ARTICLE

Combined segregation and linkage analysis of inflammatory bowel disease in the IBD1 region using severity to characterise Crohn's disease and ulcerative colitis

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Inflammatory bowel disease (IBD) is a chronic relapsing disorder affecting the gastro-intestinal tract and is subdivided into two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC). Although the aetiology of IBD is unknown, a strong genetic susceptibility is suggested and different candidate regions have been identified for both CD and UC. The IBD1 region on chromosome 16 has been confirmed to be important for susceptibility to CD, whereas conflicting evidence has been obtained for UC. We performed a combined linkage and segregation analysis in the identified IBD1 region on a sample of 82 extended families with IBD using a parametric method implemented in the computer program COMDS. This approach allows simultaneous evaluation of linkage while estimating the mode of inheritance and to include severity of the trait to characterise the CD and UC phenotypes. Our results are consistent with the presence of a major gene in the IBD1 region close to D16S408 involved in both UC and CD. Furthermore, our data support evidence that a single mutation in the gene leads more frequently to UC, whereas inheritance of two mutant alleles results in the more severe CD. In our study the IBD1 locus was found to have a major role in IBD predisposition in the Italian population. *European Journal of Human Genetics* (2000) 8, 846–852.

Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing disorder affecting the gastro-intestinal tract with a population prevalence in Western societies of 1:1000.[1] IBD is subdivided on the basis of clinical and histologic features into two subtypes: Crohn's disease (CD) and ulcerative colitis (UC).[2]

Although the aetiology of IBD is unknown, a strong genetic susceptibility is shown by epidemiological data.[3–6] Both forms of IBD can co-exist in single families, and the associated risk of developing the other form of the disease is also increased in relatives of affected subjects, suggesting the presence of shared susceptibility genes between the two diseases.[7]

Previous segregation analysis studies performed on CD and UC families separately have indicated the presence of a major

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gene leading to recessive inheritance for CD as well as one resulting in dominant and additive inheritance for UC,[8–10] although the identified major genes were estimated to account only for a small proportion of cases.

Several susceptibility loci contributing to the genetic risk for IBD have been identified in different chromosomal regions through genome-wide searches.[11–16] The importance of the candidate loci on chromosome 16 (IBD1, MIM 266600), chromosome 12 (IBD2, MIM 601458) and chromosome 6 (IBD3, MIM 604519) has been demonstrated in subsequent studies,[17–26] whereas the additional IBD loci need to be confirmed by further independent analyses.

Recently we have confirmed through a non-parametric approach the involvement of the IBD1 locus in an independent sample of Italian IBD families.[26] We now present a parametric analysis performed on an extended sample employing combined segregation and linkage analysis in the identified IBD1 region. Using this approach it is possible to test linkage and to refine the mode of disease inheritance using the information provided by the linked marker, thus allowing more accurate estimates of the genetic parameters, such as gene frequency and penetrance and, consequently, a more powerful test for linkage. Furthermore, the role of the IBD1 locus in CD and UC aetiology is investigated.

Subject and methods

Family data

Combined segregation and linkage analysis was performed on a sample of 82 extended families with IBD, ascertained through 34 CD-affected probands and 48 UC-affected probands in the multicentre programme of the GISC (Italian Group for the Study of the Colon and the Rectum). Description of families, selection scheme and total number of affected and unaffected individuals subjected to analysis are shown in Table 1. Extended pedigrees were decomposed before the analysis into nuclear families with the inclusion of

pointers, who are affected relatives through whom the nuclear family was ascertained.[27] Pointers were coded as defined in Morton *et al*.[28] The different combinations of parental phenotypes and selection criteria of the resulting nuclear families are shown in Table 2.

