



BRIEF COMMUNICATION

Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus

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Interleukin-10 (IL-10) is a pivotal immunoregulatory cytokine, influencing many aspects of the immune response. The IL-10 gene is located on chromosome 1 at 1q31-32 and is highly polymorphic. One microsatellite and three single nucleotide polymorphisms (SNPs) have been recorded within the 1.2 kb immediately upstream of the gene, with an additional microsatellite present at 4 kb upstream. The relationship between these two classes of polymorphism is poorly defined in the IL-10 gene. Haplotypes have been presented comprising alleles from the two microsatellite loci, and independently from the three SNPs, but these have not yet been brought together to define unified haplotypes. In the present report we describe the 29 IL-10 haplotypes found in 56 Dutch European families and show that they fall into four major haplotype groups, each of which spans the 4 kb upstream of the IL-10 gene and has a different distribution of IL10.G alleles. In addition, we describe three novel single nucleotide polymorphisms in the human IL-10 gene and suggest how they relate to these four haplotype families.

Keywords: IL-10; haplotype; polymorphism; interleukin; cytokine secretion

Interleukin-10 (IL-10) is an important immunoregulatory cytokine in man.¹ It is involved in the regulation of inflammatory responses through direct influence over tumour necrosis factor production.^{2,3} IL-10 is also involved in the pathology of human autoimmune disease,^{4–6} particularly in the dysregulation of B cell function in systemic lupus erythematosus leading to autoantibody production.^{7,8} In addition, its ability to induce T cell anergy⁹ and inhibit major histocompatibility complex (MHC) class-I expression¹⁰ may be important in its apparent contribution to tumour-related immunosuppression.^{11–13} IL-10 also plays an important role in the development of infectious disease. Recently, it has been shown to affect macrophage responses during mycobacterial infections.¹⁴ Furthermore, the severity to which an infection progresses is associated with serum IL-10 levels;¹⁵ recently, high serum IL-10 was observed in patients with a poor or fatal outcome to meningococcal meningitis, while patients who had mild disease and a good prognosis had lower serum IL-10 levels.¹⁶

A recent study demonstrated striking differences between individuals in their ability to produce IL-10 fol-

lowing lipopolysaccharide (LPS) stimulation of whole blood cultures *in vitro*, from first-degree family members,¹⁷ suggesting that differences in IL-10 production contained a considerable hereditary component. This was shown to be attributable in part to the structure of the IL-10 locus, as defined by haplotypes containing alleles from the two microsatellite loci.¹⁸

We and others have demonstrated that both microsatellites^{19,20} and point mutations^{21,22} exist within the human IL-10 gene; these (and other novel single nucleotide polymorphisms (SNPs) described below) are illustrated in Figure 1. The numbering shown in this figure is relative to the IL-10 promoter sequence X78437. We have previously demonstrated that strong associations exist within allele groups; for example, between the IL10.R2 and IL10.G13 alleles ($P_c < 0.00003$) and the IL10.R3 and IL10.G9 alleles ($P_c < 0.0012$).²⁰ This contribution was also shown to be related to the rare combination of alleles at the –597 and –824 SNPs ($P_c < 0.025$). In addition, Turner *et al*²² have shown strong associations between the alleles of the three proximal SNPs which predict that these three independent bi-allelic markers will fall into three haplotypes.

Recently, we studied the various genetic markers present in the 4 kb upstream of IL-10 in a population of 56 families from The Netherlands.¹⁸ One hundred and seven unrelated individuals from these families were genotyped at the IL10.R and IL-10.G microsatellite loci and at the three SNPs; in total, full genotypes for all five

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Joyce Eskdale was supported in part by the Breast Cancer Campaign.

