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RESEARCH ARTICLE

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KIR Allelic Variation and the Remission of Atopic Dermatitis Over Time

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ABSTRACT

Atopic dermatitis (AD) is a common chronic skin disease. Although generally thought to be a disease of T-cell dysregulation, recent studies have suggested that immune dysregulation of NK cells is also important. Killer cell Ig-like receptors (KIRs) are involved with NK cell regulation. The Pediatric Eczema Elective Registry is a U.S. nationwide longitudinal cohort with up to 10 y of follow-up in which 655 children had DNA available for full allelic KIR sequencing. Every 6 mo, AD activity was reported by Pediatric Eczema Elective Registry children. Using generalized estimating equations, we evaluated the association of KIR allelic variation in concert with known HLA binding ligands and whether the child reported AD in "remission" (no skin lesions and not using AD medication). KIR2DS4*001:01 (odds ratio 0.53, 95% CI [0.32, 0.88]) and KIR2DL4*001:02 (0.54, [0.33, 0.89]) in the presence of C*04:01 had the largest effect on decreasing the likelihood of AD remission. The haplotype KIR 2DL4*001:02 ∼ 2DS4*001:01 ∼ 3DL2*002:01 (0.77, [0.60, 0.99]) was also associated with a decreased likelihood of AD remission. Our findings add to the general body of evidence of a growing literature on the importance of NK cells with respect to the immunopathogenesis and natural history of AD. *ImmunoHorizons*, 2023, 7: 30–40.

INTRODUCTION

Atopic dermatitis (AD) is a common chronic skin disease. It was once thought to be a childhood illness that resolved by adolescence (1, 2). This assumption was based on limited evidence such as expert anecdotal experiences, cross-sectional studies, and longitudinal studies with limited follow-up of 1 y or less. More recent studies have shown that AD can be a lifelong chronic illness with a heterogeneous clinical course, including varying periods of remission, recurrence, severity, body site disease location, and time of onset from infancy through adulthood. Notably, comorbid illnesses such as asthma and seasonal allergies that were thought to occur after the skin findings have also been found to precede the onset of AD (3–5).

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Abbreviations used in this article: AD, atopic dermatitis; GEE, generalized estimating equation; KIR, killer cell Ig-like receptor; LD, linkage disequilibrium; OR, odds ratio; PEER, Pediatric Eczema Elective Registry; TCI, topical calcineurin inhibitor.

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Many studies have shown that AD is at least partially caused by TH2 cell dysregulation (1, 2, 6). Other types of immune dysregulation (e.g., TH1, autoimmunity, innate immunity) have also been implicated (7, 8). We previously evaluated the association between the persistence of AD symptoms and HLA class I polymorphisms and binding pockets (9–11). Specifically, we found that HLA-B*44:02 and the HLA-B residues at positions 116 (D-aspartate) and 80 (T-threonine) were associated with remission and that the magnitude of the effect varied by ancestry (e.g., White or Black race) (11). Interestingly, the *HLA-Bw4 80(T)* motif also codes for a killer cell Ig-like receptor (KIR) HLA ligand. Specific HLA ligands interact with specific KIR gene proteins that activate or inhibit the NK cells (12, 13). We were among the first to demonstrate that there may be two types of MHC-associated immune response (T cell and NK cell) that influence AD disease progression (11). In addition, using data from our Genetics of Atopic Dermatitis case-control study, we were then able to show that several *KIR* genes as well as allelic variations within these genes were associated with an increased risk of AD. Specifically, an increased risk of AD was noted for *KIR2DL5*, *KIR2DS5*, and *KIR2DS1* and the *KIR2DL5:001:01* allele (D. J. Margolis, N. Mitra, O. J. Hoffstad, R. Berna, B. S. Kim, A. Chopra, and E. J. Phillips, manuscript posted on Research Square, DOI: 10.21203/rs.3.rs-2073693/v1). As expected, individuals with the *KIR2DL5:001:01* allele, the *KIR2DS5* or *KIR2DS1* gene, and the HLA-C*C2 epitope were at an increased risk of AD (D. J. Margolis et al., manuscript posted on Research Square, DOI: 10.21203/rs.3.rs-2073693/v1). The *HLA-B*-21T (TT)* leader sequence increased the risk of AD across ethnicities. African Americans with *KIR2DL2*, *KIR2DS1*, *KIR2DL5*, and *KIR2DS5* were found to be more likely to have AD, and this risk increased in those with *KIR2DS1* and *KIR2DS5* in the presence of the HLA-C C2 epitope (10).

Although most studies have focused on T-cell dysregulation in AD, our recent studies have suggested that immune dysregulation of NK cells is also important (6, 7, 14–17). NK cells are part of the innate immune system and are thought to have a role in the surveillance of cellular self-recognition and therefore are important in immune processes related to cancers, viral and bacterial infections, and fertility. The NK cell membrane-bound KIR ligands are the primary regulator of NK cell function (13, 18, 19). The KIR family consists of 15 gene loci found on chromosome 19 that have activating (designated by "S" for short tail) or inhibitory functions (designated by "L" for long tail) for NK cells and two pseudogenes (designated by "P") (13, 20–22). Most mature NK cells express KIR, although the total number of *KIR* genes observed and expressed as well as the type of KIR ligands produced by the genes (i.e., inhibitory and activating ligands) vary (13, 18, 19). The goal of this study was to evaluate KIR allelic variation and its association with the persistence of AD using the Pediatric Eczema Elective Registry (PEER) longitudinal cohort. PEER was initially created to study potential long-term adverse effects of a topical cream used to treat AD; however, the up to 10 y of follow-up data from this cohort have been instrumental in helping to describe the longitudinal nature of the persistence of AD in a cohort of children who initially had mild to moderate disease (4, 9, 23).

MATERIALS AND METHODS

Methods

PEER (www.thepeerprogram.com) is a U.S. nationwide cohort of more than 8000 subjects with pediatric-onset AD (4, 10, 23, 24). All subjects had a diagnosis of AD confirmed by a physician. For this study, 655 PEER children, who provided a saliva sample (original PEER DNA cohort $n = 792$) and who had sufficient DNA samples for KIR genotyping, were studied (23, 25). In the PEER DNA cohort, both self-described race/ethnicity and ancestry informative markers were used to define ancestry and were previously found to be highly correlated (23). Two other AD studies have recently confirmed the observation that self-described race is highly correlated with genetic ancestry in subjects with AD (26, 27). PEER DNA enrollment occurred between November 2004 and January 2015 (23). At the time of enrollment, children were 2–17 y old, had a physician-confirmed diagnosis of mild to moderate AD, and had used pimecrolimus cream for at least 6 mo but may have used many other therapies (23). Subjects were followed for up to 10 y, and outcomes, such as persistence of AD, were measured about every 6 mo through self-reported or guardian-reported survey. Participants were not required to (and most did not) continue therapy with pimecrolimus during follow-up (24, 28). Follow-up is still ongoing and will end when all subjects have had the opportunity to provide 10 y of follow-up.

