

Polymorphism in intron 1 of the interferon-gamma gene influences both serum immunoglobulin E levels and the risk for chronic hepatitis B virus infection in Polynesians

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Abstract Intron 1 of the interferon-gamma (IFNG) gene contains two polymorphisms. The 12 CA-repeat allele of the +875 IFNGCA microsatellite and the T allele of the +A874T single nucleotide polymorphism (SNP) have been associated with increased in vitro IFNG production and a variety of clinical phenotypes. The purpose of this study was to determine whether these polymorphisms influence total serum IgE levels [tsIgE] and the outcome of a hepatitis B virus (HBV) infection. IFNGCA and +A874T were typed in 186 asthmatics of Niuean ancestry and in Polynesian women with a chronic HBV infection ($n=60$) and with natural immunity to the HBV ($n=66$). The IFNGCA genotype was associated with [tsIgE] in asthmatic children ($n=51$, $p=0.004$) but not adults ($n=135$, $p=0.87$). The data were consistent with a co-dominant influence of the 12 CA-repeat allele on high [tsIgE]. The IFNGCA genotype was also associated with the risk for chronic HBV infection ($\chi^2=11.6$, $p=0.003$) because of a dominant effect of the 12 CA-repeat allele on developing natural immunity in

homozygotes (OR=5.8, $p=0.003$) and heterozygotes (OR=2.7, $p=0.01$). Similar associations were found for the T allele of the +A874T SNP. The possibility that these associations were due to linked alleles in the adjacent 783 bp of the promoter and 3'-untranslated region of the IFNG gene was excluded by direct sequencing. In summary, high-IFNG-producing alleles in intron 1 of the IFNG locus are associated with high [tsIgE] in asthmatic children from Niue and with natural immunity to the HBV in Polynesian women. These findings are consistent with a previous report of an association between +875 IFNGCA and [tsIgE] and provide preliminary evidence of a new association with the outcome of an HBV infection.

Keywords Interferon gamma · Immunoglobulin E · Hepatitis B virus · Niue · Polynesia

Introduction

The alleles of a microsatellite polymorphism in intron 1 of the human interferon-gamma (IFNG) gene (known as +875 IFNGCA, IFNGCA or AF031844) have been associated with variation in in vitro IFNG secretion from mitogen-stimulated peripheral blood mononuclear cells (PBMC; Pravica et al. 1999), suggesting that this locus contributes to the heritability of IFNG production (Hohler et al. 2005). The highest levels of IFNG secretion have been associated with an allele with 12 CA repeats. This allele is in linkage disequilibrium with the T allele of the immediately adjacent +A874T single nucleotide polymorphism (SNP), which is part of a possible nuclear factor kappa B binding site (Pravica et al. 2000). IFNG has regulatory and effector functions within the innate and acquired human immune systems, and there is evidence that intron 1 polymorphisms

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influence phenotypes related to infection outcome (Bravo et al. 2003; Cabantous et al. 2005; Etokebe et al. 2006; Lio et al. 2002; Lopez-Maderuelo et al. 2003; Rossouw et al. 2003; Tso et al. 2005), immunoglobulin production (Demenais et al. 2002; Masutani et al. 2003), alloantigen immunity (Asderakis et al. 2001; Cavet et al. 2001; Daher et al. 2003; Prigoshin et al. 2004) and autoimmunity (Goris et al. 1999; Spriewald et al. 2005; Vandebroek et al. 2003).

Thus, these two polymorphisms have a significant influence on a wide range of phenotypes, some of which are associated with high odds ratios. The high odds ratios and high allele frequencies of the two polymorphisms indicate that significant associations with clinical phenotypes may be detectable with small numbers of subjects (Zondervan and Cardon 2004). Thus, they would be suitable for studies in Polynesia, where small population size limits the number of subjects available. We are reporting the results of two studies conducted in the Polynesian community in Auckland, New Zealand. The purpose of the first study was to confirm the previously reported relationship of high-IFNG-secreting alleles with increased serum IgE concentrations (Demenais et al. 2002). The statistical significance of this association was $p < 0.001$, which is sufficiently strong to justify follow-up studies under the BADGE criteria for assessing reproducibility suggested by Manly (2005). The purpose of the second study was to determine whether these polymorphisms influence the risk for chronic hepatitis B virus (HBV) infection.

