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LYMPHOID NEOPLASIA

The role of *KIR* genes and their cognate *HLA* class I ligands in childhood acute lymphoblastic leukemia

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Key Points

- Carrying the *KIR* A/A genotype contributes to risk of childhood ALL, particularly in Hispanics.

Killer cell immunoglobulin-like receptors (KIRs), via interaction with their cognate HLA class I ligands, play a crucial role in the development and activity of natural killer cells. Following recent reports of *KIR* gene associations in childhood acute lymphoblastic leukemia (ALL), we present a more in-depth investigation of *KIR* genes and their cognate *HLA* ligands on childhood ALL risk. Genotyping of 16 *KIR* genes, along with *HLA* class I groups C1/C2 and Bw4 supertype ligands, was carried out in 212 childhood ALL cases and 231 healthy controls. Frequencies of *KIR* genes, *KIR* haplotypes, and combinations of *KIR-HLA* ligands were tested for disease association using logistic regression analyses. *KIR* A/A genotype frequency was significantly increased in cases (33.5%) compared with controls (24.2%) (odds ratio [OR] = 1.57; 95% confidence interval [CI], 1.04-2.39). Stratifying analysis by ethnicity, a significant difference in *KIR* genotype frequency was demonstrated in Hispanic cases (34.2%) compared with controls (21.9%) (OR = 1.86; 95% CI, 1.05-3.31). Homozygosity for the *HLA-Bw4* allele was strongly associated with increased ALL risk exclusively in non-Hispanic white children (OR = 3.93; 95% CI, 1.44-12.64). Our findings suggest a role for *KIR* genes and their *HLA* ligands in childhood ALL etiology that may vary among ethnic groups. (*Blood*. 2014;123(16):2497-2503)

Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common cancer in children, accounting for ~30% of all malignancies diagnosed before the age of 15 years.[1] The etiology of this disease has not been fully elucidated, although known causes include ionizing radiation and congenital genetic syndromes, including neurofibromatosis type 1, Fanconi anemia, and Bloom syndrome.[2] The early onset of ALL suggests an important role for inherited genetic variation in increasing risk of this cancer, and several genetic associations have already been identified through candidate gene (reviewed in Vijayakrishnan and Houlston[3]) and genome-wide association studies.[4-8] In medically refractory ALL, which includes patients considered for stem cell transplantation, a spectrum of associations within the HLA complex was recently demonstrated.[9] However, these findings only account for a small proportion of childhood ALL, suggesting that other genetic associations have yet to be uncovered.

In a recent study, association was reported between variation at the killer cell immunoglobulin-like receptor (*KIR*) gene complex and childhood ALL.[10] This complex, spanning ~150 kb at chromosome 19q13.4, is a highly variable genomic region consisting of 14 *KIR* genes and 2 pseudogenes.[11] The *KIR* genes are organized head-to-tail with homologous exons and introns, peppered with varying degrees of polymorphism depending on the inhibitory or stimulatory status of the genes.[12,13] This genomic structure is conducive to nonallelic homologous recombination,[14,15] which has led to the formation of many different *KIR* gene combinations and a great amount of diversity in *KIR* gene repertoire between individuals[12,16] and populations.[17,18]

KIR genes are expressed on the surface of natural killer (NK) cells and some T cells, and regulate the development and function of these cells through interaction with HLA class I ligands. Different *KIR* genes have varied effects on NK-cell activity: those *KIR* genes with long cytoplasmic tails (indicated by L in the nomenclature) transmit inhibitory signals after binding their cognate HLA ligand, and those with short tails (S) transmit activating signals.[16] The expression of *HLA* class I alleles on the surface of cells in the body enables NK cells to recognize these as "self," and helps them to target "non-self" entities, such as some virally infected cells and cancer cells, for lysis.[19]

HLA-C is the dominant KIR ligand, and all *HLA-C* alleles can be categorized as *HLA C* group 1 (C1) or group 2 (C2) based upon polymorphic residues within the α-1 extracellular domain. The C2 ligand forms a strong inhibitory interaction with the KIR2DL1

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receptor, whereas C1 binds to both KIR2DL2 and KIR2DL3 receptors, which deliver intermediate and weak inhibitory signals, respectively (reviewed in Parham[20]). KIR3DL1 is an inhibitory receptor for *HLA-Bw4* supertype alleles, which comprise ~30% to 40% of *HLA-B* alleles.[21] By contrast, *HLA-Bw6* supertype alleles are not known to bind to any KIR. KIR3DL1/HLA-Bw4 interactions deliver weak or strong inhibitory signals to NK cells, depending upon the allelic polymorphisms within individual HLA-Bw4 ligands.[21]