All subjects (or their legal guardians) provided written informed consent approved by the University of Pennsylvania Institutional Review Board to provide DNA (protocol 706073).

Outcome

The clinical course of AD was determined as frequently as every 6 mo and defined by whether a child was in remission based on the self-reported outcome of whether a child's skin was AD symptom free during the previous 6 mo while the child was not using medication to treat their AD (23). AD disease activity was based on the survey question: "During the last six months, would you say that your child's skin disease (AD) has shown: complete disease control, good disease control, limited disease control, or uncontrolled disease?" "Symptom free" was defined as an affirmative response to "complete disease control." This response has been shown to correlate with other tools used to evaluate symptom control and is likely a marker of long-term disease severity (29–31). For the analyses looking at the association of topical drug use, the outcome measure was skin clearance based on the "complete skin control" determination (25). Drug use determination was based on whether a child had used topical steroids or topical calcineurin inhibitors (TCIs) more than infrequently during the previous 6-mo period (25). Outcome data collection for PEER is ongoing. For this study, the last date that the outcome was reported for a study

subject occurred either at 10-y follow-up or up to their last response before the end of January 31, 2022, whichever date occurred first.

HLA genotyping

DNA was collected using Oragene DNA collection kits (DNA Genotek, Ottawa, ON, Canada) as previously reported (9, 11, 23, 32). HLA class I genes (-A, -B, and -C) were sequenced using targeted amplicon-based next-generation sequencing as previously described (9, 11, 32).

For this study, we focused on HLA ligands, primarily from HLA-B and HLA-C, known to interact with KIR. Genetic variation of HLA-B results in ligand contact residues for the Bw4 epitope at locations 77, 80, 81, 82, and 83 and are best defined as 80 (amino acids I or T) and 83(R), but also included in the Bw4 epitope are HLA-A genes such as HLA-A*32, A*23, and A*24 (33, 34). The HLA-B leader sequence is defined at location −21 (amino acid T or M) and is associated with NK cell "education." The HLA-C epitope, called C1 and C2, that interacts with KIR is at position 80 and is defined by amino acids asparagine (N) and lysine (K) (i.e., C1/C2 KIR binding site) (21, 34–36). An additional HLA-C site, Cw4 (i.e., HLA-C*04:01 and *04:03) and C*05:01, was evaluated (12, 37). KIR haplotypes A and B were also evaluated. Residue locations were based on the International ImMunoGeneTics project data (37, 39). HLA estimates of the likelihood of remission of AD in PEER were previously published (11, 40).

KIR allelic sequencing

High-resolution *KIR* allele typing, requiring 15 μl of DNA in the concentration range of 20–50 ng/μl, was conducted by the Institute for Immunology and Infectious Diseases of Murdoch University in Perth, Australia. Primers were designed to target *KIR* exons 3, 4, and 5 (D0, D1, and D2 domain). Primer mixes were prepared containing two to four primers per amplicon to cover all *KIR* genes in a multiplexed assay with an amplicon size ∼400 bp. Five separate PCRs were set up for each sample to cover all targeted exons in 12.5-μl volume in 96-well plates with GoTaq DNA Polymerase (Promega) and the associated buffer system. Because each sample is uniquely indexed during the PCRs, all amplicons from the PCRs were pooled using volumes appropriate to obtain balanced read coverage for each *KIR* exon. Each pool was then ligated with unique Illumina indexes and sequencing adapters ready for Illumina sequencing. The products were sequenced on the MiSeq platform using 600V3 chemistry. After sequencing, quality-filtered paired reads were demultiplexed on the basis of unique molecular identifiers, and reads were merged using bioinformatic tools developed in-house. The merged reads were then aligned to a *KIR* locus reference sequence for all *KIR* genes using the CLC bio genomics workbench. The alignment files (bam files) were then used for assigning the *KIR* allele calls using VGAS, which generates the cluster consensus sequences, and All Class, which makes the allele calls on the basis of International ImMunoGeneTics project reference database release v2.10.0 (date December 16, 2020; https://www.ebi.ac.uk/ipd/kir/docs/version.html). The ambiguous results were condensed into i-groups. Please refer to the following link for the list of condensed alleles in the i-groups: http://www.iiid.com.au/s/iiid_KIR_iGroups_v2100.xlsx.

Analysis

Our primary focus assessed the association between alleles in the KIR gene and the likelihood of AD remission over time. We used a generalized estimating equation (GEE) approach for the binary outcome of remission, assuming an independence working correlation structure to account for the correlation among repeated measures per participant. (Outcomes were determined by survey every 6 mo for up to 10 y.) GEEs belong to a class of semiparametric regression models that measure the average response over the population and are frequently used to evaluate longitudinal data that have repeated outcome measures. The odds ratios (ORs) measured in this study reflect the odds that a subject with an exposure of interest (e.g., a specific KIR allelic variant) as compared with one who does not have that exposure will be in remission over time. We assumed an additive genetic model. Subgroup analyses were conducted for White and Black participants. ORs for association between the variant of interest and the AD clinical course (remission OR >1.0 or persistence OR ≤ 1.0) and 95% confidence intervals were estimated from these models.

We also explored interactions with the purported HLA ligands associated with specific KIRs. As is convention for evaluations of statistical interactions of known effects, $p < 0.10$ was considered significant. Missingness was previously evaluated and believed to be completely at random and consistent with GEE assumptions (41). The Bonferroni multiple testing correction was applied on the basis of the number of allelic variants evaluated that had a frequency of ≥ 0.05 per gene (7, 10, 16). This per-gene level of correction was based on prior knowledge from previous studies that KIR genes are associated with the function of NK cells and that NK cell function is associated with AD.

All statistical analyses were conducted using STATA version 17.0 (StataCorp, College Station, TX).

RESULTS

Among the 792 children enrolled in the PEER DNA cohort, sufficient DNA was available for KIR genotyping from 655 of the children. Questionnaires were returned 92,538 times from the children, and, on average, each child had 10.0 evaluations (median 10). On average, children were followed up for 8.07 (SD 2.98, median 9.5) years, or for a total of 5,287.5 person-years. Children were noted to be in remission (no AD symptoms and no AD therapy for 6 mo) 23,637 times, and 236 (41.0%) children had at least one 6-mo period of remission. The average age at onset was 2.03 (SD 2.76, median 0.75) years;

the average age of enrollment was 7.28 (SD 3.77, median 6.67) years; 52.6% were female; and 55.4% were White.

