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Lack of association between KIR and HLA-C type and susceptibility to idiopathic bronchiectasis

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Summary
Introduction: Idiopathic bronchiectasis is a poorly defined disease characterised by persistent inflammation, infection and progressive lung damage. Natural killer (NK) cells provide a major defense against infection, through the interaction of their surface receptors, including the activating and inhibitory killer immunoglobulin-like receptors (KIR), and human leukocyte antigens (HLA) class I molecules. Homozygosity for HLA-C has been shown in a single study to confer increased genetic susceptibility to idiopathic bronchiectasis. We aimed to assess whether the KIR and HLA repertoire, alone or in combination, may influence the risk of developing idiopathic bronchiectasis, in an independent replication study.
Methods: In this prospective, observational, case-control association study, 79 idiopathic bronchiectasis patients diagnosed following extensive aetiological investigation were compared with 98 anonymous, healthy, age, sex and ethnically-matched controls attending blood donor

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sessions in the same geographical location. DNA extraction was performed according to standardised techniques. Determination of presence or absence of KIR genes was performed by a sequence specific oligonucleotide probe method. Allele frequencies for the proposed KIR, HLA-B and HLA-C risk alleles both individually and in combinations were compared.
Results: We found no significant differences in allele frequency between the idiopathic bronchiectasis and control samples, whether considering HLA-C group homozygosity alone or in combination with the KIR type.
Discussion: Our results do not show an association between HLA-C and KIR and therefore do not confirm previous positive findings. This may be explained by the lower frequency of HLA-C1 group homozygosity in the control population of the previous study (27.2%), compared to 42.3% in our study, which is consistent with the genetic profiling of control groups across the UK. The previous positive association study may therefore have been driven by an anomalous control group. Further larger prospective multicentre replication studies are needed to determine if an association exists.

At a glance commentary

A single centre study was reported in 2006 that linked HLA-C and killer cell immunoglobulin-like receptor (KIR) gene type with predisposition to the development of idiopathic bronchiectasis. This finding has subsequently been cited in numerous reviews of the immunology of bronchiectasis. However, replication studies to confirm or refute this finding have not been performed. Confirmation of this association in a second independent study population would potentially lead to a significant contribution to understanding the pathogenesis of idiopathic bronchiectasis. We have, therefore, conducted a study interrogating the same candidate gene polymorphisms in a population of rigorously phenotyped idiopathic bronchiectasis patients compared with age, gender, ethnicity and geographically-matched controls. Our study was of near identical size to the original work, employing similar well-tested technology. No associations between HLA-C type and idiopathic bronchiectasis were demonstrated, whether considering HLA-C group homozygosity alone or in combination with the KIR type, hence our results did not confirm the previous study findings. The main difference in the two studies appeared to be in the frequency of the control populations. 42% had HLA-C1 group homozygosity in our study, a finding consistent with genetic profiling of control groups across the UK. This compared to 27% in the previous study. Our findings raise the possibility that the previous positive association study may have been driven by an anomalous control group population or may represent geographical differences in the UK. We conclude that further larger multicentre replication studies are needed to confirm or refute that an association exists.

Introduction

Bronchiectasis encompasses a large group of conditions that share pathological dilatation of the bronchi as a consequence of repeated, vicious cycles of infection and inflammation [1]. There is growing awareness of the importance of phenotyping bronchiectasis patients in order to identify modifiable risk factors and to engage these patients in holistic disease management programs [2]. Up to 50% of patients, however, have no defined aetiology for their bronchiectasis and are deemed idiopathic [1,2]. Although evidence clearly indicates a dysregulated immune response in the pathogenesis of bronchiectasis, the pathophysiology of idiopathic bronchiectasis remains poorly elucidated, often resulting in a palliative, untargeted approach to treatment. Previous immunogenetic studies of idiopathic bronchiectasis have demonstrated defects in both innate and adaptive immunity [3—5]. Such patients appear to have shared clinical and radiological features, suggesting a potential interplay between immunogenetic susceptibility, immune dysregulation and chronic bacterial infection [6].

Natural killer (NK) cells are a potent, rapid part of innate immunity to infection and a link to priming of adaptive immunity [7]. NK cells play an important role in the normal functioning of lung host defence through the interaction of their receptors, Killer cell Immunoglobulin-like Receptors (KIR's), with major histocompatibility complex (MHC) class I molecules on target cells [8]. KIR's are cell surface receptors that are polymorphic in both structure and function, with both activating and inhibitory receptor types being expressed on an NK cell [8,9]. KIRs exhibit substantial diversity at both the allelic and haplotypic levels resulting in significant variation in susceptibility to pathogens and disease.

