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ORIGINAL ARTICLE

HLA-DPB1 supertype-associated protection from childhood leukaemia: relationship to leukaemia karyotype and implications for prevention

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Abstract Most childhood B cell precursor (BCP) acute lymphoblastic leukaemia (ALL) cases carry the reciprocal translocation t(12;21)(p13;q22) (~25%), or a high hyperdiploid (HeH) karyotype (30%). The t(12;21) translocation leads to the expression of a novel fusion gene, *TEL-AML1* (*ETV6-RUNX1*), and HeH often involves tri- and tetrasomy for chromosome 21. The presence of *TEL-AML1*+ and HeH cells in utero prior to the development of leukaemia suggests that these lesions play a critical role in ALL initiation. Based on our previous analysis of *HLA-DP* in childhood ALL, and evidence from in vitro studies that *TEL-AML1* can activate *HLA-DP*-restricted T cell responses, we hypothesised that the development of *TEL-AML1*+ ALL might be influenced by the child's *DPB1* genotype. To test this, we analysed the frequency of six *HLA-DPB1* supertypes in a population-based series of childhood leukaemias ($n = 776$) classified by their karyotype (*TEL-AML1*+, HeH and others), in comparison with newborn controls ($n = 864$). One *DPB1* supertype (GKD) conferred significant protection against *TEL-AML1*+ ALL (odds ratio (OR), 95% confidence interval (95% CI): 0.42, 0.22–0.81; $p < 0.005$) and HeH ALL (OR; 95% CI: 0.44, 0.30–0.65; $p < 0.0001$). These negative associations were almost entirely due to a single allele, *DPB1*0101*. Our results suggest that *DPB1*0101* may afford protection from the development of *TEL-AML1*+ and HeH BCP ALL, possibly as the result of a DP-restricted immune response to BCP ALL-associated antigen(s), the identification of which could have important implications for the design of prophylactic vaccines.

Keywords *HLA-DPB1* · Childhood leukaemia · Immune response · Karyotype · *TEL-AML1* · Hyperdiploidy · Prevention

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Introduction

The majority of childhood leukaemias (85%) in Europe and the USA are acute lymphoblastic leukaemias (ALL), mainly of B cell precursor (BCP) subtype, with a characteristic age-incidence peak between 2 and 5 years [1–3]. Cytogenetic and molecular analyses have revealed that two types of clonally-acquired chromosomal changes predominate in BCP ALL. About 25% of cases carry a reciprocal translocation, t(12;21)(p13;q22), leading to the in-frame

fusion of the *TEL* (*ETV6*) gene on chromosome 12p13 and the *AML1* (*RUNX1*) gene on 21q22, respectively [4, 5]. Some 30% of cases have a high Hyperdiploid (HeH) karyotype with a modal chromosome number >50, characterised by non-random gain of chromosomes X, 4,6,8,10,14,17,18, and 21 [6, 7]. The *TEL-AML1* fusion gene is expressed in BCP ALL as a novel onco-protein [8], and is associated with a favourable treatment outcome [9, 10]. HeH is also associated with a good prognosis [11, 12], and expression profiling suggests that the majority of genes overexpressed by this subtype are localised to chromosome 21 and X [13]. Evidence that *TEL-AML1*+ and HeH pre-leukaemia cells are present in utero, prior to the onset of overt leukaemia, suggests that these lesions are associated with and perhaps critical to the initiation of BCP ALL leukaemogenesis [14–17].

