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Genetics

β-Globin locus is linked to the parathyroid hormone (PTH) locus and lies between the insulin and PTH loci in man

(DNA polymorphism/linkage analysis/gene mapping/chromosome 11p)

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ABSTRACT **Using a parathyroid hormone (PTH) cDNA probe we found a common *Pst* I polymorphic restriction site 3′ to the PTH gene in all ethnic groups examined. Because the PTH, insulin, and β-globin loci have been localized to the short arm of chromosome 11 (11p) we used DNA polymorphisms adjacent to each of these three loci to determine whether they are genetically linked and to determine their order. We found that the PTH and β-globin loci are closely linked (estimated recombination fraction, 0.07; 95% confidence limits, 0.05–0.10; lod score, 4.63; odds favoring linkage, 42,000:1). Furthermore, our findings strongly indicate that the β-globin gene cluster lies between the PTH and insulin loci. Therefore, the gene order on 11p is centromere–PTH–β-globin–insulin.**

Parathyroid hormone (PTH) is an 84-amino acid polypeptide that plays a major role in calcium homeostasis. Mature PTH is produced by two sequential enzymatic cleavages of a 115-amino acid precursor polypeptide, pre-pro-PTH (1). Human PTH cDNA and genomic fragments have been cloned and the sequences determined (2, 3). By using somatic cell hybrids this gene has been localized to the short arm of chromosome 11 (11p) (4). Among other genes localized on 11p are the β-globin gene cluster (11p12.05–11p12.08) (5), insulin gene (11p13–11p15) (6–8), and the c-Ha-*ras*-1 from human bladder carcinoma cell lines EJ and T24 (9, 10).

To determine the genetic distance between the PTH, β-globin, and insulin loci we used polymorphic restriction sites adjacent to each gene as markers in a classical linkage analysis. Our results indicate that the PTH and β-globin loci are tightly linked, but the PTH and insulin genes are not. Furthermore our data indicate that the order of these loci on 11p is centromere–PTH–β-globin–insulin.

METHODS

Subjects. Our subjects were Greek, Italian, Asiatic Indian, Chinese, and American Black couples who were at risk for β-thalassemia or sickle cell anemia. Their offspring and, in some cases, other relatives were also studied. In addition, linkage analysis was carried out on one large nuclear family. For the purpose of linkage analysis a total of 33 families were studied of which 18 (17 nuclear families and 1 three-generation family) were informative.

Restriction Endonuclease Analysis of Genomic DNA. Nuclear DNA was isolated from the leukocytes contained in 10–15 ml of EDTA-anti-coagulated blood (11). Five to 10 μg of DNA was digested with one of various restriction endonucleases using conditions recommended by the commercial suppliers. Southern blot analysis of the resulting DNA fragments was done as described (12, 13).

Radioactive Probes. The following probes were used. (*i*) An 800-base-pair (bp) *Hpa* II cDNA fragment derived from the recombinant plasmid pPTHm122 containing human PTH sequences (2). (*ii*) A 900-bp *Pst* I genomic DNA fragment derived from the recombinant plasmid pHIG900 containing human insulin gene sequences (14). (*iii*) Genomic and cDNA fragments previously described (15, 16) containing sequences of the ε-, γ-, ψβ$_1$-, and β-globin genes as well as sequences flanking the β-globin gene.

All fragments were radiolabeled with [^{32}P]dATP and [^{32}P]dCTP by the nick-translation function of *Escherichia coli* DNA polymerase I (17).

Experiments involving recombinant DNA were performed in P$_1$–EK$_1$ containment in accordance with the National Institutes of Health guidelines.

Linkage Analysis. Linkage analysis was carried out using the method of maximum likelihood (18) and the computer program LIPED (19). 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. A lod score of 3 (odds of 1,000:1 in favor of linkage) is generally considered strong evidence of linkage; a lod score of −2 (100:1 against linkage) is considered strong evidence against linkage (18).

To determine the most likely order of the three loci, multipoint analysis was done using the method of maximum likelihood (20, 21). The maximum-likelihood estimates of the three recombination fractions (and the corresponding likelihood for each of the three possible orders) are calculated simultaneously from the two-point data (all families informative for any two of the loci) and the three-point data (families informative for all three loci).

