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Association of Familial and Sporadic Rheumatoid Arthritis With a Single Corticotropin-Releasing Hormone Genomic Region (8q12.3) Haplotype

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***Objective*. Rheumatoid arthritis (RA) is a common disabling autoimmune disease with a complex genetic component. We have previously described linkage of a region of chromosome 8q12.3 with RA and association of the microsatellite marker CRHRA1 with RA in 295 affected sibling-pair families. In the current study we aimed to physically link the RA-associated marker with the corticotropin-releasing hormone (CRH) candidate gene, and to examine the genomic region for additional short tandem repeat (STR) genetic markers in order to clarify the association with RA.**

***Methods*. We examined the association of 2 STR markers with disease in the original 295 multicase families and in a cohort of 131 simplex families to refine our understanding of this genetic region in disease susceptibility in sporadic and familial RA. Genomic library screening and sequencing were used to generate physical sequences in the CRH genomic region. Bioinformatic analysis of the sequence flanking the CRH structural gene was used to screen for additional STRs and other genetic features. Genotyping was carried out using a standard fluorescence approach. Estimations of haplotype frequencies were performed to assess linkage disequilibrium. The transmission disequilibrium test was performed using TRANSMIT.**

***Results*. Physical cloning and sequencing analyses identified the genomic region linking the CRHRA1 marker and the CRH structural locus. Moreover, we identified a further STR, CRHRA2, which was in strong linkage disequilibrium with CRHRA1 ($P = 4.0 \times 10^{-14}$). A haplotype, CRHRA1*10;CRHRA2*14, was preferentially carried by unaffected parents at a frequency of 8.6% compared with the expected frequency of 3.1%. This haplotype was overtransmitted in the multiply affected families ($P = 0.0077$) and, similarly, in the simplex families ($P = 0.024$). Combined analysis of both family cohorts confirmed significant evidence for linkage ($P = 4.9 \times 10^{-4}$) and association ($P = 5.5 \times 10^{-3}$) for this haplotype with RA.**

***Conclusion*. In demonstrating significant linkage disequilibrium between these 2 markers, we have refined the disease-associated region to a single haplotype and confirmed the significance of this region in our understanding of the genetics of RA.**

Rheumatoid arthritis (RA) is the most common disabling autoimmune disease and is a typical, complex, multifactorial disorder in which as-yet-unknown environmental and incompletely characterized genetic factors interact to cause the condition. Familial aggregation is well recognized and an excess sibling recurrence risk of between 2 and 8 is reported (1–4). Concordance in monozygotic twins (~15%) is ~4 times greater than that in dizygotic twins, highlighting the importance of genetic

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factors in disease susceptibility (5,6). From these data it has been estimated that up to 60% of the population variance in RA may be genetic (7). The association of RA with the HLA region has been known for >20 years (8,9), but family studies suggest that it accounts for only 30–40% of the genetic component of the disease (10). The majority of the genetic component therefore lies outside the major histocompatibility complex, and genetic models of RA suggest that it consists of multiple loci, each with weak effect on disease susceptibility (2,3). On this basis, several genome scans have been performed or are in progress, but so far these have produced relatively inconclusive results (11–14). Several regions of possible/probable linkage have been identified, but few have been replicated and none have been reduced to a disease association, a prerequisite to localizing causal genetic variation.

The candidate gene approach in families has been more rewarding (15–17), providing, in 2 reports, evidence of chromosomal regions of linkage that have been reduced to locus association. The first report is that of the interleukin-1 gene cluster, which, through the use of the combined sib–transmission disequilibrium test (TDT) and TDT, was shown to be linked in the presence of association with erosive RA (16). The second case is our previous report of linkage and association of the corticotropin-releasing hormone (CRH) genomic region with RA (17).