Liability classes were specified prior to the analysis to correct for covariates with affection, as in the usual formulation of the liability model.[29] Age of observation was available only for 19 of the 82 families; therefore, when age was not specified, three age classes were formed corresponding to the generation of the pedigree member (Table 3). Population risks for each liability class were derived from Orholm *et al*,[10] since age-specific risk estimates based on a large population survey are not available for the Italian population. Although sporadic reports have suggested a lower incidence rate for IBD in Italy, an increasing trend has also been reported.[30]

In order to investigate the mode of inheritance of the IBD trait, the whole sample of 82 families was studied, defining affecteds as those with either CD or UC. Information on severity (see Statistical analysis) was included in the analysis of the IBD sample by forming two severity classes among affected subjects: UC-affected patients were assigned to the less severe class and CD-affected patients to the more severe class. This classification reflects the clinical features of the disease: UC is largely a mucosal disease restricted to the large intestine, whilst in CD the entire thickness of the bowel is affected and it may involve any part of the gastrointestinal tract. The relative proportions among affected individuals estimated from our sample for UC and CD were 0.618 and 0.382, respectively.

Combined segregation and linkage analysis was also performed separately for each sub-group of the whole IBD family sample (CD only, UC only, and CD/UC families) and tests of heterogeneity were carried out.

Eight markers spanning approximately 30 cM of the IBD1 region (D16S517, D16S409, D16S411, D16S419, D16S408,

Table 1 Description of families, selection scheme* and total number of affected and unaffected individuals included in the analysis

Affected relatives	CD	No. of families UC	CD/UC	Total no. affected	Total no. unaffected	Secondary proband
2 sibs	8	13	9	60	89	
2 sibs + 1 first cousin	1	–	–	3	1	1
2 sibs + 1 niece/nephew	–	1	–	3	6	
2 sibs + 1 parent	–	3	1	12	6	
3 sibs	1	–	1	6	2	
4 sibs	1	–	–	4	10	
1 aunt/uncle; 1 niece/nephew	2	1	4	14	31	
2 first cousins	–	1	1	4	11	2
2 first cousins + 1 parent	–	1	–	3	5	1
1 grandparent; 1 grandchild	1	1	1	6	9	
1 parent; 1 child	3	13	6	44	52	
Singleton	4	4	–	8	26	
Total	21	38	23	167	248	4

*Pedigree information on first and second degree relatives of affected probands was obtained when possible (single selection of pedigrees), whereas more distant branches of pedigrees were sampled only when they included an affected relative (secondary proband).

Table 2 Distribution by ascertainment and mating type of the nuclear families subjected to the analysis

Ascertainment selection	Mating type[a]		
	N×N	N×A	N×U
IBD nuclear families			
complete selection (proband as a parent)	0	22	0
complete selection (selection through a pointer[b])	19	4	1
incomplete selection (proband as a child)	57	14	1
truncate selection (secondary proband)	4	0	0
CD-only nuclear families			
complete selection (proband as a parent)	0	5	0
complete selection (selection through a pointer[b])	6	1	0
incomplete selection (proband as a child)	17	1	1
truncate selection (secondary proband)	1	0	0
UC-only nuclear families			
complete selection (proband as a parent)	0	11	0
complete selection (selection through a pointer[b])	5	2	1
incomplete selection (proband as a child)	23	9	0
truncate selection (secondary proband)	2	0	0
CD/UC nuclear families			
complete selection (proband as a parent)	0	6	0
complete selection (selection through a pointer[b])	8	1	0
incomplete selection (proband as a child)	17	4	0
truncate selection (secondary proband)	1	0	0

[a]N=normal, A=affected, U=unknown status; [b]nuclear families with the parent of the proband as a child.
Ascertainment probability was set to 0.001 for incomplete selection and to 1.0 for truncate selection.

Table 3 Liability classes[a] for IBD, CD, and UC specified in the analysis

Liability class	Age class or generation	CD	UC	IBD
1	<30 years or 3rd generation	0.000810	0.001473	0.002283
2	≥30 and <50 years or 2nd generation	0.001575	0.004742	0.006317
3	≥50 years or 1st generation	0.001203	0.004409	0.005612

[a]Derived from Orholm et al.[10]

D16S514, D16S503, and D16S421) were tested for linkage using a two-point approach and simultaneously estimating the parameters of the genetic model. When linkage to a marker was observed, further tests allowing more general genetic models were done.