The KIR proteins are members of the immunoglobulin (Ig) superfamily of receptors. Their nomenclature is based on their structure, where the number of Ig-like

extracellular domains (2D or 3D) and the length of the cytoplasmic tail (long, L or short, S) defines the name of the protein [10]. The genes encoding the KIR molecules map in a cluster (19q13.4) that contains all the genes for both activating and inhibitory molecules [7]. KIR haplotypes can broadly be classified as group A or B. The B group of haplotypes are defined by the presence of one or more of the following genes: KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1. Conversely, the group A haplotypes are defined by the absence of all of these genes and the presence of KIR2DS4, the only activating gene in this group. KIR2DS4 has a null allele which has a population frequency of approximately 84%, thus a large proportion of individuals homozygous for haplotype A may express no activating KIR [11].

KIR genes encode receptors that differ in specificity for HLA class I ligands and signalling potential. Well recognised are the specificities of KIR2DL1, 2DL2, 2DL3 and KIR2DS1 and 2DS2 for HLA-C, KIR3DL1 for HLA-B, and KIR3DL2 for HLA-A [10–12]. Both KIR and HLA loci show extreme population diversity and rapid evolution, suggesting that they are under pathogen-mediated selection and that they influence disease outcome at the individual level.

When considering HLA-C molecules as ligands for KIR receptors on NK cells, all HLA-C allotypes can be grouped into two major KIR epitopes on the basis of a dimorphism at position 80 of the α1 domain: asparagine defining HLA-C group 1 and lysine defining HLA-C group 2 alleles. Group 1 alleles bind inhibitory KIRs 2DL2 and 2DL3 and activating KIR2DS2, and group 2 alleles bind inhibitory KIR2DL1 and activating KIR2DS1 [8–11]. KIR3DL1 recognises HLA-B alleles with the Bw4 serological motif (HLA-Bw4), with those with an isoleucine at position 80 (Bw4I) showing stronger inhibition than those with a threonine at this position (Bw4T) [12]. An individual can be homozygous for Bw4T or Bw4I or carry a single copy, heterozygous and carry both, or negative for both. Certain combinations of HLA-C with activating KIRs 2DS1 and 2DS2 have been shown to correlate with autoimmune conditions, leukaemia and inflammatory diseases [4,13–16]. Furthermore, high expression of the highly inhibitory KIR3DL1 associated with HLA-B, along with Bw4-80I ligands, has been shown to correlate with reduced viral loads and slower rate of disease progression in human immunodeficiency virus [17].

Through the interaction with KIRs, HLA-B and C molecules are able to modulate NK-cell function. It has been proposed that binding of HLA-C to activating KIR molecules is 'weaker' than that to inhibitory KIR's, favouring NK cell inhibition in normal circumstances. Infected or diseased cells with abnormal MHC class I expression fail to inhibit NK activity and become targets of NK cell lytic activity [8]. This ligand-receptor pairing is thus unusual in the immune system in-so-far as inheritance of different combinations of these polymorphic germ line sequences in populations imparts differential connectivity to NK-cell activation, with different effector cell outcomes capable of exerting a strong impact on disease susceptibility.

A previous UK study on 96 patients with idiopathic bronchiectasis represents the only publication to date examining the association between KIR and HLA-C type and susceptibility to idiopathic bronchiectasis [4]. This comparison of patients with a similar number of normal controls, concluded that homozygosity for HLA-C group type was associated with increased prevalence of idiopathic bronchiectasis. It was suggested that this homozygosity might result in increased probability of mismatches between KIR and HLA-C ligand with the potential to alter NK cell regulation and function, representing a biologically plausible genetic basis for disease predisposition. However, replication studies to confirm or refute this finding have not been performed. Confirmation of this association in a second independent study would potentially lead to a significant contribution to understanding the pathogenesis of idiopathic bronchiectasis. We have therefore conducted a study interrogating the same candidate gene polymorphisms in a separate population of rigorously phenotyped idiopathic bronchiectasis patients and controls, matched according to age, gender, ethnicity and geographical location to reduce confounding. This study is of near identical size to the original study, employing similar well-tested technology, comparing allele and genotype frequencies for possible associations with KIR, HLA-B and HLA-C risk alleles both individually and in combinations.

