IL10* –3575T→A was associated with an increased risk of non-Hodgkin lymphoma (table 2), particularly with risk of diffuse large B-cell lymphoma, but not follicular lymphoma (table 3, figure 3) or with other histologies combined (p for trend=0·22). This result remained

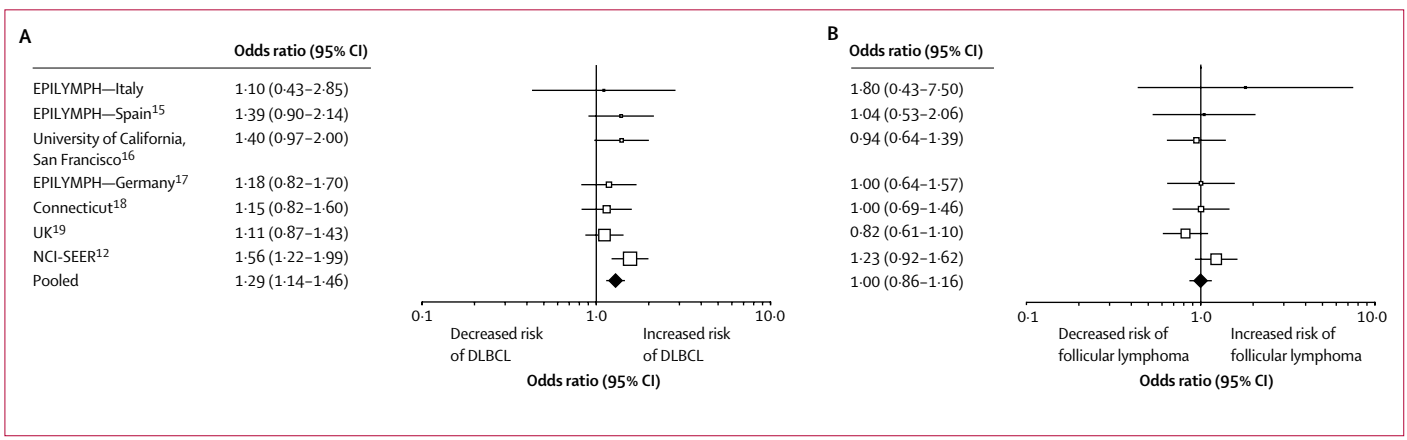

Figure 1: **Forest plots for study-specific and pooled risk estimates from additive model of *TNF* –308G→A for diffuse large B-cell lymphoma (DLBCL, A) and follicular lymphoma (B)**
DLBCL pooled estimate p<0·0001; follicular lymphoma pooled estimate p=0·98.

significant for diffuse large B-cell lymphoma when any one study was excluded (data not shown) and was generally consistent, with most studies having risks from the additive model of more than 1·0 (figure 3, table 4). The p value from the additive model was 0·056 after adjustment by the false-discovery rate method and the finding was noteworthy as determined by the false-positive report probability method for only the highest prior probability estimate of 0·05.

IL10 –3575T→A was in strong linkage disequilibrium with *IL10* –1082A→G (pooled D′=0·97 and r^2=0·63), which also was associated with increased risk of diffuse large B-cell lymphoma (table 3). We attempted to separate the effects of each polymorphism, since both could be functional.[29,30] Assuming an additive model, the haplotype with *IL10* –3575T→A and *IL10* –1082A→G (ie, AG haplotype) was associated with increased risk of diffuse large B-cell lymphoma, and this risk was consistent across most studies (figure 4). By contrast, the TG haplotype was not associated with risk of diffuse large B-cell lymphoma (figure 4). The two haplotypes AG and TG differed significantly in risk of diffuse large B-cell lymphoma (p=0·007).

Associations did not differ by age or sex, and there was no multiplicative gene–gene interaction between *TNF* –308G→A and *IL10* –3575T→A (not shown). For individuals homozygous for *TNF* –308G→A and homozygous or heterozygous for *IL10* –3575T→A, risk of diffuse large B-cell lymphoma was doubled, with no association with follicular lymphoma (table 3).

None of the other single-nucleotide polymorphisms investigated in our study was associated with risk of all non-Hodgkin lymphoma (table 2) or of diffuse large B-cell lymphoma or follicular lymphoma (table 3). We note that the homozygous *IL1B* –511C→T genotype was significantly associated with increased risk of non-Hodgkin lymphoma in one study and with decreased risk in another study, whereas the pooled analysis of all studies showed no overall association (figure 5).

