INFLAMMATORY BOWEL DISEASE

Association between K469E allele of intercellular adhesion molecule 1 gene and inflammatory bowel disease in a Japanese population

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Gut 2003;52:75-78

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Accepted for publication
16 April 2002

Background and aims: The genetic contribution to inflammatory bowel disease (IBD) is under investigation. Recent evidence indicates a significant linkage between a locus on chromosome 19p13 and IBD. We investigated the association between an intercellular adhesion molecule 1 gene (*ICAM-1*) polymorphism located on chromosome 19p13 and IBD in a Japanese population.

Methods: We compared 207 Japanese patients who had IBD (79 with Crohn's disease (CD); 128 with ulcerative colitis (UC)) with 103 unrelated Japanese controls. We determined R241G and K469E polymorphisms of the *ICAM-1* gene using polymerase chain reaction (PCR) techniques.

Results: Both frequency and carriage rate of the K469 allele were significantly higher in IBD patients than in controls (allelic frequency, $p=0.0026$; carriage rate, $p=0.0034$; odds ratio 2.59; 95% confidence interval 1.42-4.68). Furthermore, the frequency of the K469 allele was significantly increased in both CD and UC. Subgroup analysis demonstrated that both K469 allelic frequency and K469 carriage rate were significantly higher in patients with the small bowel and colon type of CD and entire colitis compared with healthy controls.

Conclusions: We identified an overall association between IBD and *ICAM-1* K469 in a Japanese population. Further studies of this chromosome region are required to elucidate the gene responsible for IBD.

The aetiological relevance of ulcerative colitis (UC) and Crohn's disease (CD) remains unknown. However, they may be associated with a genetic predisposition as familial inheritance and increased concordance rates in siblings or twins of inflammatory bowel disease (IBD) are prevalent.¹ To understand these aetiological trends, the chromosomal region encompassing the appropriate genes requires thorough investigation.

Association studies and linkage analyses have been independently performed to identify the gene responsible for IBD. Genetic association studies have examined the relationships between HLA,²⁻⁴ DRB1,⁵⁻⁷ DPB1,⁸ MHC class I chain related gene A (*MICA*),⁹ interleukin (IL)-1 α , IL-1 β , and IL-1ra,¹⁰⁻¹⁵ tumour necrosis factor,¹⁶⁻¹⁷ IL-10,¹⁸⁻¹⁹ and intercellular adhesion molecule 1 (*ICAM-1*).²⁰ Linkage analyses have shown a possible linkage between CD and a locus on chromosome 16 (*IBD1* locus),²¹⁻²³ between UC and chromosome 12q (*IBD2* locus),²⁴⁻²⁶ and between IBD and chromosomes 6p (the *IBD3* locus),²⁷⁻²⁸ 1p,²⁹ and 19p.³⁰

Any chromosomal region identified by both linkage and association analysis is likely to be closely associated with IBD and a promising source of the responsible genes. For example, microsatellite markers located on chromosome 6p, which encompasses the HLA antigen genes, indicate both a significant genetic association and linkage in IBD, and this area is being mapped to seek a candidate IBD susceptibility gene(s).³¹⁻³²

Rioux *et al* have identified a significant association between IBD and chromosome 19p13 using linkage analysis.³⁰ Because no other linkage analyses have identified 19p13 as the region responsible for IBD, association analysis of this locus is required to confirm their findings. We therefore examined the *ICAM-1* gene that is located on chromosome 19p13. *ICAM-1* plays a pivotal role in the migration of neutrophils to inflammatory sites, and it may be involved in various inflammatory

diseases. In addition, the *ICAM-1* gene has single base polymorphisms leading to amino acid changes in codons 241 and 469³³ which may help to identify genetic associations in patients. Using these polymorphism markers, Yang *et al* could not detect an overall association between IBD (neither UC nor CD) and *ICAM-1* although differences were significant when patient groups were stratified by antineutrophil cytoplasmic antibodies (ANCA).²⁰

Because few association studies of *ICAM-1* and IBD have been reported, it is not known whether *ICAM-1* has aetiological relevance to this disease. To precisely investigate the genetic associations of the chromosome region encompassing *ICAM-1*, ethnically defined populations should be analysed. A Japanese population may be suitable as the frequency of immigration is low and linkage disequilibrium decay is relatively low.³⁴ In the present study, we have examined the genetic association between *ICAM-1* polymorphism and IBD in a Japanese population. We found that the K469 allele frequency of the *ICAM-1* gene is significantly associated overall with both CD and UC, suggesting that the 19p13 region contains potential candidates for genetic susceptibility to IBD.