Methods

Study design

This regional observational, case-control association study was performed prospectively over a two-year period (October 2006 to August 2008). Ethical approval was obtained from the regional ethics committee. Written informed consent was obtained for all participants. Reporting of this observational study conforms to the recommendations of STROBE [18].

Study population

Our study population consisted of 79 consecutive unrelated patients referred from primary care to two secondary care bronchiectasis clinics in the North East of England at North Tyneside General Hospital and the Freeman Hospital. The consecutive study design meant that both new patients and established clinic attendees were included. Inclusion criteria consisted of adult Caucasian patients, aged 18 years or more, with a confirmed diagnosis of idiopathic bronchiectasis after completion of relevant investigations. Patients with traction bronchiectasis related to interstitial lung disease were excluded. Given the heterogeneity surrounding the aetiology of bronchiectasis, a comprehensive clinical evaluation using history, examination and extensive laboratory investigations with a detailed phenotyping protocol as previously published was performed to distinguish patients with idiopathic bronchiectasis from patients with bronchiectasis of known cause [2]. Patients with bronchiectasis secondary to other causes such as congenital disease, post-infectious aetiology including mycobacterial disease, chronic obstructive pulmonary disease, bronchial asthma, connective tissue disorders, interstitial lung disease, cystic fibrosis, allergic bronchopulmonary aspergillosis, inhalational lung injury, gastro-oesophageal reflux,

immunodeficiency and primary ciliary dyskinesia were excluded.

Control population

The control subjects consisted of 98 anonymous, healthy age gender and ethnically-matched blood donors from the same geographical region (Newcastle upon Tyne, UK). These samples were obtained from an anonymised historical archive of DNA samples from the academic haematology laboratory at Newcastle University where uniform written consent had already been granted for use in this and other studies. No specific respiratory history or lung function results were available from the donors.

HLA-C and KIR gene analysis

Genomic DNA was derived from buccal samples (mouthwash) or whole blood by conventional means (SDS lysis, proteinase K digestion, phenol and chloroform extraction, ethanol precipitation in the presence of ammonium acetate). Determination of the presence or absence of KIR genes was performed by a sequence specific oligonucleotide probe method (SSOP) [15,19,20]. Controls and patients were classified as to whether they were positive for C1 or C2 groups of HLA-C, HLA-B or both, and HLA-Bw4 (Bw4T or Bw4I), or both. The presence of HLA-C1 group, -C2 group and − Bw4 was also determined by the SSOP method. For purposes of this study the HLA-Bw4 motif was only considered on the HLA-B locus. Typing for the presence or absence of multiple activating and inhibitory KIR genes was performed.

Statistical analysis

Allele and genotype frequency was determined by direct counting. Statistical comparison between allele and genotypic frequencies in the study and control groups was performed using chi square or Fisher's exact test using SPSS statistical software. p values less than or equal to 0.05 were considered statistically significant.

Results

A diagnosis of idiopathic bronchiectasis was made in 79 consecutive bronchiectasis patients following careful exclusion of other known causes of bronchiectasis. The mean age of the patients was 65 years (±12.2) with 40 (51%) males and 39 (49%) females. 16 (20%) had a significant smoking history of greater than 10 pack years. Mean FEV1% was 78.0 (25.6). 12 (15%) were chronically colonised with *Pseudomonas aeruginosa*. Table 1 shows the baseline demographics of idiopathic bronchiectasis patients. A comprehensive list of aetiological screening investigations performed is included in Table 2.

HLA-C group 1 was present in 70 (89%) of idiopathic bronchiectasis patients compared to 85 (87%) controls ($p = 0.82$). There was no difference in homozygosity for HLA-C group 1 in idiopathic bronchiectasis patients compared with controls. Comparison of HLA-C group 1 and 2 alleles, whether alone or in combination, demonstrated no statistical differences between the two patient groups (all $p > 0.05$) (Table 3).

Inhibitory KIRs known to be associated with HLA-C include KIR2DL1, 2DL2, 2DL3 and KIR2DS1 and 2DS2. Interrogation of inhibitory KIRs, both alone and in combination with HLA-C, demonstrated no statistical differences between idiopathic bronchiectasis patients and healthy controls (Table 4).