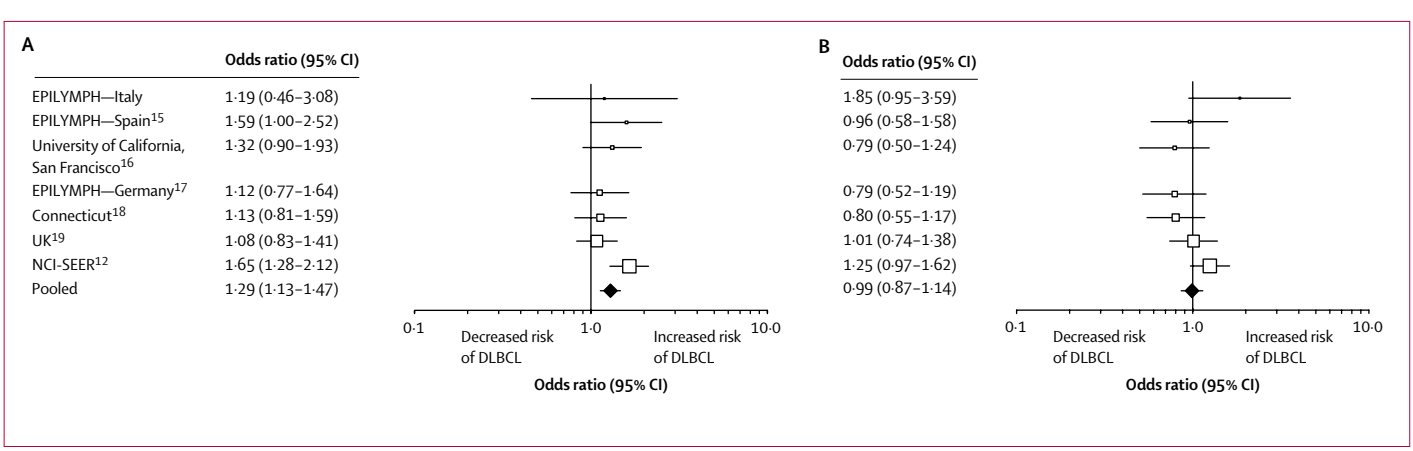

Figure 2: **Forest plots for study-specific and pooled risk estimates from additive model of haplotypes with *TNF* –308G→A and *LTA* 252A→G for diffuse large B-cell lymphoma (DLBCL)**
(A) AG versus GA. Pooled estimate p=0·00014. (B) GG versus GA. Pooled estimate p=0·95. Frequency of haplotypes: AG=0·16, GG=0·16, GA=0·68, and AA=0·003. Global omnibus test p=0·00125.

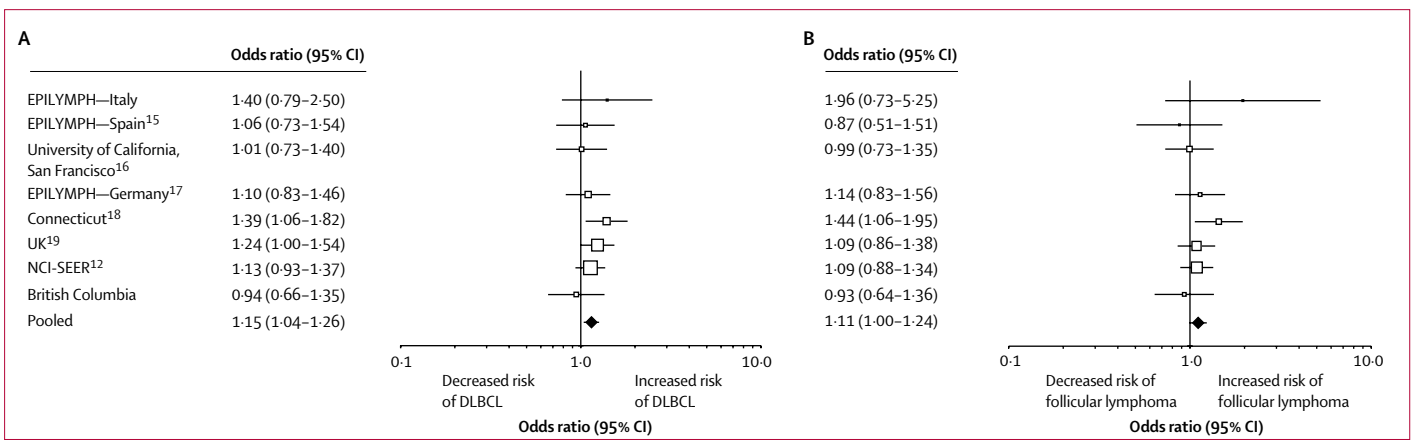

Figure 3: **Study-specific and pooled risk estimates from additive model of *IL10* –3575T→A for diffuse large B-cell lymphoma (DLBCL, A) and follicular lymphoma (B)**
(A) Pooled estimate p=0·006. (B) Pooled estimate p=0·059.

