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Deletion of the Activating NKG2C Receptor and a Functional Polymorphism in its Ligand HLA-E in Psoriasis Susceptibility

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Abstract

Psoriasis is an inflammatory, immune-mediated disease of the skin. Several studies have suggested that natural killer (NK) cells and their receptors may be important for its pathogenesis. Here, we examined whether deletion of the activating natural killer receptor gene NKG2C, which has a frequency of 20% in the European population, was associated with psoriasis susceptibility. The NKG2C deletion and a functional polymorphism in its ligand HLA-E were genotyped in a Caucasian cohort of 611 psoriasis cases and 493 controls. We found that the NKG2C deletion was significantly increased in cases compared to controls (0.258 vs. 0.200, p=0.0012, OR=1.43 [1.15–1.79]). The low-expressing HLA-E*01:01 allele was associated with psoriasis (p=0.0018), although this association was dependent on HLA-C. Our findings support a potential immunoregulatory role for NK cells in psoriasis and suggest the importance of future studies to investigate the contribution of NK cells and their regulatory receptors to the pathogenesis of psoriasis.

Keywords

Psoriasis; natural killer; NKG2C; KLRC2; HLA-E

Background

Psoriasis is a common chronic inflammatory skin disease affecting 2–3% of the population. Both T cells (1) and keratinocytes (2) are thought to play a central role in the initiation and maintenance of psoriasis. Natural killer (NK) cells may also play a role in the pathogenesis of psoriasis (3). NK cells in psoriatic lesional skin secrete excessive amounts of the Th1 cytokine interferon-gamma (4). Moreover, the psoriasis susceptibility gene *HLA-C*06:02* contains the C2 epitope which binds the activating NK cell receptor KIR2DS1, which has

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Author contribution

X.Z., R.G., and O.P. performed the research. X.Z. and H.C. analyzed the data. A.B. contributed samples. X.Z. and W.L. wrote the paper. W.L. designed the study.

Conflicts of interest

No conflicts of interest to disclose.

been genetically associated with psoriasis (5–7). Recently, we found that another activating NK cell receptor, *KIR3DS1*, was associated with psoriasis (8). The work of Gladman and others have described a role for the interaction of KIR receptors with HLA in psoriatic arthritis (9). Thus, several studies have shown that NK cells and their regulatory receptors may have in important role in psoriatic disease.

Questions addressed

The aim of this study is to investigate whether a 16 kb deletion (10) of *NKG2C* and a functional polymorphism of its ligand *HLA-E* contribute to psoriasis susceptibility.

Experimental Design

Genomic DNA were obtained from 611 Caucasian psoriasis patients and 493 Caucasian healthy controls. The *NKG2C* deletion was typed using a previously published PCR protocol (10, 11). *HLA-E*01:01* and *HLA-E*01:03* were discriminated using a previously described Taqman assay (12). Statistical analysis was performed using chi-squared test or Fisher's exact test. Further details on sample collection, genotyping, *HLA-C*06:02* imputation, and statistical analysis are described in Supplementary Methods.

Results

We obtained genotyping data for the 16kb *NKG2C* deletion in 572 psoriasis cases and 458 controls. We found that the *NKG2C* deletion was significantly more common in cases compared to controls (allele frequency 25.8% vs. 20.0%, p=0.0012, OR=1.43 [1.15–1.79], Table 1). Analysis of *NKG2C* genotypes revealed that psoriasis patients were more likely to be homozygous for the *NKG2C* deletion (Del/Del) compared to controls (p=0.0065, OR=2.65 [1.26– 6.12], Table 1). These results suggest that deletion of the activating natural killer receptor NKG2C is associated with psoriasis susceptibility.

The natural ligand for NKG2C is HLA-E. We found that the *HLA-E*01:03* allele, which has higher cell surface expression and stronger peptide binding than the *HLA-E*01:01* allele, was significantly less frequent in psoriasis cases compared to healthy controls (p=0.0018, OR=0.76 [0.64–0.90], Table 1). Individuals homozygous for low-expressing *HLAE* 01:01/01:01* were at significantly increased risk for psoriasis ($p=8.3 \times 10^{-9}$, OR=2.13 [1.63–2.78]). After conditioning the association of HLA-E with psoriasis on *HLA-C*06:02*, the association of *HLA-E*01:03* with psoriasis was mitigated (p=0.203, OR=0.89 [0.74–1.07]).

Given the ligand-receptor relationship between HLA-E and NKG2C, we analyzed the association of combined *HLA-E* and *NKG2C* genotypes with psoriasis. A significantly reduced risk of psoriasis was seen in individuals who carried *HLA-E*01:03/01:03* plus *NKG2C*Pos/Pos*, or *HLA-E*01:03/01:01* plus *NKG2C*Pos/Pos*. On the other hand, the two genotype combinations *HLA-E*01:01/01:01* plus *NKG2C*Pos/Del*, and *HLA-E*01:01/01:01* plus *NKG2C*Del/Del* were significantly associated with elevated psoriasis risk (Table 2). The five other genotype combinations did not significantly vary between psoriasis cases and controls.

Conclusions

Here, we sought to determine whether genetic variants in the activating NK cell receptor NKG2C or its ligand HLA-E were associated with psoriasis susceptibility. We found that a 16 kb deletion of *NKG2C* was associated with psoriasis. The frequency of the deletion allele was higher in cases compared to controls (p=0.0012, OR=1.43) and homozygosity for the

deletion was a strong risk factor for psoriasis (p=0.0065, OR=2.65). Deletion of *NKG2C* is correlated with decreased NKG2C cell surface expression levels (11).