There are 2 main *KIR* haplotypes, designated A and B, which depend upon the genes that are present.[16] The A haplotype contains up to 7 expressed genes (*KIR3DL3*, *KIR2DL3*, *KIR2DL1*, *KIR2DL4*, *KIR3DL1*, +/−*KIR2DS4*, *KIR3DL2*) and 2 pseudogenes (*KIR2DP1* and *KIR3DP1*). KIR B haplotypes are more variable in gene content and contain up to 14 genes, including 1 to 8 *KIR* genes unique to B haplotypes (*KIR2DS2*, *KIR2DL2*, *KIR2DL5B*, *KIR2DS3*, *KIR3DS1*, *KIR2DL5A*, *KIR2DS5*, *KIR2DS1*), of which the majority are stimulatory. Hence, the number of activating vs inhibitory *KIR* genes in an individual depends upon their *KIR* haplotype zygosity. The frequency of *KIR* haplotypes or of specific *KIR* genes has been associated with several diseases, including reproductive disorders,[22] autoimmune diseases,[23-25] susceptibility to infections,[26,27] and cancer.[28,29]

Because association between *KIR* locus variation and childhood ALL was recently reported,[10] but was not replicated in a subsequent analysis,[30] we review and extend these genetic associations in an independent data set. Due to the important role that KIR-HLA ligand interactions may have on the development and activity of NK cells, it is essential to jointly model these loci in disease association studies. We carried out analysis on the presence or absence of all 16 *KIR* genes, including both inhibitory and activating *KIR*, along with the genotyping of their *HLA-C* and *HLA-B* ligands in 212 ALL cases and 231 healthy controls from the California Childhood Leukemia Study (CCLS). This represents the first investigation of the joint role of these highly variable immune-related loci in contributing to childhood ALL risk.

Materials and methods

Ethics statement

This study was reviewed and approved by institutional review committees at the University of California Berkeley, the California Department of Public Health, and all collaborating institutions. Written informed consent was obtained from all parent respondents. Informed consent was provided according to the Declaration of Helsinki.

Study subjects

Subjects included in this study were enrolled in the CCLS as previously described.[31] Briefly, the CCLS is a continuing case-control study initiated in 1995, and includes childhood ALL cases recruited from 35 counties in Northern and Central California. One or 2 controls were selected for each case matching on age (birthdate), sex, Hispanic ethnicity, and maternal race using information from birth certificates obtained from the California Office of Vital Records. Subjects were eligible if they resided in the study area, were younger than 15 years of age at diagnosis (reference date for matched controls), had at least one English- or Spanish-speaking parent or guardian, and had no history of cancer diagnosis. Approximately 85% of eligible cases and 86% of contacted eligible controls consented to participate. Genotyping of *KIR* genes and their HLA ligands was carried out in 212 ALL cases and 231 controls who were born in California and had available neonatal bloodspots stored by the California Department of Public Health's Genetic Disease Screening Program. Of these, 114 cases and 128 controls were of Hispanic ethnicity, 76 cases and 86 controls were non-Hispanic white, and 22 cases and 17 controls were non-Hispanic "other" (a mixture of African American, Asian, and other ethnicities). This subgroup of the CCLS was chosen at random from a larger case/control series, to have sufficient power to detect a significant case-control difference in *KIR* A/A vs B/x genotype frequency.

DNA extraction

DNA was extracted from one-eighth segments of dried blood spots collected at birth using the QIAamp DNA Micro Kit (QIAGEN). Samples from cases and controls were extracted together and subsequently diluted to 2.5 ng/μL with nuclease-free water. Samples were then randomized prior to being transferred to 96-well plates.

KIR genotyping

For genotyping the *KIR* loci, we used a high-throughput single nucleotide polymorphism (SNP)-based *KIR* genotyping assay developed using the SEQUENOM MassARRAY system and the matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometer platform.[23,32] This assay tests for the presence or absence of 16 *KIR* genes (including the 2 *KIR* pseudogenes) and their common alleles, including the truncated allelic variants of *KIR2DS4* (with 22-bp deletion, producing a protein capable of being soluble but not anchored on the cell surface) and the fully expressed variants of this stimulatory receptor.[33]

Briefly, 38 primer pairs are used to amplify the region surrounding the SNPs being queried. This is followed by the use of 33 homogenous mass extend primers to differentiate individual SNP patterns for the *KIR* genes on the MALDI-TOF platform. In this assay, multiple receptor domains are queried to identify possible recombinant loci. These assays were run using the *KIR* sequence alignment in the Immuno Polymorphism Database (http://www.ebi.ac.uk/ipd/kir) IPD version 2.4.0 (4/15/11). Quality control measures included blind reanalysis of 10% of samples.