RESULTS

A Polymorphic *Pst* I Restriction Site in the PTH Gene Region. After DNA was digested with *Pst* I and hybridized to the PTH cDNA probe, we observed PTH sequences of various individuals in 2.2- or 2.7-kilobase (kb) fragments (Fig. 1*A*). Three types of patterns were observed—homozygotes for the 2.2-kb fragment, homozygotes for the 2.7-kb fragment, and heterozygotes for both fragments. Three lines of evidence indicate

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Abbreviations: kb, kilobase(s); bp, base pair(s); PTH, parathyroid hormone.

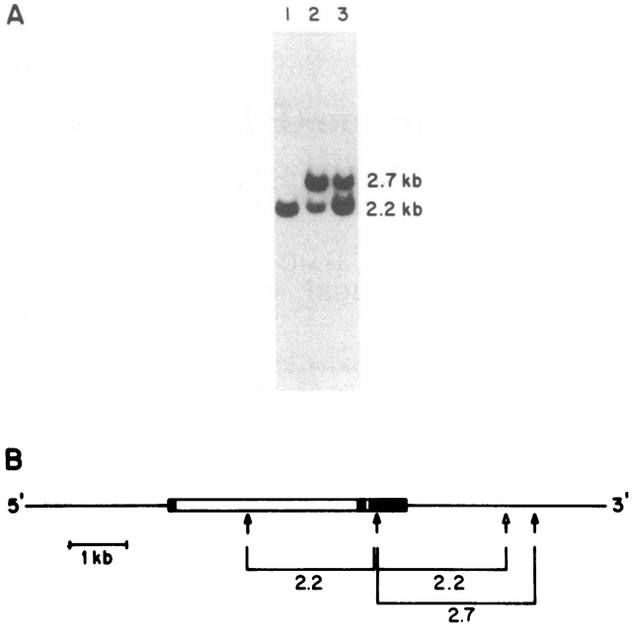

FIG. 1. (*A*) Autoradiogram of restriction endonuclease patterns produced by the *Pst* I polymorphism in the PTH gene. Genomic DNA was digested with *Pst* I and hybridized with the PTH cDNA. Lanes: 1, homozygote for the 2.2-kb fragment; 2, homozygote for the 2.7-kb fragment; 3, heterozygote for the 2.2- and 2.7-kb fragments. (*B*) Diagram of cleavage fragments produced by the presence or absence of the *Pst* I polymorphic restriction site. Black boxes represent the exons of the PTH gene; white boxes represent intervening sequences. The constant *Pst* I sites detected by the probe are shown by closed arrowheads; the polymorphic *Pst* I site is indicated by an open arrowhead.

that the 2.2- and 2.7-kb fragments result from a restriction site polymorphism. (*i*) Identical patterns are obtained using a 10-fold excess of enzyme, (*ii*) segregation patterns in more than 40 families are completely consistent with Mendelian inheritance, and (*iii*) constant cleavage patterns of other endonucleases exclude a large deletion or insertion near the PTH gene. In addition to the 2.2-kb fragment, which results from the presence of the polymorphic *Pst* I site, an invariant 2.2-kb fragment also hybridizes to the PTH cDNA. This invariant 2.2-kb fragment is observed in all individuals including those who are homozygous for the 2.7-kb fragment (Fig. 1). DNA from both types of homozygotes was digested with *Pst* I/*Bgl* II, *Pst* I/*Hin*dIII, or *Pst* I/*Eco*RI, and the polymorphic *Pst* I site was localized to the 3′ flanking region of the gene. These results indicate that the *Pst* I site in exon 3 is invariant and that the constant 2.2-kb fragment is derived mainly from the first intervening sequence (Fig. 1*B*). The presence of the polymorphic *Pst* I site leads to a 2.2-kb fragment; its absence produces a 2.7-kb fragment. When genomic DNAs from 10 unrelated individuals were individually digested with *Bam*HI, *Bgl* II, *Eco*RI, *Hin*cII, *Hin*dIII, *Hpa* I, *Sst* I, *Taq* I, and *Xba* I and hybridized with the PTH cDNA probe, no DNA polymorphisms were identified.