Materials and methods

These studies were approved by the Auckland Ethics Committees of the New Zealand Ministry of Health, and a written, informed consent was obtained from all subjects and/or their guardians.

Subjects for the IgE study

The phenotypes of the 216 asthmatic Niue Islanders who were recruited into this study have been described in detail elsewhere (Abbott et al. 2004). Briefly, we recruited 216 Niue Islanders with physician-diagnosed asthma that persisted after their sixth birthday and that was not secondary to other medical conditions or to cigarette smoking. The 6 years of age criterion was chosen to minimise the number of subjects with the 'transient early wheezing' phenotype (Martinez et al. 1995). The subjects were classified as atopic or non-atopic on the basis of a skin test to house dust mite, mixed grass and cat aeroallergens.

Subjects for the study of chronic HBV infection

Sixty Polynesian women with chronic HBV infection (two positive tests for HBsAg at least 6 months apart) and 66 Polynesian women who were sero-positive for the anti-HBs and anti-HBc antibodies (indicating a successful immune response to a naturally occurring HBV exposure) were sequentially recruited from the ante-natal clinics of the National Women's Hospital, Auckland, New Zealand. The ages and ethnicities of these subjects are summarised in Table 1.

Methods

Total serum IgE concentrations

Serum was stored at -70°C and total serum IgE concentrations ([tsIgE]) were measured by enzyme-linked immunosorbent assay (ALerCHEK, Portland, ME) following the manufacturer's instructions. Sera with [tsIgE] exceeding the highest standard included in the kit were repeated with serum diluted 1:9. Serum for measurement of [tsIgE] was available on 187 of the 216 asthmatic subjects, including 52 children (aged 6–11 years) and 135 adults and adolescents (aged 12–86 years). One child with a [tsIgE] of more than 3,500 IU/ml was excluded from the analyses. The range of [tsIgE] in the remaining subjects was from 2 to 988 IU/ml, with the widest variance in the children (Abbott et al. 2004).

Genomic DNA

Genomic DNA was extracted from PBMCs using a proteinase K/phenol/chloroform method and diluted to 50 ng/ μl .

Table 1 Polynesian subjects recruited for the study of chronic HBV infection

		Chronic HBV infection ($n=60$)	Natural immunity to HBV ($n=66$)
Age	Mean $\pm$ SEM	27.1 $\pm$ 0.7	27.9 $\pm$ 0.7
	Range	19–38	18–42
Ethnicity	Cook Island	6	4
	Maori	11	14
	Niue Island	4	9
	Samoa	15	18
	Tonga	24	21
Gender	Male/female	0/60	0/66

Genotyping of the intron 1 polymorphisms of the IFNG gene

The IFNGCA microsatellite was amplified by polymerase chain reaction (PCR) using 5'-Fam-tcttacaacacaaatcaaa and 5'-gccttctgtagggattat as primers, with the PCR conditions as described in the Human GDB Genome Database (<http://www.gdb.org/>). PCR products were analysed on an ABI Prism™ 377 DNA sequencer (Applied Biosystems, Foster City, CA). The genotype of the +874*A/T SNP in each individual was obtained using an amplification refractory mutational system assay (Pravica et al. 2000).

Re-sequencing of the IFNG gene

Primers for re-sequencing were designed using the published IFNG sequence AF375790 (SeattleSNPs 2001). The promoter region was amplified using the primers 5'-ctgaagtactatttgggatatttggttaaa and 5'-acagccaagagaaccacaaacgat. The 878-bp amplicon extended from the MER33 repeat region into exon 1, including 783 bp of the promoter upstream of the translation start site, the transcription and translation start sites and four known SNPs. The 3'-untranslated region (3'-UTR) of the IFNG gene was amplified using the primers 5'-ggtaatacggtcatttttaaaggaa and 5'-agccaacaaatgctagtggaaa. The 997-bp amplicon includes the complete exon 4 of IFNG and one known SNP. An amplicon including both intron 1 polymorphisms was generated using the primers 5'-cttaattctctcgaaac and 5'-gccttctgtagggattat. The resulting PCR products were sequenced using the original amplification primers using ABI Prism BigDye Terminator v3.1 cycle sequencing mix (Applied Biosystems) on an ABI Prism genetic analyser 3100 (Applied Biosystems).