HLA ligand genotyping

Genotyping of *HLA-C1/C2* and *HLA-Bw4/Bw6* ligand groups was performed as described in Hollenbach et al.[23] Briefly, genotyping was performed using a method developed using the SEQUENOM MassARRAY system with the primer extension reactions analyzed on the MALDI-TOF mass spectrometer. The *HLA-C* alleles are classified as C1 or C2 KIR ligand groups, depending upon 2 amino acid (aa) positions encoded in exon 2. The C1 ligand group contains serine (AGC) at aa77 and asparagine (AAC) at aa80, while the C2 ligand group encodes asparagine (AAC) and lysine (AAA) at those positions. *HLA-B* alleles fall into 2 broad groups, Bw4 or Bw6, depending upon the presence of either glycine or arginine at aa83, respectively; only Bw4 supertype alleles are ligands for KIR3DL1 receptors. Amplicons containing exon 2 of *HLA-C* or *HLA-B* genes were produced using polymerase chain reaction primers designed in regions previously used for locus-specific amplification of *HLA* class I genes.[34] Both the *KIR* and *HLA* genotyping were carried out in the Clinical Laboratory Improvement Amendments (CLIA)-approved Clinical Histocompatibility & Immunogenetics Laboratory at the Children's Hospital & Research Center of Oakland, under the supervision of E.A.T.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) analysis was carried out with 2 sets of *KIR* genes treated as alleles of the same locus: *KIR2DL2/KIR2DL3* and *KIR3DL1/KIR3DS1*. Tests for HWE were performed for these *KIR* loci along with *HLA-B* and *HLA-C* genotypes, in all controls and also stratified by ethnicity, using contingency table testing and a standard χ^2 measure, with a significance level of $P = .05$.

Unconditional logistic regression analyses were carried out for association tests and for generation of odds ratios (ORs) and 95% confidence intervals (CIs) using the *R* statistical environment (http://www.R-project.org). Several variables (sex, age at diagnosis, and ethnicity) were assessed as confounders, but were not included in the final model due to a minimal influence on risk (did not affect the OR by >5%); hence, unadjusted logistic regression models were used.

KIR haplotypes A and B were defined based on the presence or absence of specific *KIR* genes, as previously described.[16] The SEQUENOM

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MALDI-TOF assay detects presence/absence but not copy number of each *KIR* gene, thus we were unable to distinguish between *KIR* B heterozygotes and homozygotes. Haplotype analysis, therefore, involved comparison of the frequency of *KIR* A/A vs *KIR* B/x genotypes, with "x" representing either the A or B haplotype (equivalent to a recessive genetic effect of *KIR* A). We hypothesized that *KIR* A/A may be associated with increased childhood ALL risk, based on the findings of Almalte et al that presence of activating *KIR* genes is protective against ALL.[10] Power calculations were carried out (using SAS) to determine the minimal effect size this study could detect for effects of *KIR* A homozygosity on risk of ALL. Given a rare allele frequency of 0.30 for *KIR* A/A based on previous studies,[30] and an α of 0.05, we were well powered (80%) to detect an effect size of OR = 1.44 in overall cases and controls, an effect size of OR = 1.61 in Hispanics only, and an effect size of OR = 1.76 in non-Hispanic whites only. These effect sizes are in line with SNP ORs from genome-wide association studies of childhood ALL.

For analysis of the frequency of each *KIR* gene, the presence vs absence of each gene was determined in each subject, with a P value $< .003$ (0.05/16 genes) regarded as statistically significant based on Bonferroni correction for multiple testing. Analysis of the total number of *KIR* genes between subjects involved summing the number of genes present in each subject of the 14 genes and 2 pseudogenes at the *KIR* locus. Neither of these analyses incorporated information on *KIR* gene copy number. Analysis of the number of inhibitory *KIR* genes included *KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL5*, and *KIR3DL1*, but not the framework inhibitory *KIR* genes *KIR2DL4*, *KIR3DL2*, and *KIR3DL3*. For analysis of the activating *KIR* genes, in addition to *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5*, and *KIR3DS1*, we also counted nonmutant *KIR2DS4* (without the 22-bp deletion)[33] as activating. Mutant *KIR2DS4* (with 22-bp deletion) was counted neither as activating nor inhibitory.

Frequency of the 4 possible combinations between *KIR* genotype (A/A or B/x) and *HLA-C* alleles (C1 or C2) or *HLA-B* alleles (Bw4 or Bw6) was analyzed in cases and controls. Additional case-control comparisons were carried out for the frequency of known *KIR* gene-*HLA* class I ligand combinations (supplemental Table 3, available on the *Blood* Web site). After correction for multiple testing in both analyses, P values $< .0125$ (0.05/4) and $< .0056$ (0.05/9) were regarded as statistically significant, respectively.