CRH has a pivotal regulatory role in the hypothalamic–pituitary–adrenal (HPA) axis, which is key to the integration of immune inflammatory stimuli and the inflammatory response (18) and therefore is an excellent candidate gene for RA. We previously described moderate linkage of a region of chromosome 8q12.3 with RA and association of the CRH.PCR1 microsatellite marker (herein renamed CRHRA1) in 295 affected sibling-pair (ASP) families. In the current study we aimed to physically link the RA-associated marker with the CRH candidate gene. We assessed the relationship between an additional short tandem repeat (STR) genetic marker in the region and susceptibility to disease, to clarify the significance of this region in RA genetics.

PATIENTS AND METHODS

Patient and family characteristics. Genomic DNA was obtained from 295 multiple-case RA families from the UK Arthritis Research Campaign's national repository of family material and from an additional collection of 131 RA families recruited from the Oxfordshire and Southwest UK regions. This latter cohort consisted of 129 simplex families and 2 ASP families. All patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria for RA (19). For a detailed summary of the pedigree data, refer to Table 1.

Table 1. Summary of families used in association analysis*

	ASP families	Simplex families
Number of families	295	131
Total number of typed affected offspring	640	133
Families with 2 parents typed	36	110
Families with 1 parent typed	43	15
Families with ≥1 unaffected sibling typed	145	16

* ASP = affected sibling-pair.

Genomic sequence characterization. Oligonucleotide probes flanking the CRHRA1 STR marker (previously designated CRH.PCR1 [20]) were used to screen a human RP11 bacterial artificial chromosome (BAC) library. Two positive clones, 366K18 and 766A10 (accession numbers AF277371 and AF277372, respectively), were selected and sequenced using a shotgun strategy (for details, see http://genome.imb-jena.de/). A total of 14 fragments derived from clones 366K18 and 766A10 and 7 fragments from a clone AC021240 sequenced at the Whitehead Institute (http://www-seq.wi.mit.edu/) were assembled using SeqMan version 4.0.1 (DNAStar, London, UK) into a 93-kb segment of contiguous DNA. Visual inspection and application of the Repeatmasker routine in NIX (http://www.hgmp.mrc.ac.uk/NIX/) identified multiple low-complexity repeat sequences with the potential for genetic polymorphism. Polymerase chain reaction (PCR) primers were designed to amplify 4 of these with a repeat copy number of >18. The potential STRs were screened for polymorphism in a panel of 96 white RA patients, using fluorescence-based PCR genotyping. The NIX application suite was used to predict the presence of genes within this region.

PCR conditions. The oligonucleotide primers for the STR microsatellites were synthesized by Amersham-Pharmacia Biotech (Little Chalfont, UK). The microsatellite CRHRA1, which lies 25 kb downstream of the CRH structural gene, was genotyped using previously published primer sequences (17). Oligonucleotide primers for the formerly uncharacterized microsatellite marker CRHRA2 were synthesized as forward, 5′-CAGTTTCCTTGGGCTTCTACAG-3′, and reverse, 5′-AAGTCCCTATCTCCAAAGCAAT-3′. Forward PCR primers were fluorescently labeled with the phosphoramidite dye HEX attached to the 5′ end. PCR amplifications were performed using ABgene 1.1x PCR Master Mix in a total reaction volume of 12.5 μl containing 30 ng of genomic DNA and 4 pmoles of each PCR primer. The ABgene master mix contains 0.1% Tween 20, 0.2 m*M* of each dNTP, 2.5 m*M* $MgCl_2$, and 0.3125 units *Taq* DNA polymerase in a 75 m*M* Tris HCl, 20 m*M* $(NH_4)_2SO_4$ buffer (Advanced Biotechnologies, Leatherhead, UK). All PCR reactions were performed on PE 9700 thermal cyclers (PE Applied Biosystems, Foster City, CA) in 96-well microtiter plates. The thermocycler parameters for the microsatellites consisted of an initial denaturation cycle at 94°C for 4 minutes followed by 27 cycles at 94°C for 30