Statistical analysis

Statistical analysis was done using COMDS, a computer program for COMbined segregation and linkage analysis with Diathesis and Severity. Full documentation about the model can be found in Morton et al.[31]

In the COMDS program two autosomal disease loci can be considered: a *major* disease locus, linked to a marker locus, and a *modifier* locus, although the modifier locus does not necessary have the smaller effect. The two loci are biallelic with a high and a low risk allele, and their effects are assumed to be additive on the liability scale. Parameters of the genetic model are: frequency of the alleles favouring high liability (q, q_m), degree of dominance (d, d_m), ranging from 0 for a recessive trait to 1 for a dominant one, displacement between homozygotes (t, t_m), and the recombination fraction, θ, between the major gene and the marker locus.

Complex phenotypes are included in COMDS by forming severity classes among affected subjects through a sub-classification ordered by increasing severity. A severity scale is assumed for affected individuals: on this scale the effect of each locus is modelled by a scaling parameter, S for the major and S_m for the modifier locus, so that St and $S_m t_m$ represent the distance between homozygotes. Therefore, when $S = 0$ there is no genotypic difference between individuals of different severity classes and the major locus has no effect on severity of disease; when $S > 0$ the genotypic difference between individuals of two adjacent severity classes can be less ($S < 1$) or more (when $S > 1$) than the difference between affected and normal individuals. When $S > 1$, the effect of the major locus on severity is therefore greater than its effect on affection.

Parameter estimates of the genetic model are obtained by maximising the likelihood function of the observed phenotypes in families using joint likelihood. Support for a particular hypothesis is obtained by maximum like-lihood ratio test (LRT): the test statistic $2(\ln L_{general\ model} - \ln L_{restricted\ model})$ is distributed in large samples as χ^2 with degrees of freedom equal to the difference of the number of parameters estimated in the two models. LRT is based on

comparison of hierarchical models (the restricted model must be a subset of the general one). For non-hierarchical models. Akaike's information criterion[32] can be used to identify the most parsimonious model.

When testing for linkage to a marker locus, lod scores are calculated as the $\log_{10}$ of the ratio between the likelihood obtained with θ estimated and the likelihood with θ constrained to 0.5, under a specific genetic model.

Results

Results of the COMDS analysis for the whole IBD sample are reported in Table 4. In this analysis affected individuals were defined as having IBD, thus including those with either CD or UC.

When linkage and severity were not considered the major dominant and recessive modifier model provided the best fit (model 5). This two-loci model was significantly better than the single major locus (model 4 vs model 5: $\chi^2 = 9.9$, 3 df, $P = 0.0194$). Taking severity of the disease into account, a significant improvement of the likelihood was observed for the two-loci model with an estimated severity parameter > 0 for the recessive modifier locus (model 5 vs model 6: $\chi^2 = 12.87$, 1 df, $P = 0.0002$), whereas the likelihood was not improved when estimating S for the dominant major locus (data not shown), indicating a main effect of the recessive modifier gene on severity of the disease.

When linkage was tested for the eight markers spanning the IBD1 region, the best evidence of linkage was obtained at marker D16S408, where a lod score of 2.93 at a recombination fraction of 0.0 was observed for the two-loci model (model 8) and a lod score of 4.14 (for $\theta = 0.0$) was obtained for the single-locus dominant model (model 7). The two-loci model did not provide a significant better fit than the single-locus dominant model (model 7 vs model 8: $\chi^2 = 4.55$, 3 df, n.s.). Conversely, the two-loci model gave a significantly better fit than the single-locus model when the severity parameter was estimated for the modifier recessive gene

(model 7 vs model 9: $\chi^2 = 14.89$, 4 df, $P = 0.0049$). Among the different two-loci models tested a major dominant gene in linkage with D16S408 ($\theta = 0.0$) and a modifier recessive gene, with a major effect on severity of the trait, provided the most parsimonious hypothesis and therefore the best fit in our study (only the major recessive and modifier dominant model is shown: model 9 vs model 10, AIC: 279.98 vs 283.15). Under model 9 the recessive locus was found to have a major effect on severity of disease since S_m was > 1, whereas the inclusion of S for the dominant locus did not improve the likelihood (data not shown) and S was constrained to 0. The two-loci model, where the recessive modifier locus contributes not only to affection but also to severity of disease, provided a significantly better fit than the simplifying hypothesis where $S_m = 0$ (model 8 vs model 9: $\chi^2 = 10.34$, 1 df, $P = 0.0007$). Penetrances were estimated for each liability class under the identified two-loci model (model 9, Table 4): homozygous individuals at the recessive locus showed complete penetrance in each liability class, whereas a reduced penetrance was estimated for heterozygous individuals at the dominant locus, with the highest estimated penetrance obtained for liability class 2 (penetrance = 13.5% for liability class 1; penetrance = 43.1% for liability class 2; penetrance = 37.9% for liability class 3). Probability for affected subjects of having either UC or CD for each genotype was estimated under the same model: interestingly, all affected subjects with a recessive mode of inheritance were expected to develop CD, whereas approximately 70% of the patients with a dominant mode were estimated to present with UC and 30% with CD.