In Hispanic subjects, we investigated whether ancestral differences between cases and controls may explain *KIR* genotype frequency. Principal components (PCs) analysis was carried out for a recent Hispanic ALL GWAS study,[35] and the first 2 PCs were associated with Native American vs European ancestry and African vs European ancestry, respectively. We were, therefore, able to include these continuous ancestry-informative PCs as covariates in the logistic regression model to test whether population substructure biased analysis of the *KIR* A/A vs B/x genotype frequency in Hispanic case-control comparisons.

Results

Cases and controls were comparable with respect to sex, age at diagnosis (date of reference for controls), and ethnicity (Table 1).

KIR genes

Blind reanalysis of 10% of samples revealed complete concordance in *KIR* genotypes. Genotyping of all 16 *KIR* genes was successful in 435 of 443 subjects (98.2%): for each of the remaining 8 samples, only 1 *KIR* gene assay failed with the remaining 15 gene assays successful. There were no significant deviations from HWE for the *KIR2DL2*/*KIR2DL3* or *KIR3DL1*/*KIR3DS1* gene frequencies in either cases or controls, or when stratified by ethnicity ($P > .05$).

***KIR* haplotypes.** It was possible to determine *KIR* A haplotype homozygosity across all 443 study subjects. Frequency of *KIR* A/A was significantly higher in ALL cases (33.5%) compared with control subjects (24.2%), with OR = 1.57 (95% CI, 1.04-2.39; P = .03) (Figure 1 and supplemental Table 1). When subjects were stratified by ethnicity, a significant difference was seen in *KIR* A/A genotype frequency between Hispanic cases and controls only: 34.2% of Hispanic ALL cases were homozygous for *KIR* A haplotype compared with only 21.9% controls (P = .03), with OR = 1.86 (95% CI, 1.05-3.31) (Figure 1 and supplemental Table 1). In non-Hispanic whites, while there was no significant case-control difference in frequency of *KIR* A/A (P = .37), there was an increased frequency of this *KIR* genotype in cases (36.8%) compared with controls (30.2%). Restricting cases to the 204 known B-cell ALL subjects did not affect the strength of association between KIR A/A genotype and disease risk (ΔOR $<$ 5%).

Table 1. Demographic characteristics of childhood ALL cases and controls: CCLS

Characteristics	n (%)	
	Cases, n = 212	Controls, n = 231
Child's sex		
Male	117 (55.2)	134 (58.0)
Female	95 (44.8)	97 (42.0)
Mean age at diagnosis/reference, y (SE)	4.75 (0.21)	4.53 (0.20)
Child's ethnicity		
Hispanic	114 (53.8)	128 (55.4)
Non-Hispanic white	76 (35.8)	86 (37.2)
Non-Hispanic other	22 (10.4)	17 (7.4)
ALL subtype		
B cell	204 (96.2)	N/A
T cell	5 (2.4)	N/A
Subtype unknown	3 (1.4)	N/A

N/A, not available.

In Hispanic subjects, we investigated whether ancestral differences between cases and controls accounted for the significant association between *KIR* A/A genotype frequency and ALL risk. Adjusting for ancestry-informative PCs did not influence effect estimates for this association (ΔOR $<$ 5%).

***KIR* gene frequencies.** Several genes were present at a lower frequency in cases compared with controls, although these differences did not reach statistical significance after correction for multiple testing ($P > .003$). Most of the genes underrepresented in cases are specific to the *KIR* B haplotype (supplemental Figure 1), reflecting the increased frequency of *KIR* A haplotype homozygotes in these individuals. A similar pattern of *KIR* gene frequency was seen when analyses were stratified by ethnicity, although intriguingly there was an increased frequency of *KIR2DS4* and *KIR3DL1* in non-Hispanic white cases compared with controls that was not seen in Hispanics (supplemental Figure 1).

Total number of KIR genes. Assessment of the number of *KIR* genes present was carried out for 208 cases and 227 controls, as 4 cases and 4 controls were removed due to missing information. Cases had a significantly lower total number of *KIR* genes present than controls (P = .03; OR = 0.91; 95% CI, 0.84-0.99), with a median of 11 *KIR* genes present in cases and 13 in controls, reflecting the difference in *KIR* A/A genotype frequency (Figure 2). After assigning *KIR* genes to inhibitory or activating status (or neither), the total number of inhibitory *KIR* genes present was significantly lower in cases than controls (P = .038; OR = 0.78; 95% CI, 0.62-0.99). There was also a trend toward a lower number of activating genes present in cases compared with controls (P = .076; OR = 0.89; 95% CI, 0.80-1.01) (Figure 2). This again reflects the higher frequency of the *KIR* A/A genotype, in which there are fewer of both activating and inhibitory genes.