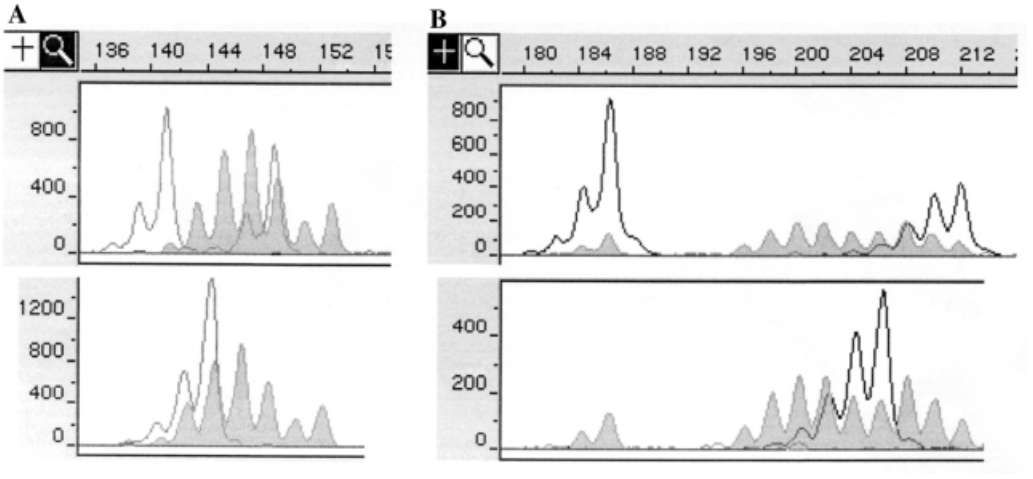

Figure 1. Use of allelic ladders for defining short tandem repeat alleles in family-based genetic association. Correct size assignment of polymorphic microsatellite alleles can be validated by using an "allele-specific ladder" labeled with a different fluorophore. The example here shows the TET-labeled CRHRA1 **(A)** and CRHRA2 **(B)** allele-specific ladders (shaded areas) superimposed on the actual allele peaks (FAM-labeled CRHRA1 and HEX-labeled CRHRA2, respectively; unshaded areas) for 2 individuals (top panels, heterozygote and bottom panels, homozygote). The ladder represents the most abundant alleles in the population, and consistent peak heights allow rapid and accurate identification of alleles across groups of unrelated individuals. The X-axis shows the size of the peaks in basepairs, and the Y-axis shows the relative peak amplitude.

seconds, 55°C for 30 seconds, and 72°C for 60 seconds, and a final extension step at 72°C for 10 minutes.

Genotyping. Semiautomated analysis of microsatellite genotypes was performed using an Applied Biosystems 377 DNA sequencer with GENESCAN analysis software, version 3.1.2 (PE Applied Biosystems). PCR products for both microsatellite markers were combined into a single pool prior to electrophoresis. Electrophoresis was performed on 0.2 mm premixed 5% polyacrylamide gels (Amresco, Solon, OH) run for 2.5 hours at 3,000V with a running temperature of 51°C. DNA fragment analysis software, GENOTYPER version 1.1 (PE Applied Biosystems), was used for semiautomated allele-size assignment in basepairs. The allele size of each PCR product was determined in reference to an internal lane size standard Tamra 500 (PE Applied Biosystems). To increase accuracy and to establish absolute allele-size assignment, samples were pooled with a ladder of common alleles for each of the markers. Genotyping was performed by visualizing the specific marker alleles for each individual (FAM- and HEX-labeled for CRHRA1 and CRHRA2, respectively) overlaid by the TET-labeled marker ladders (see Figures 1A and B) (21). All genotypes were read by 2 independent investigators to ensure accurate allele assignment, and genotyping was repeated on a random 5% of samples for both markers. Marker data were initially processed using the program PedCheck (22) to identify any marker inconsistencies within the pedigrees.