No other significant linkage was obtained for the remaining markers studied (all with lod scores < 1.0, data not shown).

In order to evaluate whether a significant difference could be detected between CD, UC and mixed families with respect to linkage to the IBD1 locus and to investigate the presence of genetic heterogeneity in our IBD sample, we performed the analysis on the following three sub-samples of families: CD

Table 4 Results of combined segregation and linkage analysis for the 82 IBD extended families (122 nuclear families)

Model	q	t	d	S	θ	q_m	t_m	d_m	S_m	$-2lnL+C^a$	Z^b
1. ML dominant	0.0075	5.16	(1)	(0)		(0)				297.84	
2. ML recessive	0.0795	7.53	(0)	(0)		(0)				330.43	
3. ML additive	0.0077	9.55	(0.5)	(0)		(0)				297.72	
4. ML general	0.0077	9.91	0.80	(0)		(0)				297.72	
5. Best 2-loci model	0.0064	7.91	1	(0)		0.0263	10.11	0	(0)	287.82	
6. Best 2-loci model+severity	0.0066	5.59	1	(0)		0.0226	18.30	0	0.84	274.95	
Best linkage (D16S408)											
7. Best ML model	0.0077	5.56	1	(0)	0.0	(0)				278.87	4.14
8. Best 2-loci model	0.0069	4.82	1	(0)	0.0	0.0217	9.47	0	(0)	274.32	2.93
9. Best 2-loci model+ severity	0.0067	5.01	1	(0)	0.0	0.0240	6.65	0	5.07	263.98	2.38
10. ML recessive and modifier dominant	0.0303	8.83	0	1.09	0.11	0.0069	8.52	1	(0)	267.15	1.69

ML=major locus; fixed parameters in parentheses; under model 9, 87% of the affected subjects were estimated to have a at risk genotype.
[a]The constant C is present because calculations relating to ascertainment probability and marker typing are ignored since they are independent of the genetic model; [b]Z is the two-point lod score calculated by taking the difference in $-2ln(L)+C$ between linked and unlinked hypotheses and dividing by $2ln(10)$.

Table 5 Results of combined segregation and linkage analysis for the IBD sub-samples

Sample – ML models	q	t	d	θ	−2lnL+C	Z
CD-only families						
1. ML dominant	0.0080	3.73	(1)		83.55	
2. ML recessive	0.0795	7.56	(0)		80.53	
3. ML general	0.0496	9.19	0.27		77.10	
4. ML dominant	0.0082	4.20	(1)	0.0	72.28	2.45
5. ML recessive	0.0795	8.79	(0)	0.10	70.63	2.15
6. ML general	0.0554	10.79	0.05	0.09	67.78	2.02
UC-only families						
7. ML dominant	0.0064	5.37	(1)		19.15	
8. ML recessive	0.0795	7.94	(0)		41.00	
9. ML general	0.0064	5.73	1		19.15	
10. ML dominant	0.0065	5.97	(1)	0.0	12.54	1.44
11. ML recessive	0.0795	8.01	(0)	0.24	39.64	0.30
12. ML general	0.0065	5.97	1	0.0	12.54	1.44
CD/UC families						
13. ML dominant	0.0090	5.60	(1)		192.41	
14. ML recessive	0.0937	5.47	(0)		206.55	
15. ML general	0.0096	9.78	0.72		191.66	
16. ML dominant	0.0092	5.09	(1)	0.04	191.05	0.30
17. ML recessive	0.0944	5.34	(0)	0.33	206.35	0.04
18. ML general	0.0098	10.84	0.44	0.05	190.23	0.31

Number of extended families: CD only=21; UC only=38; CD/UC=23.
Number of nuclear families: CD only=32; UC only=53; CD/UC=37.

only, UC only, and mixed families, where CD and UC affected members were both present. Various models were tested, under both the hypotheses of no linkage and linkage to D16S408 (Table 5).