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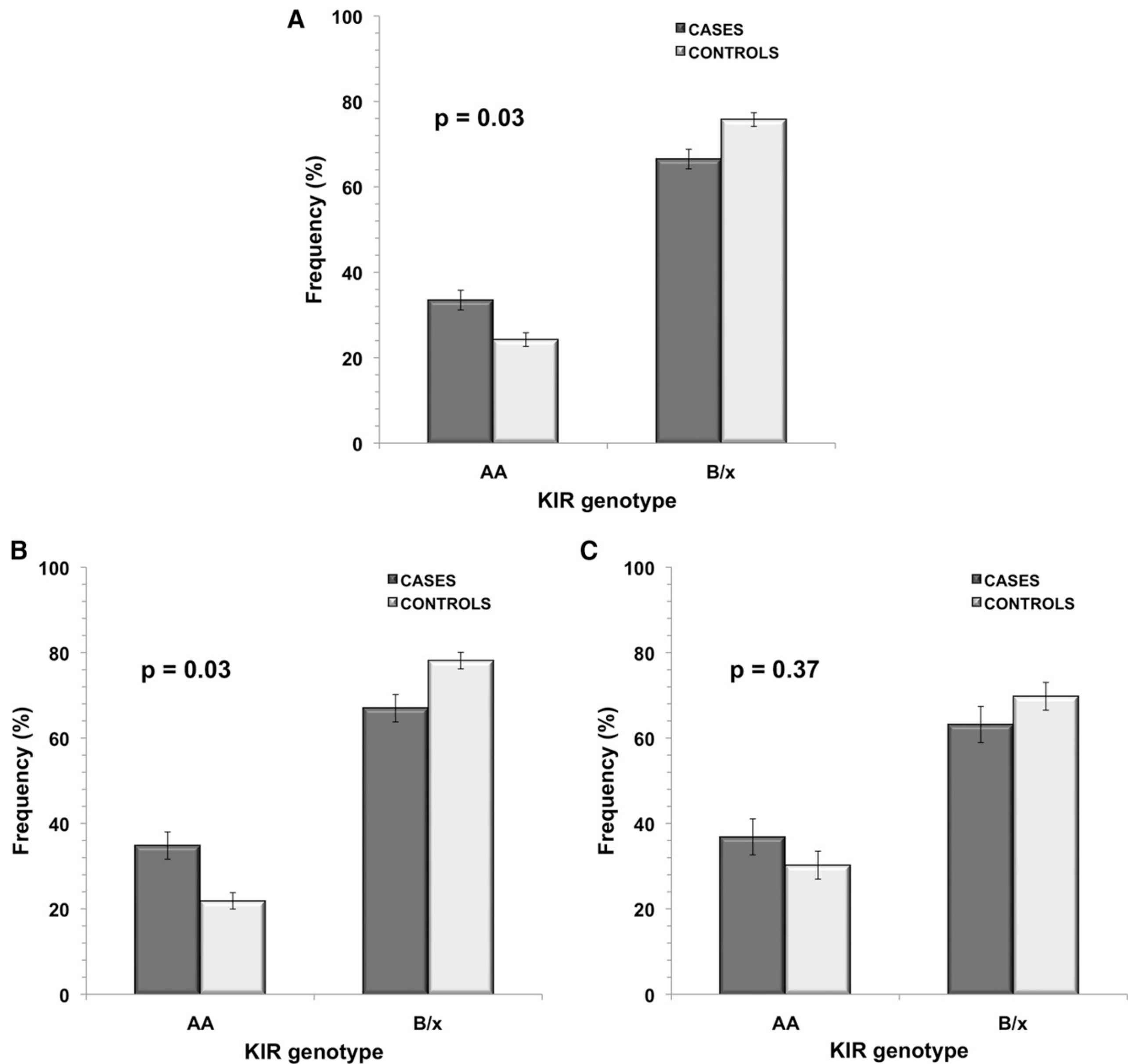

Figure 1. *KIR* genotype frequency in childhood ALL cases and controls in all subjects and in ethnic subgroups. *KIR* genotype frequency in childhood ALL cases vs controls is shown for (A) all subjects (cases, n = 212; controls, n = 231), (B) Hispanics only (cases, n = 114; controls, n = 128), and (C) non-Hispanic whites only (cases, n = 76; controls, n = 86). *P* values were calculated using unconditional logistic regression analysis between ALL cases and controls. Error bars represent SE.

