Evidence that an *HLA-DQA1–DQB1* haplotype influences susceptibility to childhood common acute lymphoblastic leukaemia in boys provides further support for an infection-related aetiology

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**Summary** Comparison of *DQA1* and *DQB1* alleles in 60 children with common acute lymphoblastic leukaemia (c-ALL) and 78 newborn infant control subjects revealed that male but not female patients had a higher frequency of *DQA1*′*0101′/′0104 and *DQB1*′*0501* than appropriate control subjects. The results suggest a male-associated susceptibility haplotype in c-ALL and supports an infectious aetiology.

**Keywords:** childhood common acute lymphoblastic leukaemia; *HLA-DQA1; DQB1*; aetiology; genetic susceptibility; infection

Evidence suggesting that childhood common acute lymphoblastic leukaemia (c-ALL) may have an infectious aetiology (reviewed by Greaves. 1997) continues to accumulate. Associations with socioeconomic status (Alexander et al. 1990), time–space case clustering (Alexander. 1992), population isolation and mixing (reviewed by Kinlen. 1995), parental occupation (Kinlen. 1997) and seasonality (Badrinath et al. 1997; Westerbeek et al 1998) all point to factors affecting the transmission of an infectious agent. However, no leukaemogenic infectious agent has yet been identified in c-ALL.

Results suggesting that infectious diseases may be more common in children before the development of ALL than they are in normal children (van Steensel-Moll et al. 1986) imply that the immune response may itself contribute to the aetiology of c-ALL (Greaves and Alexander. 1993). If differences in the efficiency of such immune responses do influence the risk of c-ALL, this may be related to the differential antigen-presenting capacity of HLA class II alleles. We previously showed that children with c-ALL typed more frequently for *HLA-DQB1*′*0501* than control subjects (Dearden et al. 1996). As *DQB1* is very tightly linked to *DQA1*, we carried out a molecular analysis of *DQA1* alleles in patients previously typed for *DQB1* to see if both genes influenced susceptibility to c-ALL.

**MATERIALS AND METHODS**

**Patients and control subjects**

The patients consisted of an unselected series of 60 children (38 boys, 22 girls) with c-ALL from the same series as described previously (Dearden et al. 1996). Control blood samples were obtained from the umbilical cords of 78 normal full-term newborn infants (38 boys and 40 girls) delivered at St Marys Hospital.

**HLA-DQA1 molecular typing**

Genomic DNA was extracted from patient and control blood samples as previously described (Dearden et al. 1996), and *DQA1* typing carried out as described by Noreen et al (1992). A 228-bp exon 2 fragment of *DQA1* was amplified by the polymerase chain reaction (PCR) using the primers DQAAMP-A (5′-ATG TTA ACT ACC AGT-3′) and DQAAMP-B (5′-TTG GTA GCA GCG GTA GAG TTG-3′), obtained from the British Society for Histo compatibility and Immunogenetics (BSHI). PCR mixtures consisted of 50 ng of genomic DNA, 0.5 μM of each primer and 0.3 mm dNTPs in 20 μl of PCR buffer. Amplifications (32 cycles) were carried out on a Thermal Cycler (Appligene, France). and PCR products dot-blotted onto nylon filters, which were hybridized with ten 18-mer sequence-specific oligonucleotide (SSO) probes (from BSHI) end-labelled with γ-32P-ATP, detecting eight *DQA1* alleles [*0101* + *0104*, *0102*, *0103*, *0201*, *03* + *0302* + *0303*, *0401*, *05* + *0502* + *0503* and *0601*]. The filters were scanned for radioactivity on an InstantImager (Canberra Packard, Berks, UK). Positive hybridization was scored by comparing test vs negative control spots (typically a tenfold difference in counts). *DQA1* alleles were assigned by comparing SSO patterns with published information (see Marsh reference to Website). The following pairs of alleles could not be distinguished from each other with the probes used in this study: *0101* from *0104*; *0301* from *0302* and subtypes of *05*: + *0601* homozygotes gave the same result as + *0401*/′*0601* heterozygotes; and + *0103* homozygotes gave the same result as + *0102*/′*0103* heterozygotes.