In vitro studies have shown that a *TEL-AML1* nonamer junctional-peptide can activate *HLA-DPB1*-restricted CD4+ helper and *HLA-A2*-restricted CD8+ cytotoxic T cell responses [18, 19]. This implies that *TEL-AML1* may serve as an onco-antigen in vivo in subjects with *HLA* alleles that bind this peptide. We previously reported that the frequency of *DPB1*0101* was significantly less in childhood BCP ALL cases than in unrelated controls [20]. We also detected a deficit of this allele in cases classified by *TEL-AML1* and HeH status, but we did not explore the reason for this putative protective effect. Here we hypothesise that if *HLA-DP*-restricted T cell responses to *TEL-AML1* inhibit the development of BCP ALL, this should be evident from the reduced frequency of the restricting allele(s) in cases, and its predicted ability to bind *TEL-AML1*. We tested this hypothesis, as part of the UK Childhood Cancer Study [21], by comparing the frequency of *HLA-DPB1* peptide-binding supertypes in a population-based series of karyotyped childhood leukaemias with unrelated controls.

Materials and methods

Cases and controls

Blood samples were collected nationally from childhood leukaemia cases ($n = 776$) between 1992 and 1998, and locally from newborn controls ($n = 864$), between 1991 and 1997 as part of the national UK Childhood Cancer Study (UKCCS) [21]. Case data (diagnoses, cytogenetics, ages, gender, ethnic background) were validated by the UKCCS data centre at the Epidemiology and Genetics Unit, University of York. Diagnostic immunophenotyping of leukaemia cases was carried out on bone-marrow as previously described [21], according to criteria for UK Medical Research Council (MRC) leukaemia trials. Leukaemia subtypes are defined as Pro-B ALL (CD10−, CD19+); BCP-ALL (CD10+, CD19+); T-ALL (CD2/CD7+, CD19−, DR−), and AML. All procedures were carried out with national and local ethical approval. Controls were drawn from a cross-sectional series of umbilical cord blood samples obtained from normal term newborns born at St Mary's Hospital, Manchester, UK. Using data obtained at birth (gender, ethnic background), cases and controls were matched for white UK ethnicity. The male:female ratio of the total case series was 1.23:1; for the controls it was 1.01:1.

Conventional cytogenetics

Cytogenetic analysis was carried out on diagnostic bone marrow and/or peripheral blood samples by local UK cytogenetic laboratories according to standard methods [22]. These data were collected and reviewed by the Leukaemia Research Fund Cytogenetics Group [23] and provided to the UKCCS [21].

Molecular cytogenetics

TEL-AML1 transcripts were detected in ALL bone-marrow and peripheral blood using RT-PCR and fluorescence in situ hybridisation (FISH), respectively, as previously described [24]. RT-PCR was carried out on cDNA from reverse transcribed RNA using a single pair of primers [25]. As the t(12; 21) is a cryptic translocation, FISH analysis provided confirmation of positive and negative RT-PCR results. HeH was identified either by conventional cytogenetic analysis or, in cases of a failed cytogenetic result, by interphase FISH to indicate extra copies of chromosomes 21 and X, or using a panel of centromeric probes [24]. Based on cytogenetics, RT-PCR, and FISH, cases were classified into three cytogenetic groups: *TEL-AML1* positive, high HeH, and combined "other". The "other" group comprised all cases with a normal or abnormal cytogenetic result that did not include either *TEL-AML1* or HeH.

HLA-DPB1 molecular typing

HLA-DPB1 molecular typing was carried out by the method previously described in detail [20]. A 327 bp fragment of *DPB1* exon 2 in each DNA sample was amplified using a single pair of *DPB1* generic PCR primers, aliquots of each PCR product spotted onto 384 sample nylon filters, and hybridised using a panel of 28 ^{32}P-labelled sequence specific oligonucleotide probes (SSOP). Probe hybridisation was detected using real-time autoradiography, and alleles assigned from published *DPB1* ideograms.

