

Linkage map of the short arm of human chromosome 11: Location of the genes for catalase, calcitonin, and insulin-like growth factor II

(linkage analysis/DNA polymorphism/recombination/gene mapping)

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ABSTRACT The following order of genes on the short arm of human chromosome 11 (11p) was determined previously: parathyroid hormone (*PTH*)—the β -globin gene cluster (*HBBC*)—*HRAS1*/insulin. Although it is generally agreed that *HRAS1* (formerly termed *c-Ha-ras-1*) and the insulin gene are close to each other [1–4 centimorgans (cM)], their order on chromosome 11p is still in question. We have now added three other genes, those for catalase, calcitonin, and insulin-like growth factor II (*IGF-II*), to this map of chromosome 11p by use of restriction site polymorphisms adjacent to these genes in classical linkage analysis. Most importantly, we find no evidence of linkage between the catalase and *HBBC* loci. In addition, our data indicate that the calcitonin gene is located between the catalase gene and the *PTH* gene. Our best estimate of the distance between the catalase and calcitonin genes is ≈ 16 cM, while that between the calcitonin and *PTH* genes is ≈ 8 cM. In agreement, very loose linkage was found between the catalase and *PTH* loci (≈ 26 cM). Since the catalase locus has been mapped to 11p13, these data support the view that the *PTH*, *HBBC*, *HRAS1*, and insulin loci are located on the distal short arm of chromosome 11. The *IGF-II* gene is tightly linked to both the *HRAS1* oncogene and the insulin gene since no recombinants were observed between the *IGF-II* and the *HRAS1*/insulin loci. Thus, based on our linkage analysis we propose that the most likely gene order for the short arm of chromosome 11 is centromere–catalase–calcitonin–*PTH*–*HBBC*–*HRAS1*/insulin–telomere and that the *IGF-II* gene is very close to both the *HRAS1* and the insulin genes.

Several genes such as the human β -globin gene cluster (*HBBC*), the parathyroid hormone (*PTH*) gene, the oncogene *HRAS1* (formerly termed *c-Ha-ras-1*), and the insulin gene have been assigned to the short arm of chromosome 11 (1–5). By using DNA polymorphisms for linkage analysis, it has been shown that *PTH*, *HBBC*, *HRAS1*, and the insulin gene are closely linked to each other (6, 7). While we have reported that *HRAS1* is close, but proximal, to the insulin gene, White *et al.* (8), with more extensive data, have placed *HRAS1* 1–2 centimorgans (cM) distal to the insulin gene (8). Meanwhile, considerable controversy has arisen regarding localization of these genes on the short arm of chromosome 11 (11p). Some investigators have suggested that the *HBBC*, insulin, and *HRAS1* genes are located at the 11p15 region (9–12), while others have placed the β -globin and insulin genes toward the centromere and *HRAS1* proximal to 11p15 (13–16). Although

most of the recent evidence favors the more distal localization of these genes, their physical position on 11p has not been conclusively determined.

Human erythrocyte catalase converts hydrogen peroxide to oxygen and water in a reaction that protects the erythrocyte membrane from oxidizing agents such as H_2O_2 and various free radicals (17). The catalase gene and the Wilms tumor-aniridia-genitourinary abnormalities-mental retardation complex (*WAGR*) have been mapped to 11p13.05-p13.06 by analysis of gene dosage in individuals with various deletions of 11p13 (18, 19). Bruns *et al.* (20) used synthetic oligonucleotide mixtures to isolate catalase cDNA and showed an absence of the gene in chromosome 11 variants containing deletions of 11p13. These chromosomes were isolated from two unrelated individuals with aniridia-Wilms tumor and one individual with the *WAGR* complex (20).

Calcitonin is a 32-amino acid polypeptide hormone secreted by parafollicular or C cells of the thyroid gland (21). Its main biological effect appears to be inhibition of bone resorption by decreased number and activity of bone-resorbing osteoclasts (21). The calcitonin gene has been mapped to the short arm of chromosome 11 by using DNA of somatic cell hybrid lines and *in situ* hybridization (22, 23).