Discussion

In a large pooled analysis of data from eight studies of non-Hodgkin lymphoma participating in the InterLymph consortium, we noted that *TNF* –308G→A and *IL10* –3575T→A were associated with an increased risk of non-Hodgkin lymphoma, particularly diffuse large B-cell lymphoma. *TNF* and *IL10* are good candidate genes for the study of lymphomagenesis because they code for important immunoregulatory cytokines that are crucial mediators of inflammation, apoptosis, and **Th1/Th2 balance**, and function as autocrine growth factors in lymphoid tumours.[5,31,32] Moreover, studies[33–35] of *TNF* and *IL10* knock-out mice have shown that each cytokine affects B-cell lymphomagenesis either indirectly or directly. Furthermore, clinical studies[9,10,30] suggest that serum concentrations of TNFα and IL10 affect prognosis of non-Hodgkin lymphoma, particularly diffuse large B-cell lymphoma. Lastly, research[36] on monozygotic twins suggests that production of TNFα and IL10 have a strong heritable basis.

Other studies[10,30,37,38] that have assessed *TNF* –308G→A and several *IL10* polymorphisms and risk of non-Hodgkin lymphoma have been small and not population-based, and could not provide conclusive results about the role of these variants in the development of this disease. The results we report here are derived from a pooled analysis that included at least ten times more study participants than these previous reports. Our findings, especially for *TNF* –308G→A, were highly significant, and effects were consistent across studies.

TNF –308G→A has been associated with increased susceptibility for cerebral malaria, and other infections and inflammatory conditions such as rheumatoid arthritis and Sjögren's syndrome.[39,40] Several studies[41–43] have shown that this allele results in higher constitutive and inducible expression of TNFα. However, conclusions based on other model systems have varied,[40] probably because of differences in cell type and stimuli used, and, as such, further work is needed to clarify the function of this polymorphism.

Th1/Th2 balance
T-helper (Th) 1 and Th 2 cells are characterised by secretion of specific cytokine patterns that direct distinct immune response pathways. Whereas Th1 cells drive cellular immunity to fight intracellular pathogens including viruses and eliminate cancerous cells, Th2 cells drive humoral immunity via upregulation of antibody production to protect against extracellular pathogens. An imbalance of the Th1/Th2 system could be responsible for both the occurrence and the progression of infectious, autoimmune, and neoplastic diseases.