Data analysis

Childhood leukaemia cases typed for *HLA-DPB1* alleles were stratified by karyotype into *TEL-AML1*+, HeH and

other karyotypes. Only results from cases with verified cytogenetic data and knowledge of *TEL-AML1* status were included in the analysis. *DPB1* allele frequencies were determined using the POPGENE (v.1.31) [26] descriptive population genetics programme. Selected *DPB1* alleles in cases and controls were then clustered into six peptide binding pocket (PBP) supertypes, based on amino acid dimorphisms at positions 11, 69 and 84 of the DPβ_1 domain, corresponding to the P6, P4 and P1 PBP. Using single letter amino-acid codes, these supertypes are designated GEG, LED, GED, GKG, LKD and GKD. Rare supertypes (LRD, GRV, GKV) with a cumulative frequency of ≤5% were excluded. Case-control *DPB1* supertype and allele frequencies were compared using univariate statistical analysis to calculate cross-product odds ratios (ORs) and 95% confidence intervals (95% CI) by the Sheehe method with the RERI program from the Linkage Utility Package, LINKUTIL [27]. Statistical significance of case-control supertype and allele frequency differences was determined using Fisher's Exact test to calculate *p* values using the 2by2 programme in LINKUTIL. Results are depicted as uncorrected 2-sided *p* values, with a *p* value of ≤0.008 being required to correct for six supertype clusters, to give significance ($p \leq 0.05$). Other corrections are discussed in the text.

Results

Cytogenetic characteristics of childhood leukaemias

The karyotypes of childhood leukaemia cases recruited during the UKCCS have previously been reported [21]. These included 1,013 (76.5%) of the 1,324 B-lineage ALL cases, comprising *TEL-AML1*+ ($n = 139$), HeH ($n = 423$), and other karyotypes ($n = 451$). Cytogenetic data were also available for 99 of 137 T ALL (72%), and 160 of 250 AML (72%) cases. Of the 1,272 UKCCS leukaemia cases with cytogenetic data, DNA samples were obtained and typed for *DPB1* alleles from 776 (61%) cases (Table 1). The majority of *DPB1*-typed B-lineage ALL cases were *TEL-AML1*+ ($n = 90$) or HeH ($n = 276$).

DPB1 supertype frequency in relation to leukaemia karyotype

The number and frequency of *DPB1* alleles in the karyotyped UKCCS cases and in controls are shown in Table 2. The 3-letter supertype assignment of each allele, based on di-allelic amino acid polymorphisms in the three peptide binding pockets at positions 11, 69 and 84 of the DP$_{\beta 1}$ domain, accommodating amino acid residues at P6-P4-P1 of a core peptide, are also shown. A total of 37 *DPB1* alleles were identified in the combined case-control series, including 18 in *TEL-AML1*+, 22 in HeH, 25 in other cases, and 31 in the controls. Six uncommon alleles (*2401, 2501, 3301, 3501, 4901, 5101*) were confined to the cases, and nine uncommon alleles (*1801, 2101, 2701, 3401, 3601, 3901, 4401, 4801, 5001*) were found only in controls. Excluding four alleles (*1101, 1501, 1801, 3401*) with rare supertypes, all other alleles (33) could be clustered into six supertypes, three with *β*69E (GEG, LED, GED), and three with *β*69K (GKG, LKD, GKD). The number of alleles with the GKG, LKD, or GKD supertypes in cases (8–12) and controls (12) was similar; the number of alleles with the GEG and GED supertypes in cases and controls (2–3) was also similar, whilst the number with the LED supertype in cases (4–6) differed from the controls (9).

Table 1 Childhood leukaemias classified by cytogenetic status

Karyotype [a]	Diagnosis[c]					
	Pro-B ALL	BCP ALL	T ALL	AML	OTHER	Total
TEL-AML1	1	89	0	0	5	95
HeH	3	273	2	0	22	300
OTHER	17	167	72	93	32	381
N[b]	21	529	74	93	59	776

[a] Karyotype based on conventional and molecular cytogenetic analysis on leukaemic bone-marrow or peripheral blood blasts. For details see "Materials and methods"