Frequency of the *Pst* I Polymorphism in Various Racial and Ethnic Groups. The *Pst* I polymorphic site near the PTH gene is found in all racial groups examined, which suggests that it may have occurred prior to the divergence of the human races. The frequency of the presence of this polymorphic site in the populations examined is shown in Table 1. Among a total of 234 PTH alleles, this frequency is 0.37. However, this frequency varies from 0.25 to 0.54 in the different ethnic groups for which significant data exist. The high frequency of the *Pst* I polymorphism suggested that it could serve as a useful marker of the PTH structural gene in an effort to map this gene in relation to other genes on 11p.

Table 1. Frequency of the presence of the polymorphic *Pst* I site 3′ to the PTH gene in various groups

Ethnic group	No. of chromosomes examined	Site present	Frequency
Greek	47	12	0.25
Italian	39	12	0.31
Asian Indian	29	10	0.34
American Black	52	28	0.54
Southeast Asian	8	5	0.62
Chinese	12	7	0.58
Other	47	12	0.26
Total	234	86	0.37

Construction of a Linkage Map on Chromosome 11p. Lebo *et al.* (22) have reported that the insulin gene is linked to the β-globin gene cluster with a recombination frequency of 0.14 and a lod score of 2.6. To determine whether the PTH gene is linked to the β-globin or insulin loci (or both) and to create a linkage map including the three loci, we used polymorphic markers for each of the loci to perform classical linkage analysis by the method of maximum likelihood. The following polymorphic restriction sites were studied. (*i*) *Pst* I sites 3′ to the PTH gene; (*ii*) *Hin*dIII sites in the γ-globin genes, *Hin*cII sites flanking the $\psi\beta_1$ gene, *Hin*fI, *Ava* II, and *Bam*HI sites adjacent to the β-globin gene (15, 23–25); and (*iii*) the polymorphic insertions 5′ to the insulin gene (26–28). In almost all the families examined previous studies done for prenatal diagnosis of various β-globin gene disorders had identified polymorphisms that enabled differentiation of parental β-globin alleles (29). Table 2 shows the results obtained for the polymorphic markers near the PTH and insulin genes. The majority of families were informative for linkage between PTH and β-globin, insulin and β-globin, or insulin and PTH loci. Several families were informative for all three loci of interest.

As shown in Table 3, the lod scores at various recombination fractions indicate that the PTH and β-globin loci are closely linked. The maximum likelihood estimate of the recombination fraction is $\hat{\theta} = 0.07$ (95% confidence limits, 0.05–0.10) with a lod score of 4.63, and the odds in favor of linkage are 42,000:1. In our study the best estimate of the recombination fraction between insulin and β-globin is $\hat{\theta} = 0.11$ (95% confidence limits, 0.07–0.13) with a lod score of 3.57 in agreement with the report of Lebo *et al.* (22) that these two loci are also linked. Finally, our data indicate that even though the insulin and PTH loci are syntenic, they are either very loosely linked or not linked at all [$\hat{\theta} = 0.25$ (95% confidence limits, 0.16–0.38) with a lod score of 0.16].

A linkage map for the three loci was derived from the data in Table 3 and from recombinants observed in three informative families (3, 13, 17 of Table 2). Using the method of multipoint analysis developed by Meyers (20), we found that the most likely arrangement of these loci is PTH–β-globin–insulin (Fig. 2). The odds in favor of this arrangement (the β-globin locus in the middle) versus either the insulin or PTH loci in the middle are 891:2:1.

DISCUSSION

Several genes have been assigned to the short arm of chromosome 11 by *in situ* hybridization or analysis of somatic cell hybrids. The β-globin gene cluster has been assigned to 11p12.05–11p12.08 (5), the insulin gene to 11p13–11p14 (8) or

Table 2. DNA polymorphisms flanking the PTH and insulin genes of members of 18 families whose β-globin alleles had been previously characterized