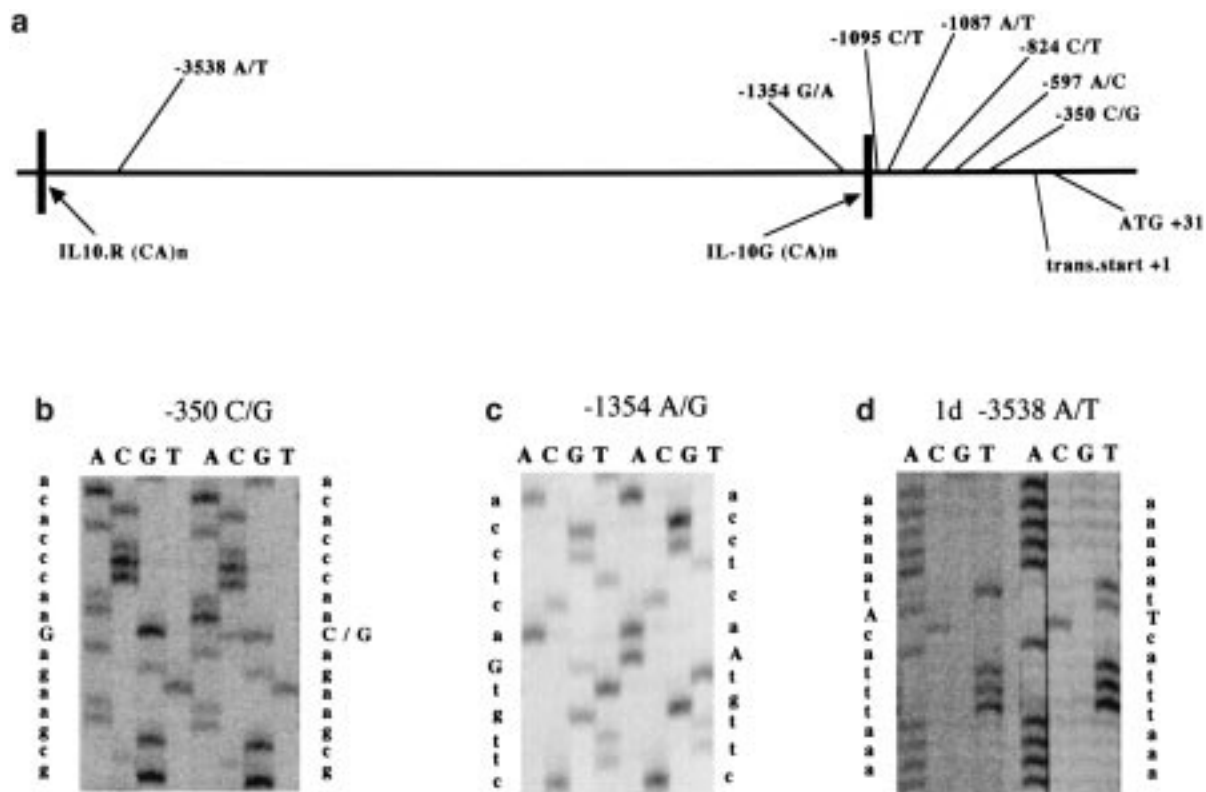


Figure 1 The polymorphic nature of the human IL-10 5' flanking region. (a) The locations of currently defined polymorphic elements in the human IL-10 gene are shown. Numbering is relative to the Kube sequence (Genbank accession number X78437). Note that the C/T at position -1095 is not observed in our test population of Dutch Caucasians. (b-d) Novel polymorphic elements within the IL-10 5' flanking sequence were defined by sequencing of the entire region. Sequences were obtained using EpiCentre reagents and separated on a LiCor 4000 sequencer. Novel SNPs were sequenced in both directions.

loci were available for 83 unrelated individuals, on whom the following analysis was based (these 83 individuals were the parents from families comprising both parents and at least one child; in one case the haplotypes of the father were derived from comparing those of the mother with those of the children). When we calculated which IL10.G alleles were present in association with the three SNP groupings on an 'observed *vs* expected' basis, it was clear that the IL10.G alleles were not distributed randomly between the three SNP groups; it was particularly apparent that the larger IL10.G alleles were preferentially associated with the ACC allele combination of SNPs (data not shown).

This observation, in combination with our previous allele associations in the original West-of-Scotland population described above, prompted us to examine the inheritance of alleles within the Dutch families. A total of 166 haplotypes were studied; no recombination was noted in any of the families. A total of 29 different haplotypes found to be present in the 83 unrelated individuals (ie, the parents in the studied families), made up from the IL10.R, IL10.G and SNP alleles. However, it was apparent to us (both from the allelic associations found in our previous work^{18,20} and observation of the haplotypes) that this large number of haplotypes might have arisen as a result of the high number of IL10.G alleles, placed on a much smaller number of 'backbone' haplotypes made from the IL10.R locus and the SNPs.

Accordingly we constructed four core haplotypes and examined their distribution (Table 1):

- R3-(IL10.G)-G-C-C,
- R2-(IL10.G)-G-C-C,
- R2-(IL10.G)-A-C-C,
- R2-(IL10.G)-A-T-A.