[b] *N* number of diagnosed leukaemia cases with a given karyotype

[c] Leukaemias diagnosed by immunophenotyping and cytomorphology: Pro-B ALL, pro-B cell acute lymphoblastic leukaemia; BCP ALL, B cell precursor ALL; T ALL, T cell ALL; AML, acute myeloid leukaemia; "other", leukaemias that could not be classified by immunophenotyping or cytology

The frequencies of all 6 *DPB1* supertype clusters in cases classified by karyotype compared with controls are shown in Table 3. Only one (GKD) of the six supertypes had OR values significantly <1.0 in all three karyotyped case series (Odds Ratio, 95% confidence interval: *TEL-AML1*: 0.42, 0.22–0.81; HeH: 0.44, 0.30–0.65; other: 0.72, 0.53–0.97). Correction for six supertypes gives significant *p* values for *TEL-AML1* (0.03) and HeH (0.00006), but not other leukaemias (0.18). Cases with two other supertypes having lysine at position 69 (GKG, LKG) were not significantly different from controls. Odds ratios were significantly >1.0 for the three *β*69E supertypes, GEG (all case series), for LED (HeH and other), and GED (*TEL-AML1* and HeH). *P* values were significant after correction for six supertypes for GEG in HeH (0.048), and other cases (0.006).

Only 3 *DPB1* alleles (*0101, 0501, 5001*) with the "protective" GKD supertype were present in the case-control series (Table 2). Table 4 compares the frequency of two of

Table 2 *DPB1* allele frequency in childhood leukaemia in relation to karyotype

*DPB1** allele	Frequency ($f \times 100$)[b]				
	Supertype[a]	*TEL-AML1*	HeH	OTHER	CONTROL
0101	GKD	2.55	3.92	6.04	7.99
0201	GEG	9.69	9.97	9.58	5.96
0202	GEG	1.53	0.82	1.05	0.64
0301	LKD	10.71	9.48	10.50	11.46
0401	GKG	44.39	47.06	46.46	45.25
0402	GKG	12.76	13.07	12.07	11.52
0501	GKD	2.04	1.47	1.97	3.01
0601	LED	3.06	1.63	2.49	0.35
0801	GED		0.33	0.26	0.06
0901	LED	1.02	0.98	0.52	1.10
1001	LED	1.53	2.78	1.44	1.45
1101	[LRD]	3.57	2.29	1.31	3.76
1301	LED	0.51	1.63	2.23	1.74
1401	LKD	2.55	0.49	0.39	0.81
1501	[GRV]	0.51	0.98	0.79	0.81
1601	GED	1.02			0.06
1701	LED		1.14	0.92	0.23
1801	[GKV]				0.41
1901	GED	1.53	1.14	0.66	0.35
2001	LKD		0.16	0.13	0.06
2101	LED				0.17
2301	GKG			0.26	0.46
2401	GKG			0.13	
2501	LKD	0.51		0.13	
2601	LKD		0.16	0.13	0.58
2701	LKD	0.51			0.23
3001	LED			0.13	0.06
3301	GEG			0.13	
3401	[GKV]				0.17
3501	LKD		0.16	0.13	
3601	LED				0.06
3901	GKG				0.64
4401	LED				0.06
4801	GEG				0.17
4901	GKG		0.16		
5001	GKD				0.06
5101	GKG		0.16		
N		95	300	381	864

[a] Supertypes designated by polymorphic amino acid residue (aa) in the P6, P4 and P1 peptide binding pockets (PBP). Single letter amino acid (aa) codes are used throughout: G, glycine; E, glutamic acid; K, lysine; D, aspartic acid; V, valine; R, arginine; L, leucine. Supertypes in parentheses are not analysed further

[b] DPB1 allele frequencies (f x 100) calculated using POPGENE (see "Materials and methods")

these alleles (*0101, 0501*) with other alleles having lysine at β69 (*0401, 0402, 0301*). *DPB1*0101* was significantly reduced in *TEL-AML1* (OR, 95% CI: 0.34, 0.15–0.78; uncorrected $p = 0.005$) and HeH cases (OR, 95% CI: 0.45, 0.28–0.70; uncorrected $p = 0.0002$), but not in the other karyotyped cases. Correction for 37 *DP* alleles removes significance from *TEL-AML1* ($p = 0.18$), but not HeH ($p = 0.007$). The odds ratios for *DPB1*0501* in *TEL-AML1* and HeH were <1.0, but were not significant.