Table I lists for the full cohort the alleles that had a frequency of ≥0.05 for each KIR gene. After correcting for multiple testing, *2DL4*001:02*, *2DS4*001:01*, *3DL1*007:01*, and *3DL3*005* were significantly associated with a decreased likelihood of AD remission, and *3DL1*001:01* was found to be associated with an increased likelihood of AD remission (Table II). The effect estimates were similar in White and Black children (Table II). *2DL4*001:02* and *2DS4*001:01* (R^2 = 0.64), *3DL1*001:01* and *2DL4*001:02* (R^2 = 0.78), and *3DL1*001:01* and *2DS4*003:03* (R^2 = 0.70) were in linkage disequilibrium (LD).

Interactions between KIR alleles and HLA alleles or epitopes are presented in Table III. Alleles significantly associated with severity (Table II) were evaluated in the presence or absence of HLA ligands known to bind to proteins coded by the KIR genes (12, 42–45). The most profound interaction was between C*04:01 (part of the Cw4 serotype) ligand, *C*05:01* allele ligand, and 2DS4*001:01 coded protein or 2DL4*001:02 coded protein ($p \leq 0.10$). Both C*04:01 and C*05:01 are part of the HLA-C2 ligand. The interactions between the receptors coded by *C*04:01*, *C*05:01*, and *2DS4*001:01* have been reported previously (42, 43). For most of the other analyses, the effect estimates of the KIR alleles minimally changed in the presence of

TABLE I. Allelic frequencies ≥0.05

	Full PEER cohort		White		Black	
Allele	n	Allele Frequencies	n	Allele Frequencies	n	Allele Frequencies
KR2DL1*004:01	122	0.11 (0.09, 0.13)	60	0.09 (0.07, 0.11)	59	0.13 (0.10, 0.16)
KR2DL1*001:01	252	0.22 (0.20, 0.24)	203	0.31 (0.27, 0.34)	44	0.10 (0.07, 0.13)
KR2DL1*003:01	610	0.53 (0.50, 0.56)	354	0.53 (0.49, 0.57)	232	0.51 (0.46, 0.56)
KR2DL2*001:01	236	0.20 (0.18, 0.23)	126	0.19 (0.16, 0.22)	106	0.23 (0.20, 0.28)
KR2DL2*003:01	213	0.18 (0.16, 0.21)	157	0.24 (0.20, 0.27)	50	0.11 (0.08, 0.14)
KR2DL3*001:01	905	0.79 (0.76, 0.81)	576	0.87 (0.84, 0.89)	297	0.65 (0.61, 0.70)
KR2DL3*005:01	103	0.09 (0.07, 0.11)	29	0.04 (0.03, 0.06)	74	0.16 (0.13, 0.20)
KR2DL4*001:02	419	0.36 (0.34, 0.39)	191	0.29 (0.25, 0.32)	211	0.46 (0.42, 0.51)
KR2DL4*002:01	147	0.13 (0.11, 0.15)	102	0.15 (0.13, 0.18)	41	0.09 (0.07, 0.12)
KR2DL4*002:02	188	0.16 (0.14, 0.19)	124	0.19 (0.16, 0.22)	52	0.11 (0.09, 0.15)
KR2DL4*005:01	312	0.27 (0.25, 0.30)	212	0.32 (0.28, 0.36)	99	0.22 (0.18, 0.26)
KR2DL5*002:01	191	0.17 (0.14, 0.19)	115	0.17 (0.15, 0.20)	72	0.16 (0.13, 0.20)
KR2DL5*001:01	247	0.21 (0.19, 0.24)	144	0.22 (0.19, 0.25)	99	0.22 (0.18, 0.26)
KR2DP1*001:02	104	0.09 (0.07, 0.11)	66	0.10 (0.08, 0.12)	34	0.07 (0.05, 0.10)
KR2DP1*002:01	571	0.50 (0.47, 0.52)	323	0.49 (0.45, 0.53)	221	0.49 (0.44, 0.53)
KR2DP1*003:01	278	0.24 (0.22, 0.27)	217	0.33 (0.29, 0.36)	55	0.12 (0.09, 0.15)
KR2DS1*002:01	239	0.21 (0.18, 0.23)	176	0.27 (0.23, 0.30)	61	0.13 (0.10, 0.17)
KR2DS2*001:01	464	0.40 (0.37, 0.43)	284	0.43 (0.39, 0.47)	170	0.37 (0.33, 0.42)
KR2DS3*001:01	210	0.18 (0.16, 0.21)	124	0.19 (0.16, 0.22)	82	0.18 (0.15, 0.22)
KR2DS4*001:01	433	0.38 (0.35, 0.40)	196	0.30 (0.26, 0.33)	219	0.48 (0.44, 0.53)
KR2DS4*003:01	248	0.22 (0.19, 0.24)	171	0.26 (0.22, 0.29)	64	0.14 (0.11, 0.18)
KR2DS4*004:01	58	0.05 (0.04, 0.06)	24	0.04 (0.02, 0.05)	34	0.07 (0.05, 0.10)
KR2DS4*006:01	177	0.15 (0.13, 0.18)	114	0.17 (0.14, 0.20)	58	0.13 (0.10, 0.16)
KR2DS4*010	116	0.10 (0.08, 0.12)	98	0.15 (0.12, 0.18)	17	0.04 (0.02, 0.06)
KR2DS5*002:01	202	0.18 (0.15, 0.20)	156	0.23 (0.20, 0.27)	40	0.09 (0.06, 0.12)
KR3DL1*015:02	135	0.12 (0.10, 0.14)	52	0.08 (0.06, 0.10)	62	0.14 (0.11, 0.17)
KR3DL1*001:01	189	0.16 (0.14, 0.19)	130	0.20 (0.17, 0.23)	47	0.10 (0.08, 0.14)
KR3DL1*002:01	115	0.10 (0.08, 0.12)	102	0.15 (0.13, 0.18)	14	0.03 (0.02, 0.05)
KR3DL1*004:01	159	0.14 (0.12, 0.16)	109	0.16 (0.14, 0.19)	45	0.10 (0.07, 0.13)
KR3DL1*005:01	145	0.13 (0.11, 0.15)	108	0.16 (0.14, 0.19)	34	0.07 (0.05, 0.10)
KR3DL1*007:01	184	0.16 (0.14, 0.18)	34	0.05 (0.04, 0.07)	151	0.33 (0.29, 0.38)
KR3DL2*001:01	461	0.40 (0.37, 0.43)	246	0.37 (0.33, 0.41)	193	0.43 (0.38, 0.47)
KR3DL2*002:01	150	0.13 (0.11, 0.15)	104	0.16 (0.13, 0.19)	32	0.07 (0.05, 0.10)
KR3DL2*003:01	89	0.08 (0.06, 0.09)	57	0.09 (0.07, 0.11)	30	0.07 (0.05, 0.09)
KR3DL2*006:01	95	0.08 (0.07, 0.10)	65	0.10 (0.08, 0.12)	32	0.07 (0.05, 0.10)
KR3DL3*002:02	117	0.10 (0.08, 0.12)	86	0.13 (0.10, 0.16)	31	0.07 (0.05, 0.10)
KR3DL3*004:02	57	0.05 (0.04, 0.06)	27	0.04 (0.03, 0.06)	23	0.05 (0.03, 0.08)
KR3DL3*001:01	418	0.36 (0.34, 0.39)	292	0.44 (0.40, 0.48)	112	0.25 (0.21, 0.29)
KR3DL3*005	71	0.06 (0.05, 0.08)	3	0.00 (0.00, 0.01)	66	0.15 (0.11, 0.18)
KR3DL3*011:01	52	0.05 (0.03, 0.06)	23	0.03 (0.02, 0.05)	31	0.07 (0.05, 0.10)
KR3DP1*003:01	126	0.11 (0.09, 0.13)	61	0.09 (0.07, 0.12)	62	0.14 (0.11, 0.17)
KR3DP1*001	750	0.65 (0.62, 0.68)	398	0.60 (0.56, 0.64)	327	0.72 (0.68, 0.76)
KR3DP1*005	209	0.18 (0.16, 0.20)	172	0.26 (0.23, 0.29)	34	0.07 (0.05, 0.10)
KR3DS1*010	236	0.20 (0.18, 0.23)	190	0.29 (0.25, 0.32)	46	0.10 (0.08, 0.13)