**DQB1 molecular typing**

Patients and control subjects were typed for *DQB1* alleles by single-strand conformation polymorphism (SSCP) analysis as described previously (Dearden et al. 1996).
Table 1  HLA-DQA1 allele frequencies in childhood common ALL compared with infant control subjects

| Allele group | DQA1 allele | Total | Males | Females |
|--------------|-------------|-------|-------|---------|
|              | c-ALL       | Infant OR 95% CI | c-ALL | Infant OR 95% CI | c-ALL | Infant OR 95% CI |
| '01          | *0101/0104  | 22.3 9.0 2.27 1.12-4.52 | 21.6 6.8 4.06 1.42-10.17 | 11.4 11.3 1.01 0.35-3.10 |
| '0102        | 11.7 15.4 0.72 0.37-1.47 | 9.0 17.9 0.45 0.19-1.17 | 15.9 12.5 1.32 0.5-6.82 |
| '0103        | 8.3 5.1 1.66 0.67-4.14 | 7.7 5.1 1.54 0.45-4.89 | 9.1 5.0 1.90 0.52-6.90 |
| '0102/0103   | 2.5 1.6 3.97 0.56-17.14 | 2.6 0 - | 2.3 1.3 1.83 0.25-13.43 |
| combined '01 | 40.8 30.1 0.21 0.07-2.62 | 41.0 29.5 1.16 0.86-3.17 | 30.6 30.0 1.16 0.69-3.19 |
| '02          | *0201       | 12.5 21.2 0.53 0.28-1.04 | 11.5 24.4 0.40 0.18-0.96 | 13.6 17.5 0.74 0.29-2.06 |
| '03          | combined '03 | 22.5 15.4 0.59 0.79-2.40 | 23.1 10.3 2.62 1.07-6.02 | 20.5 20.0 1.02 0.43-2.52 |
| '04          | *0401       | 2.5 1.3 1.97 0.4-8.38 | 0 2.6 0 | 6.8 0 | |
| *0401/0601   | 0.8 0 - | - | 1.3 0 - | 0 0 - | |
| '05          | 20.8 31.4 0.57 0.34-1.0 | 20.5 30.8 0.58 0.29-1.2 | 20.5 31.3 0.56 0.25-1.36 |
| '0601        | 0.6 0 - | - | 0 2.6 0 | 0 1.3 - | 0.05-6.75 |
| combined '04 | 24.2 33.3 0.63 0.38-1.09 | 21.8 33.3 0.55 0.28-1.14 | 27.3 32.5 0.77 0.36-1.74 |

n = 60 78 38 38 22 40

* DQA1 '0101 and '0104 were not distinguished with the SSO probes used here. Heterozygotes with DQA1 '0102/0103 and '0401/0601 could not be distinguished. Combined '01 alleles include '0301 and '0302. *Results are allele frequencies (%), OR, odds ratas; 95% CI, 95% confidence interval.

DQA1 '0101/0104: total ALL vs total infant control subjects: two-sided Fisher's P = 0.03. Male c-ALL vs male infant control subjects: two-sided Fisher's P = 0.01. *Combined DQA1 '01 alleles: total ALL vs total control subjects: two-sided Fisher's P = 0.08. Male c-ALL vs male control subjects: two-sided Fisher's P = 0.18. 'DQA1 '0201/0102: total ALL vs total control subjects: two-sided Fisher's P = 0.08. Male c-ALL vs male control subjects: two-sided Fisher's P = 0.05.

n, number of subjects in each group.

Data analysis

Differences in patient and control allele frequencies are expressed as odds ratios (OR) (Altman. 1991) together with 95% confidence intervals (CI) derived using Miettinen's method (Breslow and Day. 1980). Patient and control DQA1 allele frequencies were also compared using 2x2 analysis, and tested for significance by two-sided Fisher's exact tests. DQA1 and DQB1 exon 2 polymorphic amino acid frequencies (see Marsh) were compared in patients and control subjects using ORs and 2x2 tests. Observed and expected heterozygosity was compared using allelic diversity (h) (Nei and Roychoudhury, 1974). Sample size (i.e. statistical power) calculations were performed using nQuery Advisor release 2.0 (Statistical Solutions. Cork, Ireland).

RESULTS

Study group

The patients consisted of a prospective series of 38 boys and 22 girls with c-ALL aged between 1.6 and 12.9 years, with a mean age at diagnosis of 5 years 3 months, and a median of 4 years 4 months. There was a 26% excess of male to female patients, giving an M:F of 1.73:1. The mean (median) age of the male patients was 4 years 8 months (4 years 3 months), compared with the mean (median) age of the female patients of 6 years 2 months (4 years 10 months).