Although these groupings contained the majority of the observed haplotypes, 12 of the 166 haplotypes could not be fitted to this pattern and these are listed in Table 2. Interestingly, this set contains one representative of the rare 'G-T-A' SNP combination previously described by Mok *et al.*²³

The distribution of IL10.G alleles in the population demonstrates that two alleles are substantially more common than others, IL10.G9 and IL10.G13. In Figure 2, it will be clear that the four haplotype families have a distribution of IL10.G alleles based round IL10.G9 or IL10.G13 but not both. In the haplotype R2-(IL10.G)-G-C-C, the broadest representation of IL10.G alleles is seen, with a significant number of IL10.G8 alleles present, although the most frequent allele is IL10.G13. This broad allele distribution and presence of both G8 and G13 may indicate that this is a particularly old IL10 locus haplotype. One can easily envisage how this gave rise to the R3-(IL10.G)-G-C-C haplotype, where IL10.G9 is the dominant allele, by the lengthening of the CA repeat at IL10.R by one repeat having taken place in an individual who

Table 1 Distribution of IL-10 locus haplotypes found in unrelated Dutch individuals, fitting four haplotype families

		Locus			No. observed	Percentage in population
IL10.R	IL10.G	-1087	-824	-597		
Family IL10.01						
3	7	G	C	C	4	2.4
3	8	G	C	C	1	0.6
3	9	G	C	C	31	18.7
3	10	G	C	C	14	8.4
3	12	G	C	C	1	0.6
3	13	G	C	C	2	1.2
Total					53	31.9
Family IL10.02						
2	10	A	C	C	1	0.6
2	11	A	C	C	4	2.4
2	12	A	C	C	5	3.0
2	13	A	C	C	21	12.7
2	14	A	C	C	13	7.8
2	15	A	C	C	1	0.6
Total					45	27.1
Family IL10.03						
2	8	G	C	C	8	4.8
2	9	G	C	C	2	1.2
2	11	G	C	C	6	3.6
2	12	G	C	C	1	0.6
2	13	G	C	C	13	7.8
2	14	G	C	C	2	1.2
Total					32	19.2
Family IL10.04						
2	7	A	T	A	1	0.6
2	8	A	T	A	2	1.2
2	9	A	T	A	16	9.6
2	10	A	T	A	4	2.4
2	13	A	T	A	1	0.6
Total					24	14.4

Table 2 Distribution of IL-10 locus haplotypes found in unrelated Dutch individuals, outside the four common haplotype families

		Locus			No. observed	Percentage in population
IL10.R	IL10.G	-1087	-824	-597		
2	9	G	T	A	1	0.6
3	9	A	T	A	4	2.4
3	9	A	C	C	1	0.6
3	10	A	C	C	1	0.6
4	9	G	C	C	4	2.4
5	9	G	C	C	1	0.6
Total					12	7.2

was R2-(IL10.G9)-G-C-C, giving the R3 allele and the R3-(IL10.G9)-G-C-C haplotype which is the most common in this haplotype family. Similarly, the R2-(IL10.G13)-A-C-C haplotype might have arisen by an A-to-G substitution having taken place at -1087 in an individual who was R2-(IL10.G13)-G-C-C. Visualising the creation of the final haplotype family, R2-(IL10.G)-A-T-A is less straightforward since it not only involves two apparently simultaneous SNPs, but that this occurred on a very rare background in the R2-(IL10.G)-A-C-C family, namely the

putative R2-(IL10.G9)-A-C-C (which in fact was not observed in our population). However, this haplotype family has the most restricted distribution of IL10.G alleles and may therefore be the most recent development. The speculative discussion above notwithstanding, it should be pointed out that this scheme does not readily give rise to the other more rare haplotypes seen in our Dutch family group without invoking large numbers of absent intermediates. For this reason, we do not propose to attempt to draw any formal 'evolutionary' relationships between these four haplotype families but suggest that they be considered as independent.

Sequencing of the portion of the IL105' region immediately upstream of the IL10.G microsatellite revealed the presence of a novel SNP, a G-to-A at position -1354, relative to the transcription start site (base 4020 in sequence X78437: note that this position, ie, -1354, will change with the length of the IL10.G microsatellite. In X78437 it consists of 21 CA repeats which represents IL-10.G9. IL10.R2 is represented by 13 CA repeats). Sequencing also revealed the presence of a second novel SNP, a T-to-A at position -3538. These are illustrated in Figure 1. Sequencing of a number of individuals homozygous for other alleles has revealed that these new SNPs are in apparently strong disequilibrium with the -1087 marker such that -1087A is linked to -1354G and -3538T. The provenance of a third novel G-to-C SNP at position -350 is less clear since we have observed this in only one individual and even then in a heterozygous form. However this allele was confirmed by sequencing in both directions and appears to be a genuine, if rare, polymorphism. We believe that this is in keeping with the overall haplotypic nature of the 5' region of the IL-10 gene and speculate that additional markers will fit this pattern.