When the analysis was limited to CD families, the best fit was obtained for the general model with an estimated dominance parameter, d, of 0.05 and an estimated recombination fraction of 0.09 with a corresponding lod score of 2.02 (Table 5, model 6). Co-existence of families favouring a dominant or a recessive model in the CD sample might result in an overestimation of the recombination fraction for D16S408 when considering all the CD families jointly. In the analysis of the UC families a single dominant locus provided the best fit and positive indication of linkage was obtained at marker D16S408 under the dominant hypothesis, corresponding to a lod score of 1.44 at a recombination rate of 0.0 (Table 5, model 12). In the mixed families, where affected subjects were both CD and UC, a lod score of 0.30 only was obtained with the dominant model. The two-loci models did not fit significantly better than the major locus models in IBD sub-samples (data not shown). No significant evidence for the presence of heterogeneity could be detected (all *P* values > 0.05).

Discussion

This study, based on combined segregation and linkage analysis of 82 extended Italian families, provides a genetic model for IBD, CD, and UC, insights into their relationship, and elucidates the role of the IBD1 locus in their aetiology.

Considering CD and UC jointly, significant evidence for a two-loci model with a recessive and a dominant mode of inheritance was obtained and the inclusion of severity information allowed us to ascribe to CD, the more severe class, a recessive mode of inheritance. Furthermore, considering CD and UC families separately, evidence for the presence of a major gene with an estimated dominance parameter close to the recessive case was obtained for CD, whereas evidence for a dominant major gene was obtained for UC.

Two-point linkage tests performed in the IBD1 region gave evidence for linkage to marker D16S408 for the single dominant model (lod score of 4.14). In the two-loci hypotheses, both putative major genes were found to be linked to D16S408 when assuming either a dominant or a recessive major gene linked to the marker locus. Considering the IBD sub-samples separately, a lod score of 2.02 and a lod score of 1.44 were obtained for marker D16S408 in the CD and UC samples, respectively.

These results suggest that both putative major genes are located close to D16S408 in the IBD1 region and the possibility that they represent the same gene cannot be ruled out. A common genetic aetiology for both forms of IBD is obtained in our study with respect to the IBD1 locus and it can be postulated that a major gene, involved in both UC and CD aetiology, is present in the IBD1 region close to D16S408. From our analysis we estimated that a single mutation in the gene leads more frequently to UC, whereas a double copy of the mutant allele would result in the more severe CD.

In our previous study[26] a peak multipoint non-parametric linkage score of 2.71 (*P* = 0.0035), corresponding to a lod score of 1.59 (= $2.71^2/2\ln10$) was obtained at the same marker identified in this analysis (namely D16S408),

although evidence of linkage (*P* values < 0.05) was observed over a 27 cM interval of the IBD1 region. Comparison with the previous analysis is not straightforward since in Annese *et al* a non-parametric method based on IBD-sharing in affected relatives was utilised in a reduced sample of families presenting at least two affected subjects (other than the parent–child pair). This approach provides a low resolution mapping, since observed recombinations do not exclude a chromosome region but render it only less likely.

Our results from segregation analysis are concordant with results obtained by Orholm *et al*,[10] where a major recessive gene was found for CD and a major dominant gene was found for UC, although the two traits were analysed separately and linkage was not considered in their study.

Combined segregation and linkage analysis not only allows a more accurate estimation of the genetic parameters, since marker data provide extra information when linkage is present, but also increases the power to detect linkage. In the study of complex traits, when a segregation model cannot be defined with certainty, but the presence of major genes (or oligogenes) is suspected, combined segregation and linkage analysis at specific loci previously mapped by linkage analysis remains an attractive and a powerful approach.

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