Activating KIRS known to be associated with HLA-C include 2DS1and 2DS2, neither of which were shown to be more prevalent in patients with idiopathic bronchiectasis compared to healthy controls (Table 5).

HLA-Bw4 alleles were present in 42 (54%) of idiopathic bronchiectasis patients compared to 55 (56%) controls ($p = 0.76$) (Table 6). Consideration of the allelic groups of Bw4 revealed no significant differences in homozygosity for HLA-Bw4 in idiopathic bronchiectasis patients compared with controls and no differences between the groups in

Table 1 Baseline demographics and clinical characteristics of idiopathic bronchiectasis patients.

Baseline characteristics	Idiopathic bronchiectasis
n (%)	79
Age, yr (SD)	65.3 (12.2)
Female, *n* (%)	39 (49.4)
>10 Pack year smoking history, *n* (%)	16 (20.2)
FEV1%, mean (SD)	78.0 (25.6)
FVC%, mean (SD)	87.5 (20.7)
Ratio %, mean (SD)	70.7 (10.9)
HRCT total score, mean (SD)	13.0 (10.2)
Sputum volume, ml, mean (SD)	34.4 (53.5)
Chronic pseudomonas colonisation, *n* (%)	12 (15.2)
Exacerbation frequency/ year, mean (SD)	4.44 (3.84)

Table 2 Idiopathic bronchiectasis screening investigations.

Full blood picture including serum eosinophil count
Serum IgG, IgA, IgM, IgG subclasses
Serum IgE
Alpha-1-antitrypsin level
Aspergillus precipitins[a]
Rheumatoid factor
Anti-nuclear antibodies
Antibodies to haemophilus influenza B and pneumococcus
Cystic fibrosis genotyping and/or sweat test[b]
Screening for primary ciliary dyskinesia if indicated
Sputum for culture and sensitivity, smear and culture for AFB

[a] *Aspergillus* skin prick test was performed if elevated eosinophil count, IgE level or positive *Aspergillus* precipitins.
[b] 63 (80%) Patients underwent screening for cystic fibrosis.

Table 3 Frequency of HLA-C alleles in idiopathic bronchiectasis patients and healthy controls.

HLA-C type	Bronchiectasis $n = 79$		Controls $n = 98$	
	n +ve	% +ve	n +ve	% +ve
C1	70	89.7	85	86.7
C1 Homozygous	33	42.3	41	41.8
C2	45	57.7	57	58.2
C2 Homozygous	8	10.3	13	13.3
C1C2	37	47.4	44	44.9

Statistical comparison between genotypic frequencies in the study and control groups was performed using chi square or Fisher's exact test using SPSS statistical software. All p-values were >0.05 and were therefore not statistically significant.

combination with Bw4I or Bw4T ($p = 0.53$ and $p = 0.15$) respectively.

The overall KIR frequency in activating and inhibitory KIRs was not significantly different in patients with idiopathic bronchiectasis compared with control subjects whether considering HLA-C group homozygosity alone or in combination with the KIR type or HLA-B group homozygosity alone or in combination with the KIR type. Proposed inhibitory gene combinations of KIR and HLA-C or HLA-B were found at essentially the same frequency in both groups.

Discussion

Bronchiectasis results from a complex interplay between lung infection and immunity, encompassing facets both of host responsiveness to respiratory bacteria and of inflammatory immunopathogenesis. NK cells play a critical role at the innate–adaptive interface during lung infection, and are implicated in immunity to many of the key pathogens implicated in the respiratory infections of these patients [21,22]. Genotypic analysis of HLA-C and KIR has been used across a wide range of infectious, inflammatory, autoimmune and malignant disease phenotypes to elucidate the potential contribution of NK cell activation programmes in disease susceptibility. Depending on the inheritance of predominantly activating or inhibitory KIR genomic repertoires and on differential inheritance of the cognate HLA-C class I ligands, humans may have an inherent potential for NK cell activation ranged across a spectrum of activation [23]. Susceptibility to viral infection, has tended to be associated with inhibitory genotypes, while susceptibility to autoimmune, inflammatory and neoplastic pathology is often associated with activating genotypes.