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***HLA* ligands**

Of the 443 subjects tested, 2 cases failed the *HLA-C* assay, 1 control failed the *HLA-B* assay, and 2 control samples failed both assays. These subjects were thus removed from *HLA* genotype analysis. In the remaining subjects, there were no significant deviations from HWE for either the *HLA-C1/C2* or *HLA-Bw4/Bw6* genotype frequencies in cases or controls, or when stratified by ethnicity ($P > .05$). There was no significant difference in the frequency of *HLA-C* ligand genotypes between ALL cases and controls ($P = .34$; OR = 1.14; 95% CI, 0.87-1.51), nor when stratified by ethnicity (supplemental Table 2).

For *HLA-Bw4/Bw6*, there was also no significant difference in genotype frequency between cases and controls overall ($P = .17$; OR = 1.23, 95% CI: 0.92-1.64). However, when analysis was stratified by ethnicity there was a significant association between ALL risk and *HLA-B* genotype in non-Hispanic whites ($P = .02$; OR = 1.74; 95% CI, 1.09-2.81), and an even stronger association between Bw4 homozygosity and increased ALL risk ($P = .01$; OR = 3.93; 95% CI, 1.44-12.64) (Table 2 and supplemental Figure 2). No *HLA-B* association was seen in Hispanics ($P = .82$; OR = 1.05; 95% CI, 0.69-1.61).

***KIR-HLA* ligand combinations**

Frequency of the 4 different combinations between detectable *KIR* genotypes (A/A or B/x) with *HLA-B* alleles (Bw4 or Bw6) or with *HLA-C* alleles (C1 or C2) was determined. There was no association between *KIR* genotypes and *HLA-B* alleles (data not shown). For *HLA-C*, the combination of *KIR* A/A genotype both with C1 and with

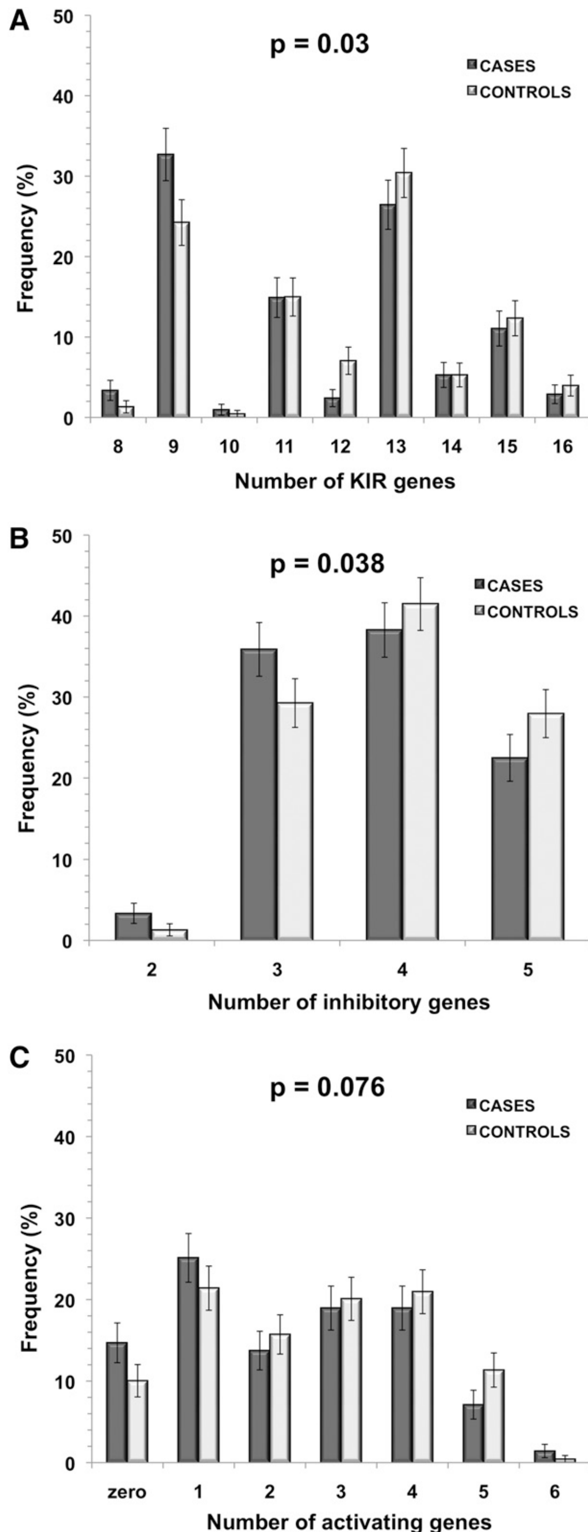

Figure 2. Number of *KIR* genes in childhood ALL cases and controls. Histograms show the number of total (A), inhibitory (B), and activating (C) *KIR* genes present in cases compared with controls (cases, n = 208; controls, n = 227). *P* values were calculated for each category using unconditional logistic regression analysis between ALL cases and controls. Error bars represent SE.