MATERIALS AND METHODS

Patients and healthy controls

The study comprised 103 unrelated healthy volunteers, 128 patients with UC, and 79 with CD. All participants were Japanese and their characteristics are described in table 1. UC or CD was diagnosed by comparison with the conventional

Abbreviations: UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; IL, interleukin; *ICAM-1*, intercellular adhesion molecule 1 gene; PCR, polymerase chain reaction; ANCA, antineutrophil cytoplasmic antibodies; OR, odds ratio.

Table 1 Characteristics of patients with Crohn's disease and ulcerative colitis

	Crohn's disease	Ulcerative colitis	Healthy controls
n	79	128	103
Sex (M/F)	42/37	68/60	47/56
Age at onset of symptoms (y)			
Mean (SD)	23.5 (10.1)	30.8 (14.5)	
Median	20	28	
Range	13–57	11–68	
Affected lesion			
Small and large bowel	31		
Large bowel	33		
Small bowel	15		
Entire colitis		84	
Left sided colitis		35	
Proctitis		9	
Surgery (yes/no)	29/50	20/108	

endoscopic, histological, and clinical criteria of the Japanese Ministry of Health and Welfare with reference to the criteria provided by both the Council for International Organizations of Medical Sciences in WHO and the International Organization for the Study of Inflammatory Bowel Disease. Patients with multiple sclerosis, systemic lupus erythematosus, or other recognised autoimmune diseases were excluded from the study. All patients and controls provided written informed consent to participate in the study.

ICAM-1 genotyping

Peripheral whole blood from patients with IBD and normal controls was collected in tubes containing EDTA. Genomic DNA was extracted from samples using the Genomic Prep™ Blood DNA Isolation Kit (Amersham Pharmacia Biotech Inc., USA) according to the manufacturer's protocol. Single base polymorphisms at codon 241 (R241G) in exon 4 and at codon 469 (K469E) in exon 6 of *ICAM-1* were determined using methods based on the polymerase chain reaction (PCR). The primers for amplification, PCR conditions, and allele specific oligonucleotides have been described elsewhere.³³ The R241G polymorphism was amplified using the primers: X4L, 5'-GATTGAAGAAGCCAGCAG-3' and X4R, 5'-GTCGTGCCATAGGTGAC-3', and two end labelled allele specific oligonucleotides for the G241 allele (5'-TCCCTGGACGGGGTGTCC-3') or R241 allele (5'-TCCCTGGACAGGGTGTCC-3') were hybridised with the amplified products. The K469E polymorphism was amplified using primers: 6442, 5'-CCATCGGGGAATCAGTG-3' and 6443,

5'-ACAGAGCACATTACGGTC-3'. The PCR products were identified by enzyme digestion with *Bst*UI which cuts the E469 allele but not K469.

Statistical analysis

Allele and genotype frequencies between each of the patient groups and controls were compared using a 2×2 contingency table and the χ^2 test. To correct for multiple comparisons in the subgroups, corrected p values (p_c) were obtained using Bonferroni's correction factor by multiplying the p value by two ($p_c=2 \times p$) because the alleles were examined simultaneously. To analyse subgroups in IBD, p_c values were corrected using Fisher's exact test when sample numbers were small. After correction, p_c was considered significant when <0.05 . The closeness of genetic association is shown as odds ratios (OR) and 95% confidence intervals (CI).