Studies analysing potential susceptibility factors for idiopathic bronchiectasis are scarce. The above mentioned UK study performed several years ago demonstrated a potential genetic susceptibility in idiopathic bronchiectasis with significantly increased frequency of HLA-C1 homozygosity in idiopathic bronchiectasis patients compared to

Table 4 Frequency of HLA-C inhibitory KIRs in idiopathic bronchiectasis patients and healthy controls.

Inhibitory KIRs HLA-C	Bronchiectasis $n = 79$		Controls $n = 98$	
	n +ve	% +ve	n + ve	% +ve
2DL1 with C2	43	54.4	52	53.1
2DL1 without C2	32	40.5	40	40.8
2DL2 with C1	40	50.6	51	52
2DL2 without C1	6	7.6	6	6.1
2DL3 with C1	63	79.7	77	78.6
2DL3 without C1	5	6.3	12	12.2

Statistical comparison between genotypic frequencies in the study and control groups was performed using chi square or Fisher's exact test using SPSS statistical software. All p-values were >0.05 and were therefore not statistically significant.

Table 5 Frequency of activating KIRs in idiopathic bronchiectasis patients and healthy controls.

Activating KIRs HLA-C	Bronchiectasis $n = 79$		Controls $n = 98$	
	n +ve	% +ve	n +ve	% +ve
2DS1	35	44.3	33	33.7
2DS2	46	58.2	57	58.2
2DS3	24	30.4	26	26.5
2DS4	75	94	94	95.9
2DS4 non-del	30	38	37	37.8
2DS4 del	59	74.7	79	80.6
2DS5	32	40.5	29	29.6

Statistical comparison between genotypic frequencies in the study and control groups was performed using chi square or Fisher's exact test using SPSS statistical software. All p-values were >0.05 and were therefore not statistically significant.

Table 6 Frequency of HLA-B alleles in idiopathic bronchiectasis patients and healthy controls.

HLA-B type	Bronchiectasis $n = 79$		Controls $n = 98$	
	n +ve	% +ve	n +ve	% +ve
Bw4	42	53.9	55	56.1
Bw4 Homozygous	12	15.4	14	14.3
Bw4T	34	43.6	39	39.8
Bw4I	15	19.2	26	26.5
3DL1	75	94	94	95.9
3DL1 with Bw4	41	52.6	53	54.1
3DL1 without Bw4	33	42.3	41	41.8
3DL1 with Bw4T	34	44.2	38	38.8
3DL1 without Bw4T	38	49.4	56	57.1
3DL1 with Bw4I	13	16.9	24	24.5
3DL1 without Bw4I	62	80.5	70	71.4
3DS1	37	46.8	33	33.7
3DS1 with Bw4	19	24.4	20	20.4
3DS1 without Bw4	16	20.5	13	13.3
3DS1 with Bw4T	14	18	10	10.2
3DS1 without Bw4T	21	26.9	23	23.5
3DS1 with Bw4I	9	11.5	12	12.2
3DS1 without Bw4I	25	32	21	21.4

Statistical comparison between genotypic frequencies in the study and control groups was performed using chi square or Fisher's exact test using SPSS statistical software. All p-values were >0.05 and were therefore not statistically significant.

controls [4]. This has subsequently been cited in review articles [6]. They concluded that approximately 50% of their idiopathic bronchiectasis cohort was HLA-C group 1 homozygous, compared with 25% of controls. The Cw*03 allele was reported to carry a 2·27-fold increased susceptibility risk and the Cw*06 allele a 0·26-fold reduced risk [4].

Our results do not confirm any of the previous findings. In our study, there was no difference in homozygosity for HLA-C group 1 and no significant difference between expression of activating KIRs and HLA-C groups alone or in combination, in idiopathic bronchiectasis patients compared with controls. It is interesting to note that the frequency of HLA-C1 homozygosity in our patients are similar to the frequency of the patients in the previous study with 42% in our study and 49% in the previous study. The main difference, therefore, seems to lie in the frequency of HLA-C1 homozygosity in the control populations of the two studies; 42% having HLA-C1 group homozygosity in the current study whilst it was 27% in the previous study. The (unusally) lower proportion of HLA-C1 homozygosity in the controls of the previous study seems most likely to account for the previously observed positive association.