Statistics

Serum IgE concentrations (presented as the mean $\pm$ SEM) were normalised by log transformation for statistical analyses. We used the general linear model (Proc GLM procedure in SAS) to perform univariate and multivariate analyses of the influence of independent variables (including recessive and co-dominant models of inheritance) on log serum IgE concentrations, with p values for type III sums of squares reported for multivariate analyses. Tukey's studentised range (honestly significant difference) test was used to assess differences between means. We also used the Type1 option of the Proc Genmod procedure in SAS to determine whether a co-dominant model of inheritance improved the fit of a recessive model. An F test was used to compare variances between groups. Frequency data were analysed using the χ^2 and the Cochran–Mantel–Haenszel

tests, and homogeneity of odds ratios was assessed using the Breslow–Day test. Odds ratios are presented with 95% confidence limits. These statistical procedures were carried out using SAS (SAS Institute, Cary, NC). Haplotype frequencies from genotypic data of unknown gametic phase were calculated using the expectation–maximization (EM) algorithm (Schneider et al. 2000).

Results

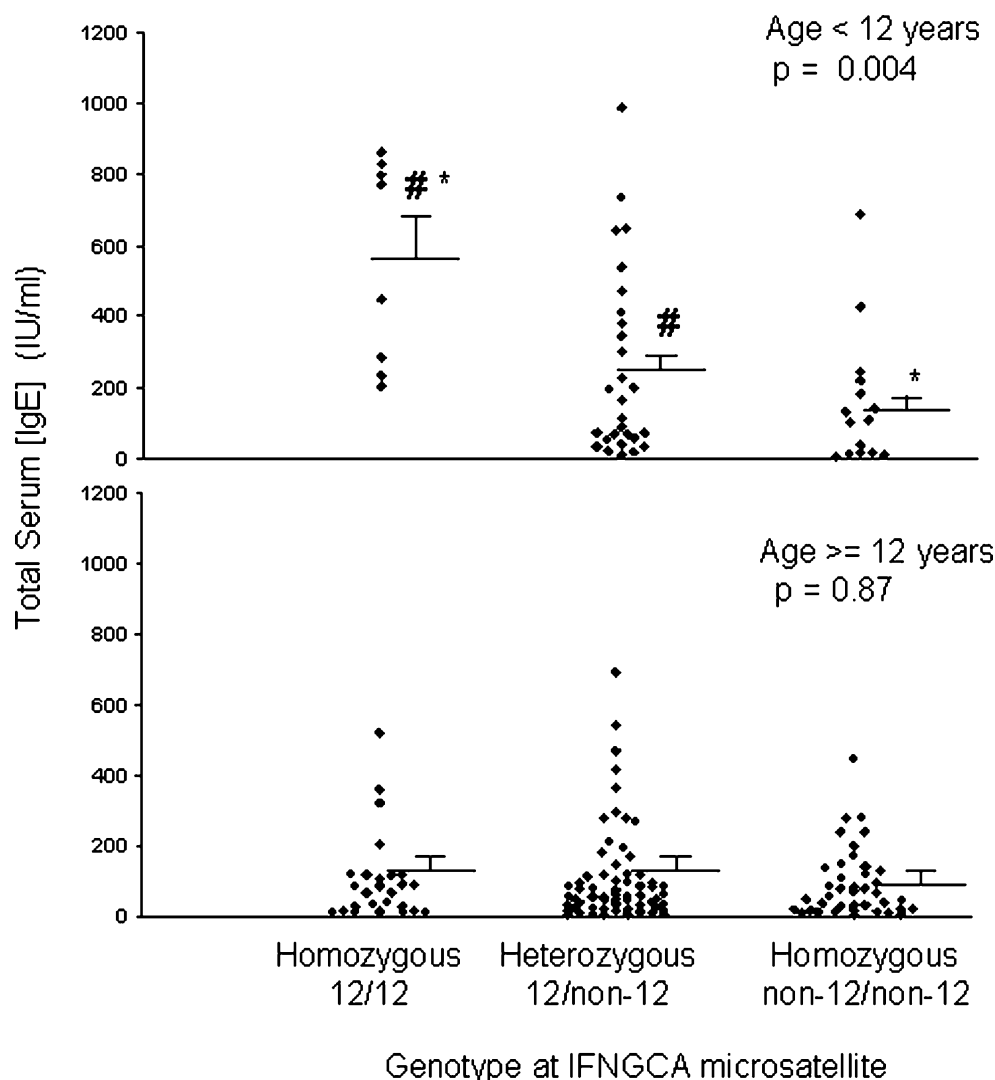
Our analyses are focused on the 12 CA-repeat allele of the IFNGCA microsatellite, as this allele is consistently associated in the literature with high IFNG production and/or phenotypes that are expected to be associated with high IFNG production. Thus, individual subjects are classified as being either homozygous for the 12 CA-repeat allele, homozygous for a non-12 CA-repeat allele (either 13, 14 or 15 CA-repeats in our populations) or being heterozygous for the 12 CA-repeat and a non-12 CA-repeat allele.