Discussion

We previously reported that the frequency of *DPB1*0101* in common (B cell precursor) ALL, and in *TEL-AML1+* and HeH cases was reduced compared with newborn controls [20], but we did not enlarge upon the possible reason. Yun et al. [19] reported that a *TEL-AML1* junctional nonamer onco-peptide, RIAECILGM, can activate an *HLA-DPB1*0501* restricted CD4+ T cell response in vitro. Since *DPB1*0101* has the same supertype as *0501*, we hypothesised that the deficit of *0101* might be related to similar peptide-binding motifs in these two alleles. One testable consequence of our result is that children typing for *DPB1*0101* may be more likely than children with other *DPB1* genotypes to generate T cell responses to the TEL-AML1 peptide. In support of this hypothesis, we show that the TEL-AML1 junctional peptide contains amino acid residues that we predict bind to appropriate peptide binding pockets of *DPB1*0101*. A similar hypothesis was proposed by Postuma et al. [28] to explain the deficit of *HLA-A3*, *A11*, and *B8* in chronic myeloid leukaemias expressing the *BCR-ABL* p210 fusion protein.

We analysed the frequency of 6 *HLA-DPB1* supertype clusters defined by amino acid dimorphisms in three critical peptide binding pockets (P6-P4-P1) at positions β11- β69-β84 (single amino acid code: GEG, GED, LED, GKG, GKD, LKD) in a population-based series of karyotyped childhood leukaemia cases in comparison with newborn controls. By clustering *DPB1* alleles into three pairs of supertypes, we were able to distinguish between allele groups with glutamic acid or lysine at β69. Castelli et al. [29] clustered *DPB1* alleles into three peptide-binding supertypes, based on amino acid dimorphisms at β84 (pocket 1) and β11 (pocket 6), but others have emphasised the importance of β69 (pocket 4) in antibody binding [30], allorecognition [31], peptide binding [32] and disease susceptibility [33]. Using in silico analysis Doytchinova and Flower [34] proposed a *DP*-supertype classification that takes account of β69. Our supertype classification is based on a synthesis of these findings, and distinguishes 3 β69E (GEG, LED, GED) from 3 β69K supertypes (GKG, LKD, GKD) (Taylor et al. submitted for publication). Supertype clusters defined by DP peptide binding predictions [35, 36] provide a better insight into the functional role of *DPB1* in BCP ALL than classical alleles [37].

Table 3 *HLA-DPB1* supertype frequency in relation to leukaemia karyotype

DPB1 supertype[a]	Control %	*TEL-AML1*				HeH				OTHER			
		%	OR	95% CI[b]	2 sided p	%	OR	95% CI	2 sided p	%	OR	95% CI	2 sided p
GKD	11.1	4.7	0.42	0.22–0.81	0.005	5.2	0.44	0.30–0.65	0.00001	8.1	0.72	0.53–0.97	0.03
GKG	58.0	57.4	0.97	0.72–1.31	0.91	61.2	1.14	0.94–1.38	0.19	58.9	1.04	0.87–1.23	0.71
LKD	13.2	13.2	1.01	0.65–1.56	0.99	10.8	0.80	0.60–1.07	0.15	11.4	0.85	0.65–1.11	0.24
GED	0.5	2.6	6.00	2.11–17.05	0.01	1.5	3.25	1.31–8.04	0.03	0.9	2.01	0.77–5.23	0.28
GEG	6.8	11.6	1.83	1.14–2.94	0.03	10.3	1.59	1.15- 2.19	0.008	10.8	1.66	1.24–2.23	0.001
LED	5.2	6.3	1.28	0.70–2.34	0.58	7.7	1.54	1.07–2.21	0.03	7.7	1.55	1.10–2.17	0.01
N[c]	864			95				300				381	