Statistical analysis. Linkage disequilibrium between markers was assessed using the estimating haplotype frequencies (EH) method (23). EH uses an independent sample of unrelated individuals to estimate haplotype frequencies under the assumptions of allelic association and of no allelic association. Association analyses were performed using the TDT (24), which tests for preferential transmission of a specific allele or haplotype, and this family-based method is robust to population stratification. The TDT was implemented using TRANSMIT (25), which uses additional genotype information from unaffected siblings to infer missing parental genotypes and reconstruct multilocus haplotypes. Using all affected siblings provides a test of linkage in the presence of association; a single offspring per family is randomly selected as a pure test of association.

RESULTS

Identification of new polymorphic markers required the compilation of sequence features for the region flanking the CRH structural gene. Because limited sequence information was available for this region of chromosome 8, we used oligonucleotide probes flanking the CRHRA1 STR marker to screen a chromosome 8 BAC library. Two positive clones (accession numbers AF277371 and AF277372) were selected and sequenced using a shotgun strategy, resulting in 19 and 25 unordered fragments for the 2 clones, respectively. The 44 fragments were assembled with 19 fragments of a recently published chromosome 8 BAC clone (accession number AC021240). The final assembly located the CRH structural gene on a 93-kb contiguous segment. Clone sequencing and assembly located the RA-associated STR marker (CRHRA1) 25 kb downstream

Table 2. CRHRA2 allele frequencies in combined affected sibling-pair and simplex families, estimated using TRANSMIT

CRHRA2 allele	Allele size (bp)	Allele frequency
2	182	0.0007
3	184	0.0007
4	186	0.037
5	188	0.0027
6	194	0.004
9	196	0.004
10	198	0.0302
11	200	0.0724
12	202	0.2554
13	204	0.1182
14	206	0.1530
15	208	0.1350
16	210	0.1114
17	212	0.0330
18	214	0.0274
19	216	0.0130
20	218	0.0014

of the CRH translation initiation site. An additional STR marker, CRHRA2, located 20 kb downstream of CRH was found to be highly polymorphic in white patients and was selected for further study. Allele frequencies for this microsatellite are shown in Table 2. Simple-sequence repeats at 26, 18, and 3 kb downstream of the CRH gene showed no polymorphism in our sample (data not shown).

Having established a fully sequenced 93-kb physical contiguous segment that contains a genetic marker associated with RA, we applied the NIX application suite (http://www.hgmp.mrc.ac.uk/NIX/) to predict the presence of genes within this region. Using the known location of CRH, expressed sequence tag (EST) alignment, gene-finding utilities, and CpG-island mapping, we confirmed the presence of the CRH structural locus but found little evidence for additional adjacent genes. Positions of the marker loci relative to the CRH structural gene and other features of the DNA are shown in Figure 2.

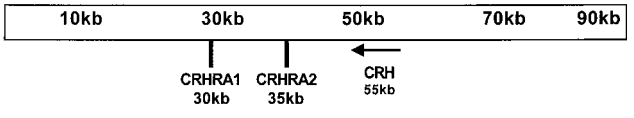

Figure 2. Genomic sequence features for the 93-kb corticotropin-releasing hormone (CRH) flanking region at 8q12.3. The CRHRA1 and CRHRA2 short tandem repeat markers lie 25 kb and 20 kb downstream of the CRH coding region, respectively. MURF-2, a muscle-specific ring finger protein gene, has recently been described in the genomic region downstream of CRH.

The CRHRA1 and CRHRA2 microsatellites were separated by only 5.5 kb of DNA. To clarify any relationship between multiple, closely located markers and a disease, it is important to investigate any linkage disequilibrium between the markers in the relevant population. An independent sample of 323 unrelated parents of RA probands was used to assess linkage disequilibrium between the CRHRA1 and CRHRA2 genetic markers. Significant linkage disequilibrium was observed between CRHRA1 and CRHRA2 ($P = 4.0 \times 10^{-14}$). Nine haplotypes were observed at a frequency of >2.5%, with the 2 most common haplotypes, CRHRA1*11;CRHRA2*12 and CRHRA1*10; CRHRA2*14, having frequencies of 22.2% and 8.6%, respectively. In the latter case, the expected frequency was 3.1%.