IFNG intron 1 polymorphisms and [tsIgE] in asthmatic Niue Islanders

There was no effect of IFNGCA genotype on [tsIgE] in the full dataset of 186 subjects ($p=0.21$). As both the mean ($p<0.0001$) and the variance ($p<0.0001$) of the [tsIgE] are lower in adults than in children, we analysed the two age groups separately. Figure 1 shows the influence of IFNGCA genotype on [tsIgE] in the children aged less than 12 years ($n=51$) and the adults ($n=135$).

In asthmatic children, the IFNGCA genotype was significantly associated with [tsIgE] (analysis of variance, $p=0.004$), and this accounted for 20.0% of the variance in [tsIgE]. On multivariate analysis, this effect was independent ($p=0.004$) of atopy status ($p=0.0008$) and gender ($p=0.60$). Atopy status was retained as a term in all subsequent multivariate analyses. Asthmatic children who were homozygous for the 12 CA-repeat allele had higher [tsIgE] than either the heterozygous children (555 ± 102 vs 251 ± 49 IU/ml, $p<0.05$) or the children who were homozygous for a non-12 CA-repeat allele (555 ± 102 vs 157 ± 48 IU/ml, $p<0.05$). The data were consistent with both co-dominant ($F=6.1$, $p=0.004$) and recessive ($F=9.5$, $p=0.003$) influences of the 12 CA-repeat allele on [tsIgE]. However, the percent of the variance in [tsIgE] accounted for by the co-dominant model of inheritance ($r^2=0.41$) was higher than that accounted for by the recessive model ($r^2=0.35$). The effect of atopy status on serum IgE ($F=19.9$, $p<0.0001$) was sequentially improved (using Proc Genmod) by adding the influence of 12 CA-repeat homozygotes ($F=7.9$, $p=0.005$) and then heterozygotes ($F=4.7$, $p=0.02$) to the

Fig. 1 The influence of the genotype of the IFNGCA microsatellite on [tsIgE] (mean $\pm$ SEM IU/ml) is shown for asthmatic children (age < 12 years) and asthmatic adults (age $\geq$ 12 years). Means that are significantly different at the $p < 0.05$ level are indicated by having the same symbol (*pound sign* or *asterisk*)



model. Although none of the eight non-atopic asthmatic children, who had low [tsIgE], had the homozygous 12 CA-repeat genotype, this did not reach statistical significance ($p=0.32$). The influence of the +874*A/T SNP genotype on tsIgE was less significant ($p=0.04$) than the influence of the IFNGCA microsatellite in the children, with the highest [tsIgE] associated with the T/T genotype (data not shown).

There was no influence of the IFNGCA ($p=0.87$) genotype on [tsIgE] in the adult group.

IFNG intron 1 polymorphisms and risk for chronic HBV infection

Table 2 shows the IFNGCA genotype distributions for the chronic HBV infection subjects ($n=60$) and the naturally immune control subjects ($n=66$). There was a significant influence of IFNGCA genotype on the outcome of a HBV infection ($\chi^2=11.6$, $p=0.003$), and this effect was similar after adjusting for ethnicity using the Cochran–Mantel–

Haenszel statistic ($p=0.002$). The odds ratios for developing a successful immune response to the HBV infection were increased in subjects who were both heterozygous (OR=2.7 [1.2–5.8], $p=0.01$) and homozygous (OR=5.8 [1.7–19.7], $p=0.003$) for the high-IFNG-producing 12 CA-repeat allele. There was no evidence for heterogeneity of the odds ratios between the five ethnic groups in either the heterozygous (Breslow–Day test, $p=0.73$) or homozygous ($p=0.76$) comparisons. The odds ratio for the comparison between the groups that were homozygous and heterozygous for the 12 CA-repeat allele did not reach statistical significance (OR=2.2 [0.62–7.6], $p=0.22$). This is consistent with a dominant effect of the 12 CA-repeat allele on the odds of having a successful immune response to a HBV infection. Table 3 shows the false-positive report probability (FPRP) calculations (Wacholder et al. 2004) for our data. Bold numbers refer to probabilities that are noteworthy at the 0.5 level, indicating that there is less than a 50% chance that our results are irreproducible because of low prior probability of association.