[a] DPB1 supertype designations as shown in Table 2

[b] OR, 95% CI: Odds ratios and 95% confidence intervals

[c] *N* number of subjects

Table 4 *DPB1* allele-associated relative risk of leukaemia in relation to leukaemia karyotype

DPB1* allele/ Supertype	Control %	*TEL-AML1*				HeH				OTHER			
		%	OR	95% CI	2-sided p	%	OR	95% CI	2- sided p	%	OR	95% CI	2 sided p
0101/GKD	8.0	2.6	0.34	0.15–0.78	0.005	3.7	0.45	0.28–0.70	0.0002	6.0	0.75	0.53–1.05	0.09
0501/GKD	3.0	2.1	0.77	0.30–1.95	0.67	1.5	0.51	0.26–1.01	0.05	2.0	0.66	0.38–1.17	0.17
0401/GKG	45.3	45.3	1.00	0.74–1.35	0.99	48.3	1.13	0.94–1.36	0.21	46.5	1.05	0.88–1.25	0.60
0402/GKG	11.5	12.1	1.08	0.68–1.69	0.88	12.5	1.10	0.83–1.46	0.56	12.1	1.06	0.81–1.37	0.73
0301/LKD	11.5	10.0	0.88	0.54–1.43	0.64	9.8	0.85	0.62–1.15	0.31	10.5	0.91	0.69–1.20	0.53

Other details as in Table 3

Because of the large number of *DPB1* alleles, we previously used Monte-Carlo (CLUMP) analysis [38] to show that several alleles with a glutamic acid residue at position 69 (β69E) were associated with susceptibility to childhood BCP ALL [20]. In this study, prior clustering of alleles allowed the number of comparisons to be reduced to 6. Univariate analysis showed that the frequency of the GKD supertype was significantly less in *TEL-AML1*+ and HeH ALL than controls. This represents a 57% reduction of this supertype in *TEL-AML1*+ cases. Because we clustered alleles into six supertypes, we corrected only for six comparisons rather than the total number of *DPB1* alleles. However, the reduction of *DPB1*0101* in HeH cases was significant ($p = 0.007$) even after correction for 37 alleles, whilst lack of significance for *DPB1*0101* in *TEL-AML1* cases ($n = 95$), can be ascribed to an insufficient number of cases. Breslow and Day [39] pointed to the conservatism of multiple allele (Bonferroni) corrections in *HLA* studies, a critical issue in studies of *DP* and disease where there is a large number of uncommon alleles that have a disproportionate effect on significance tests. Such limitations could lead to type II error, as discussed in more general terms by Perneger [40]. The key message of our study is, therefore, that our analysis requires confirmation in other karyotyped leukaemia series, particularly in populations with a high frequency of *DPB1*0101*.

The case-control differences in *DPB1* supertype and allele frequency that we report here are unlikely to be explained by ethnic stratification. We previously discussed this in detail in relation to the use of local controls for the UKCCS case series [20]. These controls were matched for white UK ethnic background with UKCCS cases. Had we chosen to ignore ethnicity, the frequency of *DPB1*0101* in our total controls (8.4%), consisting of 864 white UK and 442 newborns with other ethnic backgrounds ($n = 1{,}306$), would have differed little from the white UK series (8.0%). Furthermore the frequency of *DPB1*0101* in the previously reported UKCCS series of solid tumour cases ($n = 409$) [20] was not significantly different from the white UK newborns (OR, 95% CI: 0.8, 0.6–1.1).