We previously demonstrated disease association of CRHRA1 in 295 ASP families, with significant preferential transmission of allele 10 to affected offspring ($P = 4.4 \times 10^{-5}$) (17). We tested for preferential transmission of alleles at the CRHRA2 marker in these families, but no single allele was identified as being overtransmitted. Given the observed linkage disequilibrium, we tested for excess transmission of CRHRA1; CRHRA2 haplotypes to affected offspring in the same families. Pooling rare haplotypes with frequencies of <2.5%, significant transmission distortion was observed ($P = 0.0016$), providing global evidence for linkage in the presence of association with RA. Excess transmission of 2 haplotypes to affected offspring was observed: CRHRA1*10;CRHRA2*14 (observed transmissions 127.5, expected 113.9; $P = 0.008$) and CRHRA1*10; CRHRA2*15 (observed transmissions 50.6, expected 42.0; $P = 0.008$) (see Table 3).

In the second cohort of 131 predominantly simplex families, we tested for preferential allele transmission at each of the STR sites. Allele 10 at CRHRA1 and allele 14 at CRHRA2 showed the highest increase in transmissions to affected individuals, but the increase was not statistically significant due to lack of power (CRHRA1*10 observed 65, expected 60.1; $P > 0.1$ and CRHRA2*14 observed 46, expected 38.5; $P = 0.058$). We assessed transmission distortion of haplotypes in this cohort. The CRHRA1*10;CRHRA2*14 haplotype was again preferentially transmitted to affected offspring (observed transmissions 27.7, expected 21.2; $P = 0.02$), but no increase in transmissions was observed for the lower frequency CRHRA1*10;CRHRA2*15 haplotype (see Table 4).

Combining data from the 2 family collections provided a total sample of 426 families; a single affected

Table 3. Transmissions of CRHRA1;CRHRA2 haplotypes to affected offspring (pooling haplotypes with frequencies <2.5%) in 295 affected sibling-pair families using TRANSMIT*

Haplotype	Observed	Expected	Variance (observed–expected)	Chi-square (1 df)	*P*
11;12	227.3	231.3	49.5	0.33	NS
11;14	36.9	42.3	10.5	2.92	NS
10;14	127.5	113.9	26.0	7.10	7.7×10^{-3}
11;13	70.8	68.1	13.9	0.51	NS
10;13	62.1	60.7	16.2	0.12	NS
11;15	33.0	40.6	10.1	5.70	0.017
10;15	50.6	42.0	10.4	7.09	7.7×10^{-3}
9;15	37.3	37.5	8.8	0.002	NS
11;16	74.0	80.3	20.5	1.96	NS
11;11	39.9	44.0	12.4	1.34	NS

* df = degree of freedom; NS = not significant.

offspring was randomly selected from each family for a true test of association. After pooling rare haplotypes with frequencies of <2.5%, significant global evidence of a disease association with CRHRA1;CRHRA2 haplotypes was observed ($P = 0.027$). There was excess transmission of the common disease-associated haplotype CRHRA1*10;CRHRA2*14 to affected individuals (observed transmissions 89.0, expected 75.0; $P = 0.0014$) (see Table 5). Combined analysis of both family cohorts confirmed significant evidence for linkage ($P = 4.9 \times 10^{-4}$) and association ($P = 5.5 \times 10^{-3}$) for the CRHRA1*10; CRHRA2*14 haplotype with RA. In summary, CRHRA1;CRHRA2 haplotypes are linked to RA in multicase families and the CRHRA1*10; CRHRA2*14 haplotype is significantly linked to and associated with RA in both multiplex families and sporadic cases. The fact that the second, smaller family set has been ascertained in sporadic RA cases suggests that the association is relevant to both familial and sporadic RA. Other than the HLA region, this is the first report of confirmation of genetic linkage and association of a genomic region with RA.