Furthermore, we found that psoriasis patients were enriched for the low-expression allele *HLA-E*01:01,* though this was conditional on *HLA-C*. Individuals homozygous for *HLAE* 01:01* had a significantly increased risk of psoriasis ($p=8.3 \times 10^{-9}$, OR=2.13). Our results are in agreement with a previously published study showing that among *HLA-C*06:02* positive individuals, *HLA-E*01:03* was associated with protection from psoriasis (13).

Together, our results are potentially consistent with a recently described model in which NK cells play an immunoregulatory role in limiting excessive CD4+ or CD8+ T cell responses (14, 15). Failure to regulate these T cell responses may lead to autoimmunity (16–19). Deletion of the activating NKG2C receptor in psoriasis might lead to a relative inability to eliminate autoreactive T cells. The higher frequency of the low-expressing *HLA-E*01:01* allele in psoriasis might also lead to a diminished binding between HLA-E and activating NKG2C/CD94. However, HLA-E*01:01 might also be expected to decrease the interaction between HLA-E and the inhibitory NKG2A/CD94 receptor. Thus, the overall net effect of HLA-E*01:01 on activation or inhibition of NK cells may depend on the relative expression of NKG2C versus NKG2A on lymphocytes. Interestingly, we have previously found that there is an expansion of NKG2A+ NK cells within psoriatic skin (20). An important role for NK cell receptors in cutaneous autoimmune disease is also observed in studies of alopecia areata, in which the interaction of the activating NK receptor NKG2D with its ligands MICA and ULBP3 leads to immune attack of the hair follicle (21–23). *HLA-E*01:01* has been previously associated with several other autoimmune diseases.

*HLA-E*01:01* was found to predispose to ankylosing spondylitis (24), which shares features with psoriatic arthritis. *HLA-E*01:01* was also found to be associated with susceptibility to type 1 diabetes in a study of 199 cases and 82 healthy controls from Britain (25). On the other hand, Behcet's disease was found to be associated with *HLA-E*01:03* (26).

Interestingly, HLA-E expression has been associated with co-expression of endoplasmic reticulum aminopeptidase (ERAP) (27), a peptide-trimming protein that has been genetically associated with psoriasis (28). HLA-E has also been shown to sensitize keratinocytes to killing by CD8+ CD56+ T cells expressing NKG2C/CD94 (29). Whether the deletion of NKG2C and low expression of HLA-E in psoriasis patients results in decreased killing of HLA-E+ keratinocytes in psoriatic skin lesions and augments the hyperproliferative response requires further investigation.

In summary, we have found evidence that a common 16 kb deletion in the *NKG2C* gene is a risk factor for psoriasis susceptibility. Our findings further highlight the importance of NK cell receptors and their ligands in the pathogenesis of psoriasis, and suggest the need for additional studies to delineate the contribution of NK cells to psoriasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Allele frequencies and genotypes of NKG2C and HLA-E in psoriasis cases versus healthy controls.

	Psoriasis	Controls	P-val	OR (95%CI)	Adjustment for P	HLA-C OR (95%CI)
NKG2C						
NKG2C*Del	0.258	0.200	0.0012	1.43 (1.15–1.79)		
Del/Del	32 (5.6)	10 (2.2)	0.0065	2.65 (1.26–6.12)		
Pos/Del	232 (40.6)	163 (35.6)				
Pos/Pos	308 (53.9)	285 (62.2)				
HLA-E						
HLA-E*01:03	0.352	0.420	0.0018	0.76 (0.64–0.9)	0.203	0.89 (0.74–1.07)
01:01/01:01	259 (43.5)	163 (34.0)	8.26E-09	2.13 (1.63–2.78)		
01:01/01:03	253 (42.5)	230 (48.0)				
01:03/01:03	83 (13.9)	86 (17.9)				
HLA-C						
HLA-C*06:02	0.296	0.136	3.32E-14	2.52 (1.99–3.21)		

Table 2

Frequency of combination HLA-E and NKG2C genotypes in psoriasis cases and healthy controls.

Genotype Combination	Psoriasis	Controls	P-val	OR (95% CI)
HLA-E*01:03/01:03+NKG2C*Pos/Pos	44 (7.76)	53 (11.62)	0.041	0.64 (0.41–0.99)
HLA-E*01:03/01:03+NKG2C*Pos/Del	32 (5.64)	28 (6.14)		
HLA-E*01:03/01:03+NKG2C*Del/Del	4 (0.71)	0 (0.00)		
HLA-E*01:03/01:01+NKG2C*Pos/Pos	131 (23.10)	136 (29.82)	0.018	0.71 (0.53–0.94)
HLA-E*01:03/01:01+NKG2C*Pos/Del	101 (17.81)	84 (18.42)		
HLA-E*01:03/01:01+NKG2C*Del/Del	11 (1.94)	6 (1.32)		
HLA-E*01:01/01:01+NKG2C*Pos/Pos	133 (23.46)	95 (20.83)		
HLA-E*01:01/01:01+NKG2C*Pos/Del	98 (17.28)	51 (11.18)	0.007	1.66 (1.14–2.44)
HLA-E*01:01/01:01+NKG2C*Del/Del	13 (2.29)	3 (0.66)	0.042	3.54 (0.96–19.48)