Allelic frequencies are presented with 95% confidence intervals for the full cohort or by race. n represents sample size.

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TABLE II. Longitudinal odds ratios and 95% confidence intervals for association of AD by KIR alleles of interest (allelic frequency >0.05) with respect to skin in remission

	Full PEER cohort		White		Black	
Allele	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
KR2DL1*004:01	1.10 (0.82, 1.48)	0.5140554	1.25 (0.89, 1.74)	0.1941659	0.99 (0.56, 1.75)	0.9707114
KR2DL1*001:01	1.13 (0.91, 1.39)	0.264043	0.82 (0.65, 1.04)	0.0984398	1.85 (1.13, 3.02)	0.0145356
KR2DL1*003:01	0.97 (0.81, 1.17)	0.7663724	1.00 (0.81, 1.23)	0.9956073	1.03 (0.72, 1.47)	0.8826215
KR2DL2*001:01	1.14 (0.95, 1.37)	0.1560088	1.11 (0.90, 1.38)	0.3306981	1.16 (0.82, 1.63)	0.4149932
KR2DL2*003:01	1.02 (0.83, 1.24)	0.8672246	0.93 (0.75, 1.15)	0.5213609	1.13 (0.71, 1.81)	0.608957
KR2DL3*001:01	1.24 (0.99, 1.56)	0.0592045	0.85 (0.65, 1.11)	0.2262123	1.72 (1.11, 2.66)	0.0150986
KR2DL3*005:01	0.90 (0.65, 1.24)	0.5225138	1.43 (0.94, 2.19)	0.0986007	0.78 (0.46, 1.32)	0.3566965
KR2DL4*001:02	0.76 (0.61, 0.94)	0.0137602	0.93 (0.71, 1.21)	0.5673199	0.79 (0.52, 1.21)	0.2820558
KR2DL4*002:01	1.17 (0.87, 1.58)	0.2988927	0.87 (0.61, 1.24)	0.4409599	1.30 (0.68, 2.51)	0.426969
KR2DL4*002:02	1.32 (1.02, 1.71)	0.0326578	1.10 (0.82, 1.48)	0.5064602	1.44 (0.80, 2.60)	0.2225222
KR2DL4*005:01	0.98 (0.77, 1.24)	0.8509362	1.02 (0.79, 1.32)	0.8726327	0.79 (0.47, 1.33)	0.3725441
KR2DL5*002:01	1.09 (0.89, 1.32)	0.4127738	1.22 (0.99, 1.50)	0.0663259	0.67 (0.39, 1.14)	0.1411144
KR2DL5*001:01	1.01 (0.84, 1.22)	0.8937314	1.03 (0.84, 1.28)	0.7559859	1.10 (0.77, 1.58)	0.602766
KR2DP1*001:02	1.12 (0.82, 1.52)	0.4784845	1.17 (0.85, 1.61)	0.3372885	0.73 (0.31, 1.73)	0.4748883
KR2DP1*002:01	0.94 (0.78, 1.13)	0.5059395	0.96 (0.77, 1.19)	0.6890084	0.96 (0.67, 1.37)	0.8172527
KR2DP1*003:01	1.18 (0.97, 1.45)	0.1028114	0.90 (0.72, 1.13)	0.374797	1.90 (1.19, 3.02)	0.0067723
KR2DS1*002:01	1.12 (0.94, 1.34)	0.2106717	1.04 (0.86, 1.26)	0.6822603	1.12 (0.75, 1.69)	0.5712382
KR2DS2*001:01	1.08 (0.93, 1.26)	0.3284884	1.02 (0.86, 1.21)	0.8267459	1.11 (0.82, 1.51)	0.4901996
KR2DS3*001:01	1.07 (0.89, 1.29)	0.4924667	1.18 (0.96, 1.44)	0.1223309	0.82 (0.53, 1.26)	0.3686062
KR2DS4*001:01	0.75 (0.61, 0.92)	0.0054782	0.94 (0.75, 1.19)	0.6259422	0.69 (0.46, 1.04)	0.0754453
KR2DS4*003:01	1.20 (0.96, 1.50)	0.1056774	1.00 (0.78, 1.29)	0.9880152	1.35 (0.80, 2.28)	0.2568351
KR2DS4*004:01	0.77 (0.47, 1.28)	0.315275	0.78 (0.41, 1.50)	0.4530056	0.82 (0.37, 1.84)	0.6318728
KR2DS4*006:01	1.14 (0.88, 1.48)	0.3144937	0.96 (0.71, 1.29)	0.7718508	1.20 (0.68, 2.10)	0.5280598
KR2DS4*010	1.23 (0.93, 1.61)	0.1411239	1.03 (0.78, 1.38)	0.8159397	1.75 (0.91, 3.34)	0.0927181
KR2DS5*002:01	1.09 (0.90, 1.32)	0.3934911	1.04 (0.85, 1.27)	0.6994634	1.17 (0.74, 1.87)	0.5039832
KR3DL1*015:02	0.81 (0.59, 1.13)	0.2111338	0.91 (0.59, 1.39)	0.6630969	0.81 (0.42, 1.54)	0.5198677
KR3DL1*001:01	1.40 (1.11, 1.77)	0.0049662	1.16 (0.90, 1.51)	0.2535899	1.61 (0.90, 2.88)	0.1068173
KR3DL1*002:01	1.05 (0.78, 1.42)	0.7603527	0.89 (0.66, 1.21)	0.4613308	0.53 (0.10, 2.69)	0.4420964
KR3DL1*004:01	1.17 (0.90, 1.52)	0.2366745	0.98 (0.72, 1.31)	0.8677731	1.11 (0.60, 2.07)	0.7380509
KR3DL1*005:01	1.13 (0.85, 1.49)	0.4007563	1.01 (0.75, 1.35)	0.9482659	1.42 (0.75, 2.70)	0.2869873
KR3DL1*007:01	0.58 (0.42, 0.81)	0.0010538	0.61 (0.33, 1.15)	0.1260223	0.93 (0.60, 1.42)	0.723497
KR3DL2*001:01	1.06 (0.83, 1.34)	0.6428665	1.20 (0.90, 1.59)	0.2082537	0.97 (0.62, 1.51)	0.888881
KR3DL2*002:01	1.03 (0.78, 1.37)	0.8250675	1.01 (0.74, 1.36)	0.9709964	0.27 (0.06, 1.10)	0.0672628
KR3DL2*003:01	0.99 (0.68, 1.46)	0.9698849	0.90 (0.59, 1.38)	0.634841	0.87 (0.35, 2.16)	0.7694256
KR3DL2*006:01	0.97 (0.69, 1.36)	0.8658636	1.00 (0.71, 1.42)	0.9822959	0.95 (0.47, 1.95)	0.8935284
KR3DL3*002:02	0.98 (0.69, 1.38)	0.8950658	0.80 (0.55, 1.16)	0.2394492	0.91 (0.40, 2.05)	0.8171017
KR3DL3*004:02	0.82 (0.50, 1.34)	0.4256559	0.90 (0.50, 1.62)	0.7270519	0.84 (0.30, 2.33)	0.7371788
KR3DL3*001:01	1.18 (0.96, 1.46)	0.119563	1.06 (0.83, 1.35)	0.6268567	0.71 (0.42, 1.19)	0.1913663
KR3DL3*005	0.29 (0.14, 0.60)	0.0007863	1.09 (0.19, 6.28)	0.9206443	0.39 (0.17, 0.88)	0.0228516
KR3DL3*011:01	1.19 (0.74, 1.91)	0.4851468	1.41 (0.82, 2.41)	0.2115711	1.51 (0.72, 3.17)	0.2704815
KR3DP1*003:01	1.09 (0.79, 1.50)	0.5876947	1.44 (0.98, 2.11)	0.06465	0.92 (0.50, 1.68)	0.7889485
KR3DP1*001	0.84 (0.69, 1.04)	0.1081843	0.96 (0.75, 1.22)	0.7206196	0.83 (0.56, 1.24)	0.36038
KR3DP1*005	1.26 (0.98, 1.61)	0.0701982	0.86 (0.64, 1.14)	0.2871227	2.06 (1.20, 3.53)	0.0089682
KIR3DS1*010	1.03 (0.86, 1.24)	0.765433	1.00 (0.83, 1.21)	0.9988294	0.87 (0.51, 1.48)	0.6061565