RESULTS

Allelic frequencies, carriage rates, and genotypes of ICAM-1 alleles

Table 2 summarises allelic frequencies, carriage rates, and genotypes of the *ICAM-1* gene in IBD patient groups and controls. The allelic frequency of K469 was significantly higher in patients with IBD than in controls (allelic frequency: $\chi^2=10.37$, $p_c=0.0025$). This increase was also found in patients with CD ($\chi^2=8.93$, $p_c=0.0056$). The carriage rate of the K469 allele in patients with CD was also higher than in controls ($\chi^2=6.18$, $p_c=0.025$). Eighty nine per cent of patients with CD harboured *ICAM-1* K469, giving an OR of 2.67 (95% CI 1.24–6.15) for CD. The frequency of the K469 allele was increased in UC as in CD ($\chi^2=6.90$, $p_c=0.017$). The carriage rate of the K469 was also significantly higher in patients with UC than in controls ($\chi^2=7.08$, $p_c=0.016$). Eighty eight per cent of patients with UC had *ICAM-1* K469, giving an OR of 2.45 (95% CI 1.27–4.86).

We did not detect the R241G polymorphism in 103 individuals (50 healthy controls, 66 with UC, and 17 with CD). Because we found only the G241 allele, we did not perform an association analysis of the R241G polymorphism.

Clinical characteristics of patients with IBD harbouring the ICAM-1 K469 allele

In patients with small bowel and colon types of CD, both the allelic frequency and carriage rate of K469 allele were significantly higher than in controls (allelic frequency: $p_c=0.0041$; carriage rate: $p_c=0.027$; OR=5.51; 95% CI 1.41–21.43) (table 3).

The allelic frequency and carriage rate of the *ICAM-1* K469 allele were also significantly different in patients with the entire colitis type of UC (allelic frequency: $p_c=0.045$; carriage rate: $p_c=0.015$; OR=2.96; 95% CI 1.33–6.55). The carriage rate

Table 2 Allelic frequency and carriage rate of E/K 469 intercellular adhesion molecule 1 gene (*ICAM-1*) polymorphisms in patients with inflammatory bowel disease (IBD) and controls

	Controls (n=103)	IBD (CD+UC)		CD		UC	
		(n=207)	p_c	(n=79)	p_c	(n=128)	p_c
Genotype (n)							
EE	27	25		9		16	
EK	50	103		37		66	
KK	26	79		33		46	
Allelic frequency (%)							
E469	50.5	37.0	0.0025	34.8	0.0056	38.3	0.0171
K469	49.5	63.0	0.0025	65.2	0.0056	61.7	0.0171
Carriage rate (%)							
E469	74.8	61.8	0.047	58.2	0.0364	64.1	NS
K469	73.8	86.5	0.003	88.6	0.0257	87.5	0.0155

NS, not significant compared with healthy controls.
UC, ulcerative colitis; CD, Crohn's disease.

Table 3 Allelic frequencies and carriage rate of intercellular adhesion molecule 1 (ICAM-1) alleles in patients with Crohn's disease by affected lesions

	Controls (n=103)	Small and large bowel		Large bowel		Small bowel	
		(n=31)	p _c	(n=33)	p _c	(n=15)	p _c
Genotype (n)							
EE	27	4		2		3	
EK	50	14		15		8	
KK	26	13		16		4	
Allelic frequency (%)							
E469	50.5	28.8	0.00411	35.5	NS	46.6	NS
K469	49.5	71.2	0.00411	64.5	NS	53.3	NS
Carriage rate (%)							
E469	74.8	51.5	0.0238	58.1	NS	73.3	NS
K469	73.8	93.9	0.0278	87.1	NS	80.0	NS

NS, not significant compared with healthy controls.