HLA-DQA1 alleles in c-ALL

All DQA1 alleles detected in the c-ALL patients were also found in the infant control subjects. Analysis of heterozygosity (h) showed no significant difference observed between reported and expected values for c-ALL (obs. 71%; exp. 66.4%; P = 0.22) and infant control subjects (obs. 66.7%; exp. 75.5%; P = 0.22) or between c-ALL and control subjects.

Table 1 shows that DQA1 *01 was more frequent in patients than control subjects (OR = 1.60; 95% CI: 0.97-2.62), mainly because of DQA1 *0101/0104 (OR = 2.27; 1.12-4.52). There was also an increase in the DQA1 *03 (OR = 1.59; 0.87-2.90) and a deficit in DQA1 *0201 (OR = 0.53; 0.28-1.04).

Male c-ALL had a significantly higher frequency of DQA1 *0101/0104 (OR = 4.06; 1.42-10.17) (Table 1), a higher frequency of DQA1 *03 (OR = 2.62; 1.07-6.02) and a deficit of DQA1 *0201 (OR = 0.40; 0.18-0.96) than male control subjects. Apart from a small deficit in DQA1 *0201, there were no differences between female c-ALL and female control subjects.

DQA1 and DQB1 alleles in c-ALL

Patients and control subjects were classified according to whether they typed for both DQB1 *0501 and DQA1 *0101/0104. Table 2 shows that the greatest difference was between male c-ALL and male control subjects (OR = 3.73; 1.19-10.3). This was absent in girls with c-ALL, and in four other pairs of DQA1/DQB1 alleles known to be in linkage disequilibrium.

HLA-DQ polymorphic amino acids

We previously found that c-ALL was associated with the absence of DQB1 alleles coding for aspartic acid (Asp) at position 57 of DQB1 (DQB1Asp57: Dearden et al. 1996). As DQB1Arg52+, DQB1Asp57- is a susceptibility haplotype in juvenile insulin-dependent diabetes (IDDM: Tosi et al. 1994), we analysed its frequency in c-ALL. We found no association with c-ALL (data not shown). Further analysis revealed an increased frequency of serine at position 52 (ζSer52) of DQA1, and valine at position 57 (βVal57) of DQB1 (i.e. ζSer52, βVal57) in c-ALL (Table 3: OR = 2.34; 1.07-4.97). This was confined to boys (OR = 4.18; 1.41-11.03) and was absent from girls with c-ALL (OR = 0.83; 0.25-3.0).

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The significance of these associations disappears following correction for the number of alleles tested. We therefore performed simulations based on allele and haplotype frequencies in the present study to estimate case and control sample sizes required to obtain statistical significance. We assumed 90% power to detect a $P = 0.005$ in a two-sided test, before correction for the number of alleles in equal numbers of cases and control subjects. For $DQA1^{*}0101/0104$, the total number of patients and control subjects is 475, and for boys it is 181. For $DQA1^{*}0101/0104,DQB1^{*}0501$ haplotypes, total patient and control series require 388 in each group whereas boys require 122 patients and controls.

### DISCUSSION

Evidence suggesting that the same HLA class II polymorphic sequences contribute both to the binding of antigenic peptides and disease susceptibility (Hammer et al. 1995; Kwok et al. 1996) suggests that an HLA-$DQA1,DQB1$ haplotype association with childhood c-ALL could be construed as evidence of an infectious aetiology. In this study we found an increased frequency of $DQA1^{*}0101/0104$, and a deficit of $DQA1^{*}0201$ in c-ALL, suggesting roles in susceptibility and resistance to c-ALL respectively. Further analysis showed that $DQA1^{*}0101/0104$ was increased in boys but not girls with c-ALL, a finding which would not have been expected by chance alone. Analysis of patients and control subjects classified by the presence or absence of $DQA1^{*}0101/0104$ and $DQB1^{*}0501$ showed that an increase in this ‘haplotype’ in patients was confined to boys. This haplotype (and certain others) encodes $DQa5Ser52$ and $DQBVal57$. Both amino acids were increased in c-ALL, but this was also confined to male patients. These results need to be treated with caution as they were not corrected for the number of alleles. However, we used the results to simulate the number of cases and control subjects required to repeat the study in an independent series.