The outcome of AD in remission is based on a subject response for a 6-mo period as defined as symptom-free skin without medication.

the known HLA ligand. Notably, *Bw4* as represented by *B80T* epitope, which does not bind KIR3D receptors as tightly as the B80I epitope, was frequently found to be associated with decreased likelihood of remission of AD (Table III). A visual representation of the interaction between receptors coded by *2DL4*001:02* and *HLA-C*04:01* alleles is shown in Fig. 1.

A recent U.S. population-based study reported KIR gene allele haplotypes that included *2DS4*001:01* and *2DL4*001:02* alleles with a frequency of >0.05 (42). These telomeric haplotypes are *2DL4*001:02∼3DL1S1*002∼2DS4*001:01∼3DL2*002:01* and *2DL4*001:02∼3DL1S1*01:512∼2DS4*001:01∼3DL2*002:01*, which have a prevalence of 16.0% and 18.4%, respectively. The *2DL4*001:02∼2DS4*001:01∼3DL2*002:01* is associated with decreased likelihood of remission AD (0.77 [0.60, 0.99]; $p = 0.045$), and 2DL4*001:02∼*3DL1S1*002∼2DS4*001:01∼3DL2*002:01* is not associated with AD (0.96 [0.63, 1.45]; $p = 0.843$) (Table IV).

There were no statistically significant associations between *2DS4*001:01* or *3DL2*002:01* and the use of TCIs and symptom-free skin (Table V). There were associations between *3DL1*007:01* or *3DL3*005* and the use of TCIs and symptom-free skin (Table V), although these individuals are not able to stop using the TCIs and remain symptom free (Table II).

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TABLE III. Longitudinal odds ratios and 95% confidence intervals for association of AD, including interactions between KIR alleles and possible HLA ligands and *p* value