Family	Locus	PGF	PGM	MGF	MGM	F	M	F_1-1	F_1-2	F_1-3	F_1-4	F_1-5	F_1-6
1	β					AA	BT	AT	AT	AT			
	PTH					−−	−+	−+	−+	−+			
	Insulin					1 1	2 3	1 3	1 3	1 3			
2	β					AT	BS	AS	ST				
	PTH					+−	−−	+−	−−				
	Insulin					1 2	2 2	1 2	2 2				
3	β					AE	BT	AB	AB				
	PTH					−−	+−	+−	−−				
	Insulin					2 2	1 2	1 2	1 2				
4	β					AT	BH	TH	AB	AH			
	PTH					−+	−−	+−	−−	−−			
	Insulin					3 3	2 1	2 3	1 3	1 3			
5	β					AT	BC	CT	BT	BT			
	PTH					−−	+−	+−	−−	+−			
	Insulin					2 3	2 2	2 3	2 2	2 2			
6	β					AT_1	BT_2	AB	BT_1	T_1T_2			
	PTH					−−	+−	+−	+−	−−			
	Insulin					2 1	2 1	2 2	2 1	1 1			
7	β					AT	BC	CT	AB	AB	AC	AC	
	PTH					−−	+−	−−	−+	−+	−−	−−	
	Insulin												
8	β	AB	CD	EF	GH	BD	GF	GD	GD				
	PTH	−−	−+	−−	−−	−+	−−	−+	−+				
	Insulin	1 2	2 2	1 3	2 2	2 2	2 3	2 2	2 2				
9	β					AT_1	BT_2	AB	BT_1				
	PTH					+−	+−	+−	−−				
	Insulin					2 2	1 2	1 2	1 2				
10	β					AT_1	BT_2	T_1T_2	AT_2				
	PTH					+−	++	+−	++				
	Insulin					2 3	3 3	2 3	3 3				
11	β					AT	AT	TT	AT	AA			
	PTH					+−	+−	−−	+−	++			
	Insulin												
12	β					AT_1	BT_2	BT_1	T_1T_2				
	PTH					+−	−−	+−	−−				
	Insulin												
13	β					AB	CS	AS	AS				
	PTH					+−	+−	++	++				
	Insulin					1 2	1 2	1 2	2 2				
14	β					AT_1	BT_2	BT_1	T_1T_2				
	PTH												
	Insulin					1 3	2 3	1 3	1 2				
15	β					AT_1	BT_2	T_1T_2	AB				
	PTH												
	Insulin					1 3	2 3	2 3	1 3				
16	β					AS_1	BS_2	AS_2	S_1S_2				
	PTH												
	Insulin					1 1	1 2	1 2	1 1				
17	β					AB	CT	BC	AC	BT	AT		
	PTH					−+	−+	++	−−	++	−+		
	Insulin					1 4	2 3	1 2	1 2	3 4	1 3		
18	β					AB	CB	BB	AB	AC	BB	AB	AB
	PTH					+−	−−	−−	+−	+−	−−	+−	+−
	Insulin					2 2	1 2	1 2	1 2	2 2	1 2	1 2	1 2

PGF, paternal grandfather; PGM, paternal grandmother; MGF, maternal grandfather; MGM, maternal grandmother; F, father; M, mother; F_1-1 through F_1-6, children 1 through 6. For the β-globin gene (β) cluster A, B, C, etc. denote different β alleles. For the PTH gene + and − indicate the presence or absence of the polymorphic *Pst* I site. For the insulin gene, 1, 2, 3, and 4 denote different polymorphisms detected after digestion with *Sac* I or *Bgl* I. When data are not provided for a locus, father and mother are homozygous for the polymorphisms at that locus.

11p15–11pter (6), and the PTH locus to 11p11–11pter (4). In this study we have constructed a linkage map for these three loci using DNA polymorphisms. Originally it was proposed that a lod score of 3 was necessary to show linkage (18), but this was based on a prior probability of synteny of ≈0.05 (two markers taken at random in the population have a probability of 1/22 of being syntenic). However, in this instance, the PTH and β-globin loci are syntenic (4, 5) and have a good prior probability for linkage. Therefore, in the case of known synteny, a lod score of about 2.0 provides convincing evidence of linkage. Our lod score is 4.63 for linkage between the PTH and β-globin loci and 3.57 for linkage between the β-globin and insulin loci.

Table 3. Linkage analysis between the β-globin–PTH, β-globin–insulin, and PTH–insulin loci

Locus	θ 0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	$\hat{\theta}$	lod score	95% confidence limits
PTH–β-globin	4.43	4.52	4.12	3.51	2.81	2.08	1.37	0.73	0.07	4.63	0.05–0.10
β-Globin–insulin	3.02	3.48	3.32	2.90	2.36	1.76	1.17	0.63	0.11	3.57	0.08–0.13
PTH–insulin	−1.10	−0.30	0.01	0.13	0.16	0.14	0.09	0.05	0.25	0.16	0.16–0.38

For each pair of loci the lod scores for different values of recombination fraction (θ) and the estimated value of θ ($\hat{\theta}$) and its corresponding maximum lod score are shown.