Insulin-like growth factor II (*IGF-II*) is a member of the insulin family, which is comprised of insulin, relaxin, insulin-like growth factors I and II, and possibly the β subunit of 7S nerve growth factor (24, 25). *IGF-II* is a single-chain serum protein of 67 amino acids that is synthesized by the liver and possibly by other tissues (26). There is considerable sequence homology between precursors of human insulin, *IGF-I*, *IGF-II*, and relaxin (27, 28). The *IGF-II* gene was reported to be located on chromosome 11 by restriction analysis of DNA from somatic cell hybrids and by *in situ* hybridization (29, 30).

By using DNA polymorphisms adjacent to the catalase, calcitonin, and *IGF-II* genes in classical linkage analysis, we have mapped the locations of these genes relative to the mapped genes on chromosome 11p, *PTH*, *HBBC*, *HRAS1*, and insulin. Here we report that catalase is loosely linked to the *PTH*, *HBBC*, *HRAS1*, and insulin genes. These data support the view that the *PTH*, *HBBC*, *HRAS1*, and insulin loci are located on the distal short arm of chromosome 11. We also report that the calcitonin gene lies between the catalase and *HBBC* loci and that the *IGF-II* gene is closely linked to the insulin and *HRAS1* loci (see Fig. 1).

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Abbreviations: kb, kilobase; *PTH*, parathyroid hormone; *HBBC*, β -globin gene cluster; *IGF-II*, insulin-like growth factor II; cM, centimorgan; *HRAS1*, *C-Ha-ras-1*.

METHODS

Subjects. Our subjects were Greek, Italian, Asian Indian, Chinese, and American Black couples who were at risk for β -thalassemia or sickle cell anemia. Their offspring and, in some cases, their relatives were also studied. In addition, linkage analysis was carried out using DNA from individuals of large nuclear Utah families obtained from the Mutant Cell Repository (31) and other Caucasian nuclear families collected for linkage analysis. A total of 17 families consisting of 119 individuals was studied. Of these, 11 were two-generation and 6 were three-generation families. These families were typed for 10–13 different DNA polymorphisms and no instance of nonpaternity was observed.

Restriction Endonuclease Analysis of Genomic DNA. Nuclear DNA was isolated from leukocytes contained in 10–15 ml of EDTA-anticoagulated blood or from cultured lymphoblastoid cells contained in one small tissue culture flask (32). Five micrograms of DNA was digested with one of various endonucleases used under conditions recommended by the commercial suppliers. Southern transfer analysis of the resulting DNA fragments was performed as described (33, 34).

Radioactive Probes. The following probes were used: (i) catalase, a 2.1-kilobase (kb) *Pst* I cDNA fragment (35); (ii) calcitonin, an 860-base-pair (bp) *Msp* I cDNA fragment (36); (iii) *IGF-II*, a 713-bp *Pst* I cDNA fragment (37); (iv) *PTH*, an 800-bp *Hpa* II cDNA fragment (38); (v) insulin, a 900-bp *Pst* I genomic DNA fragment (39); (vi) *HBBC*, genomic and cDNA fragments previously used (40, 41) containing sequences of the γ -, $\psi\beta_1$ -, and β -globin genes as well as sequences flanking these genes; and (vii) *HRAS1*, a 6.6-kb *Bam*HI genomic fragment (42).

All fragments were radiolabeled with [³²P]dATP and [³²P]dCTP by the nick-translation function of *Escherichia coli* DNA polymerase I (43).

Linkage Analysis. Linkage analysis was carried out using the method of maximum likelihood (44) and the computer program LIPED (45). Lod scores were calculated at various recombination fractions, where the lod for each recombination fraction represents the logarithm of the odds in favor of linkage versus nonlinkage. When the physical location of two loci is not known by other methods, a lod score of 3 (odds of 1000:1 in favor of linkage) is considered strong evidence of linkage. The map of 11p was constructed based on these two-point lod scores. MacLean *et al.* (46) have recently shown this to give an accurate representation of the order of the loci in question under almost any circumstance. Since there were few multiply heterozygous parents, multipoint analysis was not performed (see *Discussion*).