KIR allele	Ligand	KIR	Lower	Upper	*p* Value	Ligand	Lower	Upper	*p* Value	Interaction	Lower	Upper	*p* Value
KIR2L4*001:02	C*04:01	0.8152992	0.5857915	1.134726	0.2260358	1.092527	0.6548221	1.822808	0.7347354	0.4760877	0.2194022	1.033078	0.0604316
KIR2L4*001:02	C*05:01	0.6685226	0.4801678	0.9307631	0.0170834	1.378695	0.7895264	2.407521	0.2588628	1.42726	0.6654505	3.061193	0.3608238
KIR2L4*001:02	C2	0.6472651	0.3931502	1.065629	0.0872518	0.9794563	0.6262451	1.531883	0.927521	1.1505	0.6168725	2.145745	0.6593173
KIR2L4*001:02	Bw4	0.6845128	0.4798512	0.9764647	0.0364948	1.082615	0.6672094	1.756651	0.7478877	1.111453	0.5740443	2.151972	0.7539345
KIR2L4*001:02	Bw4(T)	0.6960487	0.4784286	1.012656	0.0581966	1.494373	0.9589173	2.328824	0.0759577	1.105097	0.5939921	2.055984	0.7523878
KIR2L4*001:02	Bw4(N)	0.7323419	0.4598959	1.166187	0.1894192	1.139248	0.7327958	1.771142	0.5625441	0.9485763	0.5162622	1.742907	0.8649411
KIR2L4*001:02	A*11:01	0.7484658	0.546905	1.024312	0.0703099	1.48959	0.7320398	3.031089	0.2715874	0.5159013	0.172857	1.539736	0.235496
KIR2S4*001:01	C*04:01	0.7671993	0.5521857	1.065936	0.1142425	1.090949	0.6650122	1.789698	0.7303416	0.4584195	0.2093608	1.003762	0.0511078
KIR2S4*001:01	C*05:01	0.5848434	0.4202204	0.813958	0.0014704	1.176752	0.6775545	2.043742	0.5633484	1.995622	0.9334587	4.266397	0.0746936
KIR2S4*001:011	C2	0.6435434	0.3909537	1.059328	0.0830395	1.0431	0.6804464	1.599034	0.8464932	1.040781	0.5583944	1.939894	0.8998742
KIR2S4*001:01	Bw4	0.70401	0.4942409	1.00281	0.0518454	1.237313	0.7941269	1.927831	0.3466262	0.8090062	0.417241	1.568616	0.5304133
KIR2S4*001:01	Bw4(T)	0.6335023	0.4361177	0.9202223	0.0165561	1.423626	0.9319931	2.174598	0.1022339	1.210408	0.6511811	2.249894	0.5460199
KIR2S4*001:01	Bw4(N)	0.7542933	0.4749825	1.197851	0.2321144	1.235007	0.8080606	1.887535	0.3294282	0.7998237	0.4356591	1.468391	0.4711575
KIR2S4*001;01	A*11:01	0.6879965	0.5032369	0.9405891	0.0190865	1.361075	0.67485	2.745094	0.389095	0.6173762	0.2078213	1.834044	0.3853106
KIR3L1*007:01	C*04:01	0.4769166	0.2978804	0.7635596	0.0020467	0.7366626	0.4768714	1.137983	0.1683837	1.441054	0.5778596	3.593668	0.4332311
KIR3L1*007;01	C*05:01	0.5403334	0.3528269	0.8274886	0.0046438	1.585315	1.0599	2.371191	0.0248861	0.9101716	0.2562196	3.233213	0.8842909
KIR3L1*007:01	C2	0.62064	0.3199812	1.203802	0.1581829	1.135522	0.8089888	1.593855	0.4625442	0.7440025	0.3238463	1.709267	0.4859268
KIR3L1*007:01	Bw4	0.5738788	0.3694265	0.8914815	0.0134695	1.144783	0.8081359	1.621669	0.4466459	0.5773454	0.1926219	1.730477	0.3266908
KIR3L1*007;01	Bw4(T)	0.6115983	0.386882	0.9668386	0.0353527	1.607376	1.151348	2.24403	0.0053068	0.6059306	0.2267301	1.619335	0.317844
KIR3L1*007:01	Bw4(N)	0.5731644	0.3183536	1.031926	0.0635662	1.141613	0.8194498	1.590434	0.4336852	0.8235233	0.3685469	1.840175	0.6359913
KIR3L1*007:01	A*11:01	0.5175412	0.3417756	0.7836979	0.001863	1.062975	0.6037507	1.871496	0.8324136	0.9059768	0.1762419	4.6572	0.9059004
KIR3L3*005	C*04:01	0.3568209	0.1619748	0.7860557	0.0105471	0.838653	0.5718416	1.229954	0.3678002	0.3663113	0.0446266	3.006818	0.3497841
KIR3L3*005	C*05:01	0.3158521	0.1503749	0.6634254	0.0023373	1.586751	1.085729	2.318976	0.017087	0.2560194	0.0015017	43.64681	0.6032853
KIR3L3*005	C2	0.3898372	0.1131258	1.343399	0.1356162	1.118975	0.8177677	1.531126	0.4823105	0.6226355	0.1339793	2.893545	0.5455372
KIR3L3*005	Bw4	0.2720602	0.1155383	0.6406249	0.0028911	1.100369	0.790004	1.532666	0.5715771	1.208601	0.230232	6.34454	0.8227949
KIR3L3*005	Bw4(T)	0.2332198	0.0944126	0.5761039	0.0016039	1.436556	1.052279	1.961164	0.0225595	2.923595	0.6155682	13.8854	0.1771508
KIR3L38005	Bw4(N)	0.2334565	0.0795542	0.6850918	0.0080847	1.045727	0.7697924	1.420571	0.7748306	1.514592	0.3474073	6.603173	0.5805273
KIR3L38005	A*11:01	0.2996204	0.1433956	0.6260471	0.0013479	1.068913	0.6303544	1.812592	0.8046561	1	.	.	.

As is convention, significant interaction is associated with a *p* value <0.10 (will likely change 95% confidence interval to 99% confidence interval). All variables are dichotomous (wild type versus heterozygote or homozygote). Bw4 is a composite of all HLA Bw4 epitopes. HLA Bw4(T) or Bw4(N) represents the HLA-B epitopes with a threonine (T) or asparagine (N) in position 80.

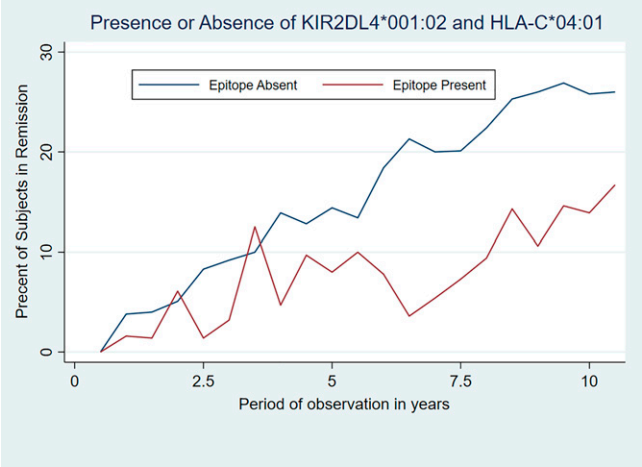

FIGURE 1. Presence or absence of *KIR2DL4*001:02* and HLA-C*04:01 status and the percentage of children in remission using surveys as described in *Materials and Methods* over time.