The patients in this study were an unselected series with c-ALL, in which 63% were boys and 37% were girls (M:F 1.73:1). Analysis of 199 cases of childhood c-ALL in the Manchester Children’s Tumour Registry (MCTR) for 1983–94 showed 118 boys and 81 girls (M:F 1.5:1), suggesting that the male excess was not due to chance. McKinney et al. (1993) found no difference in the rate of c-ALL in boys and girls aged 1–9 in a UK study, but Buckley et al. (1994) reported an M:F of 1.2:1 in 312 cases of c-ALL in a US study. We found no evidence that inclusion of only verified c-ALL patients, and exclusion of unclassified ALL favoured boys over girls. There was no difference in the age or gender of patients donating and not donating blood samples to the study.

The $DQA1$ SSO probes used here define polymorphisms confined to exon 2 but do not distinguish between $DQA1^{*}0101$ and $0104$. These alleles differ for single base substitutions in codons 2 (exon 1) and 199 (exon 4) ( Yasunaga et al. 1996). It remains to be seen whether the difference between $0101$ and $0104$ has any influence on susceptibility to c-ALL.

Our results contrast with Dorak et al. (1995) who used a restriction fragment length polymorphism (RFLP)-based method to type $DQA1$ alleles in childhood ALL. They found no increase in $DQA1$-IA in ALL, nor any difference between male and female patients.

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typing for this allele. However, they found a significant increase in allele 3 in male compared with female ALL patients. We found an increase in DQA1*03, which was confined to boys with c-ALL. However, there was no difference in patients and control subjects typing for both DQA1*03 and DQB1*0302, which are in linkage disequilibrium (Imanishi et al. 1992). Furthermore, there was no increase in the frequency of DQA1*03 homozygotes in c-ALL.

As DQA1 is tightly linked to DQB1, the DQA1*0101/DQBI*0104 association with c-ALL could be explained by linkage disequilibrium with DQB1*0501. The present study confirms an increased frequency of both alleles in boys with c-ALL, but not of other DQA1/DQB1 haplotypes. Our previous results showed a reduced frequency of aspartic acid at position 57 in c-ALL (Dearden et al. 1996), suggesting similarities with the DQB1Asp57→motif associated with IDDM (Tosi et al. 1994). However, analysis of the IDDM susceptibility haplotype DQα1Arg52.DQβ1Val57 showed no evidence of a role in c-ALL.

HLA-DQA1*0101, 0102, 0103 and 0104 all code serine at position 52, and DQB1*0501, 0604-06, 0608 and 0609 code valine at position 57 (see Marsh). However, we found that only one haplotype, DQA1*0101/DQB1*0501, predominated in c-ALL. Thirty per cent of c-ALLs compared with 17.3% of control subjects had this haplotype. Analysed by gender, 36.8% of male c-ALL compared with 13.5% male control subjects expressed DQα1Ser52.DQβ1Val57 heterodimers, but there was no difference between female patients and controls subjects.

Gene transfection studies by Kwok et al. (1993) showed that the expression of DQB1*0501-encoded β-chains is facilitated by DQA1*0101 α-chains and is influenced by amino acids coded at the 3' end of DQB1 corresponding to positions 60 and 91 of the DQB1 subunit. Furthermore, DQA1*0101/DQB1*0501 is one of the most common DQA1-DQB1 haplotypes in the UK population (Doherty et al. 1992) and the second most common haplotype in French, Danish and Spanish populations (Imanishi et al. 1992). The gene expression and population genetic data thus suggest that the DQA1*0101/DQB1*0501 haplotype may have had a selective advantage, possibly by protecting against infectious diseases.

Our results suggesting that susceptibility to c-ALL is increased in boys with a common DQA1-DQB1 haplotype could be explained by a greater contribution of DQα1Ser52/DQB1Val57 peptide-binding motifs to the protection of boys against certain types of childhood infection. There is increased susceptibility of male children to infections (Washburn et al. 1965; Purtilo and Sullivan, 1979), which suggests that certain HLA haplotypes may counteract X-linked defects in immunity in boys (Immunological Reviews, 1994), by promoting the efficiency of antigen presentation. If confirmed, our results would imply that a common DQA1-DQB1 haplotype that may increase resistance to infection in boys has an important influence on susceptibility to childhood c-ALL. This would be consistent with the predictions of the Greaves hypothesis (Greaves. 1988; Greaves and Alexander, 1993), and further suggests that the candidate infection involved in c-ALL exhibits low pathogenicity but strong immunogenicity.

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