To try and account for the differences in control populations between the two studies, we examined other available UK data for comparison. The percentage of HLA-C1 homozygosity in a Northern Ireland population ($n = 322$) was 48% and again, 48% in a population from the Orkney Islands ($n = 68$), consistent with that of our study· [23] We also looked at the frequency of HLA-C1 group homozygosity in the last 100 solid organ donors tissue typed at our centre: 49% were HLA-C1 homozygous, with 23% carrying HLA-Cw03. This compares to 27% HLA-C1 homozygous in the previous study, with 10% carrying HLA-Cw03. Furthermore we examined data available on the worldwide web; www.allelefrequencies.net of comparable English populations [24]. Although this data did not make available HLA-C1 homozygosity frequencies, we were able to determine that in data of blood donors taken at various UK centres, the ratio of HLA-C1 group frequency to HLA-C2 group frequency was 1.64 (Newcastle $n = 2739$), 1.72 (Leeds $n = 5024$), 1.75 (Liverpool $n = 1343$), 1.77 (Manchester $n = 1640$) and 1.79 (Lancaster $n = 545$) [24]. However the ratio in the original study of Boyton et al. was 0.95 in controls compared to 1.92 in patients. The main differences in frequencies of individuals with the HLA-Cw allele families between the Heart/Lung donors in the study by Boyton et al. and the blood donors was in HLA-Cw03 — 10% in the former compared to range of 23—28% in blood donors from the various UK donation centres.

There are a number of other possible reasons why our work differs from that of Boyton et al. Significant variation between age and gender between the two study groups was observed in the previous study (55 ± 1.4 years; 32% male in idiopathic bronchiectasis patients versus 24.8 ± 1.4 year; 73% male in control patients), which may also have contributed to the positive association found. Considerable variation in the composition of KIR haplotypes with geography and ethnicity has been noted. The significance of the interesting observation that some populations, such as aboriginal Australians who have very high prevalence of bronchiectasis and have some of the most activating KIR haplotypes has yet to be elucidated [25]. The difference outlined between the two studies described may therefore relate to geographical variations among control populations, perhaps with population differences in the North and the South of the UK. There is further diversity and complexity due to the fact that different individuals within a given population will carry different numbers of activating and inhibitory KIR genes and different polymorphic variants of these genes [9]. Furthermore, the impact of the KIR repertoires will vary depending on whether a given individual also carries the cognate HLA class I polymorphic variant with which the KIRs interact. Finally, there is variegated expression of KIR gene products, such that they are differentially expressed on the NK cells (and T cells) within each individual [6]. All of these factors are important considerations and, although our study cannot conclude with certainty that there is no obvious genetic susceptibility towards the development of idiopathic bronchiectasis, we have opened up the area to debate, likely prompting further investigation into the pathophysiology of this disease process to corroborate these findings.

Although precautions were taken to ensure as robust a study design as possible, there remain a number of inherent limitations to our study. Firstly our sample size is small; we failed to recruit as many idiopathic bronchiectasis patients as we initially estimated would be possible, reflecting the stringent phenotyping protocol; this may raise the possibility of a false negative result being obtained through underpowering of the study. Despite this, however, our study is of similar sample size and design to the previous study and is therefore comparable. Secondly our patients were all of Caucasian origin recruited from one area in the North East of England potentially limiting the generalisability of our results to other worldwide populations. Genetic association studies have progressed since these two studies were originally performed, but the similar sample and control sizes should enable this study to serve as a standard replication study in an independent patient cohort and may serve to encourage topical debate, which can inform further, larger studies. To date no large bronchiectasis genetic association studies have been conducted due to funding difficulties. Such studies with multicentre recruitment using standardized phenotyping protocol are now recognised as a priority [3,26]. This study recruited from two geographically close centres and a challenge is now to extend such studies to international multicentre studies.

In conclusion, we found no association between KIR and HLA type B or C and idiopathic bronchiectasis. We suggest the original report of this association was due to unusual genotype frequencies in the control population that are atypical as compared to other UK populations. Collectively these data cannot provide a definitive assessment of KIR and NK cell biology in bronchiectasis and we suggest caution is warranted regarding conclusions that available "immunogenetic analysis predicts a pathogenic role for excessive NK cell activation in bronchiectasis" [2]. The availability of techniques that factor in both polymorphic KIR variability and copy number variability will add to the usefulness and clinical application of these studies [9,27]. However, further larger prospective multi-centre replication studies to define the genetic risk factors associated with idiopathic bronchiectasis are needed.

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Conflict of interest

The authors report no known conflicts of interest relevant to this manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2014.05.017.

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