Table 4 Allelic frequencies and carriage rate of intercellular adhesion molecule 1 (ICAM-1) alleles in patients with ulcerative colitis by affected lesions

	Controls (n=103)	Entire colitis		Left sided colitis		Proctitis	
		(n=4)	p _c	(n=35)	p _c	(n=9)	p _c
Genotype (n)							
EE	27	9		4		3	
EK	50	47		16		3	
KK	26	28		15		3	
Allelic frequency (%)							
E469	50.5	38.7	0.0452	34.3	0.0377	50.0	NS
K469	49.5	61.3	0.0452	65.7	0.0377	50.0	NS
Carriage rate (%)							
E469	74.8	66.6	NS	57.1	NS	66.6	NS
K469	73.8	89.3	0.0149	88.6	NS	66.6	NS

NS, not significant compared with healthy controls.

of K469 in left sided UC was also significant as well as in the entire colitis type of UC (left sided type: 88.6%; entire type: 89.3%). However, these findings were not significant compared with the proctitis type of UC and healthy controls (proctitis type: 66%; controls: 73.8%) (table 4).

DISCUSSION

Considerable effort has been made to identify IBD susceptibility genes (either CD or UC). Most of these efforts were attempted using genetic association studies and linkage analyses. However, because IBD is highly multifactorial in terms of both environmental and genetic factors, precise mapping of the susceptible loci should be complex. Coordinate examinations using both of the methods mentioned above are needed to search for the potent IBD loci. The present study strengthens the linkage prevalence of chromosome 19p13 in IBD³⁰ by showing an overall association with the K469E polymorphism of the ICAM-1 gene and IBD in a Japanese population.

Only one study has examined a genetic association between ICAM-1 gene polymorphism and IBD. Yang *et al* examined the genetic association between K469E and R241G polymorphisms of the ICAM-1 gene and IBD in Caucasians. Although the association was significant when patient groups were stratified by ANCA status, no overall difference between patient groups and healthy controls was found in their study.²⁰ Our results are in contrast with these findings as we identified a significant association with K469E in IBD patients. One possible explanation for this conflict might be due to differences in ethnic background. A good example is the results of genetic association studies examining the relationship between ICAM-1 polymorphism and type 1

diabetes. The ICAM-1 polymorphism was significantly associated with adult onset diabetes in a Japanese population³⁵ but not in Danish³⁶ or Finnish families.³⁷ Linkage disequilibria between genes in the same chromosomal region may vary among different ethnic populations.

As the Japanese are thought to have expanded from a founder population of approximately 1000 individuals over 100 generations,³⁸ they are considered suitable for both genetic association and linkage analyses. Linkage disequilibria between ICAM-1 K469 and the responsible loci for IBD may have been highly conserved on chromosome 19p13 within this population but more detailed linkage analyses of this chromosome region are required to identify the IBD susceptibility gene(s) in this population.

Several investigators have examined the genetic association between ICAM-1 gene polymorphism and chronic inflammatory diseases. Among Polish Caucasian patients with multiple sclerosis, the allelic frequency of ICAM-1 K469 was significantly increased (68% v 49% in controls) while the distribution of the R241G polymorphism was not significant.³⁹ In Italian patients with either polymyalgia rheumatica or giant cell arteritis, the frequency of the R241 allele was significantly higher than in controls (polymyalgia rheumatica/giant cell arteritis 12.9% v 3.1% in controls) but the K469E polymorphism was not significant.⁴⁰ The allelic frequency of E469 was significantly increased among Palestinian and Jordanian patients with Behçet's disease (47.6% v 38.3% in controls) but the R241G polymorphism was not identified.⁴¹ These results are contradictory and so the significance of this gene may differ according to the type of inflammatory disease.

All of these diseases as well as IBD are characterised by intense infiltration of leucocytes at inflamed lesions. ICAM-1 is expressed on the surface of activated endothelial cells where

it plays an important role in lymphocyte migration and activation. R241G and K469E amino acid changes in the ICAM-1 gene may influence the functional role of ICAM-1 as both are located on the Mac-1 binding domain and in immunoglobulin-like domain 5, respectively. The relevance of the ICAM-1 gene polymorphism may be explained by functional alteration of this gene product.²⁰⁻³³

Recent linkage analyses have revealed that the chromosome region 19p13 is closely related to the atopy phenotype in Italian families with allergic asthma⁴² and type 1 diabetes in a British population.⁴³ This chromosomal region encompasses several important genes that regulate the immunological and inflammatory systems (complement component 3, thromboxane A2 receptor, leukotriene B4 hydroxylase, Janus protein tyrosine kinase family).³⁰ Our results should be confirmed when the genetic association of these loci is determined. In conclusion, the present study revealed an overall association between the ICAM-1 K469 allele and IBD (CD and UC) and may potentiate the significance of chromosome 19p13. Further studies are required to confirm our findings.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education and Science, Japan (08770358 and 11670484), and by a grant from the Ministry of Health and Welfare, Japan.