Table 2 Distribution and frequency of IFNGCA microsatellite genotypes in subjects with a chronic HBV infection and control subjects who were sero-positive for both the anti-HBs and anti-HBc antibodies

Genotype	Case, <i>n</i> (freq)	Control, <i>n</i> (freq)	Odds ratio	<i>p</i>	Breslow–Day, <i>p</i>
12 CA/12 CA	4 (0.07)	14 (0.21)	5.8 (1.7–19.7)	0.003	0.76
12 CA/non-12 CA	18 (0.30)	29 (0.44)	2.7 (1.2–5.8)	0.01	0.73
non-12 CA/non-12 CA	38 (0.63)	23 (0.35)	1		

The influence of the +874*A/T SNP genotype on risk for chronic HBV infection was less significant (Cochran–Mantel–Haenszel statistic, $p=0.009$) than the influence of the IFNGCA microsatellite, with the highest odds ratio associated with the T/T genotype (data not shown).

Haplotypes between the microsatellite and SNP alleles

The initial descriptions of the +875 IFNGCA microsatellite and the +874*T/A SNP indicated that the 12 CA-repeat allele of the microsatellite and the T allele of the SNP were in complete and exclusive linkage disequilibrium (Pravica et al. 2000), and thus would be expected to produce the same results in our two association studies. However, the significances of the associations with [tsIgE] and chronic HBV infection risk were both higher for the microsatellite than the SNP, indicating the existence of a new haplotype(s) in our populations. A haplotype consisting of the 13 CA-repeat microsatellite allele and the T SNP allele was identified in the Niue Island dataset using the EM algorithm (Schneider et al. 2000) and was confirmed in both datasets by direct sequencing of homozygous and heterozygous subjects. In addition, direct sequencing identified one haplotype consisting of the T allele of the 874*T/A SNP and an IFNGCA allele with 15 CA repeats.

Other SNPs in the IFNG Locus

We re-sequenced the adjacent promoter and 3'-UTRs of the IFNG gene in 50 Niuean children with asthma to determine

whether alleles at other known SNP loci within IFNG might contribute to these genetic effects. We did not detect any previously published SNP polymorphisms in the promoter (rs2069708, rs3814242, rs2069709 [-179GT], rs2069710 [-155GA]) of these subjects. There were no SNP polymorphisms detected either within or following (rs2069724) the 3'-UTR of these subjects. We detected a new C/A SNP (-25CA) with minor A allele frequency of 0.05 in the 5'-UTR of the promoter region at position -25 relative to the translation start site. We also sequenced the adjacent IFNG promoter region in 66 Polynesian women from the hepatitis B study, and two women (one Tongan, one Samoan) were heterozygous for -25CA, indicating that it is widespread in Polynesian populations.

Discussion

The microsatellite and SNP polymorphisms in intron 1 of the IFNG gene are present in Europeans (Pravica et al. 1999, 2000), Asians (Nagarkatti et al. 2002; Tso et al. 2005) and Africans (Cabantous et al. 2005) and have been associated with a wide spectrum of clinical phenotypes. However, association studies can produce false-positive results due to a low prior probability of association (Manly 2005; Wacholder et al. 2004), admixture, stratification, sampling error and ethnic variations in either linkage disequilibrium or genetic and environmental factors. Thus, there is a need to increase the confidence that these associations reflect genuine genetic effects. This can be achieved by either replicating the association in other ethnic groups, by demonstrating linkage of a specific allele to a phenotype or by studies of the molecular mechanisms that might be responsible for the association.