RESULTS

A Polymorphic *Ava* II Site in the Catalase Gene Region. When we digested genomic DNA with *Ava* II and then hybridized the fragments with the catalase probe, we observed catalase sequences of various individuals in 4.3- or 2.2-kb fragments. Three types of patterns were observed: homozygotes for the 4.3-kb fragment, homozygotes for the 2.2-kb fragment, and heterozygotes for both fragments. In addition to the 4.3-kb fragment that results from absence of the polymorphic *Ava* II site, an invariant 4.3-kb fragment was observed in all individuals, including those who were homozygotes for the 2.2-kb fragment. When we digested DNA of 20 unrelated individuals with *Msp* I, *Hgi*AI, *Rsa* I, *Taq* I, *Nco* I, *Sst* I, *Bam*HI, *Xba* I, *Hind*III, *Hinc*II, *Hin*I, *Mbo* II, *Bgl* I, *Pvu* II, *Eco*RI, and *Eco*RV and then hybridized the fragments with the catalase probe, no DNA polymorphisms were identified.

DNA from individuals of several ethnic groups was analyzed for the presence (+) or absence (–) of the *Ava* II polymorphic restriction site. The frequency of the + allele in Greeks and Italians, Chinese, and American Blacks was 0.33, 0.21, and 0.14 in 76, 18, and 14 chromosomes examined, respectively.

A Polymorphic *Msp* I Restriction Site in the *IGF-II* Gene Region. Similarly, when we digested genomic DNA with *Msp* I and then hybridized the fragments to the *IGF-II* probe, we observed *IGF-II* sequences of various individuals in 1.05- and 0.9-kb fragments. Again, three types of individuals were observed: homozygotes for the 1.05-kb fragment, homozygotes for the 0.9-kb fragment, and heterozygotes for both fragments.

The frequency of the presence of the *Msp* I site (+ allele) in Greeks and Italians, Asian Indians, Chinese, and American Blacks was 0.61, 0.75, 0.90 and 0.97 in 41, 28, 30, and 28 chromosomes examined, respectively.

Construction of a Linkage Map of 11p. To determine the linkage between the catalase, calcitonin, *IGF-II*, *PTH*, *HBBC*, *HRAS1*, and insulin genes, the following polymorphisms were used: (i) *Ava* II and *Kpn* I for the catalase gene (G. Bruns, personal communication), (ii) *Taq* I for the calcitonin gene (22), (iii) *Msp* I for the *IGF-II* gene, (iv) *Pst* I for the *PTH* gene (6), (v) *Hind*III for the γ - and $\psi\beta_1$ -globin genes, *Hinc*II for the $\psi\beta_1$ -globin sequence, *Hin*I, *Ava* II, and *Bam*HI for the β -globin gene (47), (vi) *Sst* I for the insulin gene (48), and (vii) *Msp* I for the *HRAS1* gene (40). Data obtained on the recombination fractions between these loci were added to our previously reported data for the *PTH*, *HBBC*, *HRAS1*, and insulin loci (6, 7).

As shown in Table 1, no recombinants were found between the *IGF-II* and insulin genes in 14 chances and between the *IGF-II* and *HRAS1* genes in 22 chances, giving lod scores for these two pairs of loci of 3.00 and 6.53, respectively, at $\hat{\theta} = 0.00$. Thus, tight linkage was found between the *IGF-II*, insulin, and *HRAS1* genes. Analysis of seven informative families for both *IGF-II* and *HBBC* showed one recombinant in 23 chances, giving a lod score of 3.27 at $\hat{\theta} = 0.05$. Two recombinants were found in 13 chances between *IGF-II* and *PTH* for a lod score of 0.3 at $\hat{\theta} = 0.22$. A small number of families were informative for both *IGF-II* and calcitonin. No linkage was found between these two loci.

Calcitonin was found to be ≈ 8 centimorgans (cM) from the *PTH* gene ($\hat{\theta} = 0.08$, lod score = 3.30), 10 cM from the *HBBC* locus ($\hat{\theta} = 0.10$, lod score = 3.50), and 25 cM from the *HRAS1* gene ($\hat{\theta} = 0.25$, lod score = 0.44). For these three pairs of loci the results were based on a minimum of three or four recombinants in 23 to 31 total chances for recombination (Table 1). Two-point analysis of the recombination distance between the calcitonin and insulin genes yielded $\hat{\theta} = 0.14$, lod score = 2.51 with two recombinants in 21 chances. However, given the gene order proposed below, two of the 19 nonrecombinant chromosomes were shown by analysis of intermediate loci to have two recombinants between the calcitonin and insulin loci.