The blood samples for this study were obtained in remission from UKCCS cases. This might have influenced allele frequencies if there had been selection of cases that favoured survival. With very few exceptions, however, UKCCS cases were entered into the United Kingdom UKALL XI trial (1990–1997), and this had a remission rate of 98%, only 2% deaths in remission, and an overall survival rate of 85% at 5 years [41]. The median time from

diagnosis to blood sampling in our series was 7 months, >25% of samples having been collected within 2 months, and 70% within 12 months. It is thus unlikely that there was an allele bias due to delayed blood sampling. We are proposing to examine the relationship between *DPB1* supertypes and long-term survival in a further study.

The lysine residue at position 69 (β69K) of the GKD supertype creates a positively charged P4 pocket with an affinity for ligands with a negative charge, including glutamic acid (E). Yun et al. [19] generated two *DPB1* allele-restricted T cell clones to *TEL-AML1* fusion peptides, one of which carried the *0501* allele. They found that this recognised the TEL-AML1 nonapeptide RIAECILGM, which has a negatively charged E in the P4 position. The positively charged K residue in the P4 PBP of *DPB1*0501* and *0101* (Fig. 1) should bind the E residue of the *TEL-AML1* nonapeptide [35, 36]. Whether differences between *DPB1*0101* and *0501* in the P9 pocket (β9: 0101 = Y; 0501 = L; β55: 0101 = A; 0501 = E) significantly affect binding remains to be determined. Two other *DPB1* supertypes with β69K (GKG and LKD) account for 71% of the supertype frequency in the controls, but were not deficient in *TEL-AML1+* ALL. This suggests that a glycine (G) at position 11 and aspartic acid (D) at position 84, as well as lysine at position 69 of GKD could be important in protection from *TEL-AML1+* ALL.

The GKD supertype frequency in HeH ALL was significantly less (5.2%) than in the controls (11.1%; OR, 95% CI: 0.44, 0.30–0.65; $p < 0.0001$), equivalent to a 50% reduction in *DPB1*0101*. It is unlikely that this can be explained by undetected *TEL-AML1* transcripts in HeH cases since we used interphase FISH [24] as well as RT-PCR to detect this rearrangement. This suggests that HeH ALL might also express a *DPB1*0101*-restricted onco-antigen, of which the AML1 protein whose expression is amplified in HeH ALL [42, 43] is a candidate. Six of the 9 TEL-AML1 junctional amino acid residues (RIAECILGM) are coded by the alternately spliced native AML1 variant, AML1G, including the negatively charged E with an affinity for the β69K in the P4 PBP of *DPB1*0101* (Fig. 1). In the absence of a suitable peptide-binding prediction algorithm for *DPB1*0101*, we scanned AML1G in silico for DRB1-binding peptides using TEPITOPE [44] and identified several putative DR-restricted T cell epitopes. Thus, it is possible that AML1 overexpression in HeH could lead to loss of immunological tolerance, resulting in the development of a protective *DPB1*-restricted T cell response.

We observed a marginally significant reduction in *DPB1*0101* in leukaemias with other (i.e. *TEL-AML1-*, HeH-) karyotypes, but since this series was cytogenetically heterogeneous, it could have included small numbers of undetected *TEL-AML1+* and HeH cases. *DPB1*-restricted T cell responses to other onco-proteins might also explain this mildly protective effect.

Our result does not prove a causal relationship between *DPB1*0101* and a T cell response to *TEL-AML+* BCP ALL. This will require evidence for the expression of this allele by *TEL-AML1+* leukaemias, the *0101*-restricted binding of this onco-peptide, and the generation of an *0101*-restricted T cell response in vivo, all of which require further study. Expression of *HLA-DR* is used as a diagnostic aid in BCP ALL [45, 46], and recent evidence suggests that *DR* expression is significantly greater in *TEL-AML1+* than *TEL-AML1-* BCP ALL or normal pre-B cells, implying