Previously published segregation analyses of [tsIgE] have shown strong genetic influences, with reported heritabilities of 26% (Mathias et al. 2005), 47% (Palmer et al. 2000), 49.5% (Blumenthal et al. 1981), 43% (Gerrard et al. 1978) and between 40 and 50% (Grundbacher 1975). Bazaral et al. (1971) reported higher heritability in children (79%) than adults (59%). Influences of both dominant (Blumenthal et al. 1981; Sampogna et al. 2000) and recessive (Gerrard et al. 1978; Palmer et al. 2001; Xu et al. 2000) major genes, as well as polygenic influences

Table 3 FPRP for our association between the IFNGCA polymorphism and the risk for chronic HBV infection (Wacholder et al. 2004)

Prior probability	12 CA-repeat heterozygotes possible odds ratios			12 CA-repeat homozygotes possible odds ratios		
	1.5	2.0	3.0	2.0	4.0	6.0
0.67	0.075	0.024	0.009	0.051	0.009	0.005
0.2	0.398	0.165	0.067	0.306	0.066	0.036
0.1	0.598	0.307	0.139	0.498	0.136	0.077
0.05	0.758	0.484	0.254	0.677	0.250	0.150
0.01	0.942	0.830	0.640	0.916	0.635	0.479
0.001	0.994	0.980	0.947	0.991	0.946	0.903

(Bouzigon et al. 2004; Gerrard et al. 1978), have been described. Our data show that polymorphism in the IFNG locus accounts for 20% of the variance in total serum IgE levels in asthmatic Niuean children, and the data were most consistent with a co-dominant influence of the 12 CA-repeat allele of IFNGCA on [tsIgE]. Demenais et al. (2002), in data presented in an abstract, found that 4% of the variance in serum IgE levels was accounted for by the IFNGCA microsatellite, which is lower than the estimate of 20% found in our study. Seventy-one percent of the asthmatic subjects studied by Demenais et al. were described as children between the ages of 8 and 15 years (Dizier et al. 2000), and thus they studied a group of subjects who were older than our Niuean children. If IFNGCA has a stronger influence on serum [IgE] in children than in adults, this age difference might account for the difference in the percent of variance in serum [IgE] accounted for by the two studies. Another possibility is that the low levels of genetic diversity found in Niue Islanders (Abbott et al. 2006) might eliminate the contribution of uncommon alleles that have been lost as a result of genetic drift from other regulatory loci, allowing the IFNGCA polymorphism to contribute a higher proportion of the genetic variance.

The shortest IFNGCA microsatellite allele in this population, with 12 CA repeats, was associated with higher levels of serum IgE than longer alleles with 13–15 repeats. This is also consistent with the results of Demenais et al. It is also of interest that high in vitro IFNG production in children has been associated with asthma-associated phenotypes such as high serum IgE levels (Smart et al. 2002), aeroallergen-induced wheal size (Heaton et al. 2005) and bronchial hyperresponsiveness (Heaton et al. 2005), as this 12 CA-repeat allele is also associated with high in vitro IFNG production (Pravica et al. 1999). However, there is still a need for data establishing the molecular mechanisms responsible for this association as well as evidence for linkage. The current lack of convincing reports of linkage of this locus to serum IgE levels could be due to lack of statistical power to detect an association accounting for only 4% of the variance in populations studied so far.

A higher rate of concordance for developing a chronic HBV infection in monozygotic relative to dizygotic twins (Lin et al. 1989) is consistent with a genetic influence on the risk for developing a chronic HBV infection. Replicated associations between the risk of chronicity and HLA-DRB1*13 (Ahn et al. 2000; Thio et al. 2003; Thursz et al. 1995) and HLA-DRB1*09 (Ahn et al. 2000; Meng et al. 2003) alleles provide evidence that the HLA class II locus is responsible for some of this effect. Preliminary associations have also been found with alleles in the HLA class I (Thio et al. 2003), vitamin D receptor (Bellamy et al. 1999) and tumor necrosis factor- α (Hohler et al. 1998) loci. Our data are consistent with the possibility that the high-

IFNG-producing alleles of IFNGCA and +A874T increase the probability of having a successful immune response to a naturally acquired HBV infection and adds to the list of genetic loci with preliminary evidence for genetic association. Although the significance level ($p=0.002$) for our association is not high enough to be considered reproducible according to the criteria of Manly (2005), these criteria are more suitable for associations discovered during a whole genome screen, where all haplotypes are considered to have the same low prior probability of association. The approach of Wacholder et al. (2004) is probably more appropriate for our study, as it allows estimates of prior probability of association from existing data to be considered, including diseases with related pathophysiology. For example, IFNG is a component of the immune responses to both tuberculosis and the HBV (Guidotti et al. 1996; Maini et al. 1999; Rossol et al. 1997). The initial report of association between tuberculosis susceptibility and the intron 1 polymorphisms of IFNG was significant at $p=0.02$ (Lio et al. 2002) and has been reproduced by four (Etokebe et al. 2006; Lopez-Maderuelo et al. 2003; Rossouw et al. 2003; Tso et al. 2005) and missed by two (Cooke et al. 2006; Fitness et al. 2004) subsequent studies, for a reproducibility rate of 0.67. Table 3 shows that if the prior probability of association with chronic HBV infection is as low as 0.05, there is still an acceptable probability of this being a false-positive report.