DISCUSSION

The genotyping of polymorphic STR markers in DNA from ASP families, combined with nonparametric linkage analysis, has been an important innovation in the mapping of genetic loci in common complex disorders with a polygenic background. Replication of this type of effect is problematic, and refining the region over which linkage has been detected to variation, which is disease-associated, is a major challenge (26). In this study, we were able to confirm our earlier finding of linkage and association of a single CRHRA1*10 microsatellite allele with RA and to extend this by physically mapping the marker on a 93-kb DNA contiguous segment containing the CRH gene. Our knowledge of the genomic sequence allowed us to isolate an

Table 4. Transmissions of CRHRA1;CRHRA2 haplotypes to affected offspring (pooling haplotypes with frequencies <2.5%) in 131 simplex families using TRANSMIT*

Haplotype	Observed	Expected	Variance (observed–expected)	Chi-square (1 df)	*P*
11;12	67.2	61.7	21.9	1.37	NS
11;14	8.6	9.0	4.0	0.05	NS
10;14	27.7	21.2	8.4	5.07	0.024
11;13	12.3	9.0	3.8	2.94	NS
10;13	11.6	10.9	5.0	0.08	NS
9;13	7.3	9.5	3.7	1.37	NS
11;15	17.2	19.5	7.9	0.65	NS
11;16	7.3	10.2	4.2	2.01	NS
10;16	10.2	10.5	4.5	0.02	NS
11;11	12.1	12.6	5.7	0.06	NS
10;15	11.6	10.9	5.0	0.08	NS

* See Table 3 for definitions.

Table 5. Transmissions of CRHRA1;CRHRA2 haplotypes to a single affected offspring per family in the combined set of 426 families using TRANSMIT (pooling haplotypes with frequencies <2.5%)*

Haplotype	Observed	Expected	Variance (observed–expected)	Chi-square (1 df)	*P*
11;12	173.8	172.1	54.1	0.05	NS
11;14	26.6	28.2	10.1	1.23	NS
10;14	89.0	75.0	25.2	10.17	1.4×10^{-3}
11;15	29.0	27.7	10.4	0.14	NS
10;15	37.3	31.7	11.6	2.01	NS
9;15	26.7	27.5	9.7	2.21	NS
11;13	41.8	41.1	13.7	1.60	NS
10;13	35.6	37.0	13.1	0.06	NS
11;16	55.0	57.3	21.2	0.92	NS
11;11	28.5	31.8	12.2	2.79	NS

* See Table 3 for definitions.

additional STR marker, CRHRA2. Analysis of this marker alone revealed no significant overtransmission, in contrast to that seen with CRHRA1. The associated CRHRA2*14 allele had a lower frequency than the CRHRA1*10 allele (15.3% versus 24.8%) and the study therefore had less power to detect transmission distortion of this allele. Analysis of both CRHRA1 and CRHRA2 revealed extensive linkage disequilibrium between the markers and a single haplotype associated with RA.

Rather than simply replicating the association of the CRHRA1*10 allele with RA in ASP families, we were first able to refine the region of association in these families by improving the resolution of our analysis. However, these findings were in the same population group as that used to obtain the original result, and therefore we examined the same markers in a separate collection of RA families. Our finding of the same disease-associated haplotype in this latter cohort supports the original findings and encourages confidence in the importance of this region in RA.

Replication of these findings in a further independent family collection would be welcome. We previously estimated the λ_s for CRH to be 1.14; the size of the sample chosen for replication will therefore be critical if a true effect is not to be missed. Despite notable successes, the approach of nonparametric linkage is relatively insensitive for the detection of polygenic loci (27,28). Case–control association studies can be more powerful but may be vulnerable to artifacts due to population stratification, and this in addition to phenotypic variation and genetic heterogeneity are all possible explanations for the lack of replication/confirmation studies to date in this and other complex diseases.

The association of the same haplotype with disease in both familial and sporadic cases of RA indicates the significance of this region even in cases that might have been considered "less genetic" by virtue of being nonfamilial. Similar findings have been reported in other complex diseases where loci contribute to both familial and sporadic cases of disease, for example, the HLA–DR locus in multiple sclerosis (29), the IDDM18 locus in type 1 diabetes mellitus (30), and a locus in the 2q37 region in systemic lupus erythematosus (31). In contrast, there are many examples of familial forms of complex diseases, such as breast cancer (32) and Parkinson's disease (33), in which the familial disease loci do not appear to play a direct role in the general population. As more complex disease loci are identified and adequately powered studies are carried out, the generality of these findings will become clearer.