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REFERENCES

- Yang H, Rotter JL. The genetics of ulcerative colitis and Crohn's disease. In: Kirsner JB, ed. *Inflammatory bowel disease* (5th edn). Philadelphia: Saunders, 2000;250-79.
- Mallas EG, Mackintosh P, Asquith P, et al. Histocompatibility antigens in inflammatory bowel disease. *Gut* 1976;17:906-10.
- Delpre G, Kadish U, Gazit E, Joshua H, et al. HLA antigens in ulcerative colitis and Crohn's disease in Israel. *Gastroenterology* 1980;78:1452-7.
- Asakura H, Tsuchiya M, Aiso S, et al. Association of the human lymphocyte-DR2 antigen with Japanese ulcerative colitis. *Gastroenterology* 1982;82:413-18.
- Yang H, Rotter JL, Toyoda H, et al. Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA class II) and subclinical (antineutrophil cytoplasmic antibodies) markers. *J Clin Invest* 1993;92:1080-4.
- Futami S, Aoyama N, Honsako Y, et al. HLA-DRB1*1502 allele, subtype of DR15, is associated with susceptibility to ulcerative colitis and its progression. *Dig Dis Sci* 1995;40:814-18.
- Satsangi J, Welsh K, Bunce M, et al. Contribution of genes of major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet* 1996;347:1212-17.
- Sugimura K, Asakura H, Mizuki N, et al. Analysis of genes within the HLA region affecting susceptibility to ulcerative colitis. *Hum Immunol* 1993;36:112-18.
- Sugimura K, Ota M, Matsuzawa J, et al. A close relationship of triplet repeat polymorphism in MHC class I chain-related gene A (MICA) to the disease susceptibility and behavior in ulcerative colitis. *Tissue Antigens* 2001;57:9-14.
- Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994;106:637-42.
- Bioque G, Crusius JBA, Koutroubakis I, et al. Allelic polymorphism in IL-1b and IL-1receptor antagonist (IL-1Ra) gene in inflammatory bowel disease. *Clin Exp Immunol* 1995;102:379-83.
- Kovis E, Satsangi J, Roussomoustakaki M, et al. Cytokine gene polymorphisms in inflammatory bowel disease. *Gut* 1996;39:705-10.
- Roussomoustakaki M, Satsangi J, Welsh K, et al. Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology* 1997;112:1845-53.
- Hacker UT, Gomolka M, Keller E, et al. Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. *Gut* 1997;40:623-7.
- Ishizuka K, Sugimura K, Honma T, et al. Influence of interleukin-10 on the interleukin-1 receptor antagonist (IL-1ra)/interleukin-1beta ratio in the colonic mucosa of ulcerative colitis. *Digestion* 2001;63(suppl 1):22-7.
- Plevy SE, Targan SR, Yang H, et al. Tumor necrosis factor microsatellites define a Crohn's disease-associated haplotype on chromosome 6. *Gastroenterology* 1996;110:1053-60.
- Negoro K, Kinouchi Y, Hiwatahi N, et al. Crohn's disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. *Gastroenterology* 1999;117:1062-8.
- Tagore A, Gonsalkorale WM, Pravica V, et al. Interleukin-10 (IL-10) genotypes in inflammatory bowel disease. *Tissue Antigens* 1999;54:386-90.
- Parkes M, Satsangi J, Jewell D. Contribution of the IL-2 and IL-10 genes to inflammatory bowel disease (IBD) susceptibility. *Clin Exp Immunol* 1998;113:28-32.
- Yang H, Vora DK, Targan SR, et al. Intercellular adhesion molecule 1 gene associations with immunologic subsets of inflammatory bowel disease. *Gastroenterology* 1995;109:440-8.
- Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996;379:821-3.