The best estimate of recombination distance between the catalase and calcitonin genes is 16 cM (4 recombinants in 25 chances, $\hat{\theta} = 0.16$, lod score = 1.28). In nine informative families for both the catalase and *PTH* genes, 9 recombinants were found in 36 chances ($\hat{\theta} = 0.26$, lod score = 0.54). Thirteen recombinants in 40 chances were found between the catalase and *HBBC* loci ($\hat{\theta} = 0.40$, lod score = 0.06), whereas 10 recombinants in 34 chances were found between catalase and *HRAS1* ($\hat{\theta} = 0.35$, lod score = 0.42). Thus, we have little to no evidence for linkage between the catalase locus and the *PTH*, *HBBC*, and *HRAS1* loci. From our data on these families, we propose the most likely order of these genes on chromosome 11p to be centromere–catalase–calcitonin–

Table 1. Linkage analysis between the catalase, calcitonin, *PTH*, *HBBC*, *HRAS1*, insulin, and *IGF-II* loci

Loci	Families	Rec/T	lod score at θ					$\hat{\theta}$	lod score
			0.05	0.10	0.20	0.30	0.40		
Catalase/insulin	6	9/32	-3.21	-1.06	0.47	0.76	0.50	0.25	0.81
Catalase/ <i>HRAS1</i>	9	10/34	-5.74	-3.26	-0.31	0.37	0.41	0.35	0.42
Catalase/ <i>HBBC</i>	9	13/40	-5.09	-2.61	-0.69	-0.08	0.06	0.40	0.06
Catalase/ <i>PTH</i>	9	9/36	-2.11	-0.55	0.42	0.48	0.23	0.26	0.54
Catalase/calcitonin	4	4/25	0.56	1.09	1.18	0.82	0.29	0.16	1.28
Calcitonin/insulin	4	2/21	1.67	2.35	2.38	1.75	0.80	0.14	2.51
Calcitonin/ <i>HRAS1</i>	6	4/23	-1.44	-0.31	0.41	0.43	0.18	0.25	0.44
Calcitonin/ <i>HBBC</i>	7	3/29	3.08	3.50	3.06	2.02	0.83	0.10	3.50
Calcitonin/ <i>PTH</i>	7	3/31	3.13	3.22	2.47	1.38	0.44	0.08	3.30
<i>IGF-II</i> /insulin	4	0/14	1.83	1.61	1.16	0.70	0.25	0.00	3.00
<i>IGF-II</i> / <i>HRAS1</i>	6	0/22	5.17	4.59	3.36	2.04	0.77	0.00	6.53
<i>IGF-II</i> / <i>HBBC</i>	7	1/23	3.27	3.01	2.17	1.24	0.40	0.05	3.27
<i>IGF-II</i> / <i>PTH</i>	5	2/13	-0.37	0.05	0.26	0.21	0.08	0.22	0.29
<i>IGF-II</i> /calcitonin	3	3/9	-2.44	-1.59	-0.79	-0.37	-0.13	—	—
<i>IGF-II</i> /catalase	4	2/8	-1.17	-0.52	-0.07	0.04	0.03	0.35	0.05

Lod scores for chosen recombination fractions for each pair of loci are shown, as well as the maximum estimate of the recombination fraction ($\hat{\theta}$) and its corresponding lod score. Lod scores appear less than expected because the majority of the families were phase unknown. Rec/T, minimum number of recombinant offspring/total number of informative offspring.

PTH-*HBBC*-*HRAS1*/insulin-telomere. The *IGF-II* locus appears to be close to both the *HRAS1* and insulin loci (Fig. 1).

DISCUSSION

In this paper we have made no effort to reevaluate the *HRAS1*-insulin gene order. Our previous data (7) and that of White *et al.* (8) are in agreement that these loci are very close to one another on 11p. However, the sample set of White *et al.* that places *HRAS1* 1-2 cM distal to insulin is more extensive than the sample set previously reported by us.