We therefore propose that the four most frequent haplotype families identified in Figure 2 be named:

- IL10.01: R3-(IL10.G)-G-C-C,
- IL10.02: R2-(IL10.G)-A-C-C,
- IL10.03: R2-(IL10.G)-G-C-C and
- IL10.04: R2-(IL10.G)-A-T-A

according to their frequency in our test population (Figure 2 and Table 1). Individual members of these families could be identified by the addition of the IL10.G allele present; thus, an individual haplotype which was a member of the IL10.01 family and IL10.G9 positive would be the haplotype IL10.0109, and so on. Other haplotype families may be named from populations in which they are more frequently observed. This may be particularly relevant for ethnic groups other than Caucasian. For example, it has recently been demonstrated that a Chinese population has a very different distribution of SNP alleles from that found in Caucasians.²³ In addition, there is some evidence that the two proximal SNPs may move together; Mok *et al*²³ reported instances of the SNP haplotype G-T-A (0.04%). This is extremely rare in Caucasians, although we have also seen it in our Dutch population (Table 2) where it was present in one family and inherited stably. Also, a novel C-to-T point mutation was recently reported at position -1095 in a Korean population (Figure 1),²⁴ demonstrating that sites at which SNPs are present in the IL-10 gene may well vary between ethnic groups.

Much interest has arisen round the question of whether different elements of the IL-10 locus can be associated

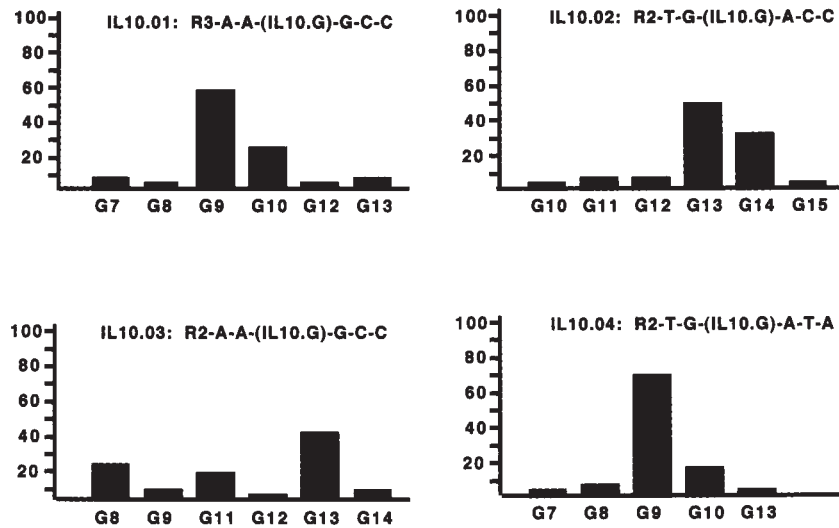


Figure 2 Distribution of IL10.G alleles in four common haplotype families. Haplotype families were identified using the IL10.R microsatellite alleles in combination with the SNPs at -1087, -824 and -597. The novel -3538 and -1354 SNPs are also shown. Naming of these families (IL10.01 etc) is in order of their relative frequency in our Dutch Caucasian test population. Note that the previously defined 'G-C-C' haplotype actually contains two distinct haplotype families, IL10.01 and IL10.03.

with the high or low IL-10 production known to take place in individuals.¹⁷ We¹⁸ have shown that different haplotypes vary in the IL-10 secretion associated with them, while others²² have suggested that the SNPs also have a role. We addressed this question in relation to the proposed IL-10 haplotype families. Figure 3a shows the median IL-10 secretion associated with each of the four IL-10 haplotype families, formally compared using the Kruskal-Wallis test. It will be seen that the lowest secretion was associated with the R3-containing family, in analogy to our previous analysis¹⁸ (1739 (1040–2308) pg/ml). The highest was associated with the R2-(IL10.G)-A-C-C (2420 (1391–3267) pg/ml). Thus, the IL-10 haplotype families are associated with differential