- Parkes M, Satsangi J, Lathrop GM, et al. Susceptible loci in inflammatory bowel disease. *Lancet* 1996;348:1588.
- Ohmen JD, Yang HY, Yamamoto KK, et al. Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. *Hum Mol Genet* 1996;5:1679-83.
- Satsangi J, Parkes M, Louis E, et al. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996;14:199-202.
- Duerr RH, Barmada MM, Zhang L, et al. Linkage and association between inflammatory bowel disease and a locus on chromosome 12. *Am J Hum Genet* 1998;63:95-100.
- Curran ME, Lau KF, Hampe J, et al. Genetic analysis of inflammatory bowel disease in a large European cohort supports linkage to chromosomes 12 and 16. *Gastroenterology* 1998;115:1066-71.
- Yang H, Plevy SE, Taylor K, et al. Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analyses. *Gut* 1999;44:519-26.
- Hampe J, Schreiber S, Shaw SH, et al. A genomewide analysis provides evidence for novel linkage in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999;64:808-16.
- Cho JH, Nicolae DL, Ramos R, et al. Linkage and linkage disequilibrium in chromosome band 1p36 in American Chaldeans with inflammatory bowel disease. *Hum Mol Genet* 2000;9:1425-32.
- Rioux JD, Silverberg MS, Daly MJ, et al. Genome wide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000;66:1863-70.
- Hampe J, Shaw SH, Saiz R, et al. Related Linkage of inflammatory bowel disease to human chromosome 6p. *Am J Hum Genet* 1999;65:1647-55.
- Yang H, Plevy SE, Taylor K, et al. Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analyses. *Gut* 1999;44:519-26.
- Vora DK, Rosenbloom CL, Beaudet AL, et al. Polymorphisms and linkage analysis for ICAM-1 and the selectin gene cluster. *Genomics* 1994;21:473-7.
- Wright AF, Carothers AD, Pirastu M. Population choice in mapping genes for complex diseases. *Nat Genet* 1999;23:397-404.
- Nishimura M, Obayashi H, Maruya E, et al. Association between type 1 diabetes age-at-onset and intercellular adhesion molecule-1 (ICAM-1) gene polymorphism. *Hum Immunol* 2000;61:507-10.
- Kristiansen OP, Nolsoe RL, Holst S, et al. The intracellular adhesion molecule-1 K469E polymorphism in type 1 diabetes. *Immunogenetics* 2000;52:107-11.
- Nejentsev S, Laine AP, Simell O, et al. Intracellular adhesion molecule-1 (ICAM-1) K469E polymorphism: no association with type 1 diabetes among Finns. *Tissue Antigen* 2000;55:568-70.
- Graham J, Thompson EA. Disequilibrium likelihood for fine-scale mapping of a rare allele. *Am J Hum Genet* 1998;63:1517-30.
- Mycko MP, Kwinkowski M, Tronczynska E, et al. Multiple sclerosis: the increased frequency of the ICAM-1 exon 6 gene point mutation genetic type K469. *Ann Neurol* 1998;44:70-5.
- Salvarani C, Casali B, Boiardi L, et al. Intercellular adhesion molecule 1 gene polymorphisms in polymyalgia rheumatica/giant cell arteritis: association with disease risk and severity. *J Rheumatol* 2000;27:1215-21.
- Verity DH, Vaughan RW, Kondeatis E, et al. Intercellular adhesion molecule-1 gene polymorphisms in Behcet's disease. *Eur J Immunogenet* 2000;27:73-6.
- Venanzi S, Malerba G, Galavotti R, et al. Linkage to atopy on chromosome 19 in north-eastern Italian families with allergic asthma. *Clin Exp Allergy* 2001;31:1220-4.
- Mein CA, Esposito L, Dunn MG, et al. A search for type 1 diabetes susceptibility genes in families from the United Kingdom. *Nat Genet* 1998;19:213-14.