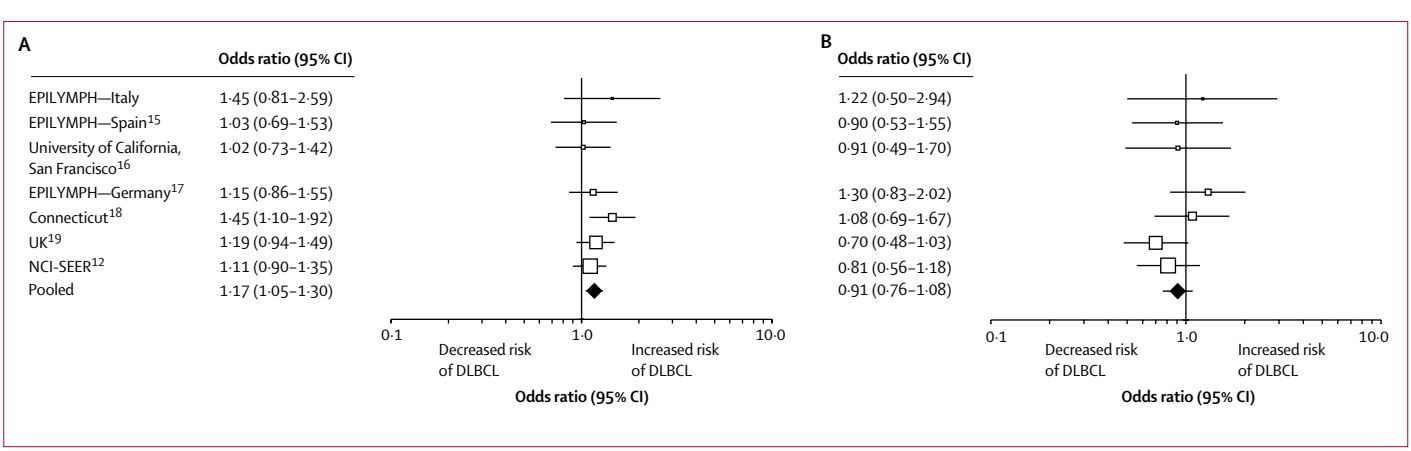

Figure 4: **Study-specific and pooled risk estimates from additive model of haplotypes with *IL10* –3575T→A and *IL10* –1082A→G for diffuse large B-cell lymphoma (DLBCL)**
(A) AG versus TA. Pooled estimate p=0·004. (B) TG versus TA. Pooled estimate p=0·29. Frequency of haplotypes: AG=0·36; TG=0·10, TA=0·53, and AA=0·006, respectively. Global omnibus test p=0·0075.

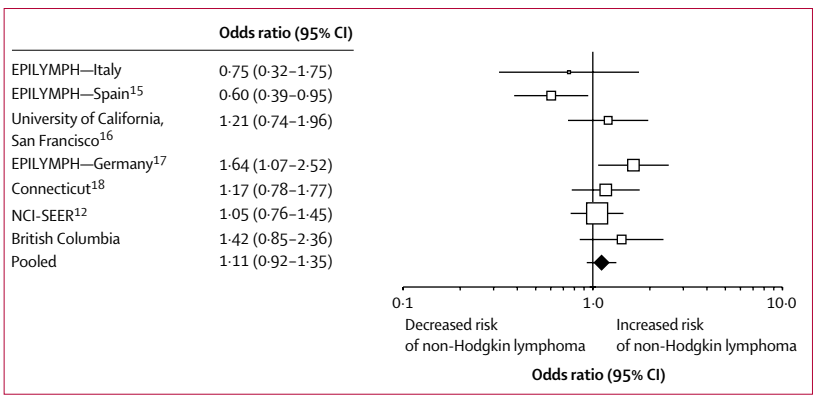

Figure 5: **Study-specific and pooled risk estimates for *IL1B* –511C→T**
TT genotype compared with CC genotype for all cases of non-Hodgkin lymphoma. Pooled estimate p=0·28. Test for heterogeneity p=0·14.

We found that the haplotype with *TNF* –308G→A and *LTA* 252A→G (ie, the AG haplotype), but not the GG haplotype, was associated with increased risk of diffuse large B-cell lymphoma, suggesting that *TNF* –308G→A could act alone or in conjunction with *LTA* 252A→G. However, we cannot exclude the possibility that its association with diffuse large B-cell lymphoma is from another variant in linkage disequilibrium within *TNF* or *LTA*[27] or in other neighbouring immunomodulatory genes such as *NFKBIL1* or *BAT1*. Furthermore, because *TNF* is located within the HLA class III region (250 kb centromeric to the HLA-B locus and 850 kb telomeric to the class II HLA-DR locus), and HLAs have a crucial function in regulation of the immune response to infection and malignant transformation, linkage disequilibrium of the *TNF* promoter polymorphism with other alleles within this region, including the extended HLA haplotype A1-B8-DR3,[44] could be responsible for increasing risk of diffuse large B-cell lymphoma.

The *IL10* –3575A allele, which results in lower production of IL10 compared with the –3575T allele,[29] was associated with increased risk of non-Hodgkin lymphoma, particularly for diffuse large B-cell lymphoma. The higher risk for diffuse large B-cell lymphoma was restricted to the haplotype containing *IL10* –3575T→A and *IL10* –1082A→G (ie, AG haplotype) rather than the TG haplotype, suggesting that *IL10* –3575T→A is more important than *IL10* –1082A→G in determining risk of non-Hodgkin lymphoma.

A possible mechanism of lymphomagenesis consistent with our findings is that higher expression of TNFα and LTα upregulates antiapoptotic regulators and proinflammatory effectors mediated via the nuclear transcription factor (NF)-κB pathway, which provides key signals to support B-cell survival and differentiation in the germinal centre.[45] NF-κB target genes are highly expressed in activated B-cell-like diffuse large B-cell lymphoma, a major subgroup of diffuse large B-cell lymphoma.[46] A tightly regulated balance between proapoptotic and antiapoptotic processes is of utmost importance and thus a slight imbalance towards increased cell survival could favour lymphomagenesis. At the same time, IL10 is a potent downregulator of the production of macrophage proinflammatory cytokines, notably TNFα.[47] Consequently, decreased expression of IL10 would less efficiently suppress proinflammatory cytokine production and, therefore, could increase risk of non-Hodgkin lymphoma. The observed association of the TNF promoter single-nucleotide polymorphism with diffuse large B-cell lymphoma underscores the role of this key cytokine in regulation of the immune response and perhaps surveillance. In this regard, the TNF pathway could be a suitable target for intervention.