	Relative amino acid position	P1	P2	P3	P4	P5	P6	P7	P8	P9
	$TEL^{334-342}$	**R**	**I**	**A**	D	C	R	L	L	W
	$AML\text{-}1G^{17-25}$	F	M	R	**E**	**C**	**I**	**L**	**G**	**M**
	TEL-AML1	**R**	**I**	**A**	**E**	**C**	**I**	**L**	**G**	**M**
*DPB1** allele	Position -> Supertype	84			69		11	55		35
0101	GKD	D			K		G	A		Y
0501	GKD	D			K		G	E		L
0401	GKG	G			K		G	A		F
0301	LKD	D			K		L	D		F
0201	GEG	G			E		G	D		F
0801	GED	D			E		G	D		F
0601	LED	D			E		L	D		F

Fig. 1 Peptide binding motifs defined by *HLA-DPB1* alleles/supertypes in relation to the TEL-AML1 junctional 9-mer core peptide (RIAECILGM) with a negatively charged E in the P4 peptide binding pocket. This peptide is known to bind to *DPB1*0501*. The homologous wild-type TEL and AML1G peptide sequences are shown for comparison. The peptide binding frame for RIAECILGM is numbered P1–P9. The TEL and AML1G peptides have been positioned so as to show the same amino acid sequences as present in TEL-AML1, but it is not known whether they have the same binding frame. Note that AML-1G shares six of the nine amino acids with TEL-AML1, including an E at P4. DPB1*0101 and 0501 are identical for PBP P6, P4 and P1, but differ for the P7 and P9 PBP. All other supertypes differ from GKD at P6, P4 and/or P1

that *TEL-AML1* leukaemias may have a distinctive antigen-presenting phenotype [47], which may be true for DP. Thus, although expression of *HLA-DP* by B-ALL is about 25% that of *HLA-DR*, DP molecules are effectively recognised by DP allo-specific cytotoxic T cells [48].

Human leukaemias express an impressive array of leukaemia-associated fusion genes and onco-fetal proteins [49], and we cannot rule out an effect due to immune responses to other leukaemia antigens. The Wilm's tumour onco-fetal protein, WT1 is expressed by a majority of childhood leukaemias [50, 51] and is known to elicit *HLA class I* and *II*-restricted T cell responses [52, 53]. Guo et al. [54] showed that the $WT1_{337\text{-}347}$ peptide elicited a *DP5*-restricted cytotoxic response to AML cells, but the highly variable expression of WT1 by BCP ALL [55] may limit its value as a target for cytotoxic T cells.

Our results showed that K → E substitution at β69 significantly increased the risk of *TEL-AML1*+ ALL in cases with the GED supertype (OR, 95% CI: 6, 2.1–17.0). This represents a 14-fold difference in relative risk compared to GKD, and raises the possibility that alleles with the GED supertype may interact with ligands, perhaps derived from infections [56], in which the negatively charged E at P4 of the *TEL-AML1* nona-peptide is replaced by a positively charged K.

There is increasing evidence to suggest that the emergence of pre-malignant cells may be prevented by the immune system [57]. Molecular evidence that *TEL-AML1*+ positive B cell precursors are present in cord blood of newborns at 100 times the rate of overt leukaemia [58, 59] raises the possibility of host control over the development of ALL. However, the frequency of *DPB1*0101* in our controls is too low to explain this difference and it may be that other *HLA* alleles restricting the immune responses to *TEL-AML1* contribute to protection. This is supported by evidence that the *TEL-AML1* nonamer elicits an *HLA*A2*-restricted cytotoxic T cell response [18]. Further studies are needed to determine whether there are other T cell epitopes in *TEL-AML1* and *AML1* peptides. However our data together with the results of in vitro studies [18, 19] suggest that modelling and testing of TEL-AML1 and AML1 peptides could be a useful adjunct to the design of a prophylactic vaccine for BCP ALL.

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