It is also known that the estimate of the recombination fraction that gives the largest lod score is in most cases higher for females than for males (30, 31). In our study there was no significant difference in recombination rate between males and females for any of the loci examined.

By using DNA polymorphisms, Lebo *et al.* (22) found that the recombination distance between the β-globin and insulin loci is about 14 centimorgans with a lod score of 2.6. If we combine our data with the data of Lebo *et al.* (22), the recombination fraction between the two loci is about $\hat{\theta} = 0.12$ with a lod score of about 6.0. Because *in situ* hybridization data (6, 7) suggests that the insulin locus is distal to the β-globin locus and our data indicate the gene order is PTH–β-globin–insulin, it follows that the PTH locus is located nearest the centromere (Fig. 2).

Chromosome 11p contains about 1.52% of the total chromosomal length in man (32). Because the human genome is about 3,000 centimorgans (33), we calculate that chromosome 11p contains ≈50 centimorgans. The three loci discussed here cover an area of ≈18 centimorgans or one-third of the theoretical map distance contained on 11p. Further studies involving other loci on 11p may demonstrate linkage with one or more of these markers. For example, the c-Ha-*ras*-1 gene from the bladder carcinoma cell lines T24 and EJ (9, 10), the locus affecting the amount of F reticulocyte production (34, 35), the catalase locus (36), the lactate dehydrogenase A locus (37, 38), and several anonymous single-copy DNA sequences from 11p (39) may be localized relative to the linkage map of Fig. 2.

The *Pst* I polymorphic site adjacent to the PTH gene region could also be useful in studying familial types of isolated hypoparathyroidism, because it can serve as a marker to differentiate between the two PTH alleles in the parents of informative families with affected members. For certain families one could use the polymorphic restriction site to determine if a particular PTH allele was associated with the disease phenotype. This type of analysis has proven very useful in elucidating the molecular basis of β-thalassemia (40) in which DNA polymorphisms in the β-globin gene cluster have enhanced the search for unrecognized mutants. In analogy to β-thalassemia this approach could be used to select certain individual samples for DNA sequence analysis to clarify the PTH gene alteration responsible for deficiency of PTH production.

Note Added in Proof. Since the submission of this manuscript, the linkage map on chromosome 11p has been improved by the localization of the c-Ha-*ras*-1 oncogene very close to the insulin gene (41, 42).

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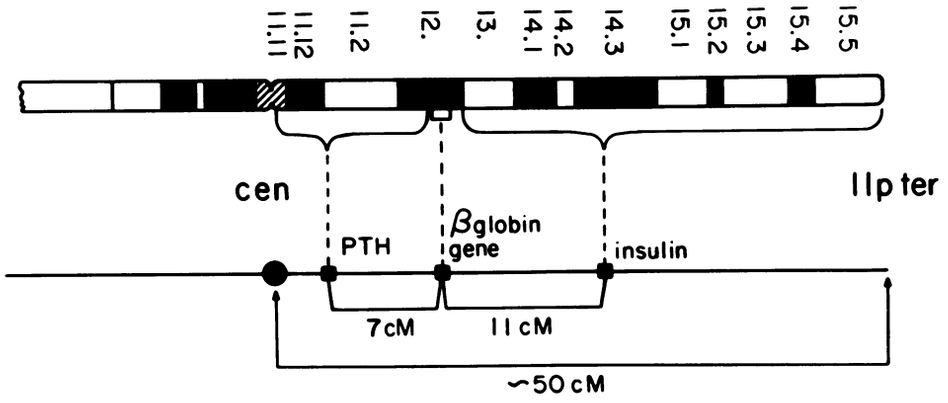

FIG. 2. Linkage map for the short arm of human chromosome 11 (11p) indicating the map distances in centimorgans (cM) between the PTH, β-globin, and insulin loci. This linkage map is shown below an ideogram of chromosome 11p. The chromosomal localizations of these loci are indicated.

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