C2 were at a higher frequency in cases compared with controls, though neither reached significance after correction for multiple testing ($P > .0125$).

For combinations of specific inhibitory and activating *KIR* genes and their *HLA* ligands, there were no significant associations in the overall case/control analysis. However, in non-Hispanic white subjects, there was an increased frequency of the inhibitory combination between *KIR3DL1* and *HLA-Bw4*. This case-control difference was more pronounced when limiting to subjects homozygous for the Bw4 allele, with an OR of almost 4; however, this was no longer significant after correction for multiple testing ($P > .0056$) (supplemental Table 3).

Discussion

In this study, we have investigated the role of *KIR* genes and their cognate *HLA* ligands in the etiology of childhood ALL, and found the *KIR* A/A genotype to be significantly associated with increased risk of disease. This association was more pronounced in Hispanic subjects, suggesting that an association at this locus may contribute to the well-documented higher incidence of childhood ALL in the Hispanic population.[36] We also report a significant association between the *HLA-Bw4* allele and risk of ALL in non-Hispanic white children, supporting a role for genetic variation at the *HLA* locus in the etiology of this disease. Moreover, these observations provide further evidence that functioning of the immune system in early life influences the development of childhood ALL.[37]

The varying effects of *KIR* and *HLA* ligand genotypes in our Hispanic and non-Hispanic white children suggest that the 2 loci may have different effects on childhood ALL susceptibility across ethnic groups. The greater case-control difference in frequency of *KIR* A/A genotype observed in Hispanics suggests that ALL incidence in Hispanics may be related to environmental factors that interact with *KIR*, for example patterns of infection, rather than simply a different allele frequency in Hispanics compared with non-Hispanic whites. The reverse might be true for the association with *HLA-Bw4* in non-Hispanic whites.

Adjusting for ancestry-informative PCs in analysis of *KIR* genotype frequency in Hispanics revealed that significant case-control differences were not influenced by underlying ancestral

Table 2. Frequency of *HLA-B* ligand genotypes in childhood ALL cases and controls in all subjects (total), Hispanics, and non-Hispanic whites: CCLS

HLA-B genotype	Cases (%)	Controls (%)	*P*	OR (95% CI)
Total				
n	210 (100)	228 (100)		
Bw6/Bw6	95 (45.2)	112 (49.1)		
Bw4/Bw6	91 (43.3)	100 (43.9)	.17	1.23 (0.92-1.64)
Bw4/Bw4	24 (11.4)	16 (7.0)		
Hispanics				
n	112 (100)	126 (100)		
Bw6/Bw6	55 (49.1)	66 (52.4)		
Bw4/Bw6	52 (46.4)	52 (41.3)	.82	1.05 (0.69-1.61)
Bw4/Bw4	5 (4.5)	8 (6.4)		
Non-Hispanic whites				
n	76 (100)	85 (100)		
Bw6/Bw6	29 (38.2)	42 (49.4)		
Bw4/Bw6	32 (42.1)	38 (44.7)	.02*†	1.74 (1.09-2.81)*
Bw4/Bw4	15 (19.7)	5 (5.9)	.01*‡	3.93 (1.44-12.64)*

P values and ORs (95% CI) calculated using unconditional logistic regression analysis.

*Significant *P* values and ORs.

†*P* value and OR for overall *HLA-B* genotype.

‡*P* value and OR for *Bw4* homozygosity.

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differences. This is in contrast to the *ARID5B*, *CEBPE*, *CDKN2A*, *PIP4K2A*, and *GATA3* ALL-risk SNPs, where risk alleles are associated with increased Native American ancestry.[35,38] This discrepancy may be explained by the rapid change (via recombination and selective pressures) thought to occur at the *KIR* locus.[18,39] Thus, the underlying *KIR* repertoire is likely to be independent of ancestry classifications defined by common SNP polymorphisms.