Disease-associated variation can be assumed to be either causal for the phenotype in question or in linkage disequilibrium with the causal variation. Optimal strategies for the reduction of complex disease to causal-associated polymorphism have to be assessed empirically but will rely on availability of DNA sequences in the region and the evaluation of polymorphism at markers across the region. Ultimately, biologic systems that read out function directly due to the DNA polymorphisms themselves will hold the key to this dilemma. The RA-associated CRHRA1*10;CRHRA2*14 haplotype could either be causal for disease or in linkage disequilibrium with the causal variation. Although the average extent of linkage disequilibrium in typical human populations is currently controversial (34,35), the 20–25-kb distance between the associated STR markers and the CRH candidate gene is well within the range of several established linkage disequilibria in white populations.

Our analysis of the sequence of the 93-kb contiguous segment suggests that the only gene present in the region is the CRH gene itself. The only CpG islands located in this region were associated with the CRH

gene, and although CpG islands are found in only 50% of mammalian genes (36), exon-finding programs (such as Grail, Genefinder, Genemark, Fex, Hexon, and Fgene) which are run using the masked sequence have shown only a marginal level of confidence for exons in regions apart from CRH. This finding, together with the relative lack of ESTs mapping to sites apart from CRH itself, makes it likely that the disease-associated variation identified by us is in linkage disequilibrium with the CRH gene or its regulatory regions. Since we have been unable to identify further polymorphic microsatellites in the region, we have reached the limit of resolution provided by these markers in this area.

The next level of informative variation is that provided by single nucleotide polymorphisms (SNPs). Characterization of genetic variation in the CRH gene and flanking regions is currently being determined using heteroduplex analysis and direct DNA sequencing. This will allow the construction of more extensive haplotypes. Once the full extent and nature of linkage disequilibrium are known and a functional assay is developed, it will be possible to make further progress in understanding the role of this region in the pathogenesis of disease. The fact that a single, relatively common haplotype appears to be responsible for the disease risk associated with this genomic region raises the possibility that the causative risk factor is a single allele at the critical locus.

The case for further characterizing polymorphic variation in this region is the excellent position of CRH as a candidate gene for RA. RA is a disease in which the pathogenesis is poorly understood but is clearly immune-mediated and inflammatory, associated with chronic synovial inflammation, cartilage erosion, and joint destruction. CRH has a pivotal regulatory role in the HPA axis, where it promotes the release of pituitary adrenocorticotropin that, in turn, stimulates the adrenal cortex to produce cortisol, a powerful, endogenous antiinflammatory agent. This axis is key to the integration of immune inflammatory stimuli and the inflammatory response (18), and has been shown to be deficient in RA patients (37). Animal models also lend support to the importance of a competent HPA axis in determining the onset and course of arthritis (38–41). It is quite plausible, therefore, that genetic variation in this region may contribute to susceptibility to RA by virtue of an inability of an individual to offer an appropriate response to an inflammatory stimulus.

To summarize, we have established a fully sequenced, 93-kb physical contiguous segment that contains 2 polymorphic genetic markers, CRHRA1 and CRHRA2. These are in strong linkage disequilibrium. The haplotype CRHRA1*10;CRHRA2*14, which was observed at a frequency of 8.6% in our study population, is associated with RA and is the best indicator of RA risk in this region. Further analysis of these markers, and additional SNPs, will enable us to determine the full extent of linkage disequilibrium around the CRH locus and to map the effect of the 8q12.3 RA association more precisely.

Addendum. Since submission of this article, MURF-2, a muscle-specific ring finger protein gene, has been described in the genomic region downstream of CRH. It is our view that the protein it encodes is unlikely to play a role in the development of RA.

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