DISCUSSION

This study uniquely focused on KIR allelic genotyping in a cohort of 655 children with AD whose clinical course was followed with respect to the persistence of their illness for ~5000 person-years. Unlike most studies of AD, Black children were overrepresented in the cohort as compared with the general U.S. population. Four alleles were associated with the prognosis of AD over time. A decreased likelihood of AD remission was noted in children with *2DL4*001:02*, *2DS4*001:01*, *3DL1*007:01*, and *3DL3*005*, and an increased likelihood of remission was noted in children with *3DL1*001:01*. *2DL4*001:02* and *2DS4*001:01* are in LD. As expected, because most KIR genes with two extracellular domains (e.g., 2DXX) code for receptor proteins that bind to the HLA-C2 ligand, the magnitude of the effect of *2DL4*001:02* and *2DS4*001:01* was modified by (i.e., interacts with) the *HLA-C*04:01* and the *HLA-C*05:01* ligands (both members of the C2 epitope), and these alleles were recently reported to be associated with the *2DS4* gene (43). *2DL4* is not known to bind to the C2 epitope. *2DL4* alleles are thought to interact with HLA-G, an HLA class I molecule of very few polymorphisms as compared with other class I molecules, such as HLA-C (46, 47). The association in our study is likely because it is in LD with *2DS4*. In general, effect estimates were similar among Black and White children, although there were four KIR alleles that could be associated with remission in Black children but not White children (*2DL1*001:01*, *3DL2*003:01*, *2DP1*003:01*, and *3DP1*005*). To our knowledge, our study is the first that evaluated the effect of specific KIR gene allelic variation with respect to the time course of AD in children.

In the PEER cohort of children with AD, *2DL4*001:02* and *2DS4*001:01* are in LD and are members of known KIR haplotypes (42). These genes are found in both the KIR A (inhibiting) and B (activating) haplotypes (12, 42). *2DL4* is telomeric, found in nearly all people, and inhibitory (12, 42). KIR gene *2DS4* is also telomeric, but it is activating, and this KIR gene is found in ~95% of the population (12, 42). *KIR2DS4* is the oldest human KIR gene and, unlike most KIR genes, has an orthologue in the chimpanzee (44). *KIR2DS4* is known to interact with *HLA-C2* and specifically *C*04:01* and *C*05:01* as well as *HLA-A*11* (37, 41, 44, 48, 49). In our study, the magnitude of the effect of *2DL4*001:02* and *2DS4*001:01* was greatest when present in a child with *C*04:01* (a C2 variant).

KIR2DL4 is also the most conserved gene in KIR evolution, being a framework gene expressed by all NK cells. KIR2DL4 has a long cytoplasmic tail typical of inhibitory KIR; however, it is associated with NK cell activation rather than inhibition. Engagement with its coded receptor hence results in NK cell activation, leading to a proinflammatory cytokine and chemokine response (12, 46). *KIR2DL4* is expressed by all NK cells as well as some T cells. It binds with HLA-G, a gene that varies much less than HLA-A, -B, or -C (47, 50). In general, unlike other KIR gene/receptors, it is not expressed on the NK cell

TABLE IV. Longitudinal odds ratios and 95% confidence intervals for association of AD with remission in the presence (+) or absence (−) of associated significant HLA ligands

Allele or haplotype	Ligand	OR (95% CI)	*p* Value
2DL4*001:02	Any	0.75 (0.61, 0.95)	0.014
2DL4*001:02	C*04:01 −	0.83 (0.64, 1.05)	0.129
2DL4*001:02	C*04:01 +	0.54 (0.33, 0.89)	0.016
2DL4*001:02	C*05:01 −	0.74 (0.58, 0.95)	0.018
2DL4*001:02	C*05:01 +	0.82 (0.51, 1.48)	0.615
2DS4*001:01	Any	0.75 (0.61, 0.92)	0.005
2DS4*001:01	C*04:01 −	0.80 (0.64, 1.00)	0.052
2DS4*001:01	C*04:01 +	0.53 (0.32, 0.88)	0.014
2DS4*001:01	C*05:01 −	0.72 (0.57, 0.90)	0.004
2DS4*001:01	C*05:01 +	0.90 (0.58, 1.38)	0.636
2DL4**001:02 ~ 2DS4*001:01 ~ 3DL2:002:01	any	0.77 (0.60, 0.99)	0.045
2DL4*001:02 ~ 2DS4*001:01 ~ 3DL2:002:01	C*04:01 −	0.96 (0.77, 1.20)	0.730
2DL4*001:02 ~ 2DS4*001:01 ~ 3DL2:002:01	C*04:01 +	0.50 (0.26, 0.98)	0.043
2DL4**001:02 ~ 2DS4*001:01 ~ 3DL2:002:01	C*05:01 −	0.84 (0.67, 1.07)	0.156
2DL4*001:02 ~ 2DS4*001:01 ~ 3DL2:002:01	C*05:01 +	1.02 (0.65, 1.61)	0.914

TABLE V. Longitudinal odds ratios and 95% confidence intervals for association of AD and use of a topical treatment

		Full cohort		White		Black	
KIR allele	Topical exposure	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
KIR2DL4*001:02	Steroid use	0.97 (0.77, 1.21)	0.7635115	0.92 (0.68, 1.24)	0.5687188	0.82 (0.56, 1.20)	0.3159582
KIR2DL4*001:02	TCI use	1.02 (0.82, 1.28)	0.8469258	0.87 (0.65, 1.16)	0.3445578	0.69 (0.46, 1.03)	0.0707266
KIR2DS4001:01	Steroid use	1.06 (0.84, 1.33)	0.6123727	0.91 (0.67, 1.23)	0.541468	1.04 (0.71, 1.52)	0.8418862
KIR2DS*4001:01	TCI use	1.11 (0.89, 1.39)	0.3516176	0.93 (0.69, 1.24)	0.6010451	0.72 (0.48, 1.07)	0.1042912
KIR3DL1*007:01	Steroid use	1.24 (0.96, 1.60)	0.0943533	1.55 (0.94, 2.56)	0.0839951	0.94 (0.67, 1.34)	0.7470756
KIR3DL1*007:01	TCI use	1.76 (1.36, 2.26)	0.0000122	2.36 (1.47, 3.79)	0.0003624	0.76 (0.53, 1.10)	0.1436235
KIR3DL3*005	Steroid use	1.41 (1.00, 1.99)	0.0486723	1.97 (0.46, 8.44)	0.3607824	1.19 (0.80, 1.75)	0.3895193
KIR3DL3*005	TCI use	1.96 (1.39, 2.78)	0.0001458	1.34 (0.31, 5.72)	0.6928777	1.17 (0.78, 1.77)	0.4446245

Subjects may use more than one therapy. The use of TCIs was required for 6 wk before study entry. Steroid use is topical steroid use. Information on the topical steroid compound was not collected.

surface; instead, it is expressed on endosomes that involve serine/threonine kinases (46). In our study, the known *2DL4*001:02∼2DS4*001:01∼3DL2:002:01* telomeric haplotype is associated with AD that is less likely to be in remission (0.77 [0.60, 0.99]; $p = 0.045$) (42). With respect to this haplotype, *KIR2DS4*001* is the only *KIR2DS4* variant that codes for a functional protein (51).

It appears that individuals with *KIR3DL3*005* and *KIR3DL1*007:01* are more likely to use TCIs and to have periods of reduced skin symptoms while using TCIs. These individuals are not more likely to have a remission (clear skin without the use of topicals). It is possible that individuals with these KIR alleles are both responsive to TCIs, so that they do not switch to topical steroids. They may be less likely to have a period of remission, thus requiring the continued use of this topical medication. However, this is a longitudinal cohort study, so, on the basis of our results, it is also possible that these associations are due to confounding by indication (i.e., the increased need for topical agent is associated with severity) or confounding due to unmeasured factors. An association with topical use was not noted for *2DL4*001:02* and *2DS4*001:01*.

NK cells are an important part of the innate immune system with a role in destroying infected and malignant cells, but NK cells also produce immunoregulatory cytokines and chemokines (52). It is likely that NK cells have important roles in inflammatory conditions. Previously, a study in Poland demonstrated that the presence of the *KIR* gene *KIR2DS1* was associated with a decreased risk of AD, and a recent study by our group demonstrated an increased risk of AD with *KIR2DS1*, *KIR2DL5*, and *KIR2DS5* (10, 53, 54). NK cell function, represented by decreased circulating numbers of NK cells and diminished NK cell function, has been shown to be associated with AD (7, 17, 17, 53, 55). The association between AD and circulating NK cell number was first noted in 1985 (16). A more recent study by Mack et al. revealed that the number of circulating NK cells in adults with moderate to severe AD was significantly less than in a healthy control population (7). This was recently replicated (16, 56). Mack et al. further noticed a diminished number of circulating mature NK cells, whereas lesional AD skin was enriched for activated NK cells (7). Furthermore, a subset of circulating NK cells may be able to suppress Th2 cytokine-producing ILC2 cells that have been associated with AD, and transferring the human cells to an AD mouse model can suppress inflammatory skin responses in the mouse (56). Increased numbers of tissue-resident NK cell have been seen in both human AD lesions and mouse lesional skin samples (7, 56). Although both tissue-resident and circulating NK cells exist, the tissue-resident NK cells are likely less cytotoxic (52). Circulating NK cells appear to express more KIR on the surface than tissue-resident NK cells (52).

Our present findings show that KIR alleles are associated with AD severity in children over time. *2DS4*001:01* and *2DL4*001:02* in the presence of *C*04:01* and the KIR B haplotype *2DL4*001:02∼2DS4*001:01∼3DL2:002:01* also in the presence of *C*04:01* is associated with decreased likelihood for AD remission (i.e., severe AD). This is likely consistent with the findings of both Mack et al. and Min et al., who indicated that the activation of circulating NK cells appears to decrease AD symptoms (7, 56). This is also consistent with a recent study by Möbus et al., who showed that NK cell numbers were elevated in AD lesion and nonlesion skin and that NK cell activation by successful treatment results in clinical improvement (52, 57).

Individuals with AD are known to have higher rates of previous infection with HSV, CMV, and EBV (58–60). NK cells are known to be part of the defense against viral infections, including the human herpesviruses (CMV, EBV, and HSV). Individuals who are seropositive for CMV infection in developed countries are also more likely to be homozygous for the KIR A haplotype, to be homozygous for the C1 or C2 ligand, and to have the *HLA-B epitope 80T* (the weaker of the Bw4 binding ligands) (61). In developing countries, the seroprevalence of CMV is higher than in developed countries, but comparable studies are not reliable (62, 63). We have previously shown that individuals with AD are more likely to have the *B80T* variant and to have KIR genes associated with the A haplotype than control subjects without AD (9, 10). Children with early-onset CMV infection are also more likely to demonstrate atopy on the basis of skin-prick testing (64). CMV infection is also known to lead to the education of inhibitory KIRs (65). It is possible that the susceptibility to herpesviruses in individuals with AD is associated with KIR-associated NK cell function or

that infection by human herpesviruses such as CMV begins the causal path toward the development of AD, because it has been implicated in many autoimmune diseases (66). Finally, NK cell activation has also been shown to be important in the defense of *Staphylococcus aureus*, which is a pathogen noted to become more prevalent on the skin of individuals with AD who are having flares of their disease (67, 68).

As with all epidemiologic studies, this study has limitations. To our knowledge, this is the largest sample of children who have been followed over time for the persistence of AD with full allelic KIR sequencing. Our study was underpowered to evaluate less common KIR allelic variants. Our initial evaluation of allelic variants with frequencies $\geq$0.05 is arguably clinically meaningful for a common disease that has a prevalence of $\sim$10% but likely limits a fuller understanding of the underlying mechanisms. However, our findings were consistent with predictions from previous studies and likely not random events associated with statistical error from multiple testing. For example, alleles on different KIR genes (i.e., *2DL4*001:02* and *2DS4*001:01*) known to be in LD had similar effect estimates; HLA ligands known to interact with these genes did interact; and a known haplotype that included these genes was associated with AD severity. The PEER DNA study is a subset of the PEER study, a national study that broadly sampled the population of the United States. Neither sample was generated using techniques to assure generalizability, so it is possible that our results do not generalize to the U.S. population. However, many studies have been published using this data resource, and the results of these prior studies appear to generalize to the general population of children with mild to moderate atopic dermatitis. An independent cohort of patients should be analyzed to replicate our findings. This is a descriptive study; hence, future studies are needed to explain the physiologic relationships between HLA genes and our KIR gene findings in AD.

In summary, our findings demonstrate that allelic variation of the *KIR-2DL4* and *-2DS4* genes are associated with more persistent AD. Our results, as expected, are augmented with respect to the classic MHC class I ligands known to be essential for the KIR allele coded proteins to bind and activate NK cells. It also seems to be specific to one common KIR B telomeric haplotype. Our findings add to the general body of evidence about the immunogenetics of AD and specifically adds to and aligns with a growing literature on the importance of NK cells with respect to the immunopathogenesis of AD, including natural history and response to therapy. Further definition of the function of NK cells in the skin at the site of AD damage will provide additional insights and could lead to modulation of the risk and course of AD as well as new therapeutics.

DISCLOSURES

D.J.M. is or recently has been a consultant for Pfizer, Leo, and Sanofi with respect to studies of atopic dermatitis and served on an advisory board for the National Eczema Association. The other authors do not report potential conflicts of interest with respect to the materials in this article.

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