Here we have added three other loci—catalase, calcitonin, and *IGF-II*—to the map of chromosome 11p. The key question we addressed is the relationship of two of these loci, calcitonin and catalase, to the known *PTH*-*HBBC*-*HRAS1*/insulin linkage group. Since good evidence of linkage was found between the calcitonin locus and the *PTH* and *HBBC* loci and no evidence of linkage was found between the catalase locus and the *PTH* and *HBBC* loci, it is most likely that the gene order is catalase-calcitonin-*PTH*-*HBBC* (Fig. 1). Further suggestive evidence for linkage between catalase and calcitonin in a small number of families also agrees with this order. Since there was only one family informative for

the three loci, catalase-calcitonin-*HBBC*, and only one recombinant child in this family, formal three-point analysis would add little to these results especially since these loci are not tightly enough linked for interference to be a major factor. It has been recently shown that, even though the support for a given order based on two-point results is often considerably less than that obtained from multipoint analyses, the order is still the same (46). Our best estimate of map distance places calcitonin 16 cM from catalase, while calcitonin and *PTH* map 8 cM apart. Our data provide further evidence suggesting that *PTH*-*HBBC*-*HRAS1*/insulin and *IGF-II* are located distal to 11p13, where catalase has been localized by mapping using interstitial deletions on chromosome 11p (19, 20) and support recent *in situ* hybridization data and analyses of translocations that have placed these loci at 11p15 (9-12).

It is interesting that *IGF-II* is very tightly linked to *HRAS1* and insulin and, therefore, these three genes can be treated as one locus in subsequent linkage analyses. Since there is significant sequence homology between the insulin and the *IGF-II* genes, it is possible that *IGF-II* is actually situated in a gene cluster or family that includes the insulin gene.

Yunis and Soreng (50) have suggested the existence of a fragile site at 11p14.2 following treatment of cultured fibro-

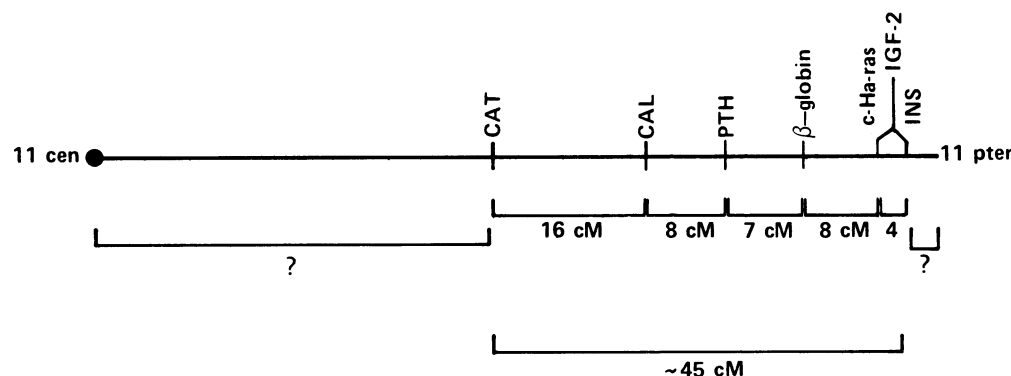


FIG. 1. Linkage map for human chromosome 11p. Genetic distances in cM found between catalase, calcitonin, *PTH*, *HBBC*, *HRAS1*, insulin, and *IGF-II* loci are shown. (*HBBC* is designated β -globin and *HRAS1* is designated C-Ha-ras.) One centimorgan is defined as the genetic distance equivalent to a recombination fraction $\hat{\theta}$ of 0.01. The distances between *PTH*, *HBBC*, *HRAS1*, and insulin genes given are those previously reported by us (6, 7). White *et al.* (8) have found 11.6 cM between *HBBC* and insulin, 12.5 cM between *HBBC* and *HRAS1*, and 3.2 cM between insulin and *HRAS1*. Gerhard *et al.* (49) have found 9 cM between *HBBC* and insulin and 10 cM between *HBBC* and *HRAS1*.

blasts with 2 mM caffeine. In addition, Szabo *et al.* (51) have suggested that there is a high rate of meiotic recombination across the fragile X site (Xq27-Xq28). We find that the genetic distance between catalase and the *HBBC* locus is greater than that which might be expected from the physical distance between 11p13 and the proximal region of 11p15. One can speculate that the inducible fragile site at 11p14.2 may give rise to an increased recombination rate in this region of the genome. Further study, including the demonstration of fragile sites on particular chromosome 11 variants that show increased rates of recombination, will be necessary to prove this hypothesis.

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