It should also be noted that this study was carried out with a relatively homogeneous group of subjects who were all female, Polynesian and of child-bearing age. The advantage of using homogeneous groups of subjects for genetic association studies is that the influence of environmental factors on the phenotype is reduced resulting in higher odds ratios for the association that are more readily detected (Zondervan and Cardon 2004). Thus, it should not be assumed that this finding applies to men or to other ethnic groups, especially as gender (Wright 2006) and ethnicity (Milne et al. 1987) are known to influence the outcome of HBV infections. It is even possible that these IFNG polymorphisms are involved in mediating these gender- and ethnicity-related effects, although further studies would be needed to demonstrate this. For example, it is possible that sex hormones or environmental influences influence the activity of signalling pathways that regulate IFNG production in such a way as to either magnify or cancel out the effects of the 12 CA-repeat allele. It is unlikely that this effect was detected because of reduced genetic diversity in the study population, as most study subjects were of Maori, Cook Island, Samoan and Tongan origins. Admixture with Melanesian and European ethnic groups is common in these Polynesian groups (Abbott et al. 2006), and they do not have the reduced level of genetic diversity found in Niue Islanders.

Most exposures to the HBV in Polynesian populations occur in childhood (Milne et al. 1987; Wainwright et al. 1986; Williamson et al. 1985), and the outcome of the infection in terms of chronicity is also determined at this time. The influence of the IFNG polymorphisms on IgE levels was also most easily detected in children. This raises the possibility that these polymorphisms have a stronger influence on the outcome of immune responses in children than in adults, and thus may contribute to the higher risk of developing a chronic HBV infection in children. Reports showing that high levels of genetically influenced IFNG production protect against both chronic tuberculosis (where initial exposures may have occurred in children; Lio et al. 2002; Tso et al. 2005) and cerebral malaria in children (Cabantous et al. 2005) lend some support to this speculation. The significance of this possibility is that further studies of this genetic effect may be most effective if carried out in phenotypes that are defined in childhood or in immune cells obtained from children.

It is still not clear whether intron 1 polymorphisms in the IFNG gene are directly responsible for regulating IFNG production or whether they are in linkage disequilibrium with other regulatory genetic loci. Our sequencing data exclude the possibility that the potentially functional -179GT (Bream et al. 2002) and -155AG (Chevallard et al. 2002) polymorphisms in the promoter region of the IFNG gene are necessary for a genetic influence on IFNG production to occur, as they were not present in any of our Niue Island subjects and were not detected in a sub-sample of pregnant women from other Polynesian islands. Although we did detect one new SNP in the IFNG promoter, it was not common enough to be responsible for the phenotypic effects reported. We have also excluded the possibility that SNPs in the 3'-UTR of the IFNG gene are directly responsible for these genetic associations. However, it is still possible that there are functional, but more distant, alleles at polymorphisms in the intronic DNA surrounding the IFNG locus that are in linkage disequilibrium with the 12 CA-repeat allele of IFNGCA (Cooke et al. 2006).

In summary, the polymorphisms in intron 1 of the IFNG gene are of interest because they may have a significant influence on a wide spectrum of clinically significant immune responses. An understanding of the molecular mechanisms that translate these genetic variations into different clinical phenotypes may lead to insights that are of therapeutic significance to a range of clinical situations. For example, it might be worthwhile designing an adjuvant for a therapeutic vaccine for chronic hepatitis B that optimised IFNG production. Our data increase the number of ethnic groups in which an influence of these ancient genetic polymorphisms has been demonstrated, are consistent with a previous study showing an association with total serum IgE levels and demonstrate a new disease association with chronic HBV infection. We also

show that the effect of these intron 1 polymorphisms is independent of polymorphism in the adjacent promoter and 3'-UTR of the IFNG gene, and we have identified two new intron 1 haplotypes. The possibility that these polymorphisms have more influence on phenotypes that are determined in childhood is of interest, as future studies of the molecular mechanisms underlying these associations may be more effective in younger subjects.

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