Two recent studies have reported conflicting data concerning the relationship between *KIR* genes and ALL risk in whites.[10,30] Almalte et al[10] reported a significantly reduced risk of childhood ALL with increasing numbers of activating *KIR* in a set of French and non-French Canadians, whereas Babor et al[30] found no significant associations with individual *KIR* genes or *KIR* haplotypes in their European (mostly German) cohort. The findings of the former are supported by the higher frequency of *KIR* A/A genotypes in cases compared with controls in our study, as the *KIR* A haplotype lacks most known activating *KIR* genes. Furthermore, the *KIR* gene associations identified by Almalte et al were consistent across both B-cell and T-cell ALL, and similarly our association with *KIR* A/A genotype did not appear to be specific to B-cell ALL. The lack of a significant association between ALL and *KIR* A/A genotype frequency in non-Hispanic white subjects may support the negative findings of Babor et al, although we did see a trend for higher *KIR* A/A frequency in cases compared with controls.

The differences in results between the two aforementioned studies may be due to cryptic differences in the ethnicity of genotyped samples, differential accuracy of genotyping methodologies due to the strong homology between *KIR* genes, and/or to potential amplification biases dependent on sample quality. The latter is less of an issue for the SEQUENOM MALDI-TOF assay used in this study, which queries at least 2 domains of each *KIR* gene and has been shown to have high accuracy and precision (>99%), independent of sample quality.[23,32] This genotyping was carried out in a CLIA-approved laboratory, and blinded repeats (10%) of our analyses demonstrated complete concordance, substantiating the assay accuracy.

An additional strength of the current study is that, in addition to *KIR* genes, we have also genotyped their main *HLA* class I ligands, alleles of *HLA-C* and *HLA-B*, enabling us to assess *KIR-HLA* combinations, which are pertinent to the development and activity of NK cells. Although no significant associations were detected in the overall data set with either *HLA-C* or *HLA-B* genotypes, there was a significant difference in *HLA-B* genotype frequency between non-Hispanic white cases and controls. Carrying 2 copies of the Bw4 allele results in an almost fourfold increased risk of childhood ALL in this ethnic group. Homozygosity for Bw4 has previously been associated with increased risk of chronic myeloid leukemia, with a trend toward increased risk of both acute myeloid leukemia and ALL,[40] and we now provide evidence that this may also play a role in childhood ALL.

HLA-Bw4 binds with *KIR3DL1* to deliver inhibitory signals to NK cells, and it is interesting to note that the frequency of this *KIR* gene was higher in non-Hispanic white cases than controls, leading to a putatively significant association between the *KIR3DL1-Bw4* combination and increased ALL risk. It is possible that this inhibitory combination may enhance the ability of leukemia cells to evade immune system detection. Unlike for some solid tumors, in which downregulation of HLA class I expression is frequently observed and is a well-documented method of immune system evasion,[41,42] the loss of HLA class I cell-surface expression is rare (<10%) in ALL.[43] Since leukemic blasts will express HLA-Bw4 in individuals carrying this allele, the subsequent inhibition of NK cells through KIR3DL1 may lead to diminished clearance of leukemia compared with individuals lacking the *Bw4* allele and/or *KIR3DL1*.

The present study supports a role for *KIR* genes in the etiology of childhood ALL; however, there are several limitations that should be considered. The finding of a Hispanic-specific association with *KIR* A/A genotype frequency and a non-Hispanic white–specific association with *HLA-Bw4* will require further investigation in a larger group of Hispanic and non-Hispanic white patients, respectively. It is possible that the slight increase in *KIR* A/A frequency we observe in non-Hispanic white cases compared with controls underlies a weaker effect on ALL risk, or a comparable effect that did not reach significance due to the smaller sample size of non-Hispanic white subjects. Due to the methods used in this study, it was not possible to differentiate *KIR* B/B genotypes from *KIR* A/B ones. Therefore, it will be important to determine whether there is an additive effect of *KIR* A haplotypes on the risk of childhood ALL, or whether only individuals homozygous for *KIR* A have increased susceptibility to this disease. In addition, only presence or absence of *KIR* genes has been investigated and not the extensive copy number variation,[44] allelic polymorphisms, and expression differences of *KIR* and *HLA* class I ligands that would further diversify the *KIR-HLA* interactions. Further work, therefore, is needed to elucidate the full role of the *KIR* and *HLA* ligand loci in childhood ALL.

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Authorship

Contribution: A.J.d.S., J.L.W., E.A.T., and P.A.B. conceived and designed the study; M.B.L., S.Z., C.X., F.C., and A.J.d.S. performed the experiments; A.P.C., C.M., P.A.B., T.B.M., J.L.W., E.A.T., K.M.W., and A.J.d.S. assisted in assembling the data; K.M.W. provided data and helped with bioinformatic analyses; A.J.d.S.

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analyzed and interpreted the data; A.J.d.S., J.L.W., and E.A.T. wrote the manuscript; and all authors critically reviewed and edited the manuscript for intellectual content and gave final approval of the manuscript.

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