IL-10 secretion and this shows a strong trend to significance in the Kruskal-Wallis test ($P = 0.06$). The construction of these families has also enabled us to consider the question of the importance of the A or G allele at position -1087. In Figure 3b, we have isolated these two nucleotides against an otherwise constant haplotypic background. We compared the IL-10 secretion associated with the haplotypes R2-(IL10.G13)-A-C-C and R2-(IL10.G13)-G-C-C by the Mann-Whitney U-test and found that the A allele was associated with higher IL-10 secretion than the G allele (2511 (1562–3330) vs 1004 (785–2718) pg/ml, $P = 0.06$). This is in contrast to a previous report²² which suggested that the A allele was associated with lower secretion. Possible reasons for this conflict are numer-

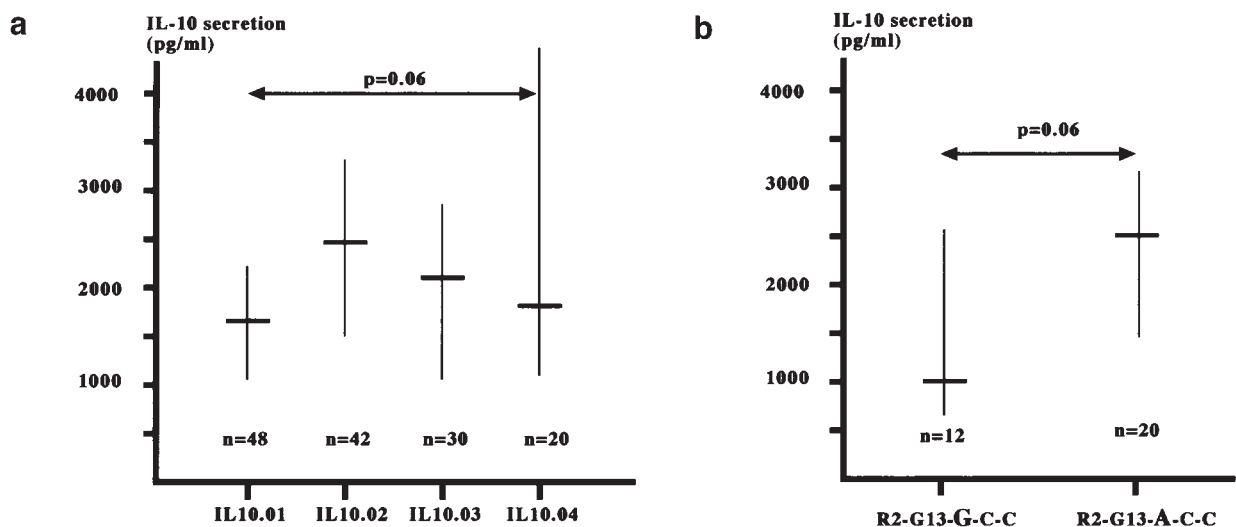


Figure 3 Relationship between haplotypes and IL-10 secretion. (a) Data from our previous study¹⁸ were re-analysed to take account of the four haplotype families. It will be seen that these haplotype families demonstrate considerable differences in IL-10 secretion, with a strong trend to significance ($P = 0.06$, Kruskal-Wallis test). (b) The relationship between the two -1087 alleles and IL-10 secretion, was compared on an otherwise constant genetic background, using data from our previous study.¹⁸ The -1087A allele was associated with higher IL-10 secretion than the -1087G allele, with a strong trend to significance ($P = 0.06$, Mann-Whitney U-test).

ous—the experimental systems were very different; our experiments used LPS stimulation, while that of Turner *et al*²² used Con-A stimulation, for example. We believe that the important point is that IL-10 regulation is likely to differ in different cell types (T cells, B cells, macrophages) and with different stimuli, and that this locus, or elements in linkage with it, may be important in affecting these differences.

In conclusion, the IL-10 locus is highly polymorphic and more polymorphic elements may well exist. These different alleles fit together into gene-haplotypes which span the 4 kb immediately upstream of the start codon, and perhaps beyond in either direction. We propose a nomenclature for these IL-10 haplotypes in which the four most frequent haplotype families in our Dutch Caucasian population are termed IL10.01, IL10.02, IL10.03 and IL10.04. Individual haplotypes within these families could be unambiguously identified by the addition of the IL10.G allele, eg, IL10.0109. These families are associated with differential IL-10 secretion, but do not in themselves account for the large variation in IL-10 secretion which exists between individuals. Considering the human IL-10 locus to be comprised of these haplotypes may help to resolve the apparent contradictions between SNP data and microsatellite data which presently exist in the literature and may also allow a rationalising of the current (apparently) overwhelming polymorphism of the locus resulting from each element being considered independently.

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