Concerns have been raised that population stratification—ie, confounding by unrecognised ethnic admixture—can lead to a test of association with a misleadingly low p value, especially in large studies.[48] We note that a small bias from any source could result in significant associations as the sample size increases. Marchini and colleagues[48] discussed the potential effects of population stratification on the test for trend for scenarios of modest differences in allele frequency and disease risk that might be present in studies of mixed European populations. Their simulations suggest that population stratification is unlikely to have biased our highly significant p value for the *TNF* –308G→A association by more than about one order of magnitude. Furthermore, the consistency of our findings across studies and across sites of the multicentred NCI-SEER study (table 4) provides additional evidence against population stratification, because this potential bias is unlikely to change risk estimates in the same direction and extent in studies done in diverse settings and in different study populations.[49]

In conclusion, our results of common cytokine single-nucleotide polymorphisms and non-Hodgkin lymphoma identified two genetic variants of probable importance in risk of this disease and showed the effectiveness of a large consortium in identification of genetic associations. The large scale of our pooled analysis helps to mitigate against false-negative and false-positive results—issues that hamper smaller studies.[23,50] Our findings provide an important clue to lymphomagenesis; nevertheless, they need to be replicated and to that end, several thousand additional cases and controls will become available for analysis in the near future from ongoing studies of non-Hodgkin lymphoma. Finally, these results suggest that exploration of additional variants in *TNF*, *LTA*, and *IL10*, in their receptors and other related genes, and in genes in linkage disequilibrium including the class III region of the MHC locus, should provide further insight into the pathogenesis and ultimately the prevention and treatment of non-Hodgkin lymphoma, the incidence of which has risen steadily worldwide over the last half of the twentieth century and for which the factors governing development remain elusive.[51,52]

Contributors

The project was conceived and led by members of the genotyping working group within InterLymph: N Rothman, C F Skibola, S S Wang, G Morgan, Q Lan, M T Smith, J J Spinelli, A Brooks-Wilson, P Hartge, S Chanock, and A Nieters. The cochairs of the InterLymph consortium at the inception of this project were P Boffetta, M Linet, and B Armstrong. Investigators who obtained and provided data from the studies are: NCI-SEER: P Hartge, S S Wang, J R Cerhan, W Cozen, S Davis, R K Severson, M Linet, and N Rothman; Connecticut: T Zheng, Q Lan, T R Holford, B Leaderer, and Y Zhang. EPILYMPH—Germany: N Becker and A Nieters; UK: E Roman, G Morgan, E Willett, S Rollinson, and C Spink; British Columbia: J J Spinelli, R P Gallagher, A Brooks-Wilson, and A Lai; EPILYMPH—Spain: S de Sanjose, X Bosch, D Whitby, and R Bosch; University of California San Francisco: E A Holly, C F Skibola, P M Bracci, and M T Smith; and EPILYMPH—Italy: P Cocco, P S Moore, A Scarpa, and M G Ennas. EPILYMPH is coordinated by P Brennan and P Boffetta. Bioinformatics support was provided by C F Skibola, A Nieters, M Yeager, and S Chanock, and quality control samples were provided by S Chanock. Genotyping was done by M Yeager and S Chanock for the NCI-SEER, Yale, and EPILYMPH—Spain studies; C F Skibola and M T Smith for the University of California San Francisco study; A Brooks-Wilson for the British Columbia study; S Rollinson and C Spink for the UK study; A Scarpa and P S Moore for EPILYMPH—Italy study; and A Nieters for EPILYMPH—Germany study. Statistical analysis was done by S I Berndt and SS Wang, with input from S Wacholder, E Willett, J J Spinelli, Q Lan, P Hartge, and N Rothman. The manuscript was drafted and revised by N Rothman, C F Skibola, S S Wang, G Morgan, Q Lan, M T Smith, E Willett, S de SanJose, P Cocco, S I Berndt, P Brennan, S Wacholder, P Hartge, E Roman, P Boffetta, S Chanock, and A Nieters. All authors reviewed and approved the manuscript.

Conflict of interest

We declare no conflicts of interest.

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