

BRIEF COMMUNICATION

Genetic polymorphism of *KIR2DL4* (CD158d), a putative NK cell receptor for HLA-G, does not influence susceptibility to asthmaM. E. L. Le Page¹, J. P. Goodridge², G. Zhang³, P. G. Holt^{4,5}, P. Sly⁵ & C. S. Witt^{1,6}¹ School of Pathology and Laboratory Medicine, University of Western Australia, Perth, WA, Australia² Fred Hutchinson Cancer Research Institute, Seattle, WA, USA³ School of Paediatrics and Child Health, University of Western Australia, Perth, WA, Australia⁴ Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, Perth, WA, Australia⁵ Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Australia⁶ Department Clinical Immunology, Royal Perth Hospital, Perth, WA, Australia**Key words**

asthma; atopy; CD158d; KIR2DL4

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Abstract

Human leukocyte antigen (HLA)-G is upregulated on the bronchial epithelium of asthma patients and genetic polymorphism affecting expression of HLA-G has been reported to influence susceptibility to asthma. As the NK cell receptor KIR2DL4 has been reported to induce interferon gamma (IFN γ) secretion when ligated with HLA-G, we postulated that the 9A/10A genetic polymorphism of KIR2DL4 which influences receptor structure may influence susceptibility to asthma. KIR2DL4 genotypes were determined in two cohorts of children ($n = 219$ and $n = 1356$) in whom total serum IgE, allergen-specific IgE, atopy, bronchial reactivity and asthma symptoms had been studied between birth and 14 years. No reproducible associations with KIR2DL4 genotype were identified, leading us to conclude that the *KIR2DL4* 9A/10A polymorphism has no influence on susceptibility to asthma.

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KIR2DL4 (CD158d) is a member of the killer cell immunoglobulin-like receptor family that is mainly expressed on NK cells and its ligand has been reported to be human leukocyte antigen (HLA)-G (1–5) though this controversial (6, 7). HLA-G has been identified as an asthma gene in family-based and genome wide association study (GWAS) studies (8–10). Although structurally almost invariant, the HLA-G gene contains many non-coding single nucleotide polymorphisms (SNPs) that are thought to affect expression of the protein (11) and it is these polymorphisms that have been associated with asthma. If HLA-G itself is an asthma gene, functional variation in receptors for HLA-G might also influence susceptibility to asthma. This study therefore aims to determine if polymorphisms in the NK cell receptor KIR2DL4, the putative target for HLA-G, influence susceptibility to asthma.

HLA-G interacts with a number of white cell receptors usually resulting in inhibitory signals that dampen immune responses and for this reason HLA-G is generally thought of as having a tolerogenic function. This idea is consistent with its restricted tissue expression being found mainly on placenta (12) and tolerogenic dendritic cells (DCs) (13).

However, its expression has been shown to be upregulated in a variety of tissues under inflammatory conditions (14) including the bronchial epithelium of asthma patients (8, 15). While most KIR receptors have either inhibitory or activating function, KIR2DL4 is unusual in that it has structural motifs associated with inhibitory and activating KIR but to date, only activating function has been convincingly demonstrated (1, 16). Although ligation of KIR2DL4 can result in weak cytolytic activity (17), robust secretion of interferon-gamma (IFN γ) is also elicited (1, 16).

KIR2DL4 has an unusual genetic polymorphism in that two common alleles of the transmembrane region exist and one allele known as the 9A-TM allele has a single nucleotide deletion in the coding region of exon 6 resulting in a defective receptor that does not induce IFN γ secretion in the presence of ligand (16). If KIR2DL4 genotype influenced the amount of IFN γ secreted during NK cell interaction with HLA-G on DCs or other tissues, this could influence susceptibility to asthma in two ways. Firstly, variation in IFN γ secretion may influence the balance between Th1 and Th2 immune responses [reviewed in Szabo et al. (18)]. Secondly, NK cell-derived

IFN γ secretion has been reported to be critical for the generation of tolerogenic DC in the placenta (19). Thus, it might be predicted that individuals with the functionally defective 9A allele of KIR2DL4 would not be able to secrete IFN γ , and might therefore be prone to producing Th2-biased immune responses and fewer tolerogenic DC. As the 9A-TM and 10A-TM (wild-type) alleles of KIR2DL4 have allele frequencies of 50% in the Australian population (20), 25% of the population is homozygous for the 9A-TM genotype and might therefore be predicted to be particularly predisposed to asthma.

In this study, we set out to determine whether the KIR2DL4 9A/10A polymorphism was associated with asthma in two populations. The Childhood Asthma Study (CAS) cohort consists of children at high risk of atopic disease based on family history studied from birth to 5 years. Details of the cohort, laboratory and clinical details have been previously published (21). Briefly, 263 children at high risk of developing asthma were followed from birth to 5 years of age. Serum total IgE concentrations and atopy, defined by skin prick testing, were determined at 6 months, 2 and 5 years as detailed in (22). Parents kept a daily symptom diary and recorded the presence of symptoms of acute respiratory tract infections (ARI), such as runny/blocked nose, cough and wheeze as well as presence of fever ($>38^{\circ}\text{C}$). If the child developed any of these symptoms, the study centre was contacted within 24 h and a home visit was arranged (within 48 h). Asthma was recorded if diagnosed by a doctor. The Raine cohort consisted of an unselected longitudinal birth cohort broadly representative of the West Australian population, as detailed previously (23). Mothers were recruited antenatally and were not selected on the basis of any asthma or atopy criteria. The cohort initially consisted of 2860 live births, with 2537 potentially available for follow-up when the children were 6 years old. Follow-up data included physical examinations at birth and 1 and 6 years of age and comprehensive questionnaires at 1, 2, 3 and 6 years of age, including data on physician diagnoses of asthma. The 6-year and 14-year follow-up included the following: physical examination; lung function tests [reported in detail elsewhere (24)]; methacholine challenge; skin prick tests to four common allergens: house dust mite (HDM), rye grass, cat dander and mould. Table 1 shows the number of individuals on whom DNA was available for KIR2DL4 genotyping and for whom clinical and laboratory data was available. When 6 year olds were surveyed for the Raine study, blood was collected from 981 children and clinical surveys were completed in most cases but were not completed in approximately 30 children for random reasons. Bronchial hyper-reactivity testing was only instigated some time after the survey commenced resulting in only 349 tests. When 14 year olds were surveyed, blood samples were collected from 1355 children, including 680 originally sampled as 6 year olds. Clinical assessments were incomplete in 44 children for random reasons.

Both cohorts are largely from metropolitan Perth but do differ in socioeconomic status (SES). The larger Raine cohort

was born between August 1989 and April 1992 and is broadly representative of the Perth population at the time, but with a trend towards lower SES, more single mothers and younger mothers relative to the CAS cohort. The smaller CAS cohort was born between 1996 and 1998 and is of higher SES. They were at high risk of asthma and allergy based on one or both parents having asthma/allergies. The prevalence of breast feeding was higher in the CAS cohort which was breast fed for longer than the Raine cohort.

DNA for KIR2DL4 genotyping was available for 223 children from the CAS cohort and 1628 children from the Raine cohort. KIR2DL4 genotyping to determine the presence of the 9A-TM and 10A-TM alleles was performed by DNA sequencing as previously described (20). The number of 9A-TM alleles present in each genotype was defined as – 0 (10A homozygous), 1 (9A/10A heterozygous) or 2 (9A homozygous). As shown in Table 1, the frequency of subjects with 0, 1 and 2 copies of the KIR2DL4 9A-TM allele was approximately 25%, 50% and 25%, respectively. The KIR2DL4 genotypes were determined to be in Hardy–Weinberg Equilibrium using a conventional chi-squared test.

The relationship between KIR2DL4 genotype and serum IgE concentrations was determined in the CAS cohort at age 5 years and in the Raine cohort at ages 6 and 14 years. The levels of serum IgE were skewed and log transformations were performed prior to further statistical analysis. The differences in IgE levels between KIR2DL4 genotypes at age 6 months and 2 and 5 years were investigated using ANOVA. Regarding zero, one or two 9A-TM alleles as a linear term, the dose–response effects of the 9A-TM allele on serum IgE were examined using linear regression models. For binary asthma-related phenotypes such as atopy, wheezing and asthma, chi-squared tests were employed to explore the associations with KIR2DL4 genotypes. The linear effects of the number of 9A-TM alleles on these phenotypes were also investigated using the Crosstabs function. Statistical analyses were performed using the SPSS statistical package Version 19 (IBM Corp, Armonk, New York). The significance level was selected as $P < 0.05$. Although several weak associations were observed between increasing number of copies of the non-membrane expressed 9A-TM allele and increasing total IgE and HDM-specific IgE, these associations were not reproduced in the larger Raine cohort. In fact, the opposite trends were often observed in the Raine cohort. This suggests that the significant associations observed were most likely type I errors. As total IgE, HDM-specific IgE and grass pollen-specific IgE concentrations are likely to be correlated with each other, the apparently high number of type I errors observed is perhaps not surprising. As only one copy of the 10A-TM allele is required for membrane expression of KIR2DL4, we repeated the analyses comparing the 9A-TM homozygotes with all others but this did not result in any greater uniformity of trends in the two cohorts.

Table 1 Number of subjects tested for KIR2DL4 genotype, IgE, atopy and clinical parameters in the Childhood Asthma Study and Raine cohorts

Variable	Age	RAINE cohort (<i>n</i> = 1628)			CAS cohort (<i>n</i> = 223)		
		Total <i>n</i>	<i>n</i> Positive	% of cohort	Total <i>n</i>	<i>n</i> Positive	% of cohort
Sex (M)	–	1356	700		219	120	
Sex (F)	–	1356	656		219	99	
KIR2DL4 10A/10A	–	1628	430		219	50	
KIR2DL4 9A/10A	–	1628	812		219	122	
KIR2DL4 9A/9A	–	1628	386		219	47	
Current wheeze	5 or 6 years	953	211		192	56	
	14 years	1313	175		–	–	
Current asthma	5 or 6 years	950	145		192	35	
	14 years	1311	135		–	–	
Bronchial hyperreactivity	6 years	349	183		–	–	
	14 years	1265	231		–	–	
Atopy	5 or 6 years	981	444		163	68	
	14 years	1355	542		–	–	
Total IgE	5 or 6 years	981	–		165	–	
	14 years	1355	–		–	–	
Phadia IgE	5 or 6 years	980	–		162	–	
	14 years	1355	–		–	–	
HDM IgE	5 or 6 years	981	–		165	–	
	14 years	1355	–		–	–	

There was neither significant relationship between *KIR2DL4* genotype and the prevalence of atopy as assessed by allergen skin prick testing in either cohort at any age, nor was there any significant relationship between *KIR2DL4* genotype and the prevalence of wheeze, bronchial hyperreactivity and asthma in the CAS and Raine cohorts at any age. As such, we were unable to find any reproducible associations between *KIR2DL4* genotype and any laboratory or clinical measure of atopy or asthma itself.

Although the CAS cohort was selected on the basis of parental atopy and the Raine cohort was a population-based cohort, it seems unlikely that this difference would be a basis for failure to replicate the weak associations identified in the CAS cohort. Nevertheless, it is possible that selection on parental atopy may result in a cohort with different genetics to a community-based cohort. Of course, we cannot exclude the possibility that other differences between the two cohorts (decade of birth, SES and prevalence and length of breast feeding) interact with *KIR2DL4* genotype to influence atopy but the interaction would have to be strong enough to result in the opposite trends in the different cohorts observed in this study.

The genetic polymorphism studied is in exon 6 of the *KIR2DL4* gene. The DNA sequencing method used to detect this polymorphism also allows detection of additional SNPs in

introns 6 that are highly correlated with common alleles of *KIR2DL4* defined by amino acid substitutions in the immunoglobulin (ligand binding) domains (16). Thus, based on the intronic SNPs, the 10A-TM allele can be subdivided into 10A-A-TM corresponding to *KIR2DL4**00102 and 10A-B-TM corresponding to *KIR2DL4**005. The 9A-TM allele corresponds to *KIR2DL4**008 and *011. The 10A-B-TM genotype is also in almost complete linkage disequilibrium with KIR3DS1 and is therefore a surrogate marker of the telomeric end of the KIR B haplotype while the 10A-A-TM and 9A-TM genotypes are in almost complete linkage disequilibrium with the telomeric ends of the KIR-A haplotypes carrying the KIR2DS4 gene with and without the 22bp deletion, respectively (25). However, repeating the analyses using the six genotypes identified by inclusion of the intronic SNPs did not reveal any additional significant associations suggesting that further studies focusing on the telomeric ends of the KIR-A and KIR-B haplotypes are likely to yield negative results.

In conclusion, we find no evidence that the *KIR2DL4* 9A/10A polymorphism has any influence on susceptibility to asthma.

Conflict of Interest

The authors